Applications of Ancillary Testing in the Cytologic Diagnosis of Soft Tissue Neoplasms

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KEYWORDS

• Soft tissue • Sarcoma • Fine-needle aspiration • Immunohistochemistry
• Fluorescence in situ hybridization

Key points

- A pattern-based approach for soft tissue neoplasms provides a practical framework for formulating differential diagnoses and applying ancillary tests.
- Ancillary tests can identify characteristic immunophenotypes and molecular alterations, enabling accurate cytologic diagnoses for soft tissue neoplasms.
- Ancillary testing allows for efficient work-up of fine-needle aspiration material, and rapid on-site evaluation has the added benefit of allowing specimen triage for ancillary testing.
- The diagnostic utility of ancillary testing relies on correlation with clinical and morphologic features and judicious application and appropriate interpretation.

ABSTRACT

Soft tissue neoplasms are diagnostically challenging, although many advances in ancillary testing now enable accurate classification of fine-needle aspiration biopsies by detection of characteristic immunophenotypes (including protein correlates of molecular alterations) and molecular features. Although there are many useful diagnostic immunohistochemical markers and molecular assays, their diagnostic utility relies on correlation with clinical and morphologic features, judicious application, and appropriate interpretation because no single test is perfectly sensitive or specific. This review discusses applications of ancillary testing for commonly encountered soft tissue neoplasms in cytopathologic practice in the context of a pattern-based approach.

OVERVIEW

Soft tissue neoplasms frequently present diagnostic challenges in cytopathology, as tumors are rare and comprise numerous and diverse entities. The World Health Organization classification includes more than 100 distinct tumor types, which are categorized according to common histogenesis.[1] The rapid rate of molecular advances in soft tissue pathology has facilitated refinements in tumor classification, recognition of novel entities, and development of diagnostic tests. Definitive cytologic diagnosis is now feasible for many tumors sampled by fine-needle aspiration (FNA) with use of ancillary tests, which can detect specific immunophenotypes and molecular alterations as well as prognostic and predictive markers. Clinical management of patients with soft tissue tumors relies on accurate reporting of biologic behavior, and in this regard ancillary testing can help refine differential diagnoses even when precise classification is not possible. Ancillary testing is also useful for tumors presenting in unusual clinical contexts (such as unexpected patient age/gender or tumor site), and molecular testing is especially helpful for tumors showing uncharacteristic cytomorphologic features or inconclusive immunophenotypes.

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The traditional role of immunohistochemistry in identifying line of differentiation is now expanded to include lineage-specific markers, protein correlates of specific molecular alterations, and novel tumor-type specific markers identified by gene expression profiling. Molecular methods have largely replaced conventional karyotype analysis. Fluorescence in situ hybridization (FISH) is used to detect translocations and amplifications. Reverse transcriptase–polymerase chain reaction detects specific fusion genes, which can be more specific than FISH given the promiscuity of many molecular alterations (eg, *EWSR1* and *FUS* rearrangements[2]). Single-gene analysis is relevant for some tumors, such as *KIT* mutations in GIST. There have been many developments in next-generation sequencing (NGS) that have specific applications in soft tissue pathology,[3] and it is expected that NGS will have wider clinical implementation and replace low-throughput techniques in the future. Most immunohistochemical antibodies and molecular assays have been clinically validated on formalin-fixed, paraffin-embedded (FFPE) material, and FFPE cell blocks are the favored substrate for ancillary testing. All cytologic preparations, however, have proved reliable substrates for FISH and sequencing-based methods,[4–6] and testing of direct smears and liquid-based preparations should be considered if material is limited. Rapid on-site evaluation gives an opportunity for specimen triage for ancillary testing during FNA.

The diagnosis of soft tissue tumors requires integration of clinical data, cytomorphology, and ancillary tests. A pattern-based approach provides a practical framework for formulating differential diagnoses and applying ancillary tests, and most soft tissue tumors fall into one of the following morphologic patterns: adipocytic, myxoid, spindle, round cell, epithelioid, and pleomorphic.[7] This review discusses updates in ancillary tests for the more commonly encountered soft tissue neoplasms on FNA in the context of this pattern-based approach.

ADIPOCYTIC NEOPLASMS

Tumors that show prominent adipocytic, or fatty, features include benign and malignant adipocytic neoplasms and several nonadipocytic tumors that have fatty components. Some adipocytic neoplasms overlap with other morphologic patterns: for instance, spindle cell lipoma, myxoid liposarcoma, and lipoblastoma have myxoid features; and pleomorphic liposarcoma and dedifferentiated liposarcoma (DDLPS) are part of the differential for pleomorphic neoplasms.

The presence of a mature fatty component and absence of cytologic atypia allows for general classification as a benign lipomatous tumor, although definitive subclassification can be more challenging on cytology.[8] The most commonly encountered benign tumors are lipoma, hibernoma, and spindle cell lipoma. Most cases of lipoma and hibernoma are diagnostically straightforward, with the former appearing as predominantly mature fat and the latter showing an admixture of mature adipocytes and brown fat with multiple cytoplasmic vacuoles.[9] Lipomas showing fat necrosis and hibernomas may mimic atypical lipomatous tumor/well-differentiated liposarcoma (ALT/WDL) and require MDM2 testing to exclude ALT/WDL (discussed later).

Spindle cell/pleomorphic lipoma shows a broad morphologic spectrum, with variable proportions of its constituent features of myxoid or collagenous stroma, uniform short ovoid-to-spindle nuclei, long ropey collagen fibers, and mature fat[10]; some cases may show multinucleated floret-like cells or small lipoblasts. Immunohistochemistry can be helpful in the diagnosis of spindle cell lipoma, which is positive for CD34 and shows loss of expression of RB1 secondary to loss of heterozygosity of 13q10,[11,12] similarly to its related tumors mammary-type myofibroblastoma and cellular angiofibroma.[13,14] Some tumors may need MDM2 and CDK4 immunohistochemistry to exclude ALT/WDL and others with more myxoid stroma may require *DDIT3* FISH to exclude myxoid liposarcoma.

ALT/WDL and DDLPS is characterized by amplification of chromosome 12q13-15. This region includes the *MDM2, CDK4,* and *HMGA2* genes, and amplification can be detected by FISH for the *MDM2* locus or immunohistochemistry for MDM2 and CDK4 (and HGMA2)[15] (**Fig. 1**). ALT/WDL shows atypical hyperchromatic stromal cells and variably sized adipocytes. These features are often subtle, and cases of lipoma-like ALT/WDL are especially challenging. Lipoblasts are rarely present and are not required for diagnosis. Fat necrosis may appear worrisome for ALT/WDL, and histiocytes can show MDM2 positivity.[16] Accurate diagnosis of ALT/WDL is important given its risk for recurrence and dedifferentiation to DDLPS. Nuclear expression of MDM2 and CDK4 is sufficiently diagnostic in many scenarios, although *MDM2* FISH has higher sensitivity for ALT/WDL in small biopsies[17] and should be considered for challenging cases. Testing for MDM2 amplification should always be performed for large (>10.0-cm) deep-seated extremity masses, tumors within body cavities (retroperitoneum and mediastinum), recurrent lesions, and tumors with equivocal atypia, because these scenarios favor ALT/WDL over lipoma.[18,19]