Chapter 2

Vulva

Benign and malignant neoplasms as well as reactive inflammatory lesions of the vulva present as ulcers, white plaques, swellings, and red or dark pigmented lesions. In view of the overlap in clinical and gross features, surgical sampling is often required to establish the diagnosis. Almost all intraoperative consultations relate to the evaluation of malignant or premalignant lesions (Table 2.1). Specimens generally fall into two categories: (a) biopsies and other limited procedures for diagnostic/therapeutic indications and (b) a variety of vulvar resections of pre-neoplastic and neoplastic lesions.

VULVAR BIOPSIES AND OTHER LIMITED PROCEDURES

Clinical Background and Specimen Handling
Small biopsies are performed either as diagnostic procedures for a suspicious area such as hyperkeratosis, or as simple local excisions of a benign solid lesion or a cyst. Biopsies are not usually submitted for immediate interpretation except under unusual circumstances, since the results of frozen sections will not change the immediate management. The biopsy should be properly oriented on a firm surface with the epidermal surface up to keep the specimen as flat as possible for examination or fixation in the laboratory (Fig. 2.1).

Other limited treatment or biopsy modalities used in vulvar disease include cryosurgery, laser surgery, electrocautery, and 5-fluorouracil. These are used for the treatment of small lesions, particularly when conservative therapy is indicated. Such specimens are not submitted for intraoperative consultation. They are not usually suitable for histopathologic evaluation in view of the complete destruction of tissues or the marked artifactual changes that preclude proper examination.
**Interpretation and Limitations**

The most critical role of intraoperative consultation in the vulva is to rule out malignancy and map the extent of resection in the context of a vulvectomy for cancer, as discussed below. Non-neoplastic epithelial disorders of vulvar skin and mucosa such as squamous cell hyperplasia, lichen sclerosus, and other dermatoses are some of the lesions that may be encountered in such a scenario, and may raise differential diagnostic issues. An adequate biopsy should include the epidermis and at least the superficial dermis. A rim of normal tissue can be helpful in identifying early changes that may be masked by necrosis or inflammatory exudate in the center of the sample. Freezing artifacts also interfere with the ability to arrive...
at a diagnosis on subsequent permanent sections. In small lesions that were completely excised, trimming to “face” the biopsy risks loss of tissue at the edge of the blade, thus permanently missing any chance to establish the nature of the lesion. Pressure to make a hasty diagnosis, in the absence of a good reason, should be resisted, since it can be a disservice to the patient.

VULVECTOMY FOR SQUAMOUS CELL CANCER
Cancer of the vulva comprises only 5% of all gynecologic malignancies. It predominantly affects postmenopausal women. The majority of malignancies arise within the squamous epithelium, most commonly on the labia majora, labia minora, clitoris, and perineum. Studies suggest that there are two etiologic types of vulvar cancer. One type is the warty or basaloid carcinomas that are seen in younger patients; these are related to human papilloma virus (HPV) and are associated with warty or basaloid vulvar intraepithelial neoplasia (VIN). The second type, keratinizing squamous cell carcinoma seen in elderly patients, is unrelated to HPV infection and is associated with differentiated (simplex) VIN. These patients have a high incidence of lichen sclerosus adjacent to the neoplasm.

Clinical Background and Specimen Types
The modern approach to the management of patients with carcinoma confined to the vulva should be individualized. Emphasis is on performing the most conservative operation consistent with cure of the disease. Several types of vulvectomy are tailored to treat squamous cell carcinoma, depending on the location, type, and extent of the tumor, stage of disease, and other clinical parameters such as risk tolerance. Wide local excision (disease-free border of at least 5 mm) is performed for the treatment of premalignant lesions or minimally invasive cancer. Partial or total vulvectomy is usually performed for invasive cancers, with inclusion of a portion of the vagina and extensions of perineum around the anus in some cases (Fig. 2.2). The depth of resection may be variable. In skinning vulvectomy, the epidermis and a variable amount of dermis are included. Radical vulvectomy is a more extensive procedure that is less frequently performed. It involves resection of the superficial aponeurosis of the urogenital diaphragm and/or pubic periosteum in addition to the vulva proper (Fig. 2.3). Pelvic exenteration, combined with radical vulvectomy, is rarely used for the treatment of vulvar cancer, and is limited to exceptional cases with the involvement of the anus, rectovaginal septum, or proximal urethra. It includes resection of several pelvic organs. Inguinal lymph nodes may also be resected for vulvar malignancies. Since the lymphatics drain primarily into
Vulvectomy for carcinoma in situ. The neoplastic process may be multifocal and bilateral, thus determining the extent of the surgery.

the superficial and deep (femoral) inguinal nodes, both levels of nodes are usually resected in cases of carcinoma.

**Specimen Handling**
The initial laboratory evaluation of any vulvar excision must include orientation of the specimen as if viewed in situ. Orientation by the surgeon is critical if the adequacy of the resection is to be assessed. Photographs and diagrams/drawings to demonstrate the margins of resection and extent of the lesion are also helpful. Deep margins should be inked, and different colors should be used for vaginal tissue or any margin toward the anal canal (Figs. 2.4 and 2.5). Margins that are macroscopically close to the tumor are preferably evaluated with sections that are perpendicular, rather than parallel, to the surgical margin. Other margins that are distant to the lesion can be evaluated with sections parallel to the margin. This approach will reduce the number of sections, intraoperative consultation time, as well as the overall operating time. In many instances, the status of the deep soft tissue margin can be assessed macroscopically. Microscopic examination of the deep margins is recommended if, on gross examination, the tumor approaches the deep soft tissue margin. The frozen section report should include the size and location of
the lesion, status of the cutaneous and mucosal margins, and status of the deep margin as well as the maximum depth of invasion.

Interpretation and Differential Diagnostic Considerations
Squamous cell carcinoma and its intraepithelial precursors are the most common malignant neoplasms of vulva, with invasive lesions accounting for about 90% of all vulvar cancers and about 5% of all gynecologic cancers. The role of the human papilloma virus in the pathogenesis of this tumor has been well documented, but a detailed discussion is beyond the scope of this text. The neoplastic process encompasses a spectrum of morphologic changes, ranging from mild cytologic atypia in intraepithelial lesions to high-grade invasive carcinomas.

**Figure 2.3** Radical vulvectomy for invasive squamous cell carcinoma. The vulva, part of inguinal skin, and soft tissues, as well as lymph nodes are included in this specimen.
**Figure 2.4** Vulvectomy for squamous cell carcinoma to demonstrate the inking of the resection margins using different colors.

**Figure 2.5** Diagram of resected vulva, identifying the resection margins and location of each section can be critical in assessing the margins.
Figure 2.6 Vulvar intraepithelial neoplasia (VIN III): (a) Involvement of the entire thickness of the squamous epithelium by the atypical cells. Surface keratinization is evident and the dermis is infiltrated by chronic inflammatory cells (H&E, medium power). (b) Higher magnification shows nuclear pleomorphism and dyspolarity in the entire thickness of the epithelium.

Vulvar Intraepithelial Neoplasia

Frozen sections should not be performed solely to evaluate the grade of vulvar intraepithelial neoplasia (VIN). However, they are necessary for the assessment of margins to exclude any high-grade VIN, in view of the increased risk of recurrence or progression to invasive disease (Fig. 2.6). Adjacent foci of VIN or hyperplasia with or without atypia are seen in many cases of invasive squamous cell carcinoma (Fig. 2.7). In cases with superficially invasive carcinomas, the frequency of adjacent VIN approaches 85% (Hoskins 2000). A thorough evaluation of all the margins in large vulvar resections is only feasible on permanent sections. Multifocal high-grade dysplasia often presents a challenge, in view of the difficulty in obtaining a clear resection margin. Foci of unsuspected invasion may occur in up to 19% of patients with VIN (Chafe 1998). In evaluating the presence and depth of invasion, involvement of adnexal structures by VIN should not be considered evidence of invasion.
FIGURE 2.7 Vulvar intraepithelial neoplasia (VIN III) and hyperplasia with atypia, seen at the margin of an invasive tumor (H&E, medium power).
(Fig. 2.8). Similarly, any tangential sectioning of the VIN lesion should not be misinterpreted. Evidence of a residual appendage, lack of a desmoplastic stromal response, and presence of circumscribed, rather than irregular, borders help in differentiating this artifact from true stromal invasion (Fig. 2.9). Sectioning the frozen tissue at more than one level can also resolve this issue.

Vulvar intraepithelial neoplasia is frequently associated with similar lesions in other pelvic organs. Approximately 50–60% of women with VIN who exhibit evidence of HPV infection have similar synchronous or metachronous lesions in the cervix, vagina, urethra, perineum, or anus. If these areas are included with the resection, they should be sampled extensively, at the time the specimen is processed, for routine microscopic examination.

**Invasive Squamous Cell Carcinomas**

Vulvar squamous cell carcinomas are often well differentiated neoplasms, with evidence of keratinization (Fig. 2.10). If they ulcerate, the stroma shows a mixture of acute and chronic inflammatory
cellular response (Fig. 2.11). Assessment of the vascular spread can be difficult at the time of intraoperative consultation. Freezing or fixation artifacts may result in the separation of nests of tumor cells from the surrounding connective tissue. Such retraction, however, is usually incomplete, leaving some parts of the tumor attached to the wall. Immunostains for endothelial markers may be necessary to settle the issue on permanent sections (Fig. 2.12). The management of invasive tumors varies with the stage and extent of the lesion. In FIGO stage IA, the tumor measures 2 cm or less, with 0.1 cm or less depth of invasion, and is associated with clinically negative nodes. These lesions tend to affect younger women and are commonly associated with multifocal pre-invasive disease and evidence of HPV infection. Such patients have a high risk of recurrence or development of new lesions. Wide local or superficial local excisions that incorporate a 1 cm normal tissue margin are the usual
Figure 2.10  Invasive squamous cell carcinoma. The tumor involves both sides of the vulva. The left side lesion is ulcerated and involves midline structures.

Figure 2.11  Invasive squamous cell carcinoma. A well differentiated tumor, showing surface ulceration with intense inflammatory reaction in the stroma.
treatment modality for such tumors. The incidence of local invasive recurrence is low if the margin is at least 0.8 cm.

For stage IB and II vulvar cancers, the usual management is radical local excision with unilateral or bilateral inguinal node dissection, contingent upon whether the lesion is laterally located or in the midline. This approach provides long-term survival, but has significant morbidity. More recent emphasis has been placed on developing individualized treatment, with limited resections for certain subsets considered to represent early or low-risk disease. In stage IB carcinoma, for example, a more conservative resection of the primary tumor with a 1–2 cm margin of normal tissue, and dissection to the deep perineal fascia, combined with inguinal lymph node dissection, is currently recommended.
Verrucous carcinoma. This highly differentiated squamous cell carcinoma is often treated by wide local excision. The neoplastic epithelium extends deeply into the subepithelial tissues by bulbous nests that have a pushing, rather than infiltrating, border. There is marked hyperkeratosis as well as parakeratosis. Unlike warty invasive carcinoma, the cytologic atypia is minimal, and the lesion is often interpreted as hyperplasia in the initial biopsies (H&E, medium power).

Verrucous Carcinoma
This uncommon, highly differentiated squamous cell carcinoma has been associated with HPV serotypes 1, 2, 6, 11, 16, and 18. Verrucous carcinoma has a tendency to involve a few specific areas, including the vulva, oral cavity, and larynx. It is a low-grade, locally invasive neoplasm that rarely metastasizes and has excellent prognosis. Consequently, treatment by a wide local excision is usually curative. The tumor has a tendency for local recurrences, especially if it has been incompletely resected. Intraoperative evaluation of these cases includes gross inspection of the lesion and its relationship to the margins of resection. Frozen section of grossly or clinically suspicious margins can be performed, and, since deep pushing penetration by bulbous epithelial masses with deep keratinization is a helpful feature, the pathologist should not hesitate in asking for deep excisions to assess the relationship with the dermis (Fig. 2.13).
Verrucous carcinomas should be differentiated from warty invasive squamous cell carcinoma and condyloma acuminatum. Invasive warty squamous cell carcinomas have a significant degree of atypia and an infiltrative border, unlike the minimal cytologic atypia and mostly pushing borders that characterize verrucous carcinomas. Condylomas lack the deep penetration by bulbous growth and keratinization at the base that are seen in verrucous carcinoma (Figs. 2.14 and 2.15).

**Differential Diagnosis of Verrucous Lesions**

**Key Histologic Features of Verrucous Carcinoma**
- Bulbous nests of neoplastic cells with pushing border
- Tumor cells have abundant cytoplasm with minimal atypia
- Hyperplastic squamous epithelium
- Prominent hyperkeratosis and parakeratosis
Key Histologic Features of Warty (Condylomatous) Carcinoma
- Papillae lined by squamous epithelium with koilocytic change on the surface
- Significant cytologic atypia, and deep keratinization at the base and in the invasive nests
- Infiltrative-jagged borders

Key Features of Condyloma Acuminatum
- Papillary architecture
- Acanthotic squamous epithelium with hyperkeratosis, parakeratosis, and hypergranulosis
- Koilocytic atypia on the surface
- No pushing border or infiltrative border
LYMPH NODE DISSECTION IN VULVAR CANCER
This is usually indicated in patients with more than 1 mm stromal invasion. The resection may be unilateral or bilateral, depending on the location and extent of the malignancy. Sentinel lymph node evaluation in vulvar carcinomas is a feasible technique that can be utilized to reduce complications and morbidity associated with inguinofemoral lymphadenectomy.

The inguinal node component can be attached to the vulvar resection or submitted separately. The superficial inguinal nodes are the most common sites of metastasis. Nodal involvement generally proceeds in a stepwise fashion from superficial to deep inguinal nodes and then to the pelvic (iliac) nodes (Fig. 2.16).

Tumor involvement of pelvic lymph nodes including internal iliac, external iliac, and common iliac lymph nodes is considered distant metastasis. Surgical resection of deep inguinal or femoral lymph nodes is usually performed in conjunction with superficial inguinal lymphadenectomy. In most cases, these samples are submitted independently from the vulvar specimen. Frozen section is not usually requested for node dissections since the results rarely have an impact on the immediate operative management. In the rare instances where an intraoperative consultation on a node is required, the initial evaluation must include a count of lymph nodes identified and the gross inspection of the cut surface. In cases where sentinel lymph node or Cloquet lymph node evaluation is requested, the entire node(s) should be submitted for frozen section. Touch imprints of the cut surface assist in the initial evaluation of metastatic disease. Areas of firmness or variation in color should raise suspicion of involvement by metastatic disease, and such areas should be selected for frozen sections, if necessary. Frozen section diagnosis, however, can be difficult in cases where only few atypical cells are noted, and, if immediate management is not altered by lymph nodes status, evaluation should be deferred until permanent sections are examined.

VULVECTOMY FOR OTHER MALIGNANCIES
Rare malignancies that may be encountered include malignant melanoma, Paget disease, adenocarcinomas, and basal cell carcinoma. Soft tissue sarcomas, such as leiomyosarcoma, angiosarcoma, liposarcoma, or embryonal rhabdomyosarcoma, are rarely seen in the vulva. In addition, metastatic tumors from other genital organs can appear in the vulvar skin or mucosa.

Malignant Melanoma
This is the second most common vulvar malignancy, usually involving the labia majora, labia minora, clitoris, and perineum, in
descending order. The main treatment modality is surgical resection. Melanoma in situ is usually treated with complete excision of the gross lesion, the full thickness of the underlying dermis, and at least a 0.5 cm rim of normal tissue. Invasive melanomas less than 0.1 cm thick are similarly managed, but with a wide (1 cm) margin.

although the utility of such wider margins for deeper melanomas is debatable (see Fig. 2.1). Radical vulvectomy with lymph node dissection is necessary in large melanomas, or for those tumors involving the urethra, clitoris, or lower vagina (Figs. 2.17 and 2.18). Sentinel lymph node evaluation is performed for tumors 0.1 cm or greater in thickness, or for melanomas with Clark level 4 or deeper, with the decision regarding lymphadenectomy based on the status of the sentinel lymph node. As in other skin sites, frozen section evaluation of margins in pigmented lesions or known melanomas is not recommended due to the difficulty of interpretation with freezing artifact and the ensuing cytoplasmic vacuolization. These artifactual changes can be misinterpreted as involvement by atypical melanocytes in the frozen section material, as well as in the permanent section of previously frozen tissue.
Malignant melanoma: Multinucleation, prominent nucleoli, intranuclear cytoplasmic inclusions, and the presence of melanin pigment help to differentiate this tumor from squamous cell carcinoma and Paget disease. Occasionally, immunostains may be necessary to confirm the diagnosis, particularly in amelanotic melanomas (H&E, medium power).
Differential Diagnostic Considerations
Nevocellular nevi are not uncommon in the vulva, particularly on the labia majora. These lesions occasionally have a verrucous appearance. Unlike malignant melanomas, these benign lesions lack nuclear atypia, mitotic figures, lymphocytic, or desmoplastic reaction. Frozen sections are not appropriate to establish the diagnosis in such cases since freezing artifacts can render evaluation extremely difficult.

Extramammary Paget Disease
This uncommon disease accounts for 1% of vulvar cancers. Paget disease of the vulva, unlike its mammary counterpart, is usually a noninvasive intraepithelial lesion that can remain unchanged for many years, and it has a low potential to progress to invasive cancer (Figs. 2.19 and 2.20). Underlying invasive carcinoma is
identified in approximately 20% of cases. The sites of origin of the invasive component include colon/rectum cervix, bladder, or urethra. Patients with only superficial Paget disease are treated with a more conservative surgery such as local excision or skinning vulvectomy, with evaluation of the margins by frozen section. Patients with Paget disease and primary adenocarcinoma of the underlying apocrine glands are treated with radical vulvectomy and inguinal lymphadenectomy.

**Differential Diagnostic Considerations**

Differentiating Paget disease from pagetoid VIN and superficial spreading malignant melanoma can be extremely difficult. In pagetoid VIN, the squamous cells in the background are dysplastic, while they appear less atypical in Paget disease or in superficial spreading melanoma (Fig. 2.21). In difficult cases, the application of a
The modified rapid periodic-acid Schiff procedure (PAS) can be useful in identifying characteristic Paget cells with their PAS-positive vacuoles. The cells also stain positively for neutral and acidic mucin (mucicarmine, Alcian blue, and Colloidal iron) and are reactive with CEA, EMA, GCDFP, and CK7. Table 2.2 summarizes the salient features differentiating vulvar intraepithelial malignancies.

**Primary Adenocarcinoma**
Most vulvar adenocarcinomas are deep lesions, arising within the Bartholin gland or skin appendages. In most cases, the diagnosis has been previously established with a biopsy of the lesion.
prior to definitive surgical resection. Approximately 20–30% have metastatic disease of the inguinal/femoral lymph nodes at time of initial diagnosis. Treatment includes a complete surgical resection and inguinal lymphadenectomy. Some benign tumors and ulcers can resemble adenocarcinoma clinically. These are discussed below. Features that suggest an adenocarcinoma include irregular infiltrating margins, desmoplastic response of the stroma, cytologic atypia, mitotic activity, and necrosis of neoplastic cells (Fig. 2.22).

**Differential Diagnostic Considerations**

Primary vulvar adenocarcinoma should be differentiated from metastatic adenocarcinoma, particularly of the cervix, endometrium, breast, and ovary (see below). In addition, a wide variety of benign lesions develop in the vulvar skin, adnexa, and soft tissues. Although a detailed discussion of these is beyond the scope of this text, a few lesions will be considered in view of their potential to be misinterpreted as malignancies in the frozen section room. These include papillary hidradenoma, rare cases of adenosis, hyperplasia of Bartholin glands, ectopic breast tissue, and endometriosis.

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<th>TABLE 2.2 Vulvar intraepithelial malignancies.</th>
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<td>VIN III</td>
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<td>Clinical and gross</td>
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<tr>
<td>Maculopapular</td>
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<tr>
<td>White or erythematous</td>
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<td>May be parakeratotic</td>
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<td>Neoplastic cells</td>
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<td>Squamous</td>
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<td>Full thickness</td>
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**Papillary hidradenoma** is a benign adnexal tumor of apocrine sweat glands that may be clinically alarming because of ulceration, bleeding, or infection. It usually forms a gray or brown 1–2 cm nodule in the labium major. The tumor consists of folded papillae, clefts, and glandular spaces surrounded by a pseudocapsule. Unlike adenocarcinoma, these spaces are lined by two layers of uniform neoplastic cells: an inner cuboidal with clear PAS positive cytoplasm and a basal layer of oval myoepithelial cells that are reactive to S-100. The uniformity of the cells and the double layer of epithelium should help in differentiating this tumor from adenocarcinoma (Fig. 2.23).

**Vulvar endometriosis** can be easily differentiated on the basis of clinical presentation, history, and location, such as in an episiotomy scar. Occasionally, it is submitted for identification when encountered as an incidental finding during surgery.

**Figure 2.22** Adenocarcinoma: This tumor involved the vulva and extended into the lower vagina. It has ulcerated through the vulvar skin. Spread from neighboring structures such as rectum or uterus should be excluded in such cases (H&E, medium power).
Basal Cell Carcinoma

Only 2–3% of vulvar carcinomas are of the basal cell type, and they typically occur in elderly patients. Basal cell carcinomas are cured by wide local excisions (Fig. 2.24). When these tumors lack a clear histomorphology, such as peripheral palisading, their differentiation from Merkel-cell carcinomas, poorly differentiated squamous cell carcinomas, and metastatic small cell carcinomas may require immunostaining of permanent sections. Basal cell carcinoma should not be confused with tangentially cut basal areas of the more common squamous VIN lesions. Proper orientation of the tissue and serial sectioning should clarify this difficulty.

Metastatic Neoplasms

Metastatic tumors to the vulva are rare and occur most often in postmenopausal women. The most common source of metastases to the vulva is the genital tract, particularly the cervix, vagina,
endometrium, and ovary. Other nongynecologic sources reported include breast, lung, kidney, colon/rectum, cutaneous melanoma, lymphomas, and genitourinary primaries. Clinical history, a review of any available material from the primary site, and additional studies using special stains and immunoprofile should establish the source in these cases.

**RECOMMENDED READING**


Frozen Section Library: Gynecologic Pathology
Intraoperative Consultation
Coffey, D.M.; Ramzy, I.
2012, XI, 240 p. 205 illus., 204 illus. in color., Softcover