



Dorothy Fox. *Housatonic Fisherman*. Watercolor, 22" × 30".

Intraoperative pathologic diagnosis of bone and soft tissue lesions is an important tool in clinical musculoskeletal oncology practice.

Practical Issues of Intraoperative Frozen Section Diagnosis of Bone and Soft Tissue Lesions

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Background: *Intraoperative pathologic diagnosis of bone and soft tissue lesions is an important yet challenging tool in clinical musculoskeletal oncology practice. There is limited information in the literature addressing the practical issues commonly encountered regarding intraoperative frozen section of musculoskeletal lesions.*

Methods: *A literature review and retrospective review of practical experience in intraoperative pathology consultation at our institute's sarcoma program were conducted to investigate the pitfalls and limitations of frozen section and potential solutions to overcome these problems.*

Results: *Frozen section evaluation is an essential and reliable procedure for guiding intraoperative decisions. Intraoperative cytology as an adjunct to frozen section enhances the accuracy of diagnosis of bone and soft tissue lesions. Cytology can accurately diagnose certain entities alone and is superior to frozen section for certain tumor types and for evaluating bone marrow margins. It is also invaluable in triaging cases for ancillary studies and for tumor banking. Practical working protocols can be developed to optimize the usefulness of intraoperative pathologic consultation.*

Conclusions: *Intraoperative pathology consultation should be done in an interdisciplinary approach by correlating clinical, radiologic, and pathologic information. As an adjunct to frozen section, cytology and gross examination enhance the accuracy of diagnosis of musculoskeletal lesions.*

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Abbreviations used in this paper: FISH = fluorescent in-situ hybridization.

Introduction

Histopathology plays an integral role in the multidisciplinary approach of treating patients with sarcoma, the accuracy of which has important therapeutic implications. Intraoperative frozen section consultation is particularly challenging. Indications for frozen section include making a diagnosis, evaluating margin status, determining tumor extent/spread, and obtaining an adequate sample for diagnosis. Frozen section provides real-time evaluation usually within 20 minutes. This

Table 1. — Distribution of Cases Based on the Type of Intraoperative Consultation

Type	No. of Cases
Gross for margin	107
Diagnosis (cytology + frozen)	160
Diagnosis (frozen only)	53
Total	320

process includes gathering clinical and radiologic information, utilizing rapid methods for tissue sampling, preparing slides, staining, performing microscopic examination, and ultimately making an accurate diagnosis. Frozen section has an overall diagnostic accuracy ranging from 89% to 98% in various studies of all tissue types.¹⁻³ Despite the high degree of accuracy, studies have shown the limitations of frozen section. For example, frozen artifact can produce inferior slides for microscopic examination, and sampling errors can result from the heterogeneity of a tumor.

Bone and soft tissue tumors present a greater challenge. Sarcomas are relatively rare compared to carcinomas. In most community hospital settings pathology services have limited exposure to bone and soft tissue tumors. Not all bone and soft tissue specimens are suitable for frozen section due to, for example, their bone and/or fatty components. Few studies have been performed evaluating the role of frozen section in the diagnosis of musculoskeletal tumors. Among several larger studies, bone and soft tissue lesions constitute a small percentage of the total lesions studied. Dalal et al² reported 133 cases of bone and soft tissue lesions (12% of total cases) with 125 accurate diagnoses (93.9%). Holaday and Assor³ reported 581 cases of bone and soft tissue frozen sections (5.8% of total cases) with 96% diagnostic accuracy. Shah et al⁴ reported that a specific diagnosis was made by frozen section in 85.9% cases with an overall diagnostic accuracy of 90.1% (82 of 91 cases). These studies excluded cases that are not suitable for frozen section. Histopathologic diagnosis on routine paraffin section is used as the gold standard.

Currently there are no consensus guidelines for the diagnostic approach to biopsy a bone or soft tissue tumor.^{5,9} Core biopsy is the most commonly used approach. Image-guided biopsy is suitable for various lesions including those not easily accessible, and it also can be utilized when an operative biopsy is not safe for the patient.^{6,9} Surgical biopsy with intraoperative frozen section has been advocated by some authors as an accurate and cost-effective method of diagnosing musculoskeletal sarcomas.⁵ Fine-needle aspiration biopsy alone or in conjunction with core biopsy has been used as the primary diagnostic modality in the evaluation of musculoskeletal tumors.^{10,11} In addition, there are no standardized protocols for performing intraoperative

frozen section of bone and soft tissue tumors. Practical working protocols should be developed to maximize the usefulness of intraoperative pathologic consultation.

In this article we discuss (1) the methods that can be used in conjunction with frozen section to enhance the diagnostic accuracy of bone and soft tissue lesions intraoperatively, (2) the methods currently used at our institute for intraoperative evaluation of musculoskeletal lesions to facilitate diagnosis, and (3) the potential pitfalls of frozen section with case illustrations. We emphasize the importance of an interdisciplinary approach to reach an accurate diagnosis and the importance of effective communication of the frozen section result.

Value of Intraoperative Cytology as an Adjunct to Frozen Section

We conducted a retrospective study to investigate the value of cytologic preparation as an adjunct to frozen section in the diagnosis of bone and soft tissue lesions and to explore the potential application of cytology techniques in the intraoperative setting. Cytology preparations typically included touch imprint and smear from a lesion. Additional unstained slides (from imprints, smears, or cytopins made from the rising of tissue fragments) can be prepared for ancillary studies when necessary.

From December 1, 2006, to August 1, 2007, a total of 320 patients had intraoperative pathologic consultation (Table 1). Among them, 160 patients (50%) underwent both cytologic and frozen section examinations, 53 had only frozen section diagnosis with no corresponding cytology examination, and 107 underwent gross examination for margins.

The final diagnoses of the 160 patients who had simultaneous cytologic and frozen section examination

Table 2. — Histopathologic Diagnosis of 160 Cases

Diagnosis	No. of Cases
Nonmesenchymal tumor	47 (29%)
Carcinoma	29
Melanoma	2
Merkel cell carcinoma	1
Lymphoma/plasmacytoma	15
Mesenchymal tumor	68 (43%)
Ewing's sarcoma	4
Synovial sarcoma	2
Giant cell tumor/lesions	15
Myxoid liposarcoma	3
Other liposarcoma	7
Peripheral nerve sheath tumor	6
Fibromatosis/fibroma	3
Angiosarcoma	5
Myxoma	12
Other	11
Mesenchymal nonneoplastic lesion	16 (10%)
Osteomyelitis	2
Nodular fasciitis	1
Benign/reactive	13
Bone marrow margins	29 (18%)

are listed in Table 2. For the 53 patients who were evaluated by frozen section only, a specific diagnosis was made in 47 (89%). For the 160 patients evaluated by frozen section with adjunct cytology, a specific diagnosis was made in 144 (91%) by frozen section only and in 156 (98%) with adjunct cytology. Overall cytology can confirm the frozen section findings; among 12 cases, a specific diagnosis would not be possible without cytology. These cases were lymphoma, plasmacytoma, Ewing's sarcoma, metastatic carcinoma, metastatic melanoma, Merkel cell carcinoma, and liposarcoma.

Cytology enhanced the diagnosis in conjunction with frozen section in several cases. For cellular neoplasms such as Ewing's sarcoma, synovial sarcoma, metastatic carcinoma/melanoma, and lymphoma/plasmacytoma (Fig 1A-B), cytology alone was diagnostic. Cytology also provided adequate specimen for confirmatory ancillary studies — molecular cytogenetic analysis by fluorescent in-situ hybridization (FISH) (Fig 2A-C) or rapid intraoperative immunohistochemistry for pankeratin or S-100 protein.

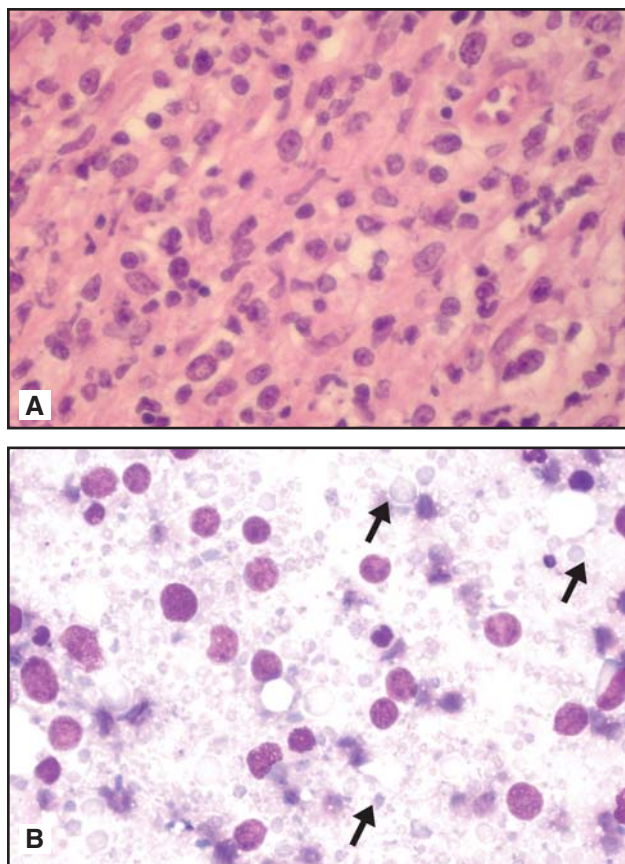


Fig 1A-B. — A 79-year-old woman with right femoral head lytic lesion clinically suspicious for metastatic disease. She had no known history of cancer. (A) Hematoxylin-eosin slide of frozen section of the tumor reveals a poorly differentiated malignant neoplasm ($\times 200$ magnification). Differential diagnosis includes metastatic disease, lymphoma and sarcoma. (B) Diff-Quik stain slide of touch imprint cytology of tumor. The cells are dyshesive. The characteristic nuclear features and numerous lymphoglandular bodies (arrows) are most consistent with a lymphoma. Follow-up flow cytometry analysis confirmed a diffuse large B-cell lymphoma.

For hypocellular or fibrotic neoplasms, the role of touch imprint cytology was limited. Smear cytology provided better cytologic and architectural details compared to frozen section. For myxoid and adipocytic lesions, cytology was more useful than frozen section for diagnosis. Myxoid liposarcomas can be easily recognized on cytology slides, facilitating confirmatory FISH testing.

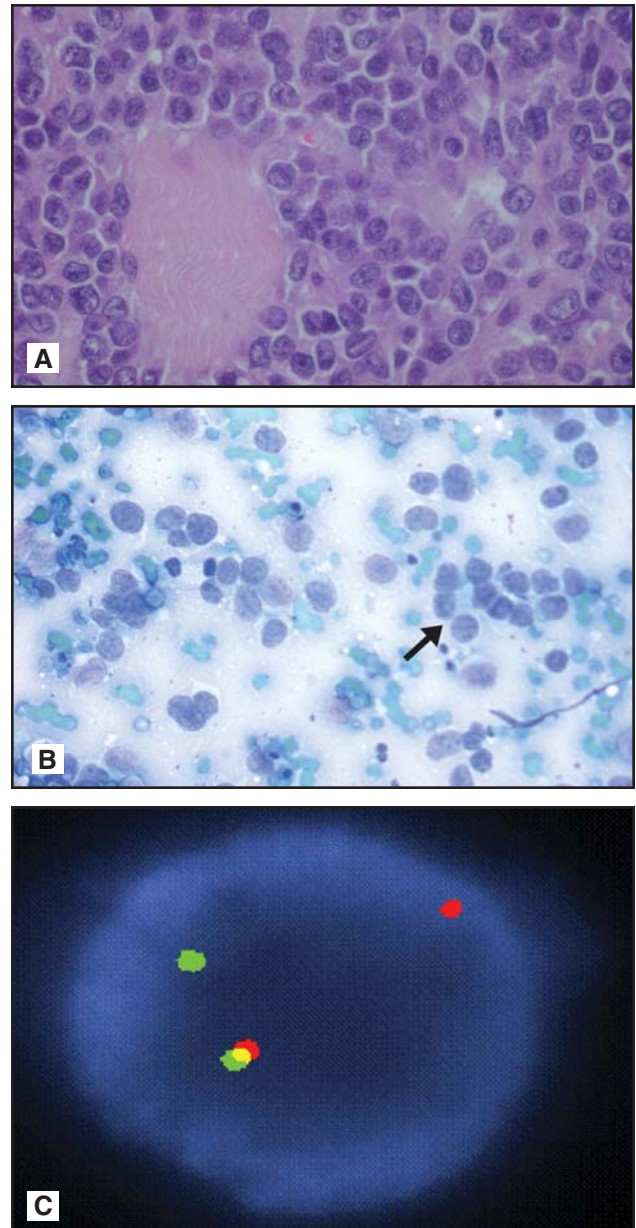


Fig 2A-C. — A 24-year-old man with left pelvic mass. Clinicoradiologic impression is that of a malignancy with top differential diagnosis including lymphoma, sarcoma, or germ cell tumor. (A) Hematoxylin-eosin stain of the frozen section shows a poorly differentiated malignant neoplasm composed of blue round cell tumor ($\times 200$ magnification). (B) Diff-Quik touch imprint cytology reveals dyshesive cells with occasional pseudorosette formation (arrow) without lymphoglandular bodies ($\times 400$ magnification). These findings favor Ewing's sarcoma/primitive neuroectodermal tumor (PNET). Unstained touch imprint cytology slide was submitted for FISH analysis. (C) FISH study is positive for $t(11;22)(q24;q12)$ chromosomal translocation, a finding that confirms Ewing's sarcoma/PNET.

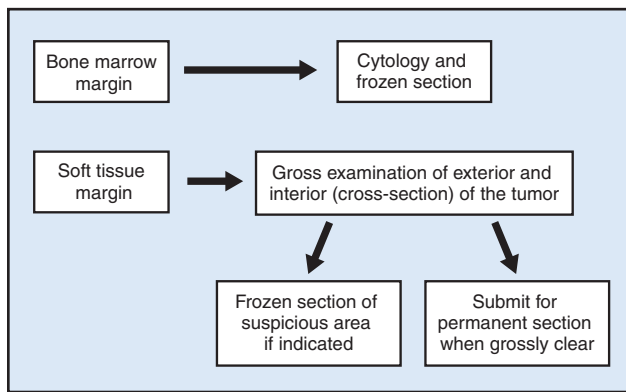


Fig 3. — Protocol for intraoperative margin evaluation.

Smears sample a larger area of the tumor than frozen section. This improves the identification of high-grade components that typically require fresh tissue of ancillary chromosomal analysis. For osseous and cartilaginous lesions, cytology improved discrimination of cellular details. Touch imprints proved to be superior to frozen sections in the assessment of bone marrow margins due to the increased sampling area and the lack of freezing artifacts. In addition, touch imprints were excellent in assessing cellular viability and overall quality of a specimen for tumor banking.

Cytology preparation is excellent for ancillary study. In one case, a woman with a history of both breast cancer and melanoma developed an osseous lesion of the right femur. Intraoperative cytology and frozen section revealed a poorly differentiated malignant neoplasm with plasmacytoid cytology. The differential diagnosis included metastatic breast cancer vs metastatic melanoma. Intraoperative cytokeratin AE1/3/CAM 5.2 staining revealed positive cancer cells confirming metastatic breast cancer. Unstained cytology preparation is routinely prepared from tumors during intraoperative consultation. These slides can be submitted for subsequent gene rearrangement study to confirm lymphoma and for FISH study to confirm sarcomas (such as Ewing's sarcoma, myxoid liposarcoma, and synovial sarcoma).

In summary, our study demonstrates the role of intraoperative cytology in evaluating bone and soft tissue lesions. Cytology alone can accurately diagnose certain entities and is superior to frozen section for certain tumor types and for the evaluation of bone marrow margins. It is invaluable in triaging cases for ancillary studies and for tumor banking. Cytologic samples are excellent for ancillary studies including FISH. However, cytology should not be used alone or to replace frozen section but rather used in conjunction with frozen section to facilitate a specific intraoperative diagnosis.

Working Protocols for Intraoperative Pathologic Consultation

At our institute, a pathologist specializing in musculoskeletal tumors performs intraoperative pathology consultation. A multimodality approach is routinely applied including gross examination, cytologic examination, and frozen section.

Our working protocol for margin evaluation is illustrated in Fig 3. For bone tumor marrow margins, the entire marrow is touched for cytology evaluation followed by frozen section. For a soft tissue mass, it is important to have clear orientation by the surgeon and careful gross examination of the exterior and interior (cross section) of the tumor to identify areas suspicious for margin involvement. If necessary, frozen sections of suspicious areas are performed; otherwise, the specimen is processed routinely for permanent section and definitive evaluation. We do not believe cytology has a practical role in evaluating soft tissue margins.

It is important to note that fresh tumor evaluation is the best opportunity to preserve tissue for ancillary studies that either cannot be performed or are performed less optimally on formalin fixed tissues. When it is indicated, the fresh tumor specimen is routinely triaged by our pathologist for ancillary study to facilitate a definitive diagnosis. Fresh tissue can be collected for flow cytometry (in RPMI), cytogenetics (in RPMI), electron microscopy (in 3% glutaraldehyde), FISH (unstained touch imprints, smears and cytopspins), and molecular studies (in RNA preservative).

Our diagnostic protocol is outlined in Fig 4. In our experience, a realistic expectation for intraoperative pathologic diagnosis includes the following: (1) determining whether lesion is benign or malignant, (2) if malignant, determining whether it is lymphoma/plasmacytoma, metastatic disease or mesenchymal primary, and (3) recognizing that the subtyping of a tumor is not

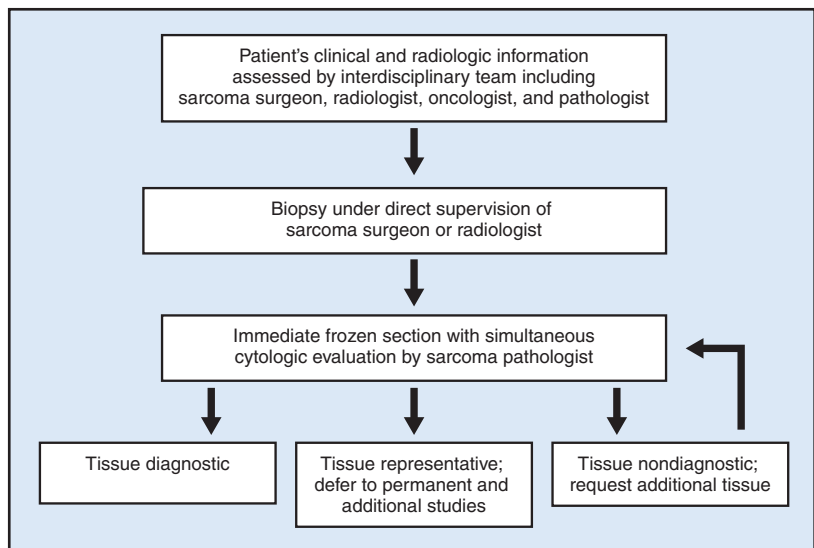


Fig 4. — Protocol for intraoperative pathology diagnosis.

always possible. It is important to obtain representative tissue, establish a differential diagnosis, and appropriately triage the tissue to facilitate a definitive diagnosis.

It is important to determine if the biopsy tissue is representative of disease and if a diagnosis can be made with the tissue provided. Touch imprint cytology evaluates the quality of the specimen. If cytology reveals a hypocellular specimen, additional tissue can be requested. It is critical to reserve tissue for ancillary studies even in cases where a specific diagnosis can be made by frozen section. Unfrozen tissue for permanent section and ancillary studies are usually needed for confirmation.

Importance of an Interdisciplinary Approach to Intraoperative Pathologic Consultation

A clinical history and the radiologic findings are vital in evaluating cytology and frozen sections of soft tissue and bone tumors. After a careful intraoperative patho-

logic evaluation is made, it is critical that the pathologist communicates effectively with clinicians. This process should include the surgeon, radiologist, and oncologist with a clear understanding of the expectation of a frozen section. Two cases are described to illustrate this interaction.

Case 3 involves a symptomatic 69-year-old man who presented to the sarcoma clinic with a 12-cm peritoneal mass. MRI images indicated a heterogenous mass with focal necrosis. He had no known history of cancer and no other detectable masses. Clinicoradiologic impression favored a malignant process. Differential diagnoses including high-grade sarcoma, lymphoma, carcinoma, and a germ cell tumor. An image-guided core biopsy was obtained for tissue diagnosis by frozen section examination. Cytology revealed mild to moderately pleomorphic spindle cells with no obvious matrix, mitoses, or necrosis. The cytoplasmic features were suggestive of a mesenchymal lesion. Frozen section demonstrated a hyper-

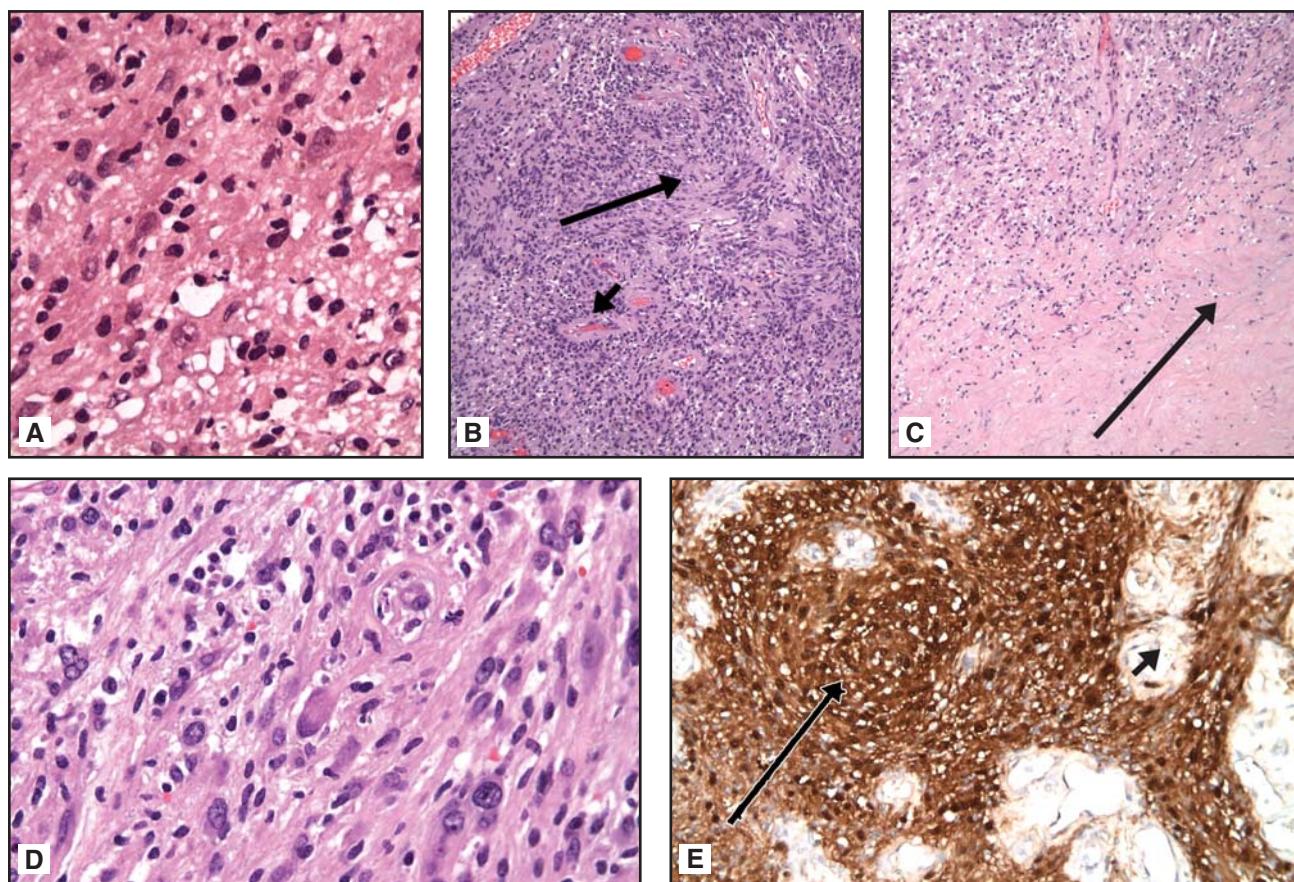


Fig 5A-E. — A symptomatic 69-year-old patient with a 12-cm peritoneal mass in sarcoma clinic. Intraoperative pathologic consultation of core biopsy was requested to determine a diagnosis or, if not possible, to rule out lymphoma. (A) Hematoxylin-eosin (H&E) stain of the frozen section of core biopsy shows atypical spindle cells suspicious for sarcoma ($\times 200$ amplification). Frozen artifact is present including hyperchromasia without nuclear detail (compared with permanent section in Fig 5D that shows hypochromasia with nuclear detail). (B) H&E stain of the formalin-fixed paraffin-embedded tissue (permanent section) reveals a spindle cell neoplasm with hypercellular areas with nuclear palisading (long arrow) and hyalinized vessels (short arrow), features commonly seen in schwannoma ($\times 100$ amplification). (C) H&E stain of permanent section demonstrates necrosis/infarction area (arrow) that mimics malignancy (amplification $\times 100$). (D) H&E stain of permanent section that is similar to frozen section showing cytologic atypia, a feature usually seen in malignancy ($\times 200$ amplification). However, there are no significant atypical mitoses identified, which does not support a malignant neoplasm. In conjunction with histologic features in Fig 5B-C, the atypical cytologic features and necrosis/infarction are the result of degenerative changes, which is a common pitfall in frozen section examination and core biopsy. (E) S-100 immunostain is strongly and diffusely positive in the nuclei of spindle cells (long arrow) and confirms the diagnosis of schwannoma ($\times 100$ amplification). Note that the hyalinized vessels are negative for S-100 (short arrow).

cellular spindle cell lesion arranged in a fascicular pattern with mild to moderate cytologic atypia (Fig 5A). No mitosis or necrosis was identified. These findings were consistent with a mesenchymal neoplasm. The cytologic atypia, in conjunction with clinicoradiologic findings, favored a malignancy. Histopathologic examination of the permanent section of this core specimen revealed similar morphologic features when compared with frozen section. Frozen section ruled out lymphoma, carcinoma, and germ cell tumor, and surgical resection was performed. The subsequently resected specimen revealed a hypercellular spindle cell neoplasm with features of schwannoma, which is a benign peripheral nerves sheath tumor (Fig 5B). Mild cytologic atypia, moderate nuclear pleomorphism, and large areas of infarction representing degenerative changes frequently seen in schwannoma were noted; however, these features are notorious mimickers of malignancy (Fig 5C-D). In this case, the overall morphologic features were most consistent with schwannoma. Immunohistochemical stains supported the diagnosis: strong and diffuse nuclear reactivity to S-100 (Fig 5E). This case illustrates the pitfalls of a core biopsy and frozen section and the importance of effective communication of a frozen section result. Tumors of this nature can be heterogeneous, and the initial core biopsy was obtained from a more cellular area with degenerative changes that mimicked a malignancy. Although the intraoperative diagnosis favored sarcoma, both the pathologist and the surgeon understood the preliminary nature of the diagnosis. The diagnosis did rule out lymphoma, carcinoma, and germ cell tumor. A mesenchymal neoplasm was most likely. However, the tumor grade and subtype could not be reliably assessed by frozen section of the core biopsy. The core sampled a tiny portion of the large mass, and this biopsy could have represented a benign mesenchymal lesion, a low-grade sarcoma, or a low-grade area of a high-grade sarcoma. In this situation, it was appropriate to resect the tumor to achieve clear margins followed by complete evaluation of the mass for a definitive diagnosis.

Case 4 involves a 56-year-old man with a right arm soft tissue mass. Clinical and radiologic impression was suspicious for a soft tissue sarcoma. Core biopsy of this mass was performed as an office procedure, and an intraoperative pathology consultation was requested. Cytology and frozen section revealed a hematopoietic neoplasm, suspicious for lymphoma. By our protocol, tissue was submitted for flow cytometry, molecular study for gene rearrangement, and permanent histologic examination. Flow cytometry revealed an overall polyclonal lymphocyte population with a subset of monoclonal larger B cells. However, the quantity of the abnormal cells was not sufficient for a definitive diagnosis of a diffuse B-cell lymphoma. In this case, gene rearrangement testing was crucial. As a result of this test being positive, a final diagnosis of B-cell lymphoma

could be made and appropriate chemotherapy prescribed. If gene rearrangement testing was negative, a diagnosis suspicious for B-cell lymphoma would be appropriate, but would warrant additional sampling for confirmation.

Conclusions

Intraoperative pathologic diagnosis of bone and soft tissue lesions is an important yet challenging tool in clinical musculoskeletal oncology practice. The pitfalls and limitations can be overcome by (1) utilizing a multidisciplinary approach to correlate clinical, radiologic, and pathologic information, (2) applying cytology and gross examination as an adjunct to frozen section to enhance the diagnosis, and (3) recognizing that intraoperative diagnosis is preliminary and warrants confirmation.

Disclosures

No significant relationship exists between the authors and the companies/organizations whose products or services may be referenced in this article.

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