Chapter 1
Biopsy Techniques and Interpretation

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The performance of a skin biopsy is an intrinsic part of the initial management of a patient suspected of having a skin cancer [1, 2]. This chapter will therefore begin with a discussion of the various skin biopsy techniques most commonly used in the diagnosis of skin cancer and their clinical indications. This will be followed by a frank discussion of the interpretation of biopsy results. Discussion of other biopsy techniques such as curettage and sentinel lymph node biopsy will be dealt with elsewhere (Chaps. 6 and 15, respectively).

Biopsy Technique

Pre-op

Before performing a biopsy, it is important to have taken a medical history and performed a physical exam. The presence of potential problems such as coagulopathies and drug allergies including lidocaine allergies, artificial joints, and heart valves should be ascertained (Chap. 8). Most biopsy procedures can be safely performed in patients on blood thinners if sufficient care is taken and hemostatic agents are available. The risks and benefits of the biopsy should be explained and consent obtained.

Site Preparation and Anesthesia

The site should next be cleansed with an antiseptic such as isopropyl alcohol, chlorhexidine, or povidone-iodine, for example. Local anesthesia is best performed with a 30-gauge needle used to slowly infiltrate a buffered lidocaine solution [3]. Most physicians utilize 1% lidocaine with 1:100,000 epinephrine. A buffered lidocaine solution can be less painful, and for larger procedures a 0.5% lidocaine solution reduces the possibility of toxicity that may occur when large amounts of lidocaine are used. One common dilution is nine parts of 0.5% lidocaine with 1:200,000 epinephrine to one part of the standard available sodium bicarbonate solution. With such a dilute concentration of epinephrine, one does not need to worry about potential interactions between epinephrine and beta-blockers, for instance, and patients do not experience the tachycardia that sometimes occurs with a stronger epinephrine solution.

Hemostasis

For biopsy sites that are not sutured, styptic agents are often used. Ferric subsulfate (Monsel's solution) may pigment the tissue, complicating histologic interpretation and 20% aluminum chloride hexahy-

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drate (Drysol) is preferable. The styptic is applied on a cotton-tipped swab with pressure to the biopsy site and held in place for several seconds and reapplied if necessary. Another alternative in a freely bleeding biopsy site is to apply a piece of hemostatic sponge, such as Gelfoam, and to bandage the site [4]. Larger wounds may require electrocoagulation for hemostasis prior to wound closure (Chap. 10). If cautery is used, care should be taken to dispose of any Drysol-impregnated gauze or applicators as this agent is highly flammable [5].

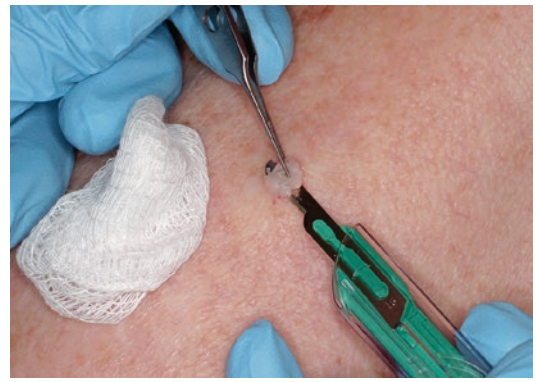

Fig. 1.1 Shave biopsy

Shave Biopsy

As the superficial layer of the skin is sampled this technique is minimally invasive and usually not associated with significant scarring. Shave biopsy can be used in the diagnosis of superficial skin cancers such as actinic keratoses (AK), squamous cell carcinoma in situ (SCCis), and basal and squamous cell carcinomas (BCC and SCC). One disadvantage of this technique is that tumor existing deep to the plane of the shave can be missed (see Table 1.1) [6].

Table 1.1 Biopsy techniques

Biopsy type	Lesion
Shave	AK, BCC, SCC
Saucerization	Pigmented lesions, SCC
Punch	SCCis to check for invasion
Incisional	Melanoma in situ to check for invasion
Wedge	Ulcerated SCC
Excisional	Atypical nevi, melanoma

Equipment

A number 15 blade, toothed forceps, hemostatic agent, cotton-tipped applicator, gauze, and bandage are the equipment used. Please note that a razor blade may also be substituted for a number 15 blade [7].

Technique

After cleansing the area, the local anesthetic is slowly infiltrated to raise a wheal. The skin is stabilized using the first and second fingers of the nondominant hand; then the belly of the blade is held against the skin in a horizontal position and a gentle sawing motion is used to slowly separate the specimen and some surrounding skin from its base (Fig. 1.1). The specimen should include full-thickness epidermis and superficial dermis. Forceps may be used to gently hold the specimen toward the end of the procedure. If the specimen is especially small and/or thin, a drop of India ink can be placed on it before transfer to the container. This will reduce the possibility of it being lost and will in no way interfere with pathologic interpretation [8]. The specimen is then transferred to the specimen container using the wooden end of the cotton-tipped applicator, sparing the forceps from being immersed in formalin. Artifactual changes occur if the specimen is not immediately and continually immersed in the formalin [9].

To assure that the correct specimen is placed in the correct pathology bottle, it is essential that the bottle label be checked. Similarly the specimen should be fully immersed in the formalin solution and the bottle shaken and visually inspected by the physician to confirm the presence of the specimen in the bottle [10].

Hemostasis is achieved; the biopsy site is then dressed with an application of antibiotic ointment or petrolatum and covered with a dressing, which is changed daily for approximately 1 week until the area has healed.

Complications

Hypopigmentation and cutaneous depression may occur if the biopsy is deep.

Saucerization

In a saucerization biopsy, a razor blade is bent into a U shape to obtain a deeper specimen. This is indicated for the biopsy of lesions reaching the upper to mid-dermis such as SCC, atypical nevi, and superficial melanoma.

Equipment

A Gillette super blue razor blade and the same equipment as used with the shave biopsy.

Technique

After cleansing and infiltrating the area as previously described, the razor blade is bent into a U shape and held between the first two fingers of the dominant hand. A sawing motion is used to obtain the biopsy (Fig. 1.2). Hemostasis and aftercare are as previously described.

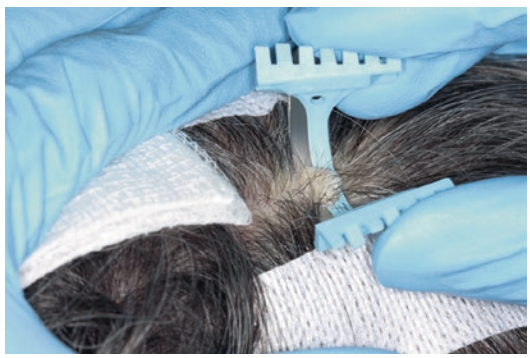

Fig. 1.2 Saucerization biopsy. Note hair is taped down to facilitate biopsy

Punch Biopsy

Punch biopsy is useful for providing information about the depth of tumor invasion as, depending on the size of punch used, it can reach subcutaneous tissue. A 3-mm punch is standard, but 6- and 8-mm punches may be used for removing larger lesions. A 2-mm punch is most often used for cosmetically sensitive areas such as the face, but may be harder to process in the lab and may give an inadequate sample for diagnostic purposes, especially for melanocytic neoplasms.

Equipment

Sterile punch, scissors, toothed forceps, suture.

Technique

Prepare and anesthetize the skin as previously described. Next, stabilize the skin by stretching it taut between the first and second fingers of the nondominant hand and perpendicular to the relaxed skin tension lines, creating an oval defect, which can be more easily sutured. Holding the punch between the first two fingers of the dominant hand, place the punch on the area to be biopsied so that all edges of the punch are in contact with the skin. Rotate the punch between the fingers pressing down at the same time until there is a loss of resistance and the subcutaneous plane is reached (Fig. 1.3). Next remove the punch and gently lift the specimen; divide its base and place it in the bottle of formalin. If forceps are used, be careful not to squeeze the specimen as this will cause "crush" artifact resulting in cellular distortion and complicating histological interpretation [9].

For esthetic and sometimes hemostatic purposes, the biopsy site may be sutured with 6-0 interrupted epidermal sutures on the face and 5-0 interrupted sutures on the body. The suture can be removed at 7–10 days depending on the site.

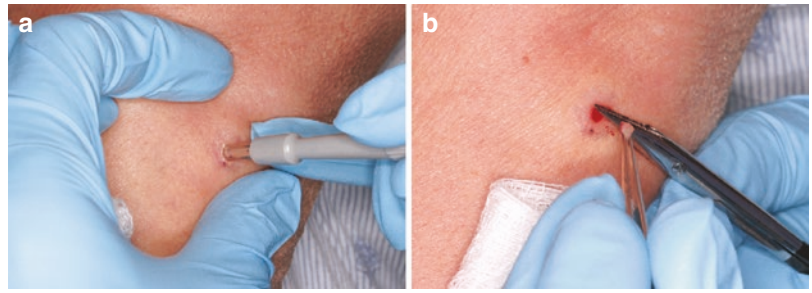

Fig. 1.3 (**a**) Punch biopsy. Note that punch is perpendicular to relaxed skin tension lines. (**b**) Punch biopsy specimen is gently handled with toothed forceps to prevent crush artifact

Biopsy Care

The biopsy site should be cleansed with water daily and covered with an antibiotic ointment and an occlusive dressing. The incidence of contact dermatitis is fairly high with certain antibiotics and this should be taken into consideration. White petrolatum may be used instead. Leaving the wound open to air or allowing it to dry will slow reepithelialization and may not optimize the final appearance [11].

Incisional Biopsy

The incisional biopsy is used when a larger specimen is needed for examination, such as with large pigmented lesions where total excision is not easily achieved [9].

Equipment

Sterilized instruments including a #15 scalpel, toothed forceps, scissors, suture, and gauze.

Technique

Prepare and anesthetize the skin as previously described. Holding the scalpel perpendicular to the skin, make a fusiform incision through the middle of the lesion down to the subcutaneous tissue (Fig. 1.4). Remove the specimen and suture the wound.

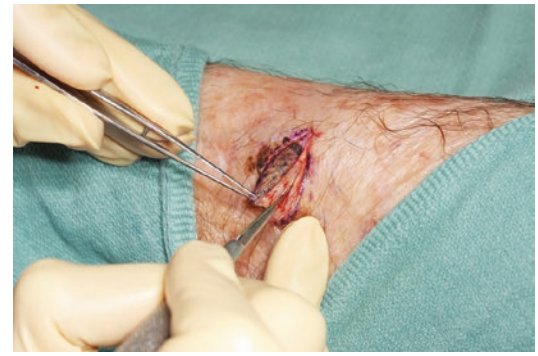

Fig. 1.4 Incisional biopsy of a suspected melanoma

Complications

Wound infection, hematoma, dehiscence, scar, and pigmentation change.

Wedge Biopsy

Wedge biopsies are used mainly to examine ulcer tissue—as with an ulcerated squamous cell cancer—and, as the name implies, are designed to include the normal tissue at the edge with the apex of the triangle pointed into the affected tissue. Thus, normal and affected tissues are sampled together and the resulting specimen is therefore pie-shaped. The defect can then be sutured or left to granulate.

Complications

Bleeding, infection, scar, and pigmentation change.

Excisional Biopsy

Excisional biopsies are defined as extending completely around the clinically apparent lesion, extending to fat, and not necessarily intended to remove the entire lesion. If the intent is to remove the entire lesion, then it is more correctly called an "excision," since the term "biopsy" means that the intent is not to remove the entire lesion. Excisional biopsies are performed for atypical nevi or when melanoma is suspected, for example [12].

Technique

The borders should be marked before the excision (Fig. 1.5). Once the area has been prepped and anesthetized as above, the specimen can be removed in a fusiform manner including subcutaneous tissue [13, 14]. To aid the dermatopathologist, a suture should be placed at the 12 o'clock position to orient the lesion with respect to the patient's body. This is only necessary for larger lesions if the surgeon wants to know more precisely where involved margins are present. For smaller excisions, or those where the entire area would be excised anyway if margins are involved, detailed orientation may not be needed. It is advisable to place this suture before excising the specimen to avoid misorientation.

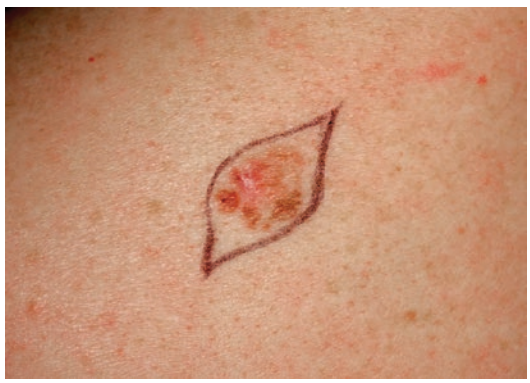

Fig. 1.5 A border is outlined around a suspected melanoma prior to excisional biopsy

Complications

Bleeding, hematoma, infection, scar.

Biopsy Log

It is the physician's responsibility to track the biopsy and a protocol must be established within the practice [10]. The importance of a biopsy log cannot be overemphasized even in these days of electronic medical records. If the biopsy is lost, it is necessary to inform the patient of the situation and to discuss whether or not to re-biopsy the lesion site. There is no credible legal defense if a skin cancer later develops at or near the site of a lesion that had been previously biopsied, the specimen lost, and the patient never informed of the situation [8].

Interpretation of Results

In general, biopsies obtained by skin punch and elliptical excision provide better specimens than those obtained by shave or tangential biopsies, as punches and ellipses are more likely to sample

Table 1.2 Advantages and disadvantages of punch, shave, and excisional biopsies [1]

Punch	Shave	Ellipse
+ Better depth	– Often too superficial	+ Best depth
– Maximum 8 mm width	+ Easier to remove wider lesions	– Difficult closing wide lesions
– More scarring (unless sutured)	– Less scarring (unless deep shave)	– Most scarring
– More equipment	– Least equipment	– Most equipment
– Slower	+ Fastest	– Slowest
– Little skill needed	+ Little skill needed	– More skill needed

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+, advantage, –, disadvantage

deeper dermis or subcutaneous tissue. There are general advantages and disadvantages to each biopsy type (See Table 1.2).

The least helpful type is that obtained by curettage. The many fragments are often difficult to process and the pathologist has to reconstruct the lesion mentally. In some instances curettage may be helpful and it is then preferable for the clinician to shave the bulk of the lesion, send this to pathology, and then to curette the base, discarding the curetting.

Elliptical excisions, both incisional and excisional, are preferred for the complete removal of dysplastic nevi and malignant skin cancers.

Tissue Orientation and Margin Evaluation

The first step in examination of a skin biopsy specimen consists of gross cutting and orientation of the specimen referred to as "grossing" [15]. All pathology reports need to contain a description of the gross examination and should specify the orientation of the specimens so that the margins seen on the slides can be appropriately determined. In addition, the report should state whether all the tissue was embedded ("in toto") or if "representative sections" were embedded.

Various decisions may be made at grossing and for this reason the process is performed by a physician or a trained pathology assistant. One decision is to determine if representative sections are to be made, just which tissue will be examined, and which will be discarded. Another grossing question to consider is whether or not to bisect punch biopsy specimens. If punches are bisected, and assuming the clinician placed the most specific changes in the center, then the initial sections are more likely to exhibit the desired histological changes. Sometimes, however, the two bisected pieces may become fragmented, difficult to orientate, or even lost. In addition, important sections may be discarded in the process of "facing" where initial incomplete sections are removed from the paraffin block and discarded until the block becomes smooth, providing complete sections. In contrast, a larger unbisected punch specimen may be easier to handle, but initial sections may be nonspecific, and deeper levels may be needed.

Various tissue orientation errors can occur. Sectioning the surface of a punch specimen will result in a round specimen with epidermis present around most of the edges, and curling of a thin shave biopsy specimen will produce a section with epidermis on opposite sides. Tangential sectioning may give the false impression of hyperkeratosis, hypergranulosis, acanthosis, an apparent increase of melanocytes and basal cells, or perhaps even a pseudomalignancy [16].

Reporting of Surgical Margins

Some pathologists like to state on the report that re-excision is indicated. However, this may place the clinician in a bind, feeling that they have to either follow this suggestion or explain why they do not in the chart. Other clinicians may appreciate this advice.

It is preferable, though not always practical, for pathologists to measure precisely in millimeters how close a tumor is to the margin, rather than to use terms such as tumor "near," "adjacent to," or "approximating the margin."

Fundamental Slide Interpretation

Low Power

Initially, the number of sections can be examined by holding the glass slide up to the light without the microscope. Next, low-power microscopy should be used to scan the slide; indeed, many cases can be diagnosed with low power alone. It is important to examine all sections or at least to look at each type of section that is different grossly.

Develop a Method for Examining Skin Specimens

It is important to develop a method for systematically examining skin sections. Some dermatopathologists will start in the dermis and later examine epidermal changes, while others will start in the stratum corneum and proceed down to the subcutaneous tissue. While observation of the architectural pattern will allow for preliminary diagnosis, it is also important to view cytologic detail such as mitoses and pleomorphism with high power. When looking at a clinical lesion, one should try to imagine what it would look like under the microscope. Similarly, when looking at a pathology specimen, one should imagine what the lesion would look like clinically. A differential diagnosis is then considered by focusing on individual histologic changes together.

Knowing the Clinician

Since many clinicians often have customary treatment habits, it is often possible for the dermatopathologist to guess who performed the biopsy or excision. The best clinicians modify their treatment based on the clinical circumstances. The following are extreme examples presented with the hope that some readers may recognize a pattern and, if applicable, maybe modify their behavior.

Too Small a Sample

Some clinicians send curettage fragments or shave biopsy specimens in more than 95% of the specimens they submit. Pieces of epidermis are submitted when there is clinical suspicion of dermal tumor. It is useful for the dermatopathologist to know if the biopsy procedure was followed by electrodesiccation and curettage, for instance, because then it is not necessary for them to comment on margin involvement. In this way confusion can be avoided if the patient gets another opinion from another physician who is unaware that the lesion has been destroyed.

Too Aggressive a Sample

Other clinicians may be too aggressive in the size of the specimen they submit for diagnosis. One instance of this would be the excision to adipose tissue of seborrheic keratoses. Another example would be the excision of a suspected melanoma with 1–3-cm margins, which is later found to be benign.

Two-Step Management

Some clinicians always perform a biopsy and then have the patient return for a subsequent visit. There are instances where one should biopsy first rather than initiate treatment at the first visit, for instance, in the case of a facial lentigo maligna. However there are other instances, such as a patient with nevoid basal cell nevus syndrome, where it is expedient and cost-effective to initiate treatment in one step when possible.

Too Little or Too Much Information

It is important to include all relevant history or diagnosis on the laboratory requisition slip. Extensive differential diagnoses are not helpful.

Know Your Laboratory

It may be helpful for clinicians to recognize certain characteristics of their dermatopathology laboratory.

One Diagnosis Only

Some pathologists may provide one specific diagnosis and rarely comment on other possible diagnoses. For some of these pathologists, there may be little doubt about the diagnosis of a Spitz nevus. For others, the possibility of a melanoma is considered with less dogmatic certainty.

Too Many Diagnoses

Some pathologists may not give a specific diagnosis, instead providing a descriptive one such as: "perivascular and spongiotic dermatitis." While these pathologists will often not elaborate further, others may at least give a differential diagnosis.

Summary

In conclusion, it behooves the clinician to understand the various biopsy techniques and to be aware of the clinical indications for each type. In the interest of patient care, accurate communication between the clinician and the dermatopathologist is important. It is also helpful for clinicians to recognize certain characteristics of the dermatopathology laboratory they use.

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