Treatment Effects in Pediatric Soft Tissue and Bone Tumors

Practical Considerations for the Pathologist

Cheryl M. Coffin, MD, Amy Lowichik, MD, PhD, and Holly Zhou, MD

Key Words: Sarcoma; Treatment effects; Therapy-induced cytodifferentiation; Osteosarcoma; Ewing sarcoma; Infantile fibrosarcoma; Rhabdomyosarcoma; Synovial sarcoma

Abstract

Dramatic improvements in survival for children with cancer have led to increased numbers of posttreatment pathologic specimens, particularly in bone and soft tissue sarcomas. Current therapeutic protocols in North America require specific pathologic classification and stratify patients based on clinical, biologic, and pathologic features. For osteosarcoma, the pathologic response to therapy predicts prognosis and modifies the treatment regimen. Ongoing studies aim to assess the response to therapy and outcome in other types of soft tissue and bone tumors. The pathologic evaluation of pretreatment and posttreatment specimens is critical for therapeutic decisions and prognostic assessment. A standardized approach to posttherapy pathologic specimens, with attention to appropriate use of ancillary tests, and assessment of clinical and biologic significance of therapy-induced pathologic changes has significance for patient management and treatment protocols.

During the past few decades, development of new therapeutic regimens for childhood bone and soft tissue sarcomas has resulted in improved survival for patients with osteosarcoma and other bone and soft tissue neoplasms. Ongoing clinical trials focus on therapeutic optimization and minimization of acute and late treatment effects. Key therapeutic decisions incorporate therapy-induced pathologic features. The pathologic interpretation of posttherapy specimens, including biopsy and resection specimens of the primary tumor following chemotherapy and radiation therapy, and biopsy specimens of suspected metastatic disease are seen by pathologists with increasing frequency. Significant challenges are encountered in these specimens, especially when there are reactive changes associated with tumor necrosis, maturation of immature elements that mimic or obscure persistent malignancy, cytodifferentiation without a clear understanding of biologic potential, or postoperative changes compounding the effects of chemotherapy and irradiation. Determining the prognostic significance of treatment-induced pathologic changes relies on careful macroscopic and microscopic evaluation, specimen triage, and interpretation of pathologic findings.

This review discusses the pathologic evaluation of posttreatment specimens among patients with common bone and soft tissue sarcomas of childhood, adolescence, and young adulthood. These include osteosarcoma, Ewing sarcoma/primitive neuroectodermal tumor (EWS/PNET), rhabdomyosarcoma, synovial sarcoma, and infantile fibrosarcoma. Optimal handling of the gross specimen, histologic assessment and differential diagnosis, and prognostic correlation and potential biologic significance of therapy-associated changes are presented.
Osteosarcoma

Osteosarcoma, the most common nonhematopoietic bone malignancy, is a high-grade malignant tumor in which the neoplastic cells produce osteoid.4 It is the prototype for evaluation of therapy-associated changes in sarcomas.5 The peak frequency of osteosarcoma occurs during the second decade of life; 60% of patients are younger than 25 years at diagnosis, and there is a male predilection. The favored sites are the metaphyses of long bones, although osteosarcoma can arise in any bone. The diagnosis is based on the identification of osteoid associated with malignant cells. A variety of classifications have been developed for osteosarcoma, and the histologic subtypes are codified in the current World Health Organization classification of bone and soft tissue tumors.4,6-8 Generally, osteosarcoma is classified as a high-grade malignant neoplasm, except for paraosteal, periosteal, and low-grade intrasosseous variants. The disease-free survival has improved greatly in recent decades, with 60% to 80% disease-free survival with use of multidisciplinary therapy that incorporates surgery and chemotherapy.4

At present, the most significant indicator of prognosis for osteosarcoma is the histologic response to preoperative chemotherapy, although other factors, such as age, sex, and tumor location, size, and stage have been included among prognostic determinants.5,8-17 Among the high-grade osteosarcomas, the fibroblastic and telangiectatic variants are more responsive than osteoblastic and chondroblastic osteosarcoma.18-20 However, despite its poor histologic response, recent studies indicate that the chondroblastic variant of high-grade osteosarcoma is associated with better long-term survival.18,19

Altered P-glycoprotein has been associated with resistance to chemotherapy and treatment failure, and some studies have indicated that P-glycoprotein positivity by immunohistochemical analysis in pretreatment specimens correlates with more rapid disease progression, a higher relapse rate, and a worse outcome, although a meta-analysis questioned the usefulness of this test.21,22

Current treatment for high-grade osteosarcoma includes biopsy followed by neoadjuvant chemotherapy, with radiologic imaging to assess tumor response and subsequent surgical resection of the treated primary tumor.11,23 A comprehensive, systematic, pathologic evaluation of the posttreatment resection specimen yields prognostic information about chemotherapeutic response and predicts potential for disease-free survival. When the pathologic response to treatment is poor, different chemotherapeutic protocols may be used.8,24,25 Grading systems for the pathologic assessment of histologic response to preoperative chemotherapy are summarized in Table 1.

The pathologic examination requires meticulous gross dissection, description, and histologic evaluation, with detailed specimen mapping. The classification of the osteosarcoma, evaluation of tumor margins, measurement of tumor dimensions, assessment of the extent of tumor, and evaluation of the percentage of necrosis after chemotherapy are required and have been summarized in various publications.5,8,24-26,30

When in doubt about the orientation and location of margins, review of the intact fresh specimen with a surgeon is essential.5 Soft tissue margins are sampled, and the pretreatment

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Grading Systems for Treated Osteosarcomas*</th>
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<tr>
<td>Reference</td>
<td>Grades</td>
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<tr>
<td>Huvos et al26</td>
<td>Viable</td>
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<tr>
<td></td>
<td>Partially necrotic</td>
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<tr>
<td></td>
<td>Largely necrotic</td>
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<td></td>
<td>Totally necrotic</td>
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<tr>
<td>Rosen et al13</td>
<td>I. Little or no effect of chemotherapy</td>
</tr>
<tr>
<td></td>
<td>II. Partial response, &lt;50% necrosis, some viable tumor</td>
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<tr>
<td></td>
<td>III. &gt;90% of tumor necrosis, foci of viable tumor</td>
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<tr>
<td></td>
<td>IV. No viable-appearing tumor cells</td>
</tr>
<tr>
<td>Raymond et al8</td>
<td>Percentage of necrosis estimated quantitatively</td>
</tr>
<tr>
<td>Picci et al27</td>
<td>Good: &gt;90% necrosis</td>
</tr>
<tr>
<td></td>
<td>Fair: 60%-90% necrosis</td>
</tr>
<tr>
<td></td>
<td>Poor: &lt;60% necrosis</td>
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<tr>
<td>Wold25</td>
<td>I. No effect</td>
</tr>
<tr>
<td></td>
<td>II. Some necrosis</td>
</tr>
<tr>
<td></td>
<td>A. &gt;50% viable tumor remaining</td>
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<td></td>
<td>B. 5%-50% viable tumor remaining</td>
</tr>
<tr>
<td></td>
<td>III. Scattered foci; &lt;5% viable tumor remaining</td>
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<tr>
<td></td>
<td>IV. No viable tumor</td>
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* Reprinted with permission from Lowichik et al.5
surgical biopsy site with skin and soft tissue is removed from the specimen and evaluated histologically. The pretreatment biopsy tract must be sampled for the presence or absence of malignancy. Joint and synovial surfaces should be inspected and sampled if “suspicious” areas are identified. Muscle widely free of the tumor and grossly uninvolved margins distant from the microscopic tumor do not require histologic evaluation.

After the external features have been examined, the bone can be cut longitudinally and examined in more detail. The section should include the 2 greatest diameters of the tumor mass. Radiologic correlation is useful to direct sectioning in the plane of tumor growth. After the initial cross-section has been cut with a saw, the margins are evaluated, and parallel cross-sections are made. Two complete cross-sections, 3.0 to 5.0 mm thick, are obtained from the longitudinally cut specimen. The extent and appearance of the tumor are documented, including the distance of the tumor from both resection margins, the size in 3 dimensions, and the presence of cortical breakthrough, soft tissue extension, direct medullary extension, involvement of epiphyseal cartilage or joint capsule, and skipped areas of tumor elsewhere in the bone. The cross-sections are fixed and decalcified, and one is selected for histologic examination and photographed or photocopied intact. The entire cross-section is submitted for histologic evaluation, and a section map is drawn onto the photograph or photocopy to document the sites of microscopic sections. At the time of surgery, a separate sample of the closest resection margin frequently is submitted as a frozen section and provides information to the surgeon for intraoperative and subsequent management.

Histologic evaluation of treated osteosarcoma focuses on the response of the tumor to treatment. Image 11. This is expressed as percentage of necrosis, with a 4-part grading system.25,29,30 The Children’s Oncology Group osteosarcoma protocol requires this type of assessment of the percentage of necrosis. Chemotherapy-induced necrosis of 90% or more has a greater than 90% disease-free survival, compared with less than 15% in patients with less than 90% necrosis.8

Qualitative assessment of the percentage of necrosis correlates well with quantitative morphometric analysis. The biggest challenge to the surgical pathologist is the interpretation of small foci of atypical cells with hyperchromatic nuclei, smudged and clumped chromatin, and vacuolated cytoplasm in a background of necrosis, calcification, or fibrosis.8,24,25 These individual atypical cells usually occupy only a small proportion of the sections. Although they are difficult to identify definitively as neoplastic or reactive, the current approach is to conservatively interpret them as “viable” tumor cells for purposes of grading and management. Larger areas of recognizable osteosarcoma, areas of complete necrosis, and focal bone and cartilage formation or increased mineralization are categorized more easily. Other therapy-related changes include ghost cells with loss of nuclear and cytoplasmic detail, granularity tissue, fibrosis, hemosiderin deposition, and inflammation.

Key elements of the pathology report for postchemotherapy osteosarcomas are summarized in Table 21. These provide information essential for prognosis and ongoing clinical management. Recent studies correlating dynamic contrast-enhanced magnetic resonance imaging with the pathologic percentage of necrosis offer a promising future method for estimating the necrotic fraction and monitoring treatment.31

**Ewing Sarcoma/Primitive Neuroectodermal Tumor**

EWS/PNET is a round cell sarcoma with varying degrees of neuroectodermal differentiation and a common cytogenetic and molecular abnormality involving the EWS gene and various chromosomal translocations.4 The most frequent chromosomal translocation is a t(11;22)(q24;q12). EWS/PNET is the second most common soft tissue sarcoma of the first 2 decades of life and also is a common bone sarcoma. While osseous lesions can occur throughout the skeleton, soft tissue EWS/PNET most frequently arises in deep truncal soft tissues.32 The diagnosis is made on the basis of histologic and immunohistochemical studies, with demonstration of a membranous staining pattern for CD99 in the majority of EWS/PNET cases and nuclear staining for the Fli-1 protein, which is overexpressed in approximately 70% of cases. Reactivity with these antibodies is not entirely specific.

The current overall survival rate for EWS/PNET is estimated at 41%,4 although survival of greater than 60% has been reported with multimodality therapy, including surgical resection with or without radiation therapy and chemotherapy.33 Prognostic factors include stage, anatomic location, tumor size, and EWS/Fli-1 fusion status. Histologic subtypes do not have major prognostic significance, although the filigree growth pattern has an association with a poorer prognosis.34-36

Recent studies of patients with EWS/PNET treated with contemporary regimens have documented a correlation between histopathologic degree of posttherapy tumor necrosis and patient survival,20,33,36-49 but a number of other studies did not reveal a correlation between histologic tumor necrosis after treatment and survival, which might have been related to specific previous treatment protocols.50-56 Table 31. Correlations between the radiographic and the pathologic responses to chemotherapy using computed tomography, magnetic resonance imaging, and other techniques have yielded variable results.36,41,44,57-60

The macroscopic appearance of treated osseous EWS/PNET may reveal predominantly reparative features, including bony widening and sclerosis, hemorrhage, necrosis, and cystic degeneration, with no grossly visible residual.
Osteosarcoma. A. Osteosarcoma of the distal femur shows irregular expansion of bone by a sclerotic mass with erosion of the cortex and extension into soft tissue. B. Hyperchromatic pleomorphic tumor cells infiltrate the bone (H&E, ×200). C. Tumor cells are associated with bright pink osteoid (H&E, ×400). D. Osteosarcoma cells show pleomorphism, hyperchromasia, and mitoses (H&E, ×400). E. Treated osteosarcoma contains blue spiculated bone and residual tumor cells (H&E, ×100). F. Treated osteosarcoma has areas of complete necrosis and dystrophic calcification simulating fungal structures (H&E, ×400). D, E, and F reprinted with permission from Lowichik et al.5

Image II Osteosarcoma. A. Osteosarcoma of the distal femur shows irregular expansion of bone by a sclerotic mass with erosion of the cortex and extension into soft tissue. B. Hyperchromatic pleomorphic tumor cells infiltrate the bone (H&E, ×200). C. Tumor cells are associated with bright pink osteoid (H&E, ×400). D. Osteosarcoma cells show pleomorphism, hyperchromasia, and mitoses (H&E, ×400). E. Treated osteosarcoma contains blue spiculated bone and residual tumor cells (H&E, ×100). F. Treated osteosarcoma has areas of complete necrosis and dystrophic calcification simulating fungal structures (H&E, ×400). D, E, and F reprinted with permission from Lowichik et al.5
tumor.\textsuperscript{5} Thorough sampling similar to that described for osteosarcoma is critical for microscopic detection of persistent tumor cells. The various grading systems for EWS/PNET vary in sampling requirements. Posttherapeutic specimens of extrasosseous EWS/PNET show macroscopic changes similar to those of osseous tumors. In addition to complete sampling of the cut surface of the tumor to assess for residual histologically recognizable tumor cells, attention to inked surgical margins is essential.\textsuperscript{5,32}

Several grading systems have been published for the histologic evaluation of postchemotherapy resections of EWS/PNET \textbf{Table 2}. A modification of the Huvos Grading System for osteosarcoma estimates the percentage of necrosis per tumor area.\textsuperscript{26,61} The Salzer-Kuntschik Grading System for osteosarcoma also has been applied to EWS/PNET.\textsuperscript{62} Picci and colleagues\textsuperscript{40} proposed an alternative grading system that samples areas more likely to contain recognizable residual tumor, including subperiosteum, soft tissue masses overlying osseous tumors, and hemorrhage foci, and this system estimates the amount of residual EWS/PNET irrespective of area of volume. A comparison of the Huvos and Picci Grading Systems found that results were similar for the 2 approaches.\textsuperscript{39} More recent studies using the Picci criteria\textsuperscript{48} or subdividing treated EWS/PNET in tumors with 100% necrosis or less than 100% necrosis\textsuperscript{49} also have confirmed the prognostic significance of the histopathologic response to induction chemotherapy.

Histologic findings in treated EWS/PNET include necrosis, inflammatory infiltrates, myxoid stromal change, decreased cellularity, and a pseudoalveolar architecture of residual tumor mimicking alveolar rhabdomyosarcoma \textbf{Image 21}. Neural differentiation has been reported in a few cases and is characterized by rosette formation, ganglion-like cells, the presence of neuropil, and a nesting pattern similar to neuroblastoma.\textsuperscript{63-65} A single case also manifested rhabdoid morphologic changes. The EWS/Fli-1 fusion transcript has been documented in pretreatment and posttreatment specimens in a single case with neural differentiation.\textsuperscript{65} Human EWS/PNET xenografts in athymic mice treated with all-trans-retinoic acid and/or interferon alfa have displayed altered nuclear/cytoplasmic ratios, calcification, and necrosis.\textsuperscript{66}

\begin{table}[h]
\centering
\caption{Correlations of Histologic Response to Chemotherapy of Ewing Sarcoma/Peripheral Neuroectodermal Tumor*}
\begin{tabular}{|l|l|l|l|}
\hline
Primary Tumor Location & Patients With Metastases Included & Histologic Grading System & Significant\textsuperscript{*} Correlation Between Histologic Features and Patient Survival \\
\hline
Bone & No & Picci\textsuperscript{47} & Yes; grade I vs II or III \\
Various & No & Picci\textsuperscript{47} & No; grade II vs III \\
Various & Yes & Salzer-Kuntschik\textsuperscript{44} & Yes; grade I vs II or III \\
Various & Yes & Salzer-Kuntschik\textsuperscript{43} & Yes; grade I vs II-VI and grades I-III vs IV-VI \\
Pelvis & Yes & Salzer-Kuntschik\textsuperscript{48} & Yes; grades I and II vs IV-VI \\
Various & Yes & Huvos\textsuperscript{42} & No; grades I-III vs IV-VI \\
Various & No & Salzer-Kuntschik\textsuperscript{46} & No; grades I-III vs IV-VI \\
Extremities & No & Salzer-Kuntschik\textsuperscript{47} & Yes; grades I and II vs III and IV \\
Various & No & Salzer-Kuntschik\textsuperscript{37} & Yes; grades I-III vs IV-VI \\
Rib & No & Salzer-Kuntschik\textsuperscript{56} & No; grades I-III vs IV-VI \\
Various & No & Picci\textsuperscript{41} & Yes; grades I and II vs III \\
Extremities & No & Picci\textsuperscript{46} & Yes; grade II and III \\
Not stated & Not stated & Huvos, Picci\textsuperscript{39} & Yes (no P values given); Huvos grades I and II vs III and IV; yes; Picci grade I vs III \\
Various & Yes & Huvos\textsuperscript{33} & Yes; grades I and II vs III and IV \\
Extremities & No & Picci\textsuperscript{47} & Yes; grade I vs II \\
Various & No & Salzer-Kuntschik\textsuperscript{38} & Yes; grades I-III vs IV-VI \\
\hline
\end{tabular}
\end{table}

\begin{table}[h]
\centering
\caption{Diagnostic Checklist for Treated Osteosarcoma*}
\begin{tabular}{|l|}
\hline
Type of resection \\
Amputation \\
Limb salvage \\
Specimen site (anatomic location) and size \\
Tumor characteristics \\
Tumor site \\
Tumor size (length plus 2 diameters) \\
Distance from tumor to margins \\
Relationship of tumor to: \\
Epiphyseal cartilage \\
Joint capsule \\
Cortex \\
Soft tissue \\
Skip metastases \\
Intravascular tumor \\
Lymph node involvement \\
Microscopic features: \\
Percent necrosis, with grade of chemotherapy \\
Status of margins \\
Comparison with pretreatment surgical pathology specimen, including histologic type \\
Specimen map \\
Results of ancillary or special studies \\
\hline
\end{tabular}
\end{table}

* Reprinted with permission from Lowichik et al.\textsuperscript{5}
At present, there is not a standardized approach for the pathologic evaluation of treated EWS/PNET. It is our practice to evaluate the tumor and provide information similar to the recommendations for osteosarcoma, to obtain ancillary immunohistochemical and cytogenetic or molecular genetic studies if indicated, and to compare the pretreatment and post-treatment specimens.

### Rhabdomyosarcoma

Rhabdomyosarcoma is a primitive malignant soft tissue sarcoma that recapitulates the phenotypic and biologic features of skeletal muscle and has 2 principal subtypes, embryonal and alveolar. The spectrum of embryonal rhabdomyosarcoma encompasses spindle cell and botryoid variants. Alveolar rhabdomyosarcoma is the most common pediatric and adolescent soft tissue tumor, accounts for about half of pediatric soft tissue sarcomas, and occurs during the first decade of life in more than 50% of cases.

Although a number of classifications have been published during past decades, the International Classification of Rhabdomyosarcoma currently is used. The botryoid and spindle cell subtypes of embryonal rhabdomyosarcoma have a highly favorable prognosis, and embryonal rhabdomyosarcoma, not otherwise specified, has an intermediate prognosis. Alveolar rhabdomyosarcoma, in contrast, has a poor prognosis. Features associated with outcome in addition to histologic type include primary tumor site, stage or clinical group, and age at diagnosis. Although the overall survival for rhabdomyosarcoma exceeds 65%, very late relapses have been reported.

Alveolar rhabdomyosarcoma has characteristic translocations involving chromosomes 1 or 2 and chromosome 13. These translocations are diagnostic and predictive of outcome. These translocations may be detected by a variety of molecular genetic techniques. Reverse transcriptase–polymerase chain reaction (RT-PCR) may be useful for monitoring treatment of alveolar rhabdomyosarcoma and detection of minimal residual disease, but it is not known whether terminally differentiated posttreatment tumors harbor the translocation and what the significance of its detection by RT-PCR in this setting might be.

Rhabdomyosarcoma treatment is stratified according to primary tumor site, patient age, histologic type, and stage or clinical group of disease, with an emphasis on organ preservation. Chemotherapy is delivered after biopsy of the primary tumor, and the treatment response is monitored by radiographic and pathologic methods. Interpretation of therapy-induced cytodifferentiation, determination of the clinicopathologic

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**Table 4**

<table>
<thead>
<tr>
<th>Author</th>
<th>Method</th>
<th>Grade</th>
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<tbody>
<tr>
<td>Huvos et al&lt;sup&gt;26&lt;/sup&gt; and Huvos&lt;sup&gt;61&lt;/sup&gt;</td>
<td>Specimens cut longitudinally in plane of tumor growth; gross abnormalities noted on anatomic sketch, along with map of histologic sections</td>
<td>I. Little or no chemotherapy effect; II. Area of acellular tumor osteoid, necrotic, and/or fibrotic material attributable to chemotherapy effect, with other areas of histologically viable tumor; III. Predominant areas of acellular tumor, osteoid, necrotic, and/or fibrotic material attributable to chemotherapy effect with only scattered foci of histologically viable tumor cells; IV. No histologic evidence of viable tumor within specimen</td>
<td>System designated for osteosarcoma; estimates tumor necrosis per unit area</td>
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<tr>
<td>Salzer-Kuntschik&lt;sup&gt;62&lt;/sup&gt;</td>
<td>Two longitudinal and various transverse sections analyzed; half of specimen cut in 2-cm blocks for paraffin embedding</td>
<td>I. No viable appearing tumor cells; II. Single vital tumor cells or 1 vital cell cluster, &lt;0.5 cm; III. Vital tumor, &lt;10%; IV. Vital tumor, 10%-50%; V. Vital tumor, &gt;50%</td>
<td>System designated for osteosarcoma; half of specimen split into 7 × 5-cm blocks for undecalcified, methacrylate embedding, and necrosis analyzed with an electron image analyzing system</td>
</tr>
<tr>
<td>Picci&lt;sup&gt;40&lt;/sup&gt;</td>
<td>Cut surface of specimen sampled with specified examination of the following: (1) soft tissue mass peripheral to peristeme, (2) subperiosteal region, (3) medullary space, (4) hemorrhagic foci; two slides per block examined</td>
<td>I. At least 1 macroscopic nodule of viable tumor (&gt;one 10× field) or scattered microscopic nodules in summation exceeding one 10× field; II. Isolated microscopic nodules of viable tumor cells in summation less than one 10× field; III. No viable tumor cell nodules; scattered, individual tumor cells permitted</td>
<td>System designated for Ewing sarcoma of extremity, nonmetastatic; estimates residual viable tumor volume; subperiosteal region of new bone formation preferred sanctuary site for residual tumor</td>
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* Adapted with permission from Lowichik et al. <sup>4</sup>
Ewing sarcoma/primitive neuroectodermal tumor (PNET). A, A tumor on the chest wall shows an infiltrative, firm, white tumor with focal hemorrhage and necrosis. B, Untreated Ewing sarcoma/PNET shows small blue cells in a nesting pattern with focal pseudorosettes (H&E, ×200). C, Membranous immunohistochemical reactivity for O13 (CD99) is typical for Ewing sarcoma/PNET (O13 [CD99], ×400). D, Treated Ewing sarcoma/PNET simulates the alveolar pattern of rhabdomyosarcoma and also shows areas of fibrosis and hemosiderin accumulation (H&E, ×100). E, Treated Ewing sarcoma/PNET shows residual tumor cells in a background of chronic inflammation, fibrosis, and foamy histiocytes (H&E, ×400). F, Treated Ewing sarcoma shows small cords of tumor cells in a background of inflammatory reactive tissue with focal hemosiderin (H&E, ×400).
significance of individual or small groups of immature myoid-appearing or atypical cells, and prognostic assessment of posttherapeutic pathologic findings are significant challenges for the surgical pathologist.

A variety of therapy-related changes in rhabdomyosarcoma have been described, and the publications are summarized in Table 5. Surgical and pathologic findings do not always correspond to the radiologic appearance of treated rhabdomyosarcoma. For example, a poor response or enlarging mass on computed tomographic scanning may not represent persistent viable tumor and might instead be due to reactive or reparative changes, cytodifferentiation, or necrosis, inflammation, and fibrosis. In addition to recognizable rhabdomyosarcoma, posttreatment specimens can show a spectrum of necrosis; inflammation with macrophages, lymphocytes, and plasma cells; fibrosis; and atrophic or regenerating nonneoplastic skeletal muscle.

It is essential for the surgical pathologist to compare the initial pretreatment and posttreatment surgical pathology specimens when evaluating treated rhabdomyosarcomas. The histologic continuum ranges from no visible effects on the tumor cells to extensive morphologic changes. At one extreme, minimal cytodifferentiation consists of more prominent but still scanty eosinophilic cytoplasm, while the nucleus remains relatively large. With increasing cytodifferentiation, the cellularity of the rhabdomyosarcoma decreases, eosinophilic cytoplasm becomes more abundant, the nuclear/cytoplasmic ratio decreases, and the shape of the rhabdomyoblasts changes. Strap cells with unipolar or bipolar eosinophilic cytoplasmic extensions and cross-striations, myotubule-like cells, and myofiber-like cells appear, and mitoses decrease. These changes are encountered most frequently in embryonal rhabdomyosarcoma.

In both types of rhabdomyosarcoma, undifferentiated areas may be interspersed with cytodifferentiated foci and do not have a predictable architectural pattern or topographic distribution. This is a potential diagnostic pitfall when a small biopsy specimen is obtained from a large mass following treatment. Chemotherapy may selectively destroy some tumor cells while it stimulates terminal differentiation of others.

Immunohistochemical analysis might be useful to confirm the myoid phenotype, but it is of little help in assessing therapy-related changes in rhabdomyosarcoma. Myoid marker expression typically is found in pretreatment and posttreatment specimens, although there may be changes in patterns of reactivity. To some extent, proliferative activity assessed by MIB-1 immunohistochemical analysis decreases after treatment and is associated with greater cytodifferentiation in embryonal rhabdomyosarcoma.

Alveolar rhabdomyosarcoma typically displays less cytodifferentiation, undifferentiated foci, and more prominent zones of tumor identical to the original pretreatment specimen and without treatment effects, and proliferative activity following treatment is unpredictable. Sparse persistent tumor cells in treated embryonal rhabdomyosarcoma and botryoid rhabdomyosarcoma do not seem to influence survival. Thus, cytodifferentiation and decreased proliferative activity are associated with embryonal rhabdomyosarcoma, but therapy-related changes for alveolar rhabdomyosarcoma are less predictable.

Small biopsy specimens of treated rhabdomyosarcoma pose particular challenges in interpretation. The significance of individual atypical cells, the recognition of small clusters of rhabdomyosarcoma in areas of fibrosis and inflammation, and the recognition of skeletal muscle atrophy and regeneration are all potential sources of difficulty. Immunohistochemical demonstration of myoid markers can distinguish between rhabdomyosarcoma and inflammation; myogenin, myo-D1, muscle specific actin, desmin, CD68, and CD45 can be useful in this context. However, regenerating skeletal muscle cells express these markers, are accompanied by inflammation, and can display an infiltrative pattern, a spectrum of cytodifferentiation, and cellular atypia. Morphologically variegated round cells with an infiltrative pattern, focal muscle fiber necrosis, the presence of myoblasts and myotubules, and inflammatory changes with edema, a lymphoplasmacytic and histiocytic infiltrate, and fibrosis all might be seen in regenerating skeletal muscle. As with treated rhabdomyosarcoma, regenerating skeletal muscle, regardless of the patient’s age, demonstrates inflammatory and myoid cell populations, and myogenin reactivity can be observed.

| Therapy-Related Changes in Rhabdomyosarcoma: Summary of Five Series |
|-----------------------------|----------------|----------------|----------------|----------------|----------------|
| Histologic Type             | Molenaar et al | d’Amore et al | Heyn et al     | Coffin et al  | Smith et al    |
| Embryonal (all types)       | 6/7            | 4/4            | 27/27          | 7/8           | 13/14          | 95             |
| Alveolar                    | 3/4            | 0              | 0              | 0             | 0              | 75             |
| Total                       | 10/15          | 4/4            | 28/28          | 11/16         | 15/19          | 83             |

* Adapted with permission from Lowichik et al. Data are given as number of cases with cytodifferentiation/total number of cases.
† Disproportionately less cytodifferentiation observed among alveolar rhabdomyosarcomas, associated with worse clinical outcome.
‡ Therapy-related maturation found in all cases, even when the original tumors had little or no cytodifferentiation.
Thus, significant challenges remain in histopathologic evaluation of treated rhabdomyosarcoma, and criteria for interpretation are understood incompletely. Extensive cytodifferentiation is seen more commonly in embryonal and botryoid rhabdomyosarcoma than in alveolar rhabdomyosarcoma, and this may reflect fundamentally different mechanisms of cellular responses to treatment and prognostic differences between favorable and unfavorable subtypes. Assessment of resection margins, measurement of tumor size in 3 dimensions, description of cytodifferentiation and its extent, estimation of the amount of necrosis, and comparison of pretreatment and post-treatment histologic features provide useful information in the pathology report and are a frequent subject of discussion between clinicians and pathologists. A complete understanding of the clinical relevance of these results awaits further studies.

**Synovial Sarcoma**

Synovial sarcoma, which accounts for 5% to 10% of all soft tissue sarcomas in children and adolescents and is one of the most common soft tissue sarcomas after rhabdomyosarcoma and EWS/PNET, is a mesenchymal spindle cell tumor with variable epithelial differentiation and a specific chromosomal translocation t(X;18)(p11;q11). Although it occurs throughout
life, synovial sarcoma has a predilection for adolescents and young adults and a 5-year survival of 31% to 83%.91 Young age, tumor diameter less than 5.0 cm, low mitotic rate, absence of necrosis, and surgical resectability are significant prognostic factors.92-95

Synovial sarcoma has 2 major histologic patterns, biphasic and monophasic. Biphasic synovial sarcoma has varying proportions of spindle cell and epithelial components with papillary, glandular, or solid patterns. Other histologic variants include poorly differentiated synovial sarcoma and calcifying synovial sarcoma. The calcifying variant is considered a favorable histologic-prognostic variant.

Information about therapy-related histologic changes in synovial sarcoma is limited, but multimodality treatment can permit limb-preserving surgery, and adjuvant chemotherapy seems to improve survival.92,94,95 The histologic response to radiation

**Image 3** (cont) E, Treated embryonal rhabdomyosarcoma shows residual, less differentiated tumor cells intermingled with strap cells and areas of hemosiderin accumulation (H&E, ×400) (reprinted with permission from Lowichik et al5). F, Treated embryonal rhabdomyosarcoma displays extensive cytodifferentiation with rhabdomyoblasts with abundant eosinophilic and clear vacuolated cytoplasm and small bland nuclei (H&E, ×400)

**Image 4** Alveolar rhabdomyosarcoma. A, Untreated alveolar rhabdomyosarcoma has a characteristic architecture of tumor cells lining fibrovascular septa and floating in alveolar spaces (H&E, ×200). B, Treated alveolar rhabdomyosarcoma contains abundant tumor cells resembling the original pretreatment specimen and rhabdomyoblastic and multinucleated tumor cells with cytodifferentiation (H&E, ×400) (reprinted with permission from Lowichik et al5).
therapy, including 100% necrosis or severe cellular alteration has been reported in a small group of patients. Caffeine-potentiated chemotherapy resulted in more than 90% necrosis to complete eradication of tumor cells in 4 of 5 patients with synovial sarcoma in another series. Studies are ongoing to correlate treatment effects with outcome in synovial sarcoma using a pathologic grading approach analogous to that for osteosarcoma.

**Infantile Fibrosarcoma**

Infantile fibrosarcoma affects neonates and infants. Although its histologic appearance simulates adult-type fibrosarcoma, its natural history is much more favorable. Most infantile fibrosarcomas are diagnosed during the first 3 months of life, and the extremities in the head and neck region are the most common sites. Rapid growth of the mass in proportion to the size of the child is an alarming characteristic. During the past decade, the effectiveness of chemotherapy has been demonstrated, although surgery remains the principal treatment. A characteristic translocation t(12;15)(p13;q25) with an ETV6-NTRK3 gene fusion is a specific cytogenetic finding, and gains of chromosomes 8, 11, 17, and 20 also are common.

The typical histologic appearance of infantile fibrosarcoma is a solid, densely cellular proliferation of spindle cells in interlacing bundles and sharply intersecting fascicles with a focal herringbone pattern. Histologic variants...
Image 5
Infantile fibrosarcoma. A, Infantile fibrosarcoma involving the distal thigh and knee appears as a large, rather grotesque mass with focal areas of ulceration. B, Untreated infantile fibrosarcoma shows sheets and vague fascicles of spindle cells intermingled with irregularly dilated blood vessels and zonal areas of coagulative necrosis (H&E, ×100). C, Untreated infantile fibrosarcoma displays foci of more primitive round and polygonal cells (H&E, ×400). D, Untreated infantile fibrosarcoma shows prominent areas of coagulative necrosis and cystic degeneration (H&E, ×200). E, Treated infantile fibrosarcoma shows a bland proliferation of spindle cells in a loose fibrous background (H&E, ×400). F, Treated infantile fibrosarcoma shows bands of fibrosis with bland cells infiltrating adipose tissue (H&E, ×100) (reprinted with permission from Lowichik et al5).
include myxoid, small round cell, and whorled patterns. Coagulative necrosis and gaping blood vessels resembling hemangiopericytoma frequently are seen. Little information is available about the pathologic features following chemotherapy. Experience with one previously published case revealed hypocellular collagenized fibrous tissue in a background of mature adipose tissue several years following chemotherapy of a previously biopsied mass.5-98,101 A clinically dramatic reduction in size following chemotherapy and occasional cases of complete remission also have been reported.104,110-113

Summary

In recent years, there has been an increasing emphasis on pathologic evaluation of treatment effects in various types of bone and soft tissue tumors, with osteosarcoma as the prototype. Recent evidence suggests that imaging studies hold future promise for pathologic and radiologic correlation of treatment effects. The importance and considerations for the pathologists are proper classification of the neoplasm, with comparison of pretreatment and posttreatment pathologic specimens; assessment of therapy-related changes, including the percentage of necrosis in selected tumors; and identification of other significant pathologic features, including assessment of margins.

National cooperative oncology groups are developing protocols to assess treatment effects in various types of nonosteosarcoma, so the surgical pathologist likely will encounter increasingly frequent requests for this type of pathologic evaluation in the future. At present, standardized approaches are available for osteosarcomas and EWS/PNET, but standardized grading systems have not been established for other types of sarcomas. Information about the existing grading systems and their applications is summarized in Tables 1, 3, and 4, and the diagnostic checklist for treated osteosarcoma, given in Table 2, can serve as a model for assessment of other types of soft tissue sarcomas until more specific approaches for nonosteosarcoma have been defined.

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References


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