Challenges of Frozen Section in Thoracic Pathology

Lepidic Lesions, Limited Resections, and Margins

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- **Context.**—The use of frozen section in thoracic pathology includes assessment of peripheral lung lesions with lepidic pattern, with greater emphasis on evaluating lung-sparing resections and margin assessment.

- **Objective.**—To review pitfalls of frozen section in thoracic pathology; in this setting, reduction of false-positive and false-negative diagnosis in lesion identification and margin assessment is critical.

- **Data Sources.**—PubMed search of frozen section lung pathology yielded specific references related to the use of frozen section in the identification of lepidic lesions and the clinical recommendation for margin distance.

**Conclusions.**—Frozen section diagnosis is overall accurate in assessment of lepidic lesions. Pitfalls include rare benign mimickers and more common reactive lesions. Standard approaches to gross assessment and margin measurement require further research with increasing use of lung-sparing resections.


The use of frozen section in many subspecialty areas of pathology has diminished and its role altered as a result of changes in surgical technique and approach. In addition, although it is overall an accurate process, experience with frozen section has identified pitfalls in its execution that have warranted reevaluation of its use. In many centers, certain types of frozen sections, such as lymph nodes during prostatectomy or lesion identification in breast carcinoma, have either dwindled significantly or become nonexistent.

In addition, the advent of small biopsy and cytologic evaluation in a variety of preoperative settings has allowed for the planning of surgical procedures without the subsequent need for intraoperative consultation. The questions have changed, and the routine use of frozen section solely for the purpose of lesion identification or nodal status has been replaced by a tailored, case-by-case approach. This preoperative treatment planning may allow for conversations to take place with the patient as to the extent of planned surgery, and as a result predetermine the scope of the procedure for which consent is given.

In thoracic frozen sections, the combination of imaging and preoperative sampling has influenced use. Mediastinal lymph nodes that are unlikely to be positive for tumor—small nodes or positron emission tomography scan-negative nodes—might not be sent for frozen section. In other instances, endobronchial ultrasound-guided sampling may be used preoperatively to evaluate lymphadenopathy prior to surgery. As a result, intraoperative nodal sampling for frozen section is increasingly directed toward nodal groups that are suspicious by imaging, or, in some procedures, potentially no nodes are sent at all.

Characterization of mass lesions is also not always required. With the definitive diagnosis of squamous cell carcinoma or patterns of invasive adenocarcinoma on small samples, a procedure can be planned as a definitive resection without wedge sample for diagnostic confirmation. Recommendations moving away from routine frozen section diagnosis of bronchial margins in lobectomy procedures allow for a lobectomy without any frozen sections at all.

Thoracic frozen sections are still frequently used, but their indication has changed. The classification of adenocarcinoma has allowed for recognition of tumors that are preinvasive or minimally invasive, and, with imaging correlates of these patterns, leads the surgeon to offer a more limited resection than lobectomy or segmentectomy. In these settings, frozen section use may include the characterization of margins. Margin assessment in lung-sparing procedures has become a more frequently requested frozen section. With the recognition that multiple pulmonary nodules may reflect (1) synchronous primary tumors, (2) invasive cancer plus a synchronous preinvasive or minimally invasive lesion, or (3) carcinoma plus an inflammatory nodule, intraoperative sampling of ipsilateral nodules in a separate lobe or segment for lesion identification and margin assessment may be needed as part of a limited resection of such secondary nodules.

In addition to these practical issues with regard to the scope of surgical procedure, frozen section remains of use in the determination of malignancy in lesions in which preoperative sampling was impractical, unsuccessful, inde-
terminate or benign. In the benign sampling, the concern for malignancy on clinical grounds may not be completely assuaged by a benign diagnosis. These frozen sections for diagnosis have many pitfalls, which include the overcalling of benign lesions as malignant; these mimickers of malignancy include rare neoplasms but, more commonly, reactive conditions. In the other direction, the recognition of malignancy in some lesions is quite difficult—for example, because of their inherently bland cytologic features or obscuring inflammation. This is a double-edged sword—both overcalling and undercalling malignancy have direct impact on the surgery chosen and therefore on patient care.

**LESION IDENTIFICATION IN GROSS EVALUATION**

The use of video-assisted thoracoscopic surgery in combination with the image detection of smaller target lesions with ground-glass attenuation has led to a more frequent problem of lesion identification during gross pathologic evaluation at frozen section. These lesions are less likely to be palpable without sectioning the tissue, and as a result the certainty that the lesion is in the specimen can be less than 100%. The success of frozen section in high-volume busy frozen section rooms, to assure both accuracy and timeliness, is vitally dependent on controlling the process from beginning to end. For the pathologist trying to meet a quality parameter of a 20-minute benchmark for frozen section completion and for the surgeon trying to properly plan a procedure, the situations in which lesions are hard to find are predictably stressful.

When receiving a lung specimen that contains a nodule, the gross evaluation is more complex when the nodule is not easily palpated. It is essential that information regarding the imaging be known at the time of the gross evaluation. Several features need to be extracted from the clinical record. The size of the lesion, its relationship to the pleura, and the shape and attenuation of the lesion are of utmost importance. The question to be answered, “Is what I am feeling or seeing consistent with the lesion?” is an important one. A solid or semisolid attenuation on imaging is more likely a palpable lesion, whereas a ground-glass lesion might not be palpable externally.

It is also important to know the plan for the procedure for that day. If the intent is to document or confirm malignancy with a lobectomy to follow, then the intraoperative consult is only for lesion identification. However, especially in ground-glass lesions, it is possible that the wedge resection is all that is planned and as a result the margin will be important. Unfortunately, the malignant lesions that are the easiest to identify externally are the ones less likely to require margin assessment. If the lesion is palpable, then its relationship to the margin can be more readily oriented with a perpendicular section through the nodule to the staple line to grossly reveal the relationship and distance to the margin (Figure 1, A). This will make it easier to demonstrate histologically on frozen section (Figure 1, B).

Microcoils have been used to improve lesion detection during video-assisted thoracoscopic surgery resections. Although this may improve lesion detection by the surgeon, the presence of numerous coils can interfere with gross evaluation and frozen section.

For the nonpalpable lesion, information about the size, shape, attenuation, and potentially location within the specimen guides the serial sections through the gross specimen needed to visualize the mass. Some authors
recommend inflation of the specimen with a mixture of optimal cutting temperature medium (OCT) before sectioning.\(^4\) Although inflation has been reported to help visualize the gross lesion,\(^5\) in my experience this is largely due to the washing away of blood, which has often engorged the specimen, making it harder to palpate and obscuring subtle color differences. Serial sectioning followed by washing the specimen with water can be equally effective at removing the blood and allowing the lung tissue to regain its natural color. At this point, radiologic ground-glass lesions that ultimately have lepidic architecture will be slightly raised relative to the adjacent lung parenchyma and have a slight difference in color. Palpation of the lung sections between index finger and thumb will reveal an area that is slightly more indurated than the adjacent spongy lung. This allows for selection of an area for lesion confirmation.

**MARGIN EVALUATION IN WEDGE RESECTIONS**

Handling the margin at this point is also important. Cutting off the staple line, although expedient, results in loss of orientation of tumor to true margin. This is problematic as there is 5.0 mm or more of lung tissue within the 3 rows of staple line. If the frozen section shows tumor on this false margin it is in a sense a documented false-positive, potentially creating a problem with the subsequent surgical evaluation. In some cases, it is not possible to take additional margin and still perform lung-preserving surgery.

It is evident that a negative resection margin has survival advantages over a positive one, and between 5% and 15% of patients have a positive margin. In a large series, Hancock et al\(^6\) showed positive margin significantly reduced 5-year survival; this result was also noted by Sawabata et al.\(^7\) However, the optimum distance of tumor to negative margin has not been definitively established.\(^8\) For small lesions (smaller than 2.0 cm), a distance of 15 mm was established by one group beyond which there was no improvement in recurrence rate.\(^9\) In one series, the recurrence rate doubled when margins were below 10 mm, again for small lung tumors.\(^10\) It may be that larger tumors require larger resection margins.\(^11\)

Specifically in ground-glass lesions, the low rate of recurrence with 10-mm margins and need for reoperation after positive margin can impact overall survival. In ground-glass lesions with 10-mm margins, reoperation or therapy at time of recurrence may be an effective strategy; this may be
Figure 4. Higher-power view of frozen section appearance of neoplastic versus reactive lesion. A, Neoplastic cells are cuboidal and, although atypical, relatively uniform from cell to cell. B, Alveolar spaces vary from ones lined by markedly atypical cells alongside enlarged but less atypical cells in a reactive lesion. C, Marked atypia with multinucleation alongside gaps along alveoli of residual type 1 cells (hematoxylin-eosin, original magnifications ×10 [A], ×20 [B], and ×40 [C]).

Figure 5. Reactive lesions that can be confused with adenocarcinoma. A, Angulated glands within an area of scarring can mimic invasive glands. B, Moving to a lower-power view can assist in recognizing the effaced but still recognizable lung architecture of regularly spaced airways and vessels. C, Collapsed bronchiolar epithelial lined alveoli (Lambertosis) can look concerning, but the relationship to the bronchiole confirms a benign metaplastic proliferation (hematoxylin-eosin, original magnifications ×10 [A], ×2 [B], and ×20 [C]).
in part due to the indolent nature of these lesions. As a result, 10 mm may be sufficient in these lesions.

Although there is no absolute agreed-upon length for adequacy of margin, it would be beneficial to establish an approach that reduces the documentation of false-positive margins while providing a more accurate measurement. One approach is to serially section the lesion perpendicular to the margin and take a small piece of lung outside of the staple line, where there is often a thin sliver of lung tissue. This would determine the true margin, but would not allow a measurement of tumor to true margin. Another approach is to grossly identify the nearest margin and to remove 9 to 12 staples in the area and cut a perpendicular section of tumor to margin. If the staple line is inked, this allows for both a gross and microscopic measurement of tumor to margin. The staple line removal does take extra time, but can be performed in about 5 minutes while the frozen section for lesion characterization is being sectioned. The removal of the staples can be performed with a very thin beaked forceps or with the point of a scissors. Cutting the staples themselves, although feasible, leads to difficult-to-remove staple fragments. Some tearing of lung tissue does occur during staple removal, and these defects can be seen in the tissue on the frozen section itself. However, despite these artifacts, measurement of tumor to true margin becomes possible and also highlights the interface between lesion and margin (Figure 2, A). In contrast, if the margin is cut off prior to inking, a false-positive margin can be inadvertently documented (Figure 2, B).

**DIAGNOSTIC CONSIDERATIONS IN LEPIDIC LESIONS**

Evaluation of lepidic lesions for lesion identification is challenging, as the tumor cells do not efface architecture and can be relatively bland. In addition, there can be associated interstitial inflammation, fibrosis, and granulomata. If the lesion is grossly identified, one important feature of section selection is the demonstration of the interface between the lesion and adjacent lung tissue. The interface is crucial, as the tumoral proliferation and its interstitial reaction show a sharp transition in neoplasms and gradual or no transition in inflammatory or reactive conditions (Figure 3, A versus B).

A second important diagnostic criterion is the morphology of the pneumocytes. In neoplastic lepidic tumors, the cells are cuboidal and enlarged, but their growth is uniform and completely replaces the normal alveolar epithelium (Figure 4, A). In reactive conditions, the pneumocytes have a wider range of size variation, from normal-sized type 2 pneumocytes to ones that are enlarged and even multinucleated (Figure 4, B and C). The presence of cilia, a lack of inclusions, mixed cell types, and cell-to-cell size variability are all in favor of reactive epithelial cells. In this setting, additional features such as hyaline membranes or fibrin may further support this benign and reactive impression.

In one series of 25 frozen section errors in thoracic pathology, inflammatory conditions with type 2 hyperplasia were confused with carcinoma, squamous metaplasia was confused with squamous carcinoma, and carcinomas with abundant inflammation or granulomas were confused with inflammatory conditions. In a study of 224 thoracic frozen sections, errors were seen in 12.1% (27 of 224) of cases, with deferrals in 6.3% (14 of 224). In addition to problems of sampling and poor-quality frozen sections, scar and inflammation were causes of diagnostic error in lung frozen sections.

Bland cytology, low cellularity, fibrosis and inflammation, and atypia in benign conditions are among reasons for frozen section error. The gross presence of a mass lesion, sharp demarcation, and cellular monotony are all in support of carcinoma. Additional features including macronucleoli, atypical mitoses, varied growth patterns, and irregular nuclear membranes are also in support of carcinoma; these are summarized in the Table.

An area of diagnostic difficulty is entrapped glands in scarred lung. The scarred area may appear as a mass lesion grossly, and the collapsed, entrapped glands can look angulated and atypical. The presence of ciliated cells in these glands is an important clue that the proliferation is benign, and the lower-power appearance may also be a hint in that the clusters of glands will have intervening scar or lung tissue around them. In addition, the presence of a regular pattern of glands with an adjacent blood vessel supports the retention of underlying architecture in these areas (Figure 5, A and B).

The presence of bronchiolar-type epithelium in alveolar spaces around bronchioles, a reactive process that is the result of bronchiolar cell proliferation traveling through canals of Lambert that connect bronchioles to alveoli (also referred to as bronchiolar metaplasia), can be sufficiently exuberant to resemble a lepidic-pattern adenocarcinoma. Here again a lower-power view can be informative as the adjacent native bronchiole and/or the presence of a similarly sized arterial blood vessel can again reaffirm retention of existing architecture (Figure 5, C).

**NEOPLASMS THAT CAN BE MISTAKEN FOR ADENOCARCINOMA**

Sclerosing pneumocytomas are unusual type 2 pneumocyte proliferations that demonstrate 4 distinct patterns: papillary, solid, telangiectatic, and sclerosing. The circumscription (Figure 6, A) and bland cellular population of the solid pattern can be mistaken for carcinoid tumor, but the nuclear features with clearing and inclusions are not those of carcinoid (Figure 6, C). The papillary pattern can be mistaken for carcinoma with lepidic or papillary pattern. The sclerosis of this lesion, although not unique to this

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**Table: Pathologic Features Differentiating Lepidic Adenocarcinoma From Reactive Hyperplasia**

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<tr>
<th>Features favoring lepidic neoplasm</th>
<th>Features favoring reactive proliferation</th>
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<td>Grossly mass lesion</td>
<td>Major</td>
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<td>Sharp interface of pneumocyte proliferation</td>
<td>Indistinct transitions</td>
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<tr>
<td>Sharp interface of interstitial inflammation/fibrosis</td>
<td>Cellular size variation</td>
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<td>Monotonous cuboidal or low columnar pneumocyte proliferation</td>
<td>Multinucleation</td>
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<td>Cilia</td>
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<td></td>
<td>No intranuclear inclusions</td>
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<td></td>
<td>Retained lung architecture</td>
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<td>Minor</td>
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<td>Absence of macronucleoli despite nuclear atypia</td>
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<td>Absence of atypical mitoses despite nuclear atypia</td>
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Figure 6.  Sclerosing pneumocytoma. A, Circumscription suggests a benign tumor but may lead to diagnosis of carcinoid tumor. B, Nuclear features of solid areas are not the salt-and-pepper chromatin of a neuroendocrine tumor. C, Papillary structures may mimic adenocarcinoma but sclerosis pattern should raise the correct diagnosis (hematoxylin-eosin, original magnifications ×1 [A], ×4 [B], and ×40 [C]).

Figure 7.  Frozen sections of glandular papilloma. A, The lower-power view of extracellular mucin and lining of spaces may mimic mucinous adenocarcinoma. Transitional-like stratified areas (B) and ciliated cells (C) are in support of a glandular papilloma (hematoxylin-eosin, original magnifications ×2 [A], ×10 [B], and ×40 [C]).
entity, should raise this lesion in the differential (Figure 6, B). The constellation of sclerosis, multiple patterns, and bland cells suggest the correct diagnosis.19

Glandular papillomas are also rare but when encountered can be mistaken for lepiderc patterns of mucinous adenocarcinoma.20 The presence of mucous cells and intra-alveolar mucin are the 2 critical mimickers of adenocarcinoma (Figure 7, A), and the notoriously bland cells of the lepiderc pattern of mucinous carcinoma are also diagnostically challenging. The presence of other cell types—transitional-appearing areas and ciliated cells (Figure 7, B and C)—is the important histologic feature, and in fact these glandular papillomas are usually small nodules, smaller than 1.0 cm.

The alveolar adenoma is a very rare lesion but may also resemble a lepiderc tumor if not considered. In addition, these lesions have dilated spaces that may superficially resemble a lymphangioma (Figure 8, A). On closer inspection, the spaces are lined by epithelial cells and potentially lined by type 2 cells (Figure 8, B). The interstitium is minimally widened by spindled cells (Figure 8, C). Although this lesion is thought to be a bland mesenchymal neoplasm with epithelial ingrowth, the inconspicuous nature of these interstitial cells and the pneumocyte hyperplasia can contribute to the misidentification of this lesion as a lepiderc-pattern adenocarcinoma.

The unique features of lepiderc-pattern adenocarcinoma and the resultant handling of these indolent tumors surgically has led to more frequent encounters with situations that combine difficult-to-identify lesions with a requirement to optimally handle surgical margins. In this situation, proper handling of margins can avoid false-positive margins in limited resections. The bland cytologic features and association with inflammatory infiltrates are also important pitfalls in lepiderc-pattern adenocarcinomas, and recognition of reactive cellular features at frozen section is important in the avoidance of false-positive frozen section diagnoses.

**DIAGNOSIS OF ADENOCARCINOMA IN SITU AND MINIMALLY INVASIVE ADENOCARCINOMA**

The frozen section diagnosis of adenocarcinoma in situ and minimally invasive adenocarcinoma from invasive carcinoma has been a debated topic. Although invasion can be definitively identified,21 there are pitfalls in the frozen section diagnosis of adenocarcinoma in situ or minimally invasive adenocarcinoma. The main issue remains sampling—invasion may be absent on one section, but found on permanent section of the entire tumor. In one series, other features that caused diagnostic challenges included lack of cytologic atypia and the presence of fibrosis or inflammation.22 However, in experienced centers, accuracy rates are high even for adenocarcinoma in situ and minimally invasive adenocarcinoma,23 despite these caveats. Nevertheless, it is difficult to uniformly recommend definitive assessment by frozen section alone, but rather a clinical and radiologic comparison with frozen section during intraoperative consultation seems a more rational approach to mitigate the effect of sampling.

**CONCLUSIONS**

The use of frozen section in the evaluation of small peripheral adenocarcinomas can lead to novel pitfalls in diagnosis. These questions have led to pitfalls in gross lesion identification and assessment of margins, especially in radiologic ground-glass nodules that are pathologically
lepidic. Given the morphologic characteristics of these lepidic tumors, reactive and benign lesions are more likely to cause incompatible frozen section diagnoses.

**References**