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Diagnostic Criteria for Differentiated Vulvar Intraepithelial Neoplasia and Vulvar Aberrant Maturation

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Objective: The aim of the study was to describe the features required for diagnosis of differentiated vulvar intraepithelial neoplasia (dVIN) and vulvar aberrant maturation (VAM).

Materials and Methods: The International Society of the Study of Vulvovaginal Diseases tasked the difficult pathologic diagnoses committee to develop consensus recommendations for clinicopathologic diagnosis of vulvar lichen planus, lichen sclerosus, and dVIN. The dVIN subgroup reviewed the literature and formulated diagnostic criteria that were reviewed by the committee and then approved by the International Society of the Study of Vulvovaginal Diseases membership.

Results: Differentiated vulvar intraepithelial neoplasia is the immediate precursor of human papillomavirus (HPV)–independent vulvar squamous cell carcinoma and shows a spectrum of clinical and microscopic appearances, some overlapping with HPV-related neoplasia. The histopathologic definition of dVIN is basal atypia combined with negative or nonblock-positive p16 and basal overexpressed, aberrant negative, or wild-type p53. The most common pattern of dVIN is keratinizing with acanthosis, aberrant rete ridge pattern, and premature maturation. The morphologic spectrum of keratinizing dVIN includes hypertrophic, atrophic, acantholytic, and subtle forms. A few dVIN cases are nonkeratinizing, with basaloid cells replacing more than 60% of epithelium. Vulvar aberrant maturation is an umbrella term for lesions with aberrant maturation that arise out of lichenoid dermatitis and lack the basal atypia required for dVIN.

Conclusions: Evaluation of women at risk for dVIN and VAM requires a collaborative approach by clinicians and pathologists experienced in vulvar disorders. Close surveillance of women with lichen sclerosus and use of these recommendations may assist in prevention of HPV-independent squamous cell carcinoma through detection and treatment of dVIN and VAM.

Key Words: vulva, differentiated VIN, vulvar aberrant maturation, HPV-independent, squamous cell carcinoma, lichen sclerosus, lichen planus, high-grade squamous intraepithelial lesion

(*J Low Genit Tract Dis* 2021;25: 57–70)

There are 2 types of vulvar intraepithelial neoplasia (VIN), both immediate precursors to vulvar squamous cell carcinoma (SCC). High-grade squamous intraepithelial lesion (HSIL, usual VIN), is human papillomavirus (HPV)–related, usually shows warty-basaloid morphology, and comprises more than 80% of VIN but less than 50% of SCC.[1–6] Differentiated VIN (dVIN) is HPV-independent and usually shows keratinizing morphology on a background of lichen sclerosus (LS).[7–11]

High-grade squamous intraepithelial lesion commonly displays full-thickness atypical cells with large dark nuclei and scant basophilic cytoplasm, giving the epithelium a blue appearance on hematoxylin and eosin (H&E)–stained slides, obviously different to nonneoplastic skin conditions.[10,11] In contrast, dVIN often recapitulates the pink-colored maturation pattern seen in benign conditions such as LS and lichen simplex chronicus (LSC). The atypia of dVIN is subtle compared with HSIL for 3 reasons—it is often confined to basal and parabasal layers, abnormal mitoses are uncommon, and some or all enlarged nuclei are vesicular rather than hyperchromatic. As a result of these difficulties, traditional definitions of dVIN focused on a distinctive pattern of parakeratosis (PK), elongated branched rete ridges, marked intercellular prickles, and keratin pearls.[7,12]

Over the past 2 decades, multiple publications highlighted deficiencies in this construct of morphology guiding diagnosis. Researchers identified keratinizing HSIL that mimics dVIN, basaloid dVIN that mimics HSIL, and atrophic dVIN that mimics LS.[11,13–18] Researchers also described lesions of uncertain malignant potential adjacent to dVIN and SCC; the term "vulvar aberrant maturation" (VAM) captures their unifying histopathologic characteristic.[19–21] Immunohistochemistry (IHC) for p16 emerged as a reliable marker for HPV-related neoplasia, and description of distinctive p53 IHC patterns helped distinguish dVIN and VAM from HSIL.[15,22–29] These advancements revealed that 10%–25% of vulvar neoplasia is misclassified when diagnosis relies on a traditional combination of clinical risk factors and morphologic categorization.[13,22,30–33]

Distinguishing between HPV-related and HPV-independent precursors has important implications for treatment and prognosis. Treatment of dVIN and steroid-resistant VAM is excision, whereas options for HSIL include imiquimod, LASER, and excision. Differentiated VIN is more likely than HSIL to progress to cancer and more often associated with a prior, synchronous, or subsequent SCC.[1,34,35] Human papillomavirus–independent SCC is less radiosensitive with higher disease-related mortality.[1,4,34–38] High-grade squamous intraepithelial lesion surveillance is within the scope of most gynecologists, whereas evaluation for dVIN and VAM requires skill and experience in vulvar dermatoses.[1,39] Correct diagnosis is essential to direct clinical care. The aims of this document are to critically appraise the literature and to formulate consensus recommendations for clinicopathologic diagnosis of dVIN and VAM.

METHODS

The International Society for the Study of Vulvovaginal Diseases (ISSVD) tasked the difficult pathologic diagnoses committee with development of consensus documents for diagnosis of lichen planus (LP), LS, and dVIN. The dVIN subgroup performed a literature search in PubMed-Medline from database inception

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The authors have declared they have no conflicts of interest.

DOI: 10.1097/LGT.0000000000000572

through March 2020 using terms: "vulvar" and "vulval" "intraepithelial neoplasia," "VIN," "differentiated," "simplex," "histology," "pathology," and "histopathology." Review of selected studies' references identified additional relevant publications. The subgroup appraised and summarized pertinent studies, synthesized them into a critical review, and then generated diagnostic criteria and recommendations. The manuscript was disseminated within the committee and underwent revisions to achieve consensus, and then was approved by ISSVD membership. Signed written consents were obtained for use of clinical photographs.

Incidence and Epidemiology

Mean age at diagnosis of HPV-independent neoplasia, to include invasive and intraepithelial disease, ranges from 67 to 78 years.[1,4,7,12,18,20,24,35,36,40,41] Although characterized as a disease of older women, multiple cases have occurred in women aged 17–39 years.[18,42–45] The age-standardized incidence of HPV-independent SCC has fallen for the past 30 years from 0.76 to 0.54/100,000.[1] Rates fell from 2.53 to 1.62/100,000 in women older than 50 years; meanwhile, the incidence in younger women remained steady since 1991 at 0.14/100,000. Median interval between biopsy-proven dVIN and SCC is reported as 23–44 months (range = 6–102).[8,14,35,46]

There is strong epidemiologic, histopathologic, and clinical evidence that LS is the major underlying cause of HPV-independent neoplasia. A Finnish registry study found women with LS have a standardized incidence ratio of 40.3 for vulvar SCC, with a total of 160 cancers.[47] Retrospective cohorts of keratinizing and/or HPV-negative SCC identify peritumoral LS in 40% to 88%.[14,27,36,40,48–51] A review of 43 cases of HPV-independent SCC found that all were associated with LS when results from previous and subsequent vulvar specimens were incorporated.[52] Historically, 5% of women with LS develop neoplasia and this risk seems reduced by tailored long-term topical steroids.[1,53] Uncertainties around primary prevention include optimal maintenance regimens, frequency and mechanism of follow-up, and circumstances under which steroid cessation is appropriate.[18,52–54] Presence of symptoms and architectural change does not predict neoplasia.[20,55] There are minimal data on secondary prevention of SCC. A molecular "point of no return" might negate the impact of steroids on a particular clone, but therapy may slow carcinogenesis in other areas.

Association between erosive LP and dVIN/SCC remains uncertain. Cohort studies report SCC in 1%–3% of affected women but fail to exclude HPV-related disease.[52,56] Finnish registry data found the standardized incidence ratio of LP for vulvar cancer of any histologic type is 1.99 (total of 18 cancers), low enough to be explained by misdiagnosis and comorbid HPV-related disease.[57–59] Reasons for possible misattribution of HPV-independent neoplasia to LP include underrecognition of comorbid LS and LP, histopathologic similarity of nonsclerotic LS and LP, and disappearance of sclerosis under neoplasia.[60–64]

The rate of dVIN diagnosis before SCC development varies by health care setting and study methodology, but the trajectory suggests improvements in detection.[1] A cohort in 2000 found that 95% of dVIN cases were adjacent to SCC.[65] Subsequent expert reviews identified in 24%–28% of dVIN cases preceding SCC.[7,35] In 2018, Jin and Liang[66] found that 34% of dVIN cases lacked associated SCC, matching the rate documented by Day et al in 2020.[20]

Clinical Assessment of dVIN and VAM

Symptoms. Symptoms reflect the underlying dermatosis, so most women report pruritus or pain.[20] Ten percent of women with LS are asymptomatic with neoplasia occurring in this setting.[11,20,31] Some women report focally severe itch or pain at the site of dVIN or VAM.

Vulvar Examination. Differentiated VIN and VAM look different to surrounding abnormal skin, but detection may be challenging. Lichen sclerosus produces changes to vulvar architecture, color, and texture, sometimes accompanied by superimposed mycotic, bacterial, or viral infection. Postinflammatory hyperpigmentation, melanosis, and postoperative changes further complicate assessment. Some specialists find a colposcope facilitates detection through light and magnification. Acetic acid application is not recommended for evaluation of HPV-independent neoplasia.[67] Differentiated VIN and VAM do not demonstrate acetowhite uptake nor enhancement in margin visualization.[68] Moreover, acetic acid is painful and produces false-positive results in areas of dermatitis and physiologic uptake at the mucocutaneous junction.

Differentiated VIN and VAM most often occur on periclitoral structures and labia minora, areas encompassing hairless skin, mucocutaneous junction, and nonkeratinized squamous epithelium.[4,20] The next most common site is perineum and perianus, in keeping with LS distribution.[20] Characterization of dVIN as unifocal and HSIL as multifocal is an oversimplification. Clinical photographs of HPV-independent neoplasia often show concurrent lesions with diverse morphologies, and 20%–50% of women have 2 or more noncontiguous disease sites.[4,12,18,20,28,69]

Vulvar aberrant maturation presents as a well-demarcated white papule or plaque, often with an irregular or verruciform surface[19] (see Figure 1). Findings consistent with dVIN include a white papule or plaque, "gray-white discoloration with a roughened surface," pink-red plaque, glazed red patch, and flat pink center with a raised white border[12,16–18,28,31,46,50,65] (see Figures 2–4). Clinicians may describe pink-red patches as ulcers or erosions, but these labels do not uniformly concur with histopathologic findings. Pink-red areas may contain white papules or have a smooth surface, mosaic pattern, or gravel-like texture.[16,17] Lesions may be less than 1 cm or extend across the vulva.[12,46] The unifying description is any treatment-resistant lesion in a field of dermatosis-affected skin.

Biopsy—Indications and Recommendations. Lichen sclerosus guidelines recommend biopsy for diagnostic uncertainty and suspicion for neoplasia but provide few additional details.[70–72] Survey of 23 expert pathologists identified clinical features thought to support dVIN: visible lesion, history of LS/LP, previous SCC, older than 40 years, and steroid nonresponsiveness; none of these distinguish between leading differential diagnoses nor reliably exclude HSIL.[73] Clinicians engaged in LS supervision require (1) familiarity with subtle manifestations of dVIN, (2) equipment and skill to biopsy in the outpatient setting, and (3) a mechanism for prompt operating room access for periclitoral or periurethral disease, multiple lesions, or refusal of office procedures. Adequate tissue sampling and labeling maximizes the chance of correct diagnosis. Accurate, pertinent information on pathology request forms is fundamental to clinicopathologic correlation.

Recommendations for tissue sampling of suspected precursor lesions[67,74]:

- Optimal specimens require a minimum 4-mm width with 5-mm depth for hair bearing skin and 3-mm depth for hairless skin; this may be achieved with punch, suture-assisted snip, or excisional techniques,
- Biopsy each morphologically distinct site at the most suspicious part of the lesion(s),
- In the case of ulcer or fissure, biopsy where there is intact epithelium, and
- In the case of presumed erosion, obtain a biopsy within the red-pink patch.

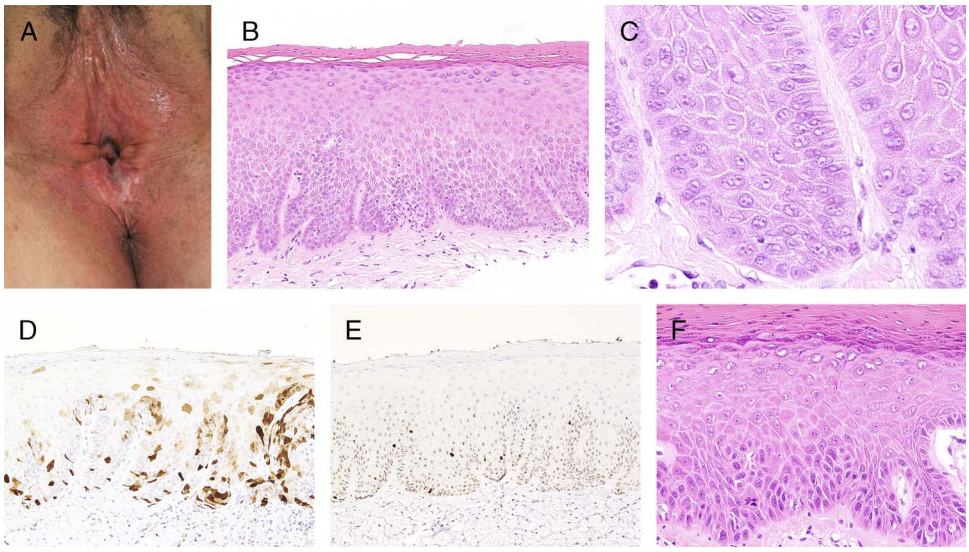

FIGURE 1. A, Vulvar aberrant maturation—white plaques with a rough surface over posterior fourchette and perineum, background of LS-associated architectural change. B, Parakeratosis, hypergranulosis, acanthosis with clubbed rete ridges, premature maturation, prominent intercellular prickles, and vesicular basal nuclei, H&E ×100. C, Uniform vesicular nuclei with intranuclear vacuoles and occasional prominent nucleoli, H&E ×400. D, p16 is nonblock positive with focal variable cytoplasmic and nuclear staining, ×200. E, p53 is wild type, ×200. F, Two years later, excision of the white plaque shows traditional keratinizing dVIN with PK, anastomosing rete ridges, premature maturation, and basal atypia seen as hyperchromasia, pleomorphism, enlargement, and an abnormal mitosis, H&E ×200.

Recommendations for labeling and communication with pathologists include[53,60,62]:

- Note laterality and location with anatomic terms, not an unoriented "clock-face" position,
- Obtain and store clinical photographs with consent,
- Write the underlying dermatosis and differential diagnosis on the request form,
- Document concern for neoplasia and any previous HSIL, VAM, dVIN, and/or SCC, and
- Flatten and pin excisional specimens with labels of surrounding structures.

Mapping biopsies may be required to outline margins and guide excisional procedures. Rates of margin positivity for dVIN range from 45% to 75%.[29,35] To preserve capacity for sentinel nodes, generalists should avoid wide local excision unless SCC was recently excluded.

Clinical Differential Diagnosis. The differential diagnosis for a white plaque within abnormal skin includes lichenified LS, LS with mycotic superinfection, the border of erosive LP, VAM, and HSIL.[14,53,62] Indicators of mycosis include erythema, labial edema, vaginal discharge, satellite lesions, erosions, fissures, and keratin debris.[75] White plaques associated with erosive LP are thin, well demarcated, located beside a glazed red patch, and homogenous in color and texture.[59,60] Vulvar aberrant maturation is well demarcated and thick or verrucous. High-grade squamous intraepithelial lesion is variable in size, shape, number of lesions, and thickness but usually presents as a white, gray, red, pink, tan, brown, and/or black plaque, sometimes showing punctuation and mosaicism.[6,11] Despite these clues, expert physical examination may not reliably distinguish between diagnoses, so histopathologic assessment is necessary.

Summary of Clinical Findings Consistent With dVIN and VAM

Differentiated VIN presents as treatment-resistant lesions different to surrounding abnormal skin. Color, texture, location, size, and focality are variable. The 3 most common appearances are a thick white plaque, a thin pink-red plaque, and a red glazed patch. Vulvar aberrant maturation manifests as a white nodule or plaque in a field of lichenoid dermatitis. If areas identified histologically as VAM persist despite potent topical and/or intralesional steroids, excision is recommended.

Histopathology of dVIN and VAM

General Principles for Evaluation of Vulvar Squamous Neoplasia. The traditional approach begins with H&E and

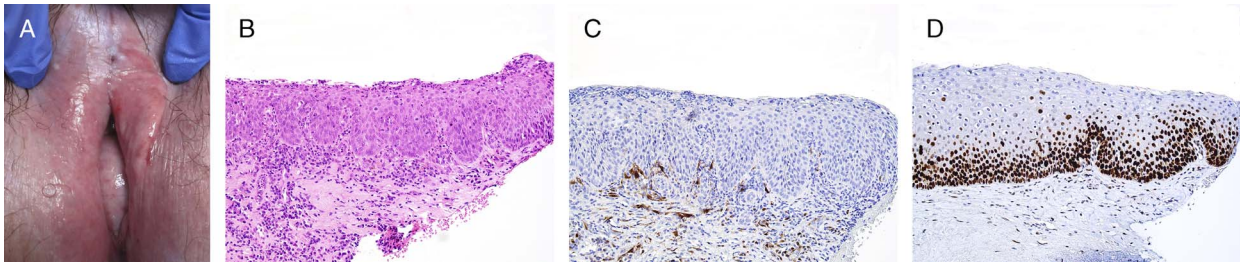

FIGURE 2. A, Differentiated vulvar intraepithelial neoplasia—glazed red macule at left clitoral frenulum on a background of LS. B, Basaloid dVIN with erosion, full-thickness atypical nuclei, multiple mitoses, and moderate lymphoplasmacytic infiltrate, H&E ×200. C, p16 is negative, ×200. D, p53 is overexpressed at basal and suprabasal layers, ×200.

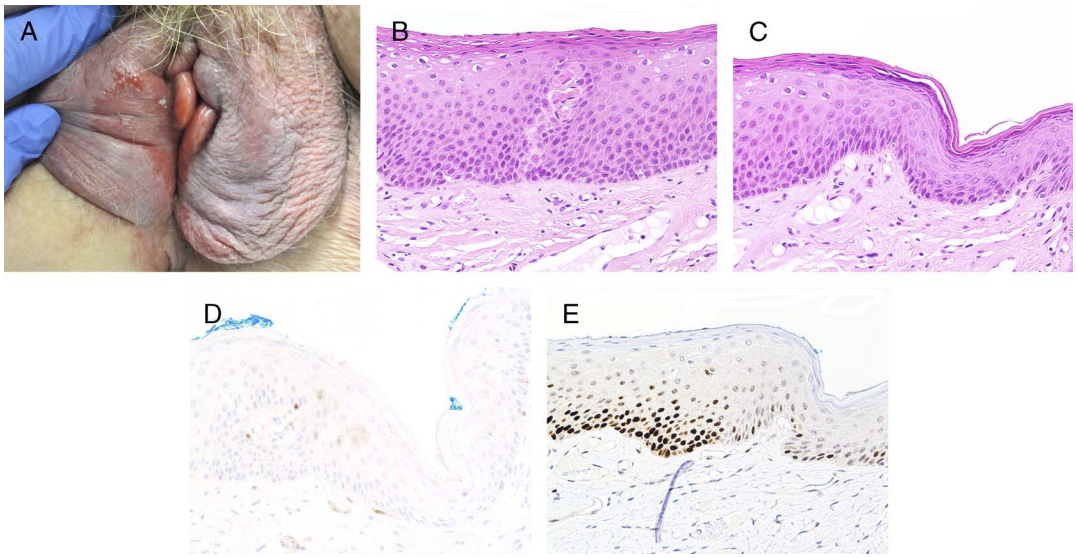

FIGURE 3. A, Differentiated vulvar intraepithelial neoplasia—large glazed red patch over bilateral labia minora and right interlabial fold. B, Subtle keratinizing dVIN with PK, flat acanthosis, and mildly hyperchromatic enlarged nuclei extending halfway up the epithelium, H&E ×200. C, Junction between dVIN and thinner nonneoplastic epithelium, H&E ×200. D, p16 is negative, ×200. E, Basal and suprabasal overexpressed p53 in dVIN contrasts with wild-type pattern in adjacent epithelium, ×200.

periodic acid–Schiff (PAS)–stained slides, reviewed first at low power and then sequentially higher power. At each step, interpretation is associated with greater interobserver disagreement. At low power, pathologists identify architectural features such as epithelial thickness, rete ridge length and shape, stromal collagen, and lymphocytic infiltrate (Table 1). At medium power, pathologists assess for surface features, intercellular breakdown, and epithelial maturation (Table 2). Evidence of dyskeratosis emerges, to include intracellular vacuoles, suprabasilar apoptotic bodies, and premature maturation defined as large suprabasilar cells with eosinophilic cytoplasm. At high power, pathologists inspect nuclei for atypical features: abnormal chromatin, pleomorphism, increased and/or abnormal mitoses, and enlargement (Table 3).[20] A decision about IHC occurs after review of H&E and PAS.

This process provides opportunities for errors that account for dVIN's poor interobserver reproducibility and 25%–61% rate of nondiagnosis.[8,29] On low power, dVIN often shows a lichenoid or acanthotic reaction pattern, leading to presumption of LS, LP, or LSC. At high power, the spectrum of atypical nuclear features overlaps with reactive changes, so identification requires a keen eye and comparison with normal. Lack of suspicion for dVIN results in failure to order p16 and p53; clinicians contribute to this through inadequate documentation on request forms. In contrast, diagnosis of warty-basaloid HSIL is straightforward because

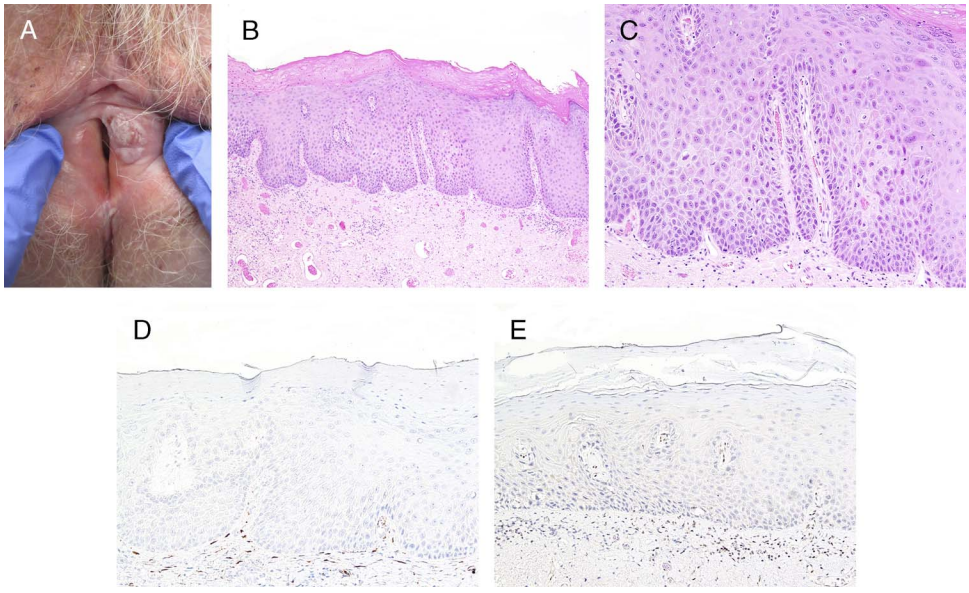

FIGURE 4. A, Squamous cell carcinoma and dVIN—central tumor surrounded by a white heterogeneous plaque. B, Hypertrophic keratinizing dVIN with thick PK and wide elongated rete ridges, H&E ×100. C, Vesicular nuclei with marked enlargement and multiple nucleoli, H&E ×400. D, p16 is negative ×100. E, p53 is aberrant negative, ×100.

TABLE 1. Spectrum of Architecture in dVIN and VAM[20]

Diagnosis	Epithelial thickness	Rete ridges—size	Rete ridges—shape	Stroma	Lymphocytic infiltrate
Nonkeratinizing dVIN					
Basaloid dVIN	Normal to thick 0.1–0.5 mm	Reduced	Flat acanthosis Blunted or bulbous	Normal Fibrosis and/or sclerosis	Moderate to dense
Intermediate dVIN	Normal to thick 0.08–0.6 mm	Reduced to enlarged	Flat acanthosis Blunted or bulbous	Normal Fibrosis and/or sclerosis	Variable
Keratinizing dVIN					
Atrophic	Thin <0.2 mm	Reduced	N/A	Sclerosis and/or fibrosis Normal	Variable
Subtle	Normal 0.18–0.35 mm	Reduced to normal	Normal	Sclerosis and/or fibrosis Normal	Scant to moderate
Traditional	Normal to thick 0.1–0.9 mm	Enlarged	Branched Clubbed	Sclerosis and/or fibrosis Normal	Variable
Acantholytic	Normal to thick 0.14–1.5 mm	Enlarged	Branched Clubbed	Sclerosis and/or fibrosis Normal	Variable
Hypertrophic	Markedly thick 0.6–2.2	Enlarged	Branched Complex	Fibrosis and/or sclerosis	Moderate to dense
VAM	Thick 0.35–2.5	Reduced to enlarged	Flat acanthosis Branched	Sclerosis and/or fibrosis Normal	Scant to moderate

architectural and maturational abnormalities are evident at low power, and at high power, the atypia and abnormal mitoses are obviously neoplastic.[11]

Yang and Hart's landmark description of dVIN focused on a morphologic pattern uncommon in nonneoplastic disorders that combines architectural features such as elongated complex rete ridges with maturational abnormalities such as PK, keratin whorls, and pearls and premature maturation. They de-emphasized basal atypia by stating "the range of nuclear atypia in these cells was variable."[12] Multiple authors subsequently endorsed that "pathologists should not be fixated on nuclear atypia in the diagnosis… but should look for supporting features of altered architecture and cell changes," in an effort to prevent overdiagnosis.[7,14,46]

However, 20 years of knowledge accumulation yields the conclusion that morphology is an unreliable indicator of diagnosis. This calls into question the framework of low-medium-high power assessment and optional IHC. Basal layer atypia is the single unifying feature of neoplasia, and a panel of p16 and p53 is the best way to distinguish between HSIL and dVIN. Mechanisms to mitigate challenges posed by subversion of long-standing concepts include detailed definitions of terms, standardized descriptions of IHC patterns and their significance, and a categorization system encompassing the spectrum of dVIN and VAM. In practice, clinicopathologic correlation, expert consultation, or multidisciplinary review is often required to arrive at the correct diagnosis.

TABLE 2. Spectrum of Dyskeratosis in dVIN and VAM[20]

Diagnosis	Surface features	Intercellular breakdown	Premature maturation	% of epithelium with cellular maturation
Nonkeratinizing dVIN				
Basaloid dVIN	Thin PK Erosion	Uncommon	None	<10
Intermediate dVIN	Thin PK Erosion	Uncommon	None	10–40
Keratinizing dVIN				
Atrophic	Thin PK Normal	Variable	None	50–80
Subtle	Normal Thin PK	None	None	60–90
Traditional	Thin to thick PK/HK	Frequent	Frequent	40–90
Acantholytic	Thin to thick PK	Marked	Variable	40–90
Hypertrophic	Thin to thick PK/HK	Frequent	Frequent	40–90
VAM	Thick PK/HK	Variable	Frequent	>80

Pathogenesis of HPV-Independent SCC. Pathogenesis involves overlap of the scar-cancer and itch-scratch-cancer hypotheses; these propose that non-HPV–related SCC arises from traumatized epithelium over scarred stroma. They derive from observations that extragenital LS is not associated with SCC, pruritic conditions such as vulvar psoriasis are not linked to SCC, and hidradenitis suppurativa scars represent an HPV- and LS-independent cancer pathway.[10,76,77] The mechanism of LS is T-cell–mediated attack on basal keratinocytes, yielding a cycle of damage and repair associated with oxidative injury, increased cell turnover, and abnormal collagen production.[11,78,79] Excoriation may fuel this process. Gradual accumulation of genetic aberrations means that there is no clear histopathologic dividing line between benign and neoplastic.

Molecular underpinning of HPV-independent neoplasia is an area of ongoing investigation. Somatic *TP53* mutations occur in 41%–79%, but correlation between histopathology, p53 IHC, mutational analysis, and prognosis remains unclear.[5,10,11,29,30,78–80] *TP53* mutations in SCC and adjacent epithelium are concordant in less than half of cases and noncontiguous dVIN lesions show different genetic aberrations, suggesting tandem development of multiple clones.[11,14,78,81] Twenty percent of HPV-independent neoplasia results from pathways related to hypermethylation, chromosome gains, and mutations in *NOTCH1* (28%–41%),

TABLE 3. Definition of Basal Nuclear Atypia in Vulvar Squamous Neoplasia

Nuclear feature	Common feature of atypia	Less common feature of atypia
Chromatin	Hyperchromatic • Dense chromatin • Nucleoli not well seen	Vesicular • Open chromatin • Enlarged eosinophilic nucleoli • Double or multiple nucleoli • Occasional binucleation
Enlargement	Diffuse marked enlargement • More than triple the size of a lymphocyte nucleus Obviously different to nuclei in nonneoplastic epithelium	Variable enlargement • Most nuclei double the size of a lymphocyte nucleus Difference between abnormal and normal nuclei highlighted by overexpressed p53
Pleomorphism	Marked cell-to-cell variation Irregularly shaped cells	Cell-to-cell variation present Majority of cells show uniform shape with enlarged vesicular nuclei
Mitoses	Increased • >1 per 1-mm basal layer length	Abnormal mitotic figures • Y-shaped, X-shaped • Pieces of extra chromosomes separate to the main mitotic spindle

HRAS (3%–31%), *PIK3CA* (0%–19%), and *CDKN2A* (11%–36%).[5,10,11,19,82–85] These findings concur with clinical observations of simultaneous VAM, dVIN, verrucous SCC, and conventional SCC in the same woman or specimen.[29,53]

Previous Work on Histopathologic Diagnosis of dVIN and VAM. Descriptions of dVIN are influenced by methodological approach. Studies of dVIN adjacent to SCC often encounter marked acanthosis, atypia, fibrosis, and lymphocytic infiltrate.[26,40,77,78] Research comparing archetypal dVIN cases to selected dermatoses may exclude subtle iterations.[12,69,86,87] Cohorts of VIN without SCC overrepresent HPV-related disease.[88,89] Publications lacking stringent HPV determination or expert pathologic review may produce misclassification.[90–92] Reassessment of pre-SCC biopsies is most likely to identify a morphologic spectrum.[8,18,46]

Several authors attempted to gain consensus on diagnostic features of dVIN. A Dutch group considered 5 items most predictive: (1) abnormal mitoses in the basal layer, (2) basal cell atypia defined as pleomorphism and enlargement, (3) dyskeratosis, (4) prominent nucleoli, and (5) elongated and anastomosing rete ridges.[46] Education on these features improved recognition in gynecologic but not general pathologists. The survey of pathologists found that basal layer atypia and negative p16 were the only essential criteria, whereas premature maturation was the sole nonnuclear feature reaching consensus as "strongly supportive."[73] Dermatopathologists identified acanthosis and branched rete ridges as strongly supportive, but these had uncertain significance for gynecologic pathologists.

A North American group described 4 categories of dVIN occurring in isolation or combination: (1) dVIN resembling LS—thin epidermis, prominent basal atypia, and stromal hyalinization, (2) dVIN resembling LSC—thick epidermis with basal cell expansion and atypia, (3) dVIN with prominent dyskeratosis, and (4) dVIN with marked spongiosis or acantholysis.[65,66] The LS-like pattern has been called "atypical LS" and associated with p53 overexpression.[14,40] Subsequent documentation of basaloid and hypertrophic patterns of dVIN further supports calls for broader diagnostic criteria.[16–18,53,73]

Vulvar aberrant maturation is an umbrella term for HPV-independent lesions combining aberrant maturation with minimal nuclear atypia. Multiple names for this have been proposed: differentiated exophytic verruciform intraepithelial lesion, vulvar acanthosis with altered differentiation, atypical epithelial acanthosis, LS with acanthosis or hyperplasia, verruciform LSC, and squamous cell hyperplasia.[8,28,40,50,73,93] It is impossible to retroactively describe squamous cell hyperplasia beyond the ISSVD definition of "a hyperplastic process of unknown etiology."[8,10,27,28,78,93,94] The survey of pathologists could not identify a preferred nomenclature and one third of participants wrote in their own unique terms.[73] Several proposed names were too narrow to encompass the spectrum of VAM, which extends from lichenified LS and hypertrophic LP at one end to verrucous SCC and dVIN at the other.[19–21,53] Where one melds into the next, a clearer understanding of molecular events in the cancerization field should aid in diagnosis and prognosis.[10,14,19]

Background and Recommended Definitions of Nuclear Atypia, p16, and p53

Nuclear Atypia. In absence of invasion, atypia of squamous keratinocytes has 3 potential etiologies: dVIN, HSIL, and reactive change. Pathologists must itemize features of (1) abnormal chromatin, seen as hyperchromatic or vesicular nuclei, (2) pleomorphism, (3) mitoses, and (4) enlargement.[6,20] The most common form of atypia is hyperchromatic and pleomorphic. Often, there are dark narrow nuclei in elongated cells surrounded by edema and intercellular bridges (see Figure 5). These "spindle-shaped" or "angulated" cells occur in more than 30% of keratinizing dVIN but are infrequently seen in benign conditions.[20,69] Vesicular nuclei occur in up to 20% of dVIN.[12,20,69] These are large and round, contain multiple or bizarre nucleoli, and may have intracellular and intranuclear vacuoles (see Figure 4). Despite epithelial maturation, suprabasilar cells are often atypical with vesicular nuclei.[12,69] This may be difficult to detect when combined with premature maturation and poor intercellular cohesion. Increased basal mitoses occur in 40%–80%; thus, absent or rare mitoses do not exclude dVIN.[20,69] Abnormal mitoses help exclude an inflammatory process; these are common in HSIL but rare in dVIN.[95]

Recommended Definition of Nuclear Atypia in Vulvar Squamous Neoplasia

Diagnosis of squamous intraepithelial neoplasia requires a systematic assessment of nuclear atypia. The 4 features are (see Table 3):

- Abnormal nuclear chromatin,
  - ○ hyperchromatic—dark with inapparent nucleoli
  - ○ vesicular—open chromatin with visible bizarre or multiple eosinophilic nucleoli, binucleation, and/or fluid-filled spaces

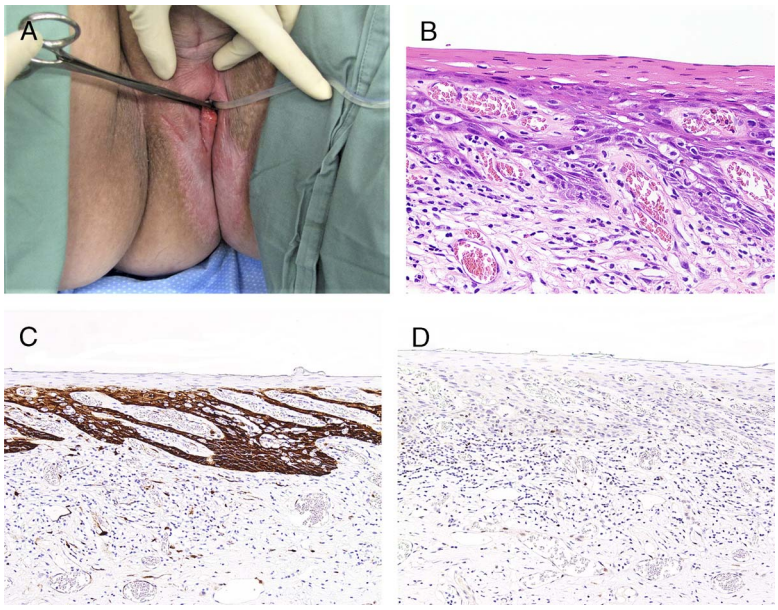

FIGURE 5. A, Exophytic red HPV-related SCC on a background of uncontrolled LS. B, Excision from the contralateral side with traditional keratinizing dVIN seen as PK, spiky rete ridges, and spindle-shaped hyperchromatic, pleomorphic, enlarged nuclei, H&E ×200. C, p16 is block positive, ×100. D, p53 is aberrant negative suggesting dual carcinogenic etiology, ×100.

- Pleomorphism,
- Increased and/or abnormal mitoses,
  - abnormal mitoses show Y-shaped, X-shaped, or bizarre spindle morphology, or contain small pieces of extra chromosomes separate to the main mitotic spindle
- Enlargement.

Immunohistochemistry for p16 and p53. Block-positive p16 identifies high-risk HPV genomic integration and serves as a reliable biomarker of HPV-related neoplasia. The Lower Anogenital Squamous Terminology project defined block-positive as continuous intense staining across nuclei and cytoplasm.[11,96] The CERTAIN trial defined diffuse nuclear and/or cytoplasmic staining as representative of transforming cervical infection and defined focal, noncontiguous, or isolated clusters of stained nuclei as negative.[97] Focal or noncontiguous p16 staining, also called nonblock positive, is unrelated to high-risk HPV and corresponds to deletions, point mutations, or promoter hypermethylation.[98] The p16 false-negative rate in vulvar and cervical HSIL is less than 5%; manifestations include intense cytoplasmic staining with nuclear sparing and moderate patchy nuclear and cytoplasmic staining.[62,99] In rare cases of simultaneous dermatosis-associated neoplasia and HPV integration, p53 may help identify the dominant pathway[62] (see Figure 5).

p53 is more complicated to interpret than p16. Basal overexpression is defined as intense nuclear staining in more than 90% of cells in the lower third of epithelium.[11] This pattern occurs in 45%–80% of dVIN, usually relating to a missense *TP53* mutation.[5,14,20,26,27,69,100,101] Staining may extend into mid-epithelium, fading where maturation begins[10,20,24,69] (see Figures 2D, 3E). The p53 pattern is wild type in 17%–42%, defined as weak to moderate nuclear staining in less than 50% of cells in the lower third of epithelium.[5,20,69] Thirteen to thirty percent of dVIN is aberrant negative for p53, because of a frame-shift, splice site, deletion, or truncating mutation[5,20,29,50,69] (see Figures 4–6). The correlation between p53 IHC and *TP53* mutation status is imperfect, with a positive predictive value of 67%.[5,84]

In contrast to dVIN, HSIL shows a suprabasilar dominant p53 pattern of strong nuclear uptake in mid-epithelium, accompanied by absent or weak noncontiguous basal staining.[15,23–25] When this pattern is present in combination with nonblock-positive p16, it may reflect HPV-related disease with false-negative p16.[62] Rates of p53 basal overexpression in LS and LSC are difficult to determine because of possible misclassification and different grading mechanisms used by researchers. This methodologic and diagnostic variation has produced rates ranging from 0% to 60%.[8,26,69,78,101,102] p53 expression in LS is not impacted by topical corticosteroid use.[103]

Basal overexpression of p53 in dVIN is useful for diagnostic confirmation and margin assessment. At the junction, there is vivid contrast between dVIN's large dark nuclei extending into suprabasilar layers, and the small, regular, lighter-stained basal nuclei of nonneoplastic skin (see Figure 3E). The distinction between null p53 in dVIN and wild-type pattern of benign epithelium is visible but less striking.[29] When dVIN has wild-type p53, margin assessment defers to standard histopathology.[12,20,28]

Studies of other stains have not encountered a test for dVIN that excludes HSIL and nonneoplastic disorders.[11] Ki-67 is a proliferation marker that stains most dVIN and HSIL more intensely than nonneoplastic epithelium. However, the Ki-67 pattern in dVIN with wild-type p53 resembles normal skin.[24,86,104] Ki-67 staining in warty-basaloid HSIL extends to the upper third of epithelium, but the keratinizing HSIL pattern is not well documented.[9] Investigators have assessed GATA3, phosphorylated-S6, CK-13 and CK-17, ProEx C, SOX2, and e-cadherin and b-catenin. None of these reliably distinguishes dVIN from diagnoses with similar appearances, so their use remains investigational.[11,69,81,86,105–107] No single reliable immunomarker for atypia exists.

Recommended Definitions for p16 Staining Patterns in Vulvar Epithelium

1. Block positive = continuous and intense staining of basal nuclei and cytoplasm
   - supports HSIL

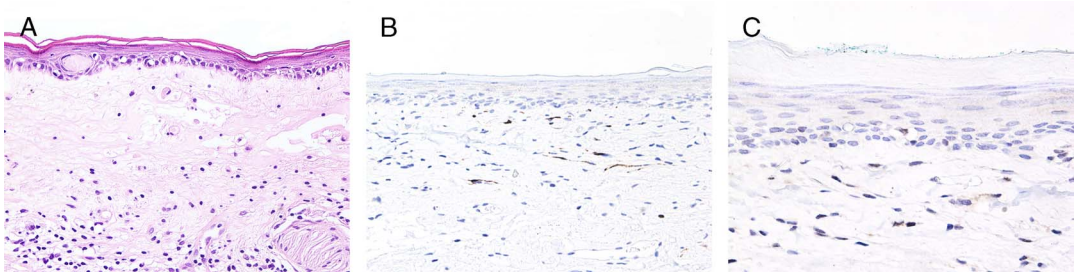

FIGURE 6. A, Atrophic keratinizing dVIN with LS-like appearance—thin epithelium, absent rete ridges, basal atypia comprising half the epithelium, and band of edematous and hyalinized collagen overlying moderate lymphocytic infiltrate, H&E ×200. B, p16 is negative, ×200. C, p53 is aberrant negative, ×400.

- ○ rarely, this may occur in dermatosis-associated neoplasia
  - ▪ aberrant negative or basal overexpressed p53 supports a dual carcinogenic etiology
  - ▪ clinicopathologic correlation is advised

2. Nonblock positive = focal intense nuclear and/or cytoplasmic staining or diffuse weak to moderate staining of cytoplasm and nuclei
   - ○ supports dVIN
   - ○ rarely, this occurs in HPV-related disease
     - ▪ suprabasilar dominant p53 supports an HPV-related etiology
     - ▪ HPV genotyping may be useful
     - ▪ clinicopathologic correlation is advised

3. Negative = total absence of staining
   - ○ supports dVIN

Recommended Definitions for p53 Staining Patterns in Vulvar Epithelium

1. Basal overexpression = continuous, intense nuclear staining, often extending upwards until the layer at which maturation begins
   - ○ most common pattern in dVIN, but occasionally mimicked in LS and LP

2. Wild type = continuous or intermittent weak to moderate nuclear staining, with scant suprabasal extension
   - ○ most common pattern in LS and LP, but does not exclude dVIN

3. Suprabasilar dominant = intermittent, variable staining of enlarged suprabasilar nuclei with basal layer sparing
   - ○ supports HSIL and excludes dVIN

4. Aberrant negative = total absence of staining
   - ○ supports dVIN

Summary of Histopathologic Diagnostic Criteria of dVIN

Diagnosis of dVIN requires basal atypia in combination with negative or nonblock-positive p16 and supportive p53 (see Table 4). Three p53 staining patterns are consistent with dVIN: basal overexpression, wild type, and aberrant negative. Suprabasilar dominant p53 pattern supports HSIL and raises suspicion for false-negative p16. Rarely, lesions may be p16 block-positive with basal overexpressed or aberrant negative p53; this supports dual neoplastic etiology (see Figure 5).

TABLE 4. Diagnostic Features, p16, and p53 in dVIN and VAM

	Diagnostic features			Supportive features		
	Basal atypia	**p16**	**p53**	**% of epithelium showing maturation**	**Architecture**	**Dyskeratosis**
dVIN	Marked abnormalities in 2 or more features	Negative or Nonblock-positive	Basal overexpression or Wild-type or Aberrant negative	Keratinizing: >40–90% Nonkeratinizing: <40%	Variable Keratinizing subtypes • Atrophic • Subtle • Traditional • Acantholytic • Hypertrophic Nonkeratinizing types • Basaloid • Intermediate	Variable Keratinizing subtypes • Premature maturation • Intercellular breakdown • Intracellular vacuoles Nonkeratinizing types • Minimal
VAM	Subtle abnormalities • Vesicular nuclei • No abnormal mitoses • No suprabasilar nuclear atypia	Negative or Nonblock-positive	Basal overexpression or Wild-type	>80%, matures just above basal layer	Acanthosis Variable rete ridge shape	Premature maturation Thick PK and/or HK

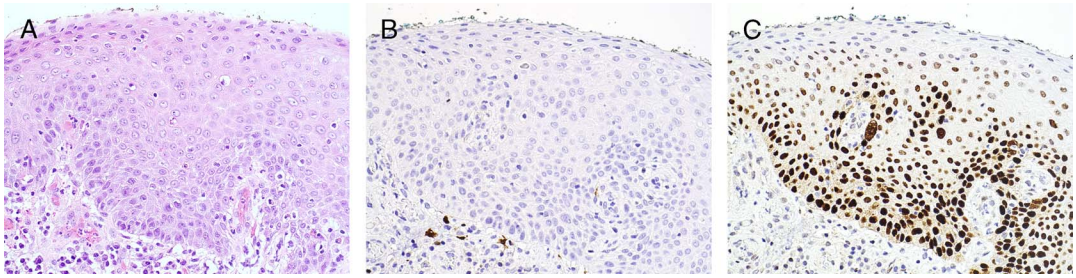

FIGURE 7. A, Intermediate type of nonkeratinizing dVIN—amphiphilic appearance with PK, uniform acanthosis, and atypical nuclei with cellular maturation occurring at the superficial 30% of epithelium, H&E ×200. B, p16 is negative, ×200. C, p53 is overexpressed at basal and suprabasal layers, ×200.

Summary of Histopathologic Diagnostic Criteria of VAM

The diagnostic features of VAM are (Table 4, Figures 1A–E)[20]:

1. Aberrant maturation seen as thick hyperkeratosis (HK) or PK and/or premature maturation
2. Acanthosis
3. Minimal basal nuclear atypia identified through qualitative assessment of the 4 features
   - vesicular nuclei with visible nucleoli
   - pleomorphism—absent to minimal
   - mitoses—occasional mitotic figures with normal spindle morphology
   - enlargement—subtle to moderate
4. p16 is negative or nonblock-positive
5. p53 is wild type or overexpressed with staining confined to the basal layer.

Morphologic Subsets of dVIN

Morphology does not reliably indicate etiology of vulvar neoplasia; 37%–43% of HPV-related cancers show keratinization, whereas 5%–12% of HPV-independent cancers show nonkeratinizing warty/basaloid morphology.[22,26,33] However, these 2 categories remain useful as a starting point for the morphologic subsets of dVIN. Keratinizing dVIN divides into types delineated by epithelial thickness and dyskeratosis. Nonkeratinizing dVIN divides into intermediate and basaloid forms based on percentage of epithelium showing cellular maturation (see Figures 2, 7, 8).[20]

Morphologic Patterns of Keratinizing dVIN—Definitions and Differential Diagnosis

Traditional Keratinizing dVIN. Abell and Gosling[108] described this common pattern of dVIN in 1961, refined by Yang and Hart in 2000.[12] There is HK and PK, acanthosis, and premature maturation (see Figures 1, 5). Rete ridges are elongated, clubbed, anastomosing, and/or branched.[9] Marked intercellular prickles produce a mosaic pattern of keratinocytes with circumferential edema because of loss of cohesion, rather than spongiosis. Suprabasilar findings include enlarged squamous cells with large, sometimes binucleate, vesicular nuclei and abundant eosinophilic cytoplasm, squamous whorls, keratin pearls, mitoses, and apoptotic bodies. The impression of atypia ranges from overt to subtle; usually, all 4 features are seen. Basal overexpressed p53 highlights atypia that appears subtle on H&E. Stroma shows variable infiltrate and often fibrosis or sclerosis; the latter seen in 40%–70%.[20,69]

Hypertrophic Keratinizing dVIN. The hypertrophic type represents 13% of keratinizing dVIN and shows marked acanthosis with dramatic abnormalities across epithelial layers[20,53] (see Figure 4). The surface shows thick HK, PK, and/or scale crust. There may be alternating columns of HK over hypergranulosis and PK over clusters of necrotic keratinocytes within hypogranulosis.[109] Rete ridges are deep and irregular with complex branching patterns called "reticular" or "cobblestone."[50,69] Basal layer degeneration at tips or tops of the rete ridges signals a background lichenoid dermatitis.[61] Stroma shows moderate to dense infiltrate and fibrosis and/or sclerosis, manifestations of the underlying dermatosis, and itch-scratch cycle.

Atrophic Keratinizing dVIN. Atrophic dVIN occurs in 13% and displays thinned epithelium with flat or reduced rete ridges.[20] There are 5 or fewer cell layers, so basal atypia replaces about half of the epithelium. A band of sclerotic collagen overlying scant to moderate lymphocytic infiltrate provokes confusion with LS (see Figure 6). Moderate to dense infiltrate and absent sclerosis produce an appearance resembling lichenoid dermatitis or erosive LP.

Acantholytic Keratinizing dVIN. The acantholytic type occurs in 8% and usually shows PK and acanthosis with complex rete ridges.[20] The prominent feature is acantholysis—an extreme form of cellular noncohesion seen as accumulation of intercellular vacuoles, focal disarray of separated cells, and areas replaced by fluid and cellular debris (see Figure 9).[69] This limits assessment of premature maturation and suprabasilar atypia.[50,69] Intracellular vacuoles are a dyskeratotic feature that enhances the acantholytic appearance.[20] Stroma shows variable infiltrate, often with fibrosis and/or sclerosis.

Subtle Keratinizing dVIN. Five percent show normal to mildly increased epithelial thickness, thin PK or stratum corneum, unremarkable rete ridge morphology, minimal dyskeratosis, and nearly normal maturation (see Figure 3).[20] In these challenging cases, atypia is the only feature that distinguishes dVIN from benign. This pattern may replace large areas of vulvar epithelium, as if clonal expansion travels rapidly along the basal layer. This may be accompanied by band-like lymphocytic infiltrate and stromal sclerosis/fibrosis, mimicking LS.

Differential Diagnosis of Keratinizing dVIN. Nonneoplastic diagnoses confused for traditional and hypertrophic keratinizing dVIN include LS, LP, LSC, and psoriasis. Assessment for atypia is more challenging when inflammation and excoriation raise the possibility of reactive change; features in keeping with this include dense infiltrate, marked exocytosis, and squamatization.[17,20,110] As epithelial thickness increases, diagnoses under consideration are lichenified LS, hypertrophic rather than classic LP, nodular prurigo or severe LSC, and lichenified psoriasis. Superinfection contributes to acanthosis; swabs, scrapings, and PAS facilitate

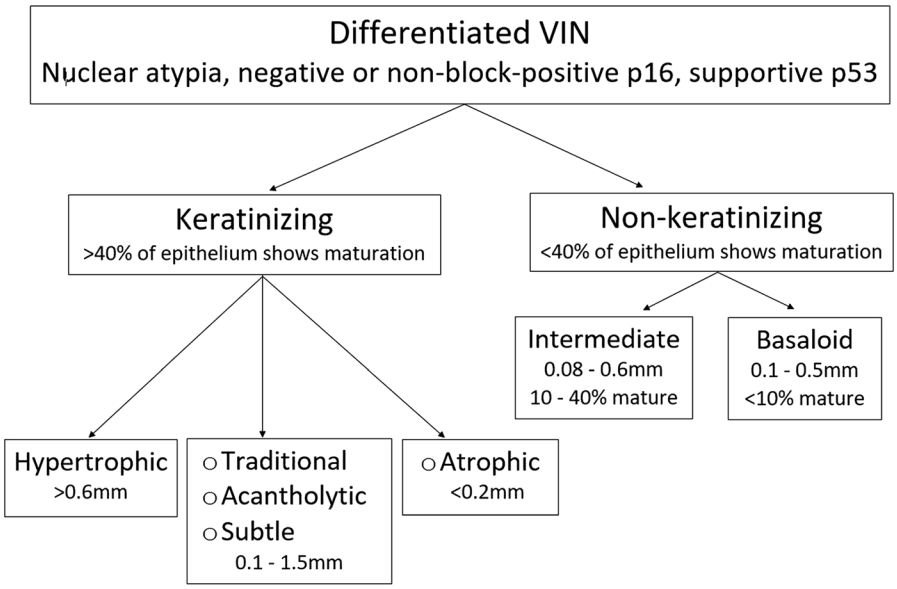

FIGURE 8. Algorithm for the morphologic subsets of dVIN.

identification of organisms.[75] Hypertrophic LP is a morphologic mimic for hypertrophic dVIN, the latter distinguished by atypical nuclei located away from the inflamed dermoepidermal junction.[61] Severe LSC and lichenified psoriasis show similar architecture to hypertrophic dVIN but have bland organized basal cells, small nuclei with open chromatin, normal maturation, and minimal vertically oriented papillary dermal fibrosis.[28]

Vulvar aberrant maturation, verrucous SCC, and keratinizing HSIL are the other mimics for dVIN. Basal nuclear changes in VAM reflect its position on the spectrum between dermatosis and dVIN. Verrucous SCC is a well-differentiated HPV-independent nonmetastasizing neoplasia with minimal atypia and spread through an expansile blunt interface.[21,111,112] This downward growth pattern is the salient difference between verrucous SCC versus VAM and dVIN. Cohorts of HPV-related SCC suggest that keratinizing HSIL represents 5% of precursors, but this rate is higher when comorbid with LS/LP.[13,26,35,62] When HSIL replaces LS-affected epithelium, 43% of cases retain dermal sclerosis, provoking confusion with dVIN and underscoring the need for p16 and p53.[62]

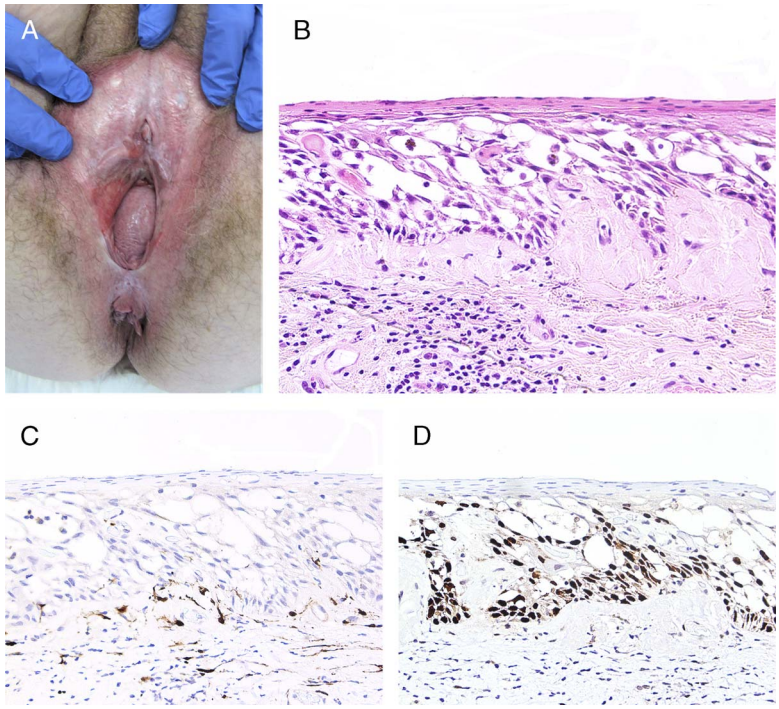

FIGURE 9. A, Squamous cell carcinoma and dVIN—red plaque at right clitoral frenulum on a background of uncontrolled LS. B, Acantholytic type of keratinizing dVIN—PK, normal thickness, marked intercellular prickles, intercellular and intracellular vacuoles with fluid-filled spaces, and sclerosis, H&E ×200. C, p16 is negative, ×200. D, p53 is overexpressed at basal and suprabasal layers, ×200.

The other morphologies of keratinizing dVIN have a limited list of imitators. Atrophic dVIN looks like LS if sclerosis is present and lichenoid dermatitis if sclerosis is absent.[8,14] Hailey-Hailey and Darier's disease shows acantholysis and dyskeratotic nuclear enlargement potentially confused with acantholytic keratinizing dVIN. Clinical correlation is helpful because Hailey-Hailey and Darier's affects other intertriginous areas and infrequently occur with LS. Subtle keratinizing dVIN is rare but should be entertained if clinical suspicion is high, and there is no inflammation or excoriation to explain the nuclear changes.

Nonkeratinizing dVIN—Definition and Differential Diagnosis

Intermediate dVIN. Intermediate dVIN reflects the biological continuum between keratinizing and basaloid morphologies and comprises less than 10% of cases.[20] It shows crowded basaloid cells replacing more than 60% of epithelium, transitioning to a narrow band of maturation underneath a thin keratin layer (see Figure 7). Epithelial thickness is normal to slightly increased and dyskeratosis is minimal. Rete ridge length is reduced to normal, with clubbed or branched morphology. Two thirds have moderate to dense infiltrate and abnormal collagen.[20]

Basaloid dVIN. Basaloid dVIN occurs adjacent to 8%–21% of HPV-independent SCC and sometimes is the only precursor lesion present.[20,53] It contains full-thickness undifferentiated keratinocytes with scanty cytoplasm and frequent mitotic figures[16,17] (see Figure 2). The surface usually shows PK with focal erosion. There is flat acanthosis or reduced clubbed/coalescent rete ridges and minimal intercellular breakdown. There is moderate to dense infiltrate in 93%, and collagen is sclerotic and/or fibrotic in 43%.[20]

Differential Diagnosis of Nonkeratinizing dVIN. The basaloid morphology of nonkeratinizing dVIN mimics the appearance of regenerative erosive LP and HSIL.[17] Erosive LP has thinned epithelium, sometimes with erosion and surface neutrophils. A band-like lymphocytic infiltrate underlies regenerative epithelial changes of maturational disarray, increased mitoses, enlarged nuclei, and nuclear-cytoplasmic ratio reversal. Block-positive p16 identifies HSIL, but basaloid dVIN and erosive LP may be difficult to distinguish. Mild acanthosis, PK, hair bearing site, and prior SCC support dVIN. Aberrant negative p53 confirms dVIN, but basal overexpression and wild-type status are nondiscriminatory. If clinical appearance is consistent with erosive LP, it is reasonable to provide potent topical steroids and rebiopsy if response to therapy is inadequate.

SUMMARY AND RECOMMENDATIONS FOR PRACTICE

Clinicopathologic assessment of dVIN is challenging, and there are serious consequences to erroneous diagnosis. Evaluation of women at risk for dVIN and VAM requires a collaborative approach by clinicians and pathologists experienced in vulvar disorders. Long-term LS surveillance and use of consensus recommendations may decrease vulvar SCC through detection and treatment of dVIN and VAM.[1]

- Aim for universal clinical photography of suspected dVIN and VAM.
- Obtain biopsies from morphologically distinct areas, as dVIN and VAM may be multifocal and have a different appearance at each site.
- Document presence of LS and/or LP and previous diagnoses of HSIL, VAM, dVIN, or SCC on pathology request forms.
- Pathologists may need to solicit additional information from clinicians if clinical notes are insufficient as to history and examination findings.
- Universal p16 and p53 in cases of suspected squamous neoplasia are advisable because dVIN and HSIL cannot be reliably distinguished by routine microscopy. If this is not possible, p16 and p53 are essential in:
  - biopsies obtained from treatment-resistant lesions within LS
  - suspected dVIN and VAM
  - presumed HSIL in women older than 45 years, with comorbid LS/LP, or nonresponse to LASER or imiquimod.
- Indicate type of dVIN and presence of LS/LP in pathology reports.
- Communication between clinician and pathologist or expert multidisciplinary review is recommended before embarking on cytotoxic, ablative, or extirpative procedures.

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