Frozen Section of Skin Specimens

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Context.—Skin cancers are the most common malignancies in this country. Treatment of these tumors often involves assessment of margins, which may be performed by frozen section.

Objective.—This article discusses indications for frozen section, various approaches to gross examination of specimens, Mohs micrographic surgery, diagnostic pitfalls, methods to improve diagnostic accuracy, and special techniques.

Data Sources.—The authors' extensive experience and review of the published literature.

Conclusions.—Frozen sections play a vital role in the evaluation of margins of basal cell carcinomas and squamous cell carcinomas. The role of frozen sections in evaluation of soft tissue tumors is controversial. With rare exception, they have no role in the evaluation of melanocytic tumors.

Although most frozen section (FS) examinations of the skin are used for evaluation of margins of
basal cell carcinoma (BCC) or squamous cell carcinoma (SCC), FS may also be used for primary diagnosis of skin lesions or for margin evaluation of other skin neoplasms. Frozen section of malignant melanoma and atypical melanocytic neoplasms is controversial and is addressed in this paper.

Traditional FS examination is accurate in evaluating margins for BCC in approximately 90% to 95% of cases. In most cases, the margins are negative for tumor in the primary resection, whether or not margins are evaluated by FS, and require no further treatment. However, tumors that are not completely removed on initial excision are more likely to be located on a cosmetically important part of the face, such as the orbit, forehead, and cheek; be recurrent lesions; or require reconstructive techniques to close. In these instances, FS to evaluate margins is often indicated.

**TYPES OF SPECIMENS**

Skin specimens submitted for FS evaluation may be shave biopsies, punch biopsies, elliptical or round excisions, or an unusually shaped excision. Intraoperative re-excisions may be thin strips of skin or may be a large excision with a central defect.

**GROSS EXAMINATION**

The initial step in the gross examination is to orient the specimen and ink the margins. Optimally, the skin excisions should be properly oriented. Therefore, appropriate communication with the surgeon and/or dermatologist is important. Once oriented, several different ink colors are used to identify the different margins. Small or unoriented specimens may be inked in a single color.

There are 3 basic methods for sectioning skin for margin evaluation. The first method, representative perpendicular margins, is presented solely as an example of improper handling of a specimen (Figure 1). This method is inadequate because it does not fully survey the peripheral margins.

The second method, horizontal margins or bread-loafing, is best used on smaller specimens (<1–2 cm), in which the entire cross section will fit on a single chuck (Figure 2). This technique is the most straightforward for gross examination and the easiest to embed. The slides will demonstrate tumor in relation to uninvolved skin and the margin, which is often helpful if the tumor has unusual histology or significant inflammation. The tips or peripheral-most sections may either be frozen en face, or perpendicular sections from the tips may be submitted. The tips for the en face method should be thin to minimize the possibility of margins falsely positive for tumor. If the en face method is used, the tissue can either be embedded margin-side up or margin-side down. The advantage to margin-side up (cutting the true margin first) is that minimal levels are needed. The disadvantage is that these specimens are more difficult to correctly orient. The advantage of margin-side down is that it allows for a complete section from the margin most proximal to the tumor. If tumor is present in the tip sections, but not at the inked margin, then the tips should leveled through to ascertain whether the tumor is actually present at the true tip margin. The disadvantage is that if tumor is present in the initial levels, then subsequent leveling may be time consuming.

The third method, margins submitted en face, is similar to the technique used in Mohs surgery (Figure 3) and is best used on large (>2 cm) tumors or on tumors that tend to be multifocal, such as superficial BCC or sebaceous carcinoma. This method is especially inadequate for evaluating malignant melanoma margins because it does not allow assessment of how closely tumor extends to the margin. The entire peripheral margins are typically submitted systematically (eg, serially and clockwise) and diagramed. The deep margin can also be submitted entirely.
Although inking the margins is not necessary when evaluating en face margins, it is advisable because the ink ensures that the true (inked) margin is properly oriented on the chuck. The specimen should be placed on the chuck with the cut-section down and the inked true-margin up. It should subsequently be fully faced, but not deeply leveled, to prevent margins falsely positive for tumor. If the initial section is incomplete, recuts should be obtained and be labeled as such, because they partially no longer represent the true margin.

If a margin is positive for tumor as evaluated by any of the previously described techniques, the surgeon will often re-excise a small strip of skin that represents the new margin. It is important that the surgeon designate the new margin either in person, with suture, or by another method, so the proper margin can be inked. Ink that is placed erroneously may result in a margin falsely positive for tumor. The additional material can then be submitted en face or horizontally sectioned. If the additional tissue is less than 0.5 cm in width and greater than 1 cm in length, it is recommended to submit the margins en face, as numerous small pieces of tissue are difficult to embed and fully face in a timely manner.

In general, all of the sections on a particular chuck should be of the same thickness and oriented at the same level. Small pieces of skin are easier to orient if they are placed on a base. The base is prepared by quickly freezing a thin layer of media on a chuck. The tissue submitted should not be larger than 1 × 1 cm, because of the difficulty producing a completely faced, adequate section. It is better to review more slides of good quality than less slides of poor quality.

**CYTOLOGIC PREPARATIONS**

Cytologic preparations may be useful for the evaluation of a nodule or mass. They are not routinely used in the evaluation of margins.

**INDICATIONS FOR FS EXAMINATION**

Not all skin excisions need to be frozen or should be frozen. Both conventional FS examination and Mohs micrographic surgery (MMS) are expensive and time-consuming. Routine FS examination of all excised BCCs is not a cost-effective use of resources. The recurrence rate for incompletely excised BCC and SCC is between 30 and 67%. The high recurrence rate after incomplete excision is one argument for examining margins by FS. Factors that contribute to margins positive for tumor and, therefore, local recurrence, include the location of the primary lesion, the histologic appearance, and the subclinical extension of disease at the margins.

Lesions that are well suited for FS have poorly defined clinical margins, have an infiltrative growth pattern histologically, are long-standing large lesions, are recurrent, or occur in areas where skin preservation is desirable. Tumors arising near the planes of embryonic closure are also more prone to recur. Frozen sectioning is often necessary to ensure that margins are negative for tumor before extensive reconstructive surgery in those tumors with poorly defined clinical margins, such as infiltrating or morpheaform BCCs.

Conversely, complete excision without FS is possible in approximately 90% of patients with clinically well-defined, nodular BCC, therefore, the routine use of FS for margin verification is of less use in this situation. Some would argue that FS is still valuable for the remaining 10% of patients. The rate of margin involvement is proportional to the size of the primary tumor; long-standing large lesions often need reconstructive closures.
Recurrent tumors are more difficult to adequately excise. Approximately one quarter of patients with recurrent tumors will have an incomplete re-excision if FS is not used, which serves as the justification for FS in the surgical treatment of recurrent tumors. Because lesions on the face can have significant cosmetic consequences, the surgeon may try to preserve critical anatomic landmarks and minimize tissue loss, and, as a result, compromise surgical margins. Facial skin excisions are also more likely to need reconstruction for appropriate cosmesis and, therefore, FS examination of the margins is often warranted. Basal cell carcinomas that occur near the planes of embryonic closure have an increased rate of recurrence and, when they do recur, they tend to track along those planes and probably contribute to the high recurrence rates for BCC on the nose, ears, and around the eyes. Therefore, FS is usually indicated for these lesions. The usefulness of FS is well documented for eyelid tumors, and FS verification of margins has been advocated on a routine basis for eyelid tumors.

**FROZEN SECTION OF MELANOCYTIC LESIONS**

Frozen section of malignant melanoma is rarely indicated. The standard of care for surgical treatment of primary melanomas is resection of at least 5-mm wide margins for in situ lesions and at least 10-mm wide margins for invasive lesions, which usually negates the argument for tissue-sparing surgery. However, there may be clinical circumstances in which immediate evaluation of the margins is desirable, such as a hemodynamically unstable patient. Immunocompromised patients might be at additional risk for wound infection, making immediate reconstruction valuable.

Primary intraoperative diagnosis of a melanocytic lesion is almost never warranted because the histologic detail and prognostic factors may be obscured. Depth of invasion and degree of cytologic atypia cannot be interpreted accurately on FS. Additionally, the section may be distorted by the physical process of freezing, so that the lesion also cannot be accurately measured on the permanent sections. The depth of invasion is of paramount importance for management and prognosis and its assessment should not be compromised. An important limitation encountered in the FS of pigmented lesions is the inability to accurately assess intraepidermal spread of single melanocytes. Many patients with malignant melanoma often have a background of atrophic sun-damaged skin with increased melanocytes, which may be atypical. The distinction of this actinically related atypical melanocytic hyperplasia from malignant melanoma in situ is difficult enough on permanent sections, let alone with the superimposed artifacts of FS.

Frozen sectioning of melanocytic lesions should, therefore, be strongly discouraged. If a melanocytic lesion is frozen, the surgeon/dermatologist should be made aware of the many limitations. This topic is further discussed in the section on MMS.

**MOHS MICROGRAPHIC SURGERY**

Mohs micrographic surgery is a technique used by some specially trained dermatologic surgeons for the treatment of various skin malignancies. One of its major advantages is tissue sparing. It is an accepted and effective technique for excision of BCC and SCC. Controversy arises about its use to evaluate margins in malignant melanoma and soft tissue neoplasms, such as dermatofibrosarcoma protuberans (DFSP).

During MMS, the specimen is mapped using a system of color coding. Relaxing incisions are made to conform a 3-dimensional specimen from a patient to a 2-dimensional slide, and to allow a more continuous external margin. Classically, oblique sections, also called 45-degree, horizontal, tangential, and beveled sections, are taken, allowing epidermis, dermis, and subcutaneous tissue to be visualized on...