1 PRINCIPLES AND PRACTICE FOR BIOPSY DIAGNOSIS AND MANAGEMENT OF MUSCULOSKELETAL LESIONS

INTRODUCTION TO THE DIAGNOSIS OF MUSCULOSKELETAL LESIONS

Fine needle aspiration and core biopsy are used at some oncology centers as the initial technique to obtain a tissue diagnosis for mass lesions occurring within the musculoskeletal system. A majority of such lesions are in actuality metastatic deposits from a primary malignancy arising elsewhere. Metastatic neoplasms usually present little difficulty in diagnosis. Primary lesions of the musculoskeletal system are a significantly greater diagnostic challenge and many authorities in histopathology of bone and soft tissue tumors have recommended against the use of small biopsy techniques for the diagnosis of these lesions. The resistance to the use of small volume specimens, especially fine needle aspiration (FNA), results from a combination of factors including the relative rarity of these lesions, the potential for radical deforming surgery and the young age of the patients in which many of these lesions occur. Musculoskeletal sarcomas account for less than 1% of all malignant neoplasms. The infrequency of these tumors results in limited experience with their morphologic appearances among surgical pathologists and cytopathologists except for those practicing at orthopedic oncology centers. Contributing to this reduction in the utilization of small biopsy procedures is the relatively high percentage of benign proliferations (pseudosarcomas) closely resembling true sarcomas. An additional issue which has slowed the implementation of FNA for the investigation of musculoskeletal neoplasms is concern that the technique may not procure sufficient sample to obtain reliable results with a variety of ancillary techniques including immunohistochemistry and molecular diagnostics.

Modern techniques and increasing familiarity with the cytologic appearance of these lesions has negated many of the above concerns. Despite reservations among many surgical pathologists and cytopathologists regarding small biopsy techniques for the diagnosis of musculoskeletal lesions, familiarity with the appearance of these tumors in cytologic and small histopathologic specimens is desirable. Many primary musculoskeletal lesions are inadvertently biopsied in the work-up of suspected metastatic disease and knowledge of their appearance is necessary for appropriate post-FNA follow-up and treatment. The relatively low cost, low patient morbidity, and reduced likelihood of biopsy related complications all favor the use of small biopsy techniques including FNA. The diagnostic biopsy must separate benign from malignant tumors, establish a grade in sarcomas, and in bone neoplasms and some soft tissue neoplasms establish a specific histologic type. Diagnostic accuracy and issues concerning the choice of biopsy technique are different for lesions arising within the bone or the soft tissues. Importantly, radiographic analysis greatly facilitates the diagnosis of osseous lesions, but has relatively little input for diagnosis of soft tissue tumors.

PRE-BIOPSY CONSIDERATIONS

The biopsy of a musculoskeletal tumor is not a trivial undertaking and requires pre-biopsy evaluation of the patient including the evaluation of clinical and radiographic features. The pre-biopsy staging studies are critical to the outcome of subsequent therapeutic maneuvers for the treatment of both benign and malignant neoplasms. Optimal diagnosis and treatment are achieved when appropriate staging studies are performed before biopsy and careful consideration is given to the choice of the appropriate biopsy technique whether FNA, needle, trochar, or incisional. Appropriate placement of the incision or needle puncture site frequently has considerable impact on subsequent incisions for definitive treatment. The selection of the biopsy technique as well as path of the biopsy may have significant impact upon potential tumor bed and biopsy tract contamination and its impact on subsequent definitive surgery. When trochanter or incisional biopsies

are performed on bone lesions adequate consideration must be given to avoiding stress concentrating defects in load bearing bones. Such defects may result in pathologic fractures. Similarly, optimal results of surgery are obtained by operating in a bloodless field in which a prior biopsy has not resulted in contamination or disruption of the tumor bed. In some cases, this goal requires immediate operation following frozen section diagnosis by surgical pathologists expert in musculoskeletal lesions. The selection of biopsy technique is influenced by the need for ancillary techniques including flow cytometry, immunohistochemistry, and molecular diagnostics. The chosen biopsy technique must be capable of obtaining adequate and appropriate tissue for potential ancillary studies so that a repeat biopsy may be avoided (except perhaps more FNA passes). The choice of the appropriate type of biopsy requires evaluation of tumor location, probable tumor type, and the need for certain types of ancillary testing (cytogenetics). In the case of osseous tumors, radiographic analysis can yield a preliminary diagnosis and determine if the lesion is inappropriate for FNA. Cystic lesions and heavily ossified lesions are usually suboptimal for diagnosis by FNA and either incisional or core biopsy is more appropriate. The selection of biopsy technique should also consider diagnostic accuracy of the alternative biopsy methods.

FACTORS IMPORTANT IN SELECTION OF BIOPSY METHOD

Formal surgical incisional biopsy has been and remains the predominant biopsy technique for the investigation of musculoskeletal lesions. Such biopsies, when properly performed, yield sufficiently abundant material for the surgical pathologist to analyze architecture, intratumoral heterogeneity, and nuclear features as well as supplying adequate tissue for a number of ancillary studies. However, incisional biopsy is not without disadvantages including higher rates of intraoperative and post-operative complications, potential disruption of the tumor bed, relatively high patient morbidity, and higher cost. Approximately 20% of incisional and large core needle biopsies are associated with significant clinical problems. Post-biopsy wound healing complications are a frequent issue. Eight percent of incisional and large core biopsies produce significant adverse effects on prognosis, and 5% of such biopsies have contributed to otherwise unnecessary amputations. Approximately 10% of biopsies in the study by Mankin et al were either non-representative or technically poor. Biopsies performed at musculoskeletal oncology centers are more likely to be correctly interpreted and less likely to be associated with technical problems and adverse outcomes. Minimal biopsy techniques including small core biopsy and fine needle aspiration possess a number of advantages over incisional biopsy because they minimize the likelihood of certain biopsy complications.

In the past two decades, interest in cost containment, reduced length of hospital stay, and a desire to minimize patient morbidity have resulted in an increased interest in the use of minimally invasive biopsy techniques. Foremost among these techniques are fine needle aspiration (22 to 25 g needles) and small core needle biopsy (Tru-cut® or 18 to 20 g needles). While these are generally acknowledged as more difficult to interpret and associated with a reduced diagnostic accuracy, any meaningful comparison of biopsy safety and diagnostic accuracy must be made between the reported diagnostic accuracies of these minimally invasive techniques and that of traditional incisional biopsy. The reported diagnostic accuracy for incisional biopsy at orthopedic oncology centers is approximately 95%. This diagnostic accuracy is less at institutions not specializing in musculoskeletal lesions.

When compared to open biopsy, both core needle biopsy and FNA are less invasive, associated with fewer complications, and are less expensive. However, both techniques provide a more limited sample and have been associated with a somewhat lower diagnostic accuracy. In a number of series, the diagnostic accuracy of small core biopsy and/or fine needle aspiration has approached that obtainable with open biopsy. Reported accuracy rates for small core biopsy have ranged between 74% and 98% for the separation of benign from malignant lesions. Accuracy of FNA for the separation of benign from malignant lesions appears relatively similar with accuracy reports from orthopedic oncology centers utilizing FNA showing accuracy rates of approximately 80 to 95%. Given the increasingly favorable experience with small core needle biopsy, it has largely replaced incisional biopsy as the initial diagnostic technique at many institutions. On the other hand, the use of FNA remains limited to selected medical centers with experienced cytopathologists, musculoskeletal radiologists, and oncologic orthopedic surgeons.

Despite the more limited material obtainable by FNA, the technique has some advantages over core needle biopsy. It is less expensive and associated with lesser degrees of patient discomfort and morbidity. FNA also has the ability to sample more easily multiple areas of a mass potentially providing more representative material than one or two core needle biopsy specimens. FNA specimens may also be immediately evaluated to assess specimen adequacy and

the need to obtain additional tissue for ancillary studies. If the diagnostic accuracy of FNAs was similar to that of core needle biopsy, the cytologic technique would be preferable as the initial biopsy method. Several studies have evaluated the relative accuracies of small core needle biopsy and FNA for separation of benign from malignant lesions, grading and subtyping of musculoskeletal lesions. In the majority of studies, core biopsy is slightly more accurate in the determination as to whether a lesion is benign or malignant and is also superior for the accurate determination of histologic grade and subtype. Nonetheless, the accuracies are sufficiently close such that FNA may be the better initial technique either in combination with small core biopsy or alone.

Since the 1990s there has been increased use of small biopsy techniques for the investigation of musculoskeletal mass lesions. Initially, core needle biopsy and FNA were utilized independently and frequently in exclusion of the other. More recently, core needle biopsy and FNA have been used in a coordinated manner resulting in increased accuracy for both diagnosis and grading, as well as improved procurement of adequate tissue for ancillary testing. The combination of FNA and core needle biopsy appears to speed diagnosis, increase the number of correct diagnoses, and improve the accuracy of subtyping and grading of sarcomas. Moreover, the combination of these two techniques usually allows a preliminary diagnosis to be made during the same clinic visit as the biopsy. The addition of small core biopsy to FNA at the same biopsy attempt may be optimal in that core biopsies supply formalin fixed paraffin embedded material. Such material is superior to cytology smears, filter preparations, and cytospin preparations in that many immunohistochemical and FISH techniques are optimized for formalin fixed paraffin embedded specimens and appropriate positive controls are almost invariably paraffin block specimens.

BIOPSY TECHNIQUE, NEEDLES, AND ANCILLARY DEVICES

The fine needle aspiration technique uses standard hypodermic needles varying in size from 27 to 22 gauge for superficial soft tissue lesions. Longer Chiba needles of similar design are available in 22 and 20 gauge sizes with lengths up to 15 or 20 cm. These are useful for deeply situated bone and soft tissue lesions. In many cases, a coaxial needle of larger gauge is used in conjunction with the Chiba needle when aspirating deeply situated soft tissue or bone lesions. These coaxial needles allow multiple passes into a lesion without having to relocalize for each pass. This saves time and reduces patient discomfort. In some cases, FNA practitioners will use a section of IV tubing interposed between the needle and the syringe to increase needle mobility. A variety of devices have been developed to hold the syringe and allow a single hand to guide the aspirating needle and develop suction by retraction of the syringe plunger. This allows fixation of a palpable nodule by the free hand. While these devices vary in design (Figure 1.1), all work by allowing the aspirator to hold and control the syringe while retracting the plunger creating a vacuum for aspiration.

Figure 1.1 Handles for Stabilization and guidance of syringe with needle used in fine needle aspiration.

Despite variations in needle size and the potential use of mechanical devices for control of the syringe, the basic aspiration technique remains the same as at other body sites. The needle is introduced into the mass and multiple rapid back and forth oscillations are made with slight changes of needle angle when possible. Generally, five to ten oscillations are made over a period of 2-10 seconds. Aspiration is stopped after this period of time or when material appears in the hub of the needle. This technique maximizes cellular yield while minimizing blood contamination. FNA may be performed with and without suction. In general, lesions felt to be richly vascular are best initially approached without vacuum applied by the syringe. This minimizes blood contamination while maximizing cellular yield. If insufficient material is obtained, vacuum generated by retraction of the syringe plunger is used on subsequent aspiration attempts. When suction is applied at the time of aspiration, the vacuum must be released before removal of the needle from the target undergoing FNA.

Aseptic precautions are taken by either cleaning the area with alcohol or other commercially available topical antiseptics. The question of topical anesthesia for needle aspirates is controversial with no satisfactory data supporting its use published in the literature. However, a majority of authors appear to recommend that local anesthesia not be used for superficial palpable nodules. Deep-seated nodules and those occurring within bone require local anesthesia generally obtained by the use of 2% lidocaine with or without epinephrine 1:100 000. The anesthetic is placed within the skin and along the planned biopsy track. In general, local anesthetic is adequate analgesia for most patients, but some will also require conscious sedation.

The choice of needle size remains controversial with proponents of ultrafine (25–27 gauge) needles stating that they obtain similar amounts of material with reduced patient discomfort. The risk of local hemorrhage and blood contamination of FNA specimens bears some relationship to needle diameter. Thus, some authorities recommend beginning with the smallest diameter needles (25–27 gauge) and progressing to larger sizes if initial smears do not contain diagnostic material. This approach has been supported by one study of thyroid aspirates in which 23 and 25 gauge needles were shown to have no significant differences in adequacy of material obtained. Similarly, Unver et al found that 18, 22, and 25 gauge needles were not associated with significantly different diagnostic yields.

Controversy exists as to whether suction is necessary (aspiration vs. non-aspiration). Most studies have focused on the thyroid and a recent series of 200 patients with thyroid aspirates were scored for blood, numbers of follicular epithelial cells, and preserved architecture. This study found no statistically significant differences for FNA biopsies performed with and without suction. A meta-analysis of four trials using aspiration and non-aspiration demonstrated an odds ratio favoring non-aspiration but the difference was not significant. Unfortunately, no well designed similar studies have been performed for FNA involving bone and soft tissue lesions. In the author's personal experience, aspiration (suction) appears to yield superior results especially for spindle cell lesions.

SPECIMEN PREPARATION

Following the aspiration procedure, material trapped within the needle can be expressed directly onto glass slides and smeared or may be placed in normal saline or liquid based preparations for cytologic examination. Additionally, material may be expressed into formalin to prepare cell blocks. Conventional or direct smears are the basis for most cytologic descriptions and criteria published for the diagnosis of both bone and soft tissue lesions. Such smears are also significantly less costly than alternative techniques. The smearing technique is well described in a variety of publications. Immediate assessment of air-dried smear preparations is helpful in judgment of specimen adequacy and in triage of additional FNA passes for ancillary techniques including flow cytometry and molecular techniques.

LARGE CORE BIOPSY

A variety of large core needles are available for biopsy of bone and soft tissue lesions. These include the Vim-Silverman needle and the TRU-CUT needle (14–18 gauge). A variety of newer smaller core needles (19-20 gauge) have been developed, each having characteristic operational features (Figure 1.2). Two basic needle types exist. One has a notch located on the side of the needle covered by a retractable sheath which cuts the specimen leaving it in the notch. A second type does not use a laterally situated cleft and cuts a rounder core. These needles come in a variety of sizes and lengths. While these needles increase the relative risk of complications including hemorrhage, patient discomfort, and anxiety, they improve overall diagnostic accuracy when combined with a traditional FNA. This improved diagnostic accuracy is secondary to preservation of architecture and the collection of larger tissue fragments for

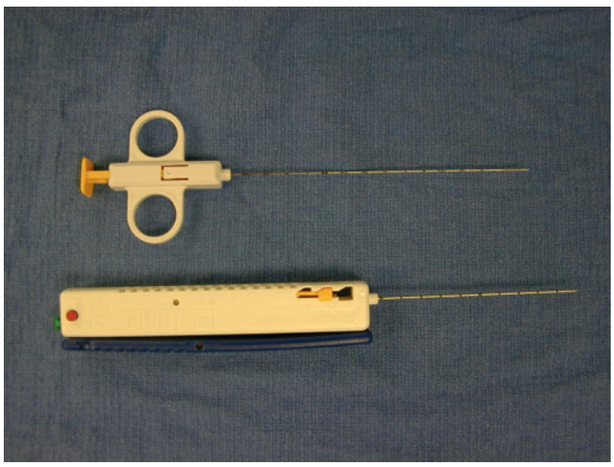

Figure 1.2 Core needle devices. Upper needle type contains a slot and cutting device yielding a small core which is approximately half the diameter of the needle. Lower core system yields a larger, more robust specimen preferable for musculoskeletal biopsies.

formalin fixation and paraffin embedding. Such tissue is superior to smear and liquid based preparations for immunohistochemical studies.

POST-BIOPSY CONSIDERATIONS: GRADE, STAGE, AND CHOICE OF THERAPEUTIC PROTOCOL

Selection of post-biopsy therapy depends on accurate assessment of the following:

1. Benign versus malignant.
2. Small cell versus non-small cell malignancies.
3. Grade of non-small cell sarcoma.
4. Stage of sarcoma (particularly size of sarcoma).

Accurate assessment of the above features allows appropriate therapy. For small cell malignancies, categorization of a neoplasm to a particular histologic type (rhabdomyosarcoma, Ewing sarcoma/primitive neuroectodermal tumor (PNET), lymphoma) is necessary for selection of the appropriate chemotherapy protocol. Fine needle aspiration, especially in combination with small core biopsy is usually sufficient to address the histopathology issues necessary for appropriate therapy. Pre-operative estimates of tumor size are achieved by CT or MRI examination. Both FNA and small core biopsy are inadequate to estimate accurately percent tumor necrosis, but such data is easily obtainable from MRI or CT evaluation.

ASSIGNMENT OF MORPHOLOGIC SUBTYPE AND CYTO/HISTOLOGIC GRADING OF SARCOMAS

Grading of sarcomas is based predominately on how closely a neoplasm resembles a normal adult or fetal tissue. Indeed, the majority of benign and malignant mesenchymal tumors are named after their supposed direction of differentiation. Thus, smooth muscle tumors are designated leiomyoma and leiomyosarcoma and fatty tumors are designated lipoma and liposarcoma. While the majority of soft tissue sarcomas reveal an apparent direction of differentiation, not all malignant mesenchymal tumors are easily categorized and a number of neoplasms are given names which do not correspond directly with an adult or fetal tissue (e.g. epithelioid sarcoma, clear cell sarcoma, and rhabdoid tumor). Sarcomas closely resembling a normal cell type are considered low grade (well differentiated) while those not resembling their assumed direction of differentiation are considered high grade (poorly differentiated). Current grading concepts do not require a grade to be assigned to certain sarcoma types which show little morphologic variability from case to case (Ewing sarcoma). Some soft tissue sarcoma types are automatically considered high grade based on their morphologic subcategorization. This convention is congruent with the primary purpose of grading which is to separate sarcomas into those with a favorable prognosis (well differentiated) from those with a poor prognosis (high grade). The information contained within the tumor grade is important in the clinical decision as to the use or not of chemotherapy and radiation. Thus, the value of a histologic or cytologic grading system is not only to predict patient outcome, but also to identify patients who may benefit from neoadjuvant or adjuvant therapy.

Two widely accepted grading schemes for bone and soft tissue sarcomas exist. With the exception of osteosarcoma and chondrosarcoma, bone neoplasms are not graded. The majority of soft tissue sarcomas should be graded using either the National Cancer Institute system or the Federation Nationale des Centres de Lutte Contre le Cancer (FNCLCC) system. In addition, the National Comprehensive Cancer Network has established practice guidelines in oncology for bone and soft tissue sarcomas. These guidelines make recommendations for the work-up, biopsy, grading, and therapy of soft tissue sarcomas at a variety of body sites. They recommend that pathologists with sarcoma expertise review biopsies and resected specimens especially for initial pathologic classification. They also recommend the use of optimal cytogenetic and molecular diagnostic techniques.

The NCI and FNCLCC grading systems are relatively similar and both utilize three histologic grades. The recently updated NCC Network guidelines use only low grade and high grade designations for selection of appropriate therapy. Both the NCI and FNCLCC systems utilize histologic subtype as an important component of grading but use tumor necrosis and mitotic count in slightly different ways to assess grade (Tables 1.1 and 1.2). The NCI system uses tumor type and mitotic count to establish a set of sarcomas as grade I. Similarly, rhabdomyosarcomas, extraskeletal osteosarcomas, mesenchymal chondrosarcomas, synovial sarcomas, pleomorphic liposarcomas, and Ewing sarcoma/primitive neurectodermal tumors are automatically assigned a grade of III. The remaining types of sarcomas are assigned to either grade II or III based on the degree of necrosis.

Table 1.1. Assigned histologic grade according to histologic type in the NCI system

Histologic type	*Grade 1*	*Grade 2*	*Grade 3*
Well differentiated liposarcoma	+		
Myxoid liposarcoma	+		
Round cell liposarcoma		+	+
Pleomorphic liposarcoma			+
Fibrosarcoma		+	+
Pleomorphic sarcoma		+	+
Myxofibrosarcoma		+	
DFSP	+		
Malignant giant cell tumor		+	+
Leiomyosarcoma	+	+	+
Rhabdomyosarcoma (all types)			+
Chondrosarcoma	+	+	+
Myxoid chondrosarcoma	+	+	
Mesenchymal chondrosarcoma			+
Osteosarcoma			+
Extraskeletal Ewing sarcoma/PNET			+
Synovial sarcoma			+
Epithelioid sarcoma		+	+
Clear cell sarcoma of soft tissues		+	+
Superficial MPNST		+	
Deep MPNST		+	+
Angiosarcoma		+	+
Alveolar soft part sarcoma			+
Kaposi's sarcoma		+	+

DFSP, dermatofibrosarcoma protuberans; MPNST, malignant peripheral nerve sheath tumor.
Reproduced with permission from: Costa J, Wesley RA, Glatstein E, et al. The grading of soft tissue sarcomas: results of a clinicopathologic correlation in a series of 163 cases. Cancer 1984;53:530. NCI, National Cancer Institute.

Table 1.2. Definitions of grading parameters for the FNCLCC system

Parameter	*Criterion*
Tumor differentiation	
Score 1	Sarcoma closely resembling normal adult mesenchymal tissue (e.g., well differentiated liposarcoma, well differentiated leiomyosarcoma)
Score 2	Sarcomas for which histologic typing is certain (e.g., myxoid liposarcoma, alveolar soft part sarcoma)
Score 3	Embryonal and undifferentiated sarcomas; sarcoma of uncertain type
Mitosis count	
Score 1	0–9/10 HPF
Score 2	10–19/10 HPF
Score 3	≥20/10 HPF
Tumor necrosis (microscopic)	
Score 0	No necrosis
Score 1	≤50% tumor necrosis
Score 2	>50% tumor necrosis
Histologic grade	
Grade 1	Total score 2, 3
Grade 2	Total score 4, 5
Grade 3	Total score 6, 7, 8

Reproduced with permission from: Coindre JM, Trojani M, Contesso G, et al. Reproducibility of a histopathologic grading system for adult soft tissue sarcomas. Cancer 1986;58:306. FNCLCC, Fédération Nationale de Centres de Lutte Contre le Cancer; HPF, high power field.

Those cases showing more than 15% necrosis are categorized as grade III. The FNCLCC system is more complex and utilizes a numerical scoring system. This system assesses tumor differentiation on a 1 to 3 basis along with a tumor necrosis and mitotic count. Sarcomas showing no evidence of necrosis are given a score of 0 while those with 50% or less necrosis are given a score of 1. Sarcomas with greater than 50% necrosis are assigned a point value of 2. Similarly, mitotic count is scored on a 1–3 basis with 0–9 mitoses per 10 high power fields being assigned 1 point, 10–19 mitoses per 10 high power fields receiving 2 points, and greater than or equal to 20 mitoses per 10 high power fields receiving a score of 3 points. Scores for each of the components of grading are summed and a histologic grade of 1 is assigned to those sarcomas with a total score of 2 or 3. Grade 2 comprises those sarcomas with a score of 4 or 5, while grade 3 sarcomas are those with a score of 6, 7, or 8. Accuracy of both the NCI and FNCLLC systems depends on sufficient tissue to accurately determine mitotic index and percent tumor necrosis. The requirement for accurate assessment of tumor necrosis and mitotic count limits the use of either of these grading systems for small biopsy samples especially FNA and small core biopsy.

A number of authors have investigated the potential for accurate grading of soft tissue sarcomas by cytologic means. Characteristics inherent in the cytologic method and limiting its ability to recapitulate histopathologic grades precisely are: (1) cytology's imperfect ability to subtype morphologically many spindle cell sarcomas; (2) inability to reproduce mitotic indices; and (3) inability to assess percent necrosis directly within a sarcoma. This latter deficiency of the cytologic method extends to small core needle biopsy. However,

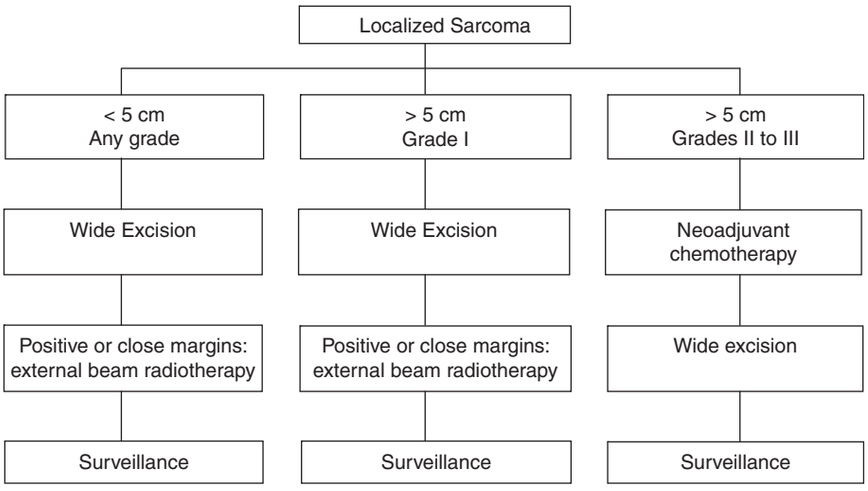

Figure 1.3 Adult soft tissue sarcoma treatment algorithm used at the University of Utah.

this can be overcome by utilizing percent necrosis estimates obtained from CT or MRI examinations as a component of grading for small sample techniques. Kilpatrick et al have extensively reviewed the issue of grading in soft tissue sarcomas and its relationship with subtyping. Three studies have specifically addressed the issue of cytologic grading and its relationship to histologic grading for soft tissue sarcomas. Weir et al assigned grades by cytologic means to 36 sarcomas and reported only one major and two minor non-correlations between the cytologic and histopathologic grades. Jones et al reviewed a series of 77 sarcomas accurately subtyping 55% of these neoplasms and achieving concordance between cytologic and histologic grade in slightly less than half of cases. Palmer et al achieved a 90% concordance between histopathologic grade and cytologic grade for soft tissue sarcomas when assigning them to low or high grade categories. Categorization of sarcomas as low or high grade is in compliance with the NCCN practice guidelines. In their study, the presence or absence of necrosis and mitotic figures were the most important features for separating low and high grade sarcomas. Nuclear atypia and the degree of nuclear overlap determined the grade when necrosis and mitotic figures were absent in cytologic specimens. They found that myxoid and spindle cell sarcomas were most difficult to subcategorize and grade. In the study by Palmer et al, the pleomorphic, small round cell and epithelioid/polygonal groups corresponded to high grade sarcomas. In their study, major errors in grading occurred in half of myxoid sarcomas and 9% of spindle cell sarcomas. Despite the success of Palmer et al in characterizing soft tissue sarcomas as low grade or high grade, they were able to subtype accurately only 14% of cases.

SELECTION OF THERAPEUTIC PROTOCOL FOR SOFT TISSUE SARCOMAS

The selection of therapeutic protocols for primary sarcomas of bone depends predominately on histologic type of sarcoma present. Selection of appropriate therapy for soft tissue sarcomas depends predominately on the grade, size, and stage of the neoplasm. In general, low grade sarcomas do not receive adjuvant chemotherapy or radiation therapy prior to their resection. High grade sarcomas ≤5 cm in greatest dimension do not require pre-operative chemotherapy or radiation therapy. High grade sarcomas >5 cm in greatest dimension usually undergo neoadjuvant radiation and chemotherapy prior to limb salvage or wide excision. Figure 1.3 demonstrates a commonly utilized protocol for treatment of soft tissue sarcomas and is similar to that recommended by the NCCN. While grade is appropriately assessed on initial FNA or small core biopsy specimens, pathologic staging is most appropriately delayed until assessment of the resection specimen.

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2 ANCILLARY TECHNIQUES USEFUL IN THE EVALUATION AND DIAGNOSIS OF BONE AND SOFT TISSUE NEOPLASMS

INTRODUCTION

Ancillary studies including immunohistochemistry, molecular diagnostics, and cytogenetics play important roles in the diagnosis, subtyping, and prognostication of hematopoietic, epithelial, and mesenchymal neoplasms. The advent of clinically useful techniques for the detection of mutations, translocations, and copy number alterations has greatly expanded the utility of molecular diagnostics in the work-up of malignant neoplasms. Ancillary studies appear to be particularly helpful in the investigation of musculoskeletal lesions including lymphomas. Chromosomal translocations and mutations appear to be of greater diagnostic aid in bone and soft tissue lesions than in neoplasms of epithelial tissues. A subset of sarcomas bears chromosomal anomalies including reciprocal translocations, deletions, mutations, and amplifications which appear to be specific for certain histopathologic types. Mutations such as those occurring in the KIT and platelet derived growth factor alpha genes are important for the diagnosis of gastrointestinal stromal tumors as well as the prediction of response to directed therapy (Gleevec). Similarly, the SYT-SSX fusion transcript resulting from the t(X;18)(p11;q11) appears to be specific for synovial sarcoma. Of equal interest, both diagnostically and pathogenetically, are the translocations and fusion genes involving the EWS gene (22q12) which appear to define a Ewing family of sarcomas comprising the entities intra-abdominal desmoplastic small round tumor, myxoid chondrosarcoma, Ewing sarcoma, and primitive neuroectodermal tumor. These findings have facilitated the development of a molecular approach to soft tissue sarcomas. On this basis, soft tissue sarcomas currently can be divided into two groups. One group has specific chromosomal abnormalities (gene mutations and translocations) while the other shows complex often non-specific karyotypic abnormalities. The specific chromosomal abnormalities can be identified in many cases by FISH (Figure 2.1) or PCR techniques performed on paraffin embedded tissue. In some cases, demonstration of specific genetic abnormalities may allow the selection of tumor specific therapy (Gleevec®), as well as improving prediction of patient prognosis. Cytogenetics utilizing chromosomal banding techniques is helpful in detecting complex karyotypes associated with a number of soft tissue sarcomas including leiomyosarcoma, pleomorphic liposarcoma, malignant peripheral nerve sheath tumor, and some well differentiated liposarcomas. As the demonstration of karyotypic abnormalities usually requires cell culture and karyotypic abnormalities are often non-specific, their utility is less clear for the diagnosis and management of soft tissue sarcomas. Currently, molecular diagnostics is most useful in the diagnosis of synovial sarcoma, myxoid/round cell liposarcoma, Ewing family of sarcomas, alveolar rhabdomyosarcoma, dermatofibrosarcoma protuberans, and gastrointestinal stromal tumors.

Immunohistochemistry has had a long history of use in the evaluation of bone and soft tissue sarcomas. The demonstration of protein products such as desmin, actin, myogenin, keratins, melan-A, and S-100 protein is of aid in determining the cell lineage for soft tissue sarcomas. This is valuable in histomorphologic diagnosis. As soft tissue sarcomas are a relatively heterogeneous group of neoplasms which may share similar morphologic features, immunohistochemical detection of characteristic protein products is helpful in accurate and precise diagnosis. Unfortunately, many soft tissue sarcomas are promiscuous in their expression of antigens complicating the diagnostic interpretation of immunohistochemically detected protein expression profiles. Table 2.1 lists some of the immunohistochemical findings helpful in the differential diagnosis of bone and soft tissue tumors. Keratins are expressed focally or diffusely by a number of soft tissue sarcomas including synovial sarcoma, epithelioid sarcoma, rhabdoid tumor, and even some examples of epithelioid angiosarcoma. Antigen categories commonly utilized for the immunohistochemical investigation

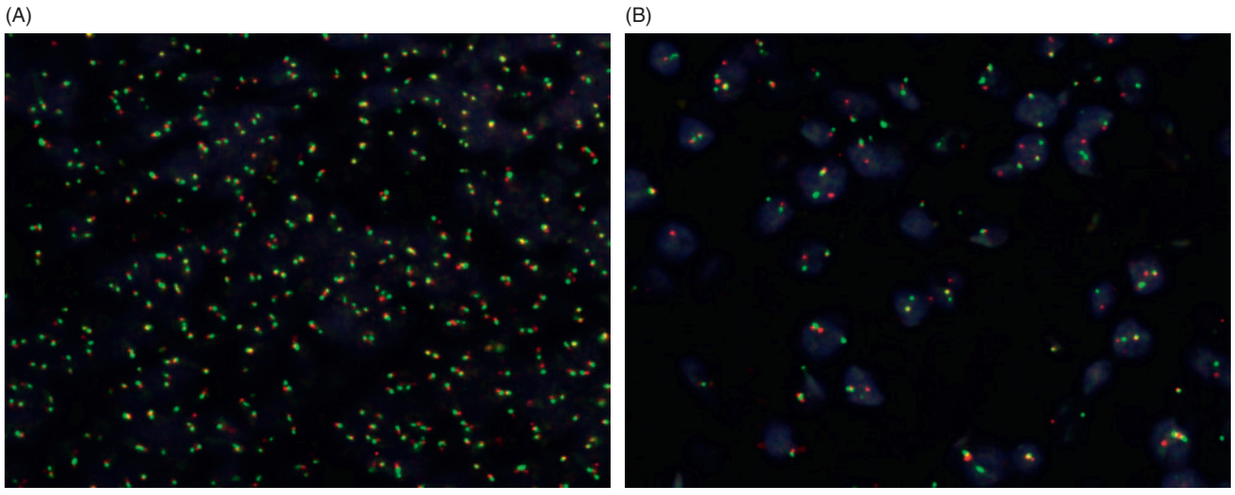

Figure 2.1 (A) Two fused sets of probes characteristic of normal (non-translocated) tissues negative for EWSR-1. (Fluorescence in situ hybridization, x 100) (B) Ewing sarcoma specimen demonstrating split probes characteristic of EWSR-1 translocation. (Fluorescence in situ hybridization, x 100)

of soft tissue sarcomas include intermediate filaments (cytokeratins, vimentin, desmin, neurofilament, and glial fibrillary acidic protein), transmembrane glycoproteins (CD31, CD34), and a variety of structural proteins or enzymes including HMB-45, melan-A, epithelial membrane antigen, synaptophysin, chromogranin, WT1, FLI-1, CD99, and CD117. While many of these antigens were initially believed to be specific for certain types of sarcomas, increased experience with immunohistochemistry has demonstrated that most are non-specific and are often expressed by a number of apparently unrelated bone and soft tissue sarcomas. Thus, cytokeratins are not only expressed by synovial and epithelioid sarcomas, but are also found in angiosarcomas, rare osteosarcomas, and rhabdomyosarcomas. Similarly, CD99, which was initially reported as unique to Ewing sarcoma, has now been shown to occur in a majority of mesenchymal chondrosarcomas, some osteosarcomas, synovial sarcomas, and rhabdomyosarcomas. Complicating the use of immunohistochemistry for fine needle aspiration specimens is the potential lack of relevant controls. Smear and cytospin preparations may not be optimal for immunohistochemistry. Issues regarding antigen sensitivity and specificity are aggravated by these techniques and neither type of preparation usually has a convenient technique specific control. The majority of controls utilized today for immunohistochemistry are formalin fixed paraffin embedded materials. The appropriate application of such controls to other specimen types remains controversial.

Similar issues appear to complicate molecular testing for bone and soft tissue sarcomas. The spectrum of neoplasms demonstrating the Ewing sarcoma translocation, t(11;22)(q24;q12), has expanded to include examples of mesenchymal chondrosarcoma, embryonal and alveolar rhabdomyosarcomas, intra-abdominal desmoplastic small round cell tumor, myxoid chondrosarcoma, and clear cell sarcoma. Thus, this translocation is found in a number of morphologically distinct but potentially pathogenetically related neoplasms. The SYT-SSX fusion transcript resulting from the t(X;18)(p11;q11) translocation was originally thought to be specific for synovial sarcoma. However, the t(X;18) has been detected in a group of malignant peripheral nerve sheath tumors, raising concerns regarding its specificity. Immunohistochemical and molecular diagnostic findings must be correlated with morphologic findings and should never be used in isolation for diagnosis.

IMMUNOHISTOCHEMISTRY

Immunohistochemistry is a well established technique for the detection of cell antigens indicative of their direction of differentiation. As noted previously, few if any antigens are entirely specific for a given histopathologic type of bone or soft tissue sarcoma, but patterns of antigen expression or lack thereof can contribute significantly when coupled with morphologic analysis to establish a