Biopsy Techniques for Pigmented Lesions of the Skin

JOHN K. GEISSE, M.D.

Malignant melanoma of the skin can vary from eminently curable superficial lesions to deeply invasive disease with a high risk of distant metastases and a poor prognosis. Early detection and treatment of malignant melanoma are critically important to prevent advanced disease. In many instances careful skin examination and appropriate biopsy of suspicious pigmented lesions, particularly in high-risk individuals, can prevent this deadly disease.

This chapter is intended as a guide for clinicians on proper biopsy techniques for pigmented skin lesions. Excisional biopsy is usually the most appropriate surgical approach to a suspicious pigmented lesion. Complete excision allows evaluation of size, symmetry, circumscription, and maturation. The prognosis of malignant melanoma varies with the depth of invasion; thus complete excision provides the pathologist with the material needed to render accurate diagnoses and microstage measurements. This in turn provides the clinician with appropriate information from which further treatment decisions can be made and allows the patient to be better informed of his or her options and long-term prognosis.

Partial biopsies may result in misinterpretation of a given specimen, because sampling error or inadequate architectural detail prevents the histopathologist from fully characterizing...

Official Publication of the California Society of Pathologists
Philadelphia, Hanley & Belfus, Inc.
the melanocytic proliferation in question. In specific instances in which complete excision of such lesions is either impractical or inappropriate, modification of this approach may be warranted. There is little indication or utility for the frozen-section diagnosis of melanoma, because artifacts make accurate interpretation difficult. Rare exceptions include large, clinically obvious melanomas and lymph node metastases. This chapter briefly reviews standard techniques for excisional biopsy of pigmented lesions and addresses special clinical scenarios in which incisional or partial biopsies may be necessary. Unusual locations, such as mucous membranes and acral sites, may require special surgical skills to provide optimal material for histologic processing.

EXCISIONAL BIOPSY OF PIGMENTED LESIONS

In most instances the clinician is prompted to biopsy a pigmented lesion because of concern over its clinical appearance and the need to rule out malignant melanoma. Cosmetic removal of nevi is a common request of patients and accounts for most of the melanocytic lesions reviewed in a pathology or dermatopathology laboratory. The surgical approach to these lesions is entirely different; in fact, they are often transected intentionally by clinicians to provide a better cosmetic result for the patient. The suspicious pigmented lesion, on the other hand, is biopsied or sampled to rule out malignant melanoma. Most such lesions are 1 cm or less in size and thus can be easily excised with 1–2 mm of normal-appearing skin surrounding the clinical margins. Elliptical excisions are commonly performed by all physicians who do surgery of the skin and, except for a few basic guidelines, need not be further detailed in this chapter.

Clinicians should always outline the clinical margins of the lesion in indelible ink and the planned surgical margin, preferably using a sterile pen, after adequate skin preparation but before instillation of anesthetic fluid (Fig. 1). Local anesthetic solutions containing epinephrine may cause distortion and blanching of the anesthetized skin, thus preventing adequate visualization of the clinical margins. Surgical marking pens with indelible ink are easily sterilized or may be purchased sterile.

An important part of planning elliptical excisions is to mark the patient’s relaxed skin tension lines (RSTLs) for better orientation of the ellipse while the patient is in a relaxed sitting pose or standing position. Relaxed skin tension lines usually lie perpendicular to the underlying musculature and vary from patient to patient (Fig. 2A). Orienting such excisions to parallel the RSTLs results in a narrower, more cosmetically acceptable scar. The RSTLs vary from site to site and can be evaluated in each location by pinching small amounts of skin and observing the formation of straight skin tension lines (Fig. 2B). If one pinches the skin opposite to the relaxed skin tension lines, uniform wrinkles parallel to the skin tension lines do not develop (Fig. 2C). If one is slightly askew, curvilinear lines develop (Fig. 2D). This simple
FIGURE 1. Irregular pigmented lesions with clinical margins and surgical margins marked with a sterile pen after skin preparation but before anesthesia.

The maneuver allows the clinician to design the ellipse and thus to place scars in the most favorable position for the best cosmetic and functional results. The patient must be in a relaxed sitting or standing position for correct orientation of the excision.

FIGURE 2. A, Relaxed skin tension lines (RSTLs) are highlighted in the neck. B, Pinching skin produces straight skin tension lines and demonstrates the optimal direction to orient an ellipse. C, Pinching skin opposite the RSTLs. D, Pinching skin askew to RSTLs produces curvilinear lines.
Simple excision of a suspicious melanocytic lesion to diagnose or to rule out malignant melanoma requires only narrow margins—usually a few millimeters around the clinical extent of the lesion. After obtaining the diagnosis of melanoma with microstaging measurements, reexcision with appropriate margins can be performed. Wide excision of a pigmented lesion on the basis of its initial presentation is not normally recommended. Dermatologists’ sensitivity for the clinical diagnosis of malignant melanoma is only 84%, with a positive predictive value of 72.4%; thus a substantial number of melanomas are missed or unsuspected by expert clinicians. Initial wide excision may result in unnecessarily aggressive surgery for benign disease. Excision of melanoma carried out before obtaining the microstaging measurements may also result in inadequate or excessive margins, thus necessitating a second procedure or increasing the morbidity beyond what is necessary or indicated.

**Punch Excisions**

Small suspicious pigmented lesions can be excised completely with punches that range between 1 and 2 mm greater in diameter than the longest dimension of the lesion in question. Sharp disposable punches are available from various sources in sizes ranging from 2–6 mm, often in 0.5-mm increments. Nondisposable punches, although available in larger sizes (≥8 mm), are rarely a practical alternative to a simple ellipse. Punches under 4 mm in diameter allow quick and efficient biopsy of lesions with reasonable cosmetic closures. Closures of punch excisions over 4 mm in size often cause small dogears or standing cones of tissue, thus resulting in less than optimal cosmesis compared with a small ellipse. As a biopsy technique, punches save time and may allow complete excision of small pigmented lesions. The clinical margins should be marked preoperatively as described above, and the punch should exceed the diameter of the lesion by 1–2 mm.

Tension should be held opposite to the relaxed skin tension lines before using the punch in a slow, twisting, back-and-forth motion to excise skin into the subcutaneous fat (Fig. 3). This technique often turns a circular punch into an ovoid or nearly elliptical defect that parallels the skin tension lines because of the tension exerted by the surgeon on the skin opposite the direction of these lines.

Once again, complete excision is recommended for pigmented lesions; thus the use of punches to perform excisional biopsies should be limited to small pigmented lesions. Occasionally punches are used for incisional biopsies of larger lesions, as discussed below.

**Shave Excisions**

Suspicious pigmented lesions or unusual appearing nevi of the central trunk sometimes can be excised adequately by a tangential excision or saucерization. This approach offers some advantages, because it creates a partial thickness defect that should exceed the diameter of the lesion in question by only 1–2 mm. The patient is spared a much larger ellipse, which by necessity
FIGURE 3. Tension is applied opposite to the RSTLs for a punch biopsy, which forms an ovoid defect after the tension is relieved.

would be 3 times as long as the lesion is wide to avoid the development of dogears or standing cones of tissue. If one suspects that the diagnosis is likely to be malignant melanoma or if the pigmented lesion contains an area of induration or nodule, complete elliptical excision should be performed. If it is a thin, nearly macular pigmented lesion or "atypical mole," shave or tangential excisions, when done properly, may allow complete excision and thus complete histologic examination of the lesion.

Tangential excisions are best performed by incising around the lesion in question, including a 1–2-mm margin of normal-appearing, nonpigmented skin, with the tip of a no. 15 or no. 15c scalpel blade (Fig. 4). Super-sharp, Teflon-coated blades make this procedure much easier. The procedure can be done with a 30–45° angle, carrying the incision into the midreticular dermis (Fig. 4B). This approach is facilitated by intradermal injection of anesthesia, which provides a firm surface to incise and allows countertraction by the surgeon (Fig. 4A). These maneuvers allow better control of the depth and lateral margins of the excision (Fig. 4C). Once an incision has been made around the diameter of the lesion, the skin can be pinched on either side and a scalpel or sharp scissors used to excise tangentially the bulk of the pigmented lesion at the depth determined by the initial circumferential incision (Fig. 4C). This method allows better control of the depth and saucerization or deep shave excision of pigmented lesions well into the mid-dermis to prevent transecting the lesion. The disc- or saucer-shaped specimen should be flattened on paper before fixation to prevent excessive curling (Fig. 4D).

Hemostasis can be obtained with aluminum chloride (20–33% solution), Monsel's solution (ferric subsulfate), gelatin powder (Gelfoam), or simple pressure. The result is a disc-shaped defect that will heal by second intention, which results in approximately 50% wound contraction. Thus the final scar is approximately one-half the size of the original defect; normally it is a flat or slightly depressed, hypopigmented scar over the central trunk. Hypertrophic scars or keloids can be induced by this method, especially over the shoulders.

or central back. The final result often is more cosmetically appealing than an elliptical excision of the central trunk, which inevitably spreads into an ovoid or diamond-shaped scar, particularly in young patients with good skin elasticity. This procedure is most useful for thin or macular lesions located on the back, where the thick dermis more easily allows complete excision while maintaining a partial thickness wound. This method is technique-dependent and may result in transection of the lesion and a partial biopsy, which sometimes make interpretation difficult.\textsuperscript{1,2,32}

When excisions are performed in the above manner, the pathologist needs appropriate instructions from the clinician to process the disc of tissue as an excision. Bread-loafing the specimen in its entirety to obtain tips that allow good representation of the lateral and deep margins should be as routine with shave or tangential excisions as with a full-thickness specimen.

Partial Biopsies of Malignant Melanoma

Occasionally patients present with clinically obvious malignant melanomas of large diameters or size with foci of induration that probably represent vertical growth. Complete excision of these lesions is recommended to confirm the diagnosis of malignant melanoma. Excision with wide margins may be considered as initial definitive therapy,\textsuperscript{12} but the result may be over- or undertreatment.\textsuperscript{5,9,14,19,20}
Incisional biopsies have been associated with a worse prognosis in some studies and with the spread of tumor into the surrounding circulation in animal studies.7,12,26-31,33,34,41,42 Other studies refute such findings and suggest that incisional biopsies of melanoma do not affect prognosis or therapeutic outcome.4,17,18,21,25,39 Complete excision is preferred if it can be performed without undue morbidity, because it provides better histologic representation of the tumor and thus allows accurate judgment of microstage measurements and prognosis.1,2,5,9,14,19-23,32,33,36,38 Large melanomas may have areas that either represent precursor nevus or are not easily interpreted as malignant melanoma. Incisional biopsies of such large pigmented lesions may lead to sampling errors, possibly missing diagnostic material and delaying definitive diagnosis and treatment.1,2,5,9,14,19-23,32,33,36,38

The exception to this rule may be malignant melanoma in situ of the lentigo maligna or acral lentiginous type, which often presents as large lesions over critical anatomic structures, such as the central face or digits. In such cases, initial complete excision is problematic.5,11,14,32-34,38 Because these lesions may remain in a radial growth phase for years, partial biopsy with either a punch or a deep shave technique may be warranted in selected cases to confirm the diagnosis.11,32,33,38 Because nonsurgical treatment of malignant melanoma in situ is currently less accepted than in past years, consideration should be given for complete excision on initial presentation.1,2,5,9,14,19-23,32,33,36,38

Occasionally there is a rationale for following such lesions in elderly patients with poor health, because development of invasive disease may be prolonged for many years.33 This may not always be the case, however, and in fact invasion and metastases in such lesions has been reported to occur rapidly.16

Large, clinically obvious, advanced melanomas of the extremities, particularly of the digits, palmar-plantar surfaces, and subungual regions, may necessitate a partial biopsy to confirm the diagnosis before more drastic surgery, such as amputation of the involved digit or limb, occasionally supplemented by limb perfusion with oncologic drugs.10,38 This scenario fortunately is becoming less and less common as public awareness of the risks of malignant melanoma and the importance of early diagnosis and therapy continues to improve.

SPECIAL CONSIDERATIONS FOR BIOPSY OF ACRAL AND MUCOSAL PIGMENTED LESIONS

Biopsy of Mucous Membranes

Pigmented lesions involving mucous membranes are not uncommon and usually represent benign lentiginous proliferations, seborrheic keratoses, or nevi. When the need arises for a diagnostic biopsy of such lesions to rule out malignant melanoma, simple elliptical excisions are preferred and are usually easily obtained, even when the labia or penis are involved.

Large pigmented lesion involving the glans penis or clitoris may represent special circumstances in which partial biopsies are indicated to confirm
diagnosis before definitive surgery to avoid excess morbidity. Many large pigmented patches prove on histopathologic examination to be examples of genital melanosis, a benign condition. Large, possibly unresectable, clinically obvious melanoma of the genitalia also may necessitate partial biopsy to confirm the diagnosis before definitive or palliative treatment.

The skin of the perineum and the mucous membranes heal rapidly with minimal scarring. Closure or partial closure of even large defects, with minimal undermining, is usually easily performed. The use of stiff monofilament sutures may cause discomfort in these regions; thus a soft, braided nylon or buried absorbable suture, such as Vicryl or Dexon II, should be selected if the wound is to be closed. Rapidly absorbable gut (Ethicon) or mild chromic gut (Davis and Geck) will suffice if there is no tension; such sutures obviate the need for removal because of their rapid resorption. Synthetic absorbable sutures, such as Vicryl or Dexon II, may be used percutaneously when the need arises for a soft, braided suture instead of a braided nylon. Like other suture, these can be removed after 1 week, or the knots can be cut out, allowing the rest of the stitches to be absorbed.

Large wounds of the perineum or mucous membranes can often be allowed to heal by second intention if closure proves problematic, as with defects on the shaft of the penis, where dehiscence is a common problem. Such wounds usually heal with minimal scarring and relatively quickly, although rates of infection may be slightly higher in these areas. Wound contraction during second intention healing may distort free tissue margins, such as the labia.

Because the skin of the perineum and the mucous membranes are quite friable, careful tissue handling is important to preserve the histologic features. Avoiding crush injury to the specimen with rough handling on removal is sometimes difficult; care must be taken to grasp the subcutaneous tissues rather than the dermis as the biopsy is cut free. Bleeding is often quite brisk in these regions. The use of epinephrine-containing anesthetics, with dilute concentrations between 1:100,000 to 1:400,000, provides vasoconstriction and improves intraoperative visualization. The use of epinephrine on the penis and possibly the clitoris has been reported to be safe by some authors, but it is not recommended. Bleeding during such biopsies usually can be controlled with spot-electrodesiccation or electrocautery and rarely requires suture ligation. Suturing wounds expediently also minimizes bleeding and tamponades all but significant arterial bleeders.

The Nail

Pigmented bands of the nailplate are not uncommon, particularly on darkly pigmented patients. Subungual malignant melanoma carries a grave prognosis with even minimal invasion. Diagnosis of such lesions early in their radial growth phase, particularly when still in situ, minimizes the morbidity of definitive surgery and improves survival.

Punch biopsies of the nail are commonly suggested in surgical texts and publications describing nail biopsies. Adequate biopsies of pigmented lesions of the nail matrix are difficult to obtain, particularly with standard
surgical approaches such as a punch biopsy. Small punch biopsies of the nail matrix are more likely to contribute to misdiagnosis through sampling error than complete excision of the pigmented band in question.\textsuperscript{33,43} Larger punch biopsies may result in ridging of the nailplate or even pterygium formation. Small pigmented bands 2 mm or less can be completely excised with the involved matrix and closed, usually with little or no permanent ridging of the nailplate.\textsuperscript{10,43} When pigmented lesions are greater than 2 mm in size, partial biopsy of the most darkly pigmented area may be necessary to minimize the morbidity of the diagnostic procedure before definitive therapy. A partial biopsy of nail matrix should be oriented transversely to avoid a split nail.\textsuperscript{43} Large masses that clinically are highly suspicious for malignant melanoma may require partial biopsy to confirm the diagnosis before amputation. Once again, incising melanomas may contribute to the risk of metastasis, although this issue is controversial.\textsuperscript{7,12,26–31,33,34,41,42}

A simple, effective solution to the problem of obtaining adequate tissue for pigmented bands of the nailplate is to excise the band completely, along with its associated nail matrix, with or without overlying proximal nail fold (Fig. 5A). The proximal nail fold should be excised if Hutchinson’s sign is present. This sign is best observed using magnification with good lighting to

![Figure 5](image_url)

**FIGURE 5.** Complete excision of a suspicious pigmented band diagnosed as squamous cell carcinoma. *A*, Suspicious pigmented lesion of the digit. *B*, Prepared, anaesthetized digit with tourniquet. The initial incision is made around band. *C*, Periosteal elevator is used for careful removal of intact specimen. *D*, Intact nail matrix, bed, and plate as en bloc resection for optimal nail unit biopsy.
detect small amounts of pigment in the cuticle and proximal nail fold (or dorsal skin of the digit) overlying the pigmented band. Hutchinson's sign is highly suspicious for the diagnosis of malignant melanoma and warrants a full thickness biopsy.\(^8\)

Digits are usually anesthetized with sensory nerve blockade because of their simple anatomy and the pain associated with local infiltration. The use of 2% lidocaine combined with an equivalent amount of 0.5–0.25% bupivacaine, both without epinephrine, provides excellent, prolonged digital blocks. Once anesthesia is obtained, bleeding is controlled and visualization optimized by the use of a tourniquet (Fig. 5B). The tourniquet must be used with care to avoid injury to the digital arteries and risk of prolonged ischemia or even necrosis of the involved digit. A 1.25-inch (3.5-cm) Penrose drain can be applied to the base of the digit, with just enough pressure to minimize arterial bleeding, using a long, wide hemostate. Care must be taken not to clamp the underlying skin. Standardized pressure cuffs are available for digital blocks and are recommended by hand surgeons to minimize the risk of excess pressure and ischemic injury to the digit.

Often removal of the nail plate is recommended before biopsy.\(^{10,43}\) We specifically advise against this procedure, because it can avulse the epithelium of the underlying nailbed, with potentially permanent loss of diagnostic material. Most nails can be incised with a no. 15 blade, first by cutting through the proximal nail fold into the matrix to the underlying bone and then by extending the excision distally through the nailplate with minimal margins around the pigmented band (Fig. 5B). The procedure must be done with care to avoid excessive injury to the underlying bone as well as injury to the surgeon; hard nailplates may prove particularly difficult to incise in this manner. In addition, lacerations may occur to the digital tip as the nailplate is incised distally, unless great care is taken to avoid slippage of the knife as it leaves the hard nail surface. Once these incisions are carried out on either side of the pigmented band, a periosteal elevator, preferably a narrow, fine instrument, may be used proximally to remove the nail matrix and nailbed from the underlying bone in one piece, with minimal crush (Fig. 5C). This approach provides complete excision of the pigmented band with the nailplate attached, including the involved matrix and proximal nail fold. Such a specimen can be submitted to the pathologist intact for excellent representative sectioning (Fig. 5D). Requisitions must clearly point out the fact that the nailplate has been submitted with the specimen so that the pathologist ensures adequate fixation and takes appropriate steps to allow good sectioning of the nailbed and matrix with the attached plate. Marked artifact from sectioning through a hard nailplate may occur if such precautions are not taken.

The wound is then approximated with 0-0 synthetic absorbable sutures (e.g., Vicryl), using a small, high-quality needle, such as an Ethicon PS-6. Small excisions (2–3 mm) of the nail matrix rarely require undermining, and the matrix may be approximated with fine, interrupted absorbable sutures tied loosely to avoid strangulation. Then the proximal nailfold may be approximated with the same suture material. The defect in the nailplate and nailbed can be
left open or partially approximated, particularly along the lateral nail fold, by suturing with 6-0 or 5-0 absorbable sutures and a high-quality needle to pierce the nailplate. Hemostasis is obtained by pressure once the tourniquet is removed. Occasionally, if the nailbed is exposed, hemostatic agents, such as a gelatin powder (Gelfoam), are applied to the wound before removing the tourniquet to help in the control of bleeding. This rarely results in a split nail if the excision is less than 3 mm in thickness. A longitudinal ridge is a common but acceptable sequela.

The above technique can be modified by using any number of nail splitters instead of a scalpel to incise the nailplate on either side of the pigmented band, although most of these devices cause some crush injury to the underlying nailbed. Nail splitters, however, make the procedure safer and more easily mastered than the method described above.

If the cuticle or proximal nailfold is not involved, the incision can be made directly over the pigmented band to expose the underlying matrix. With an assistant’s help, two small skin hooks are used to retract the intervening skin and to allow adequate visualization of the matrix. With larger pigmented bands or larger masses involving the nail matrix, incisions into either side of the proximal nailfold may be made to provide nearly complete exposure of the underlying matrix with adequate retraction. Partial transverse biopsies of the proximal matrix can be obtained and closed as described above. If done carefully, complete excision of the involved matrix provides excellent functional and cosmetic results.

**Eyelid and Lid Margin**

Pigmented lesions of the eyelid can be excised in most instances with minimal morbidity as long as the incision is vertically oriented, perpendicular to the lid margin. Partial biopsy, as described above, may be indicated for large pigmented lesions, which often represent malignant melanoma in situ (of the lentigo maligna type) and involve extensive areas of the lid.

The lid margin and conjunctival surfaces are frequent sites for pigmented lesions, particularly in dark-skinned patients. Small pigmented lesions of the lid margin may be excised with narrow margins and allowed to granulate, usually with minimal functional and cosmetic impairment unless significant portions of the tarsus are removed. Small exophytic pigmented lesions of the lid margin and conjunctival surfaces can be sampled completely or nearly completely by the use of very sharp, curved iris scissors designed specifically for this purpose. Often such scissors with small serrations provide optimal results because of their ability to grasp and hold the target tissue in place as the scissors is closed. Larger lesions of the conjunctival surface, particularly near the limbus, should be referred to ophthalmic surgeons for proper biopsy procedures.

**CONCLUSION**

Complete excisional biopsies are recommended for most suspicious pigmented lesions except in a few special circumstances, as outlined above.
Complete excision usually can be obtained with a minimum of morbidity and with reasonable cosmesis. Even large suspicious pigmented lesions should be completely excised with narrow margins to obtain a definitive diagnosis. If primary closure is problematic, most excisions may be allowed to granulate or only partially closed, with the knowledge that further treatment is likely to be necessary. Delayed reconstruction or scar revision is commonplace for large defects of the skin. Defects created near free-tissue margins (e.g., lip, eyelid, and nasal ala) should probably not be left to heal by second intention because of potential distortion of important anatomic structures from wound contraction. Clinicians should refer patients with large pigmented lesions for excisional biopsy if such procedures surpass their skill or experience. Suspicious pigmented lesions optimally should be submitted in their entirety for pathologic review.

In special circumstances, individualized biopsy techniques are preferred for adequate tissue sampling; if the clinician is unfamiliar or uncomfortable with such techniques, appropriate referral should be made to optimize the patient’s care.

References

1. Ackerman AB: Shave biopsies: The good and right, the bad and wrong. Am J Dermatopathol 5:211–212, 1983.
20. Ledwig PA, Robinson JK: Should the excisional biopsy of clinically probable melanomas include a margin that might also serve as adequate for treatment? Arch Dermatol 126:877–888, 1990.