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Clinicopathologic Diagnosis of Differentiated Vulvar Intraepithelial Neoplasia and Vulvar Aberrant Maturation

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Objective: The aim of the study was to describe the demographic, clinical, and histopathologic features of differentiated vulvar intraepithelial neoplasia (dVIN) and vulvar aberrant maturation (VAM).

Methods: Specimens from 2010 to 2020 reported as dVIN or VAM were reviewed. Clinical data included age, rurality, symptoms, and evidence of lichen sclerosis (LS). Histopathologic data included epithelial thickness, keratinization, architectural and dyskeratotic features, stroma, p16, and p53. Differentiated vulvar intraepithelial neoplasia and VAM were distinguished by assessment of basal nuclear chromatin, enlargement, pleomorphism, and mitoses.

Results: One hundred twenty women with a median age of 71 years had 179 examples of dVIN and VAM. Squamous cell carcinoma was concurrent in 66% and associated with rurality. Ten percent were asymptomatic, and all but 3 had evidence of LS. Differentiated vulvar intraepithelial neoplasia showed a range of thickness, architecture, and dyskeratosis; its unifying feature was basal atypia. Differentiated vulvar intraepithelial neoplasia displayed hyperchromasia in 83% and easily observed mitoses in 70%. Nonkeratinizing morphology, subcategorized into basaloid and intermediate, occurred in 24% of women with dVIN. Traditional dVIN represented 62% of keratinizing cases; the remainder were atrophic (13%), hypertrophic (13%), acantholytic (8%), or subtle (5%). Vulvar aberrant maturation had abnormal stratum corneum, acanthosis, premature maturation, and enlarged vesicular nuclei. Null p53 helped distinguish dVIN from VAM and dermatoses.

Conclusions: The morphology of dVIN encompasses nonkeratinizing and keratinizing types, the latter subdivided into traditional, acantholytic, atrophic, hypertrophic, and subtle. Diagnosis relies on basal atypia with supportive p16 and p53. Atypia exists on a biologic spectrum with mild abnormalities of VAM and reactive change. Identification of dVIN and VAM requires collaboration between clinicians and pathologists experienced in vulvar disorders.

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

(*J Low Genit Tract Dis* 2020;24: 392–398)

Vulvar squamous cell carcinoma (SCC) comprises 2 distinct diseases with different etiologies, management approaches,

and prognoses.^{1,2} High-grade squamous intraepithelial lesion (HSIL) precedes human papillomavirus (HPV)-related SCC, occurs across the lower genital tract, has well-described clinicopathologic features, and responds to multiple treatments including imiquimod, LASER, and excision. Differentiated vulvar intraepithelial neoplasia (dVIN) is the immediate precursor of HPV-independent SCC and demonstrates a variable, sometimes subtle clinicopathologic appearance; excision is the preferred treatment.^{3–8} In contrast to HPV-related disease, HPV-independent SCC usually occurs on skin affected by lichen sclerosis (LS), is more likely to persist and recur, less radiosensitive, and has higher disease-related mortality.^{4,9–11} Human papillomavirus-independent neoplasia may be adjacent to or arise from acanthotic epithelium with abnormal maturation; multiple names have been applied to these lesions. The proposed term “vulvar aberrant maturation” (VAM) encapsulates their unifying feature, whereas their neoplastic potential remains uncertain.^{4,12,13}

Correct categorization of vulvar neoplasia into HPV-related and HPV-independent relies on histopathology in combination with immunohistochemistry (IHC) for p16 and p53. Although most dVIN are keratinizing and most HSIL are warty basaloid, use of morphology alone will misclassify at least 20%.^{7,14–16} Block-positive p16 identifies transforming HPV infection, so it usually excludes dVIN. p53 shows different staining patterns in the 2 types of VIN, with a suprabasilar pattern in HSIL and a basal overexpressed, null, or wild-type pattern in dVIN.^{16–19} Expanded use of IHC exposed a morphologic spectrum of dVIN with multiple forms that depart from the traditional description of elongated anastomosing rete ridges with premature maturation and variable basilar atypia.^{20,21}

This study aims to detail the morphologic spectrum of dVIN and VAM and describe their demographic, clinical, and histopathologic features.

METHODS

Vulvar biopsies and excisions from 2010 to 2020 reported as dVIN or described as acanthosis with altered maturation were identified in the NSW Health Pathology, Hunter New England database. The local research ethics and governance unit approved this retrospective histopathological case series (HREC 15/11/18/5.02). Cases were excluded if slide review confirmed an HPV-related process, defined as squamous atypia with a block-positive p16 and suprabasilar p53.¹⁶ Other exclusions were unavailable slides and insufficient tissue for IHC. Focality, location, and margin status of initial excision were extracted from histopathology reports.

On slide review, site was identified as squamous mucosa, mucocutaneous junction, hairless skin, hair-bearing skin, or unsure. Features recorded were epithelial thickness, epithelial maturation defined as percentage with keratinization, and premature maturation seen as enlarged suprabasilar cells with eosinophilic cytoplasm. Stratum corneum (SC) was classified as parakeratosis (PK), hyperkeratosis (HK), normal, or eroded. Rete ridge size was reduced, normal, or enlarged; shape was blunted, clubbed/bulbous, elongated/anastomosed, or spiky. Basal nuclei were assessed for 4 features: pleomorphism, enlargement, mitoses identified over several high-power fields amounting to 1 mm of basal layer length, and chromatin described as primarily hyperchromatic or vesicular.²² Stromal collagen was labeled as sclerotic, fibrotic, or normal.

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The authors have declared they have no conflicts of interest.

This study was approved by Hunter New England Research Ethics and Governance unit (HREC 15/11/18/5.02).

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.jlgt.com).

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DOI: 10.1097/LGT.0000000000000569

Subepithelial infiltrate was semiquantitatively assessed as sparse, moderate, or dense. Diagnosis of LS required evidence of basal layer damage, seen as vacuolar change, apoptotic bodies, and/or squamatization, accompanied by sclerosis. Erosive, classic, and hypertrophic lichen planus (LP) were identified according to the International Society for the Study of Vulvovaginal Disease consensus statement.²³

Decision regarding diagnosis of dVIN versus VAM was based on degree of nuclear change; this subjective assessment incorporated the combination of features and severity of abnormality. Cases identified as VAM had abnormal maturation but insufficient atypia to be classified as dVIN. Morphology of dVIN was categorized nonkeratinizing or keratinizing, with the latter subclassified according to epithelial thickness and prominent architectural or dyskeratotic characteristics.

p53 confirmed dVIN when it was null or when overexpression contrasted large pleomorphic nuclei against small, uniform nuclei in adjacent nonneoplastic epithelium. Persistence of overexpression into suprabasilar layers was quantified as a percentage of epithelial staining, from 10% being basal cells only to 100% being full thickness. p53 was noncontributory when it was wild type or basal overexpressed without contrast to adjacent benign epithelium. p16 was negative, nonblock positive reported as diffuse or focal staining, or block positive.

Demographic data included age, rurality, body mass index (BMI), diabetes mellitus (DM), immunosuppressive conditions, and current tobacco use. Clinical features recorded were symptoms, lesion appearance, diagnosis and treatment of LS, management of initial concurrent cancer, outcome, and follow-up duration. Analysis involved descriptive statistics, group comparisons with Fisher exact test, and comparison of means with Student *t* test.

RESULTS

There were 179 examples of dVIN and VAM from 120 women with a median age of 71 years (range = 37–93 years). Of 102 women with dVIN diagnosed during the study period, 76.5% (78) had keratinizing dVIN, 10% (10) had nonkeratinizing dVIN, and 14% (14) had both (see Figure 1, Supplemental Figure 1, <http://links.lww.com/LGT/A177>). There was no difference in age or concurrent SCC between keratinizing and nonkeratinizing types.

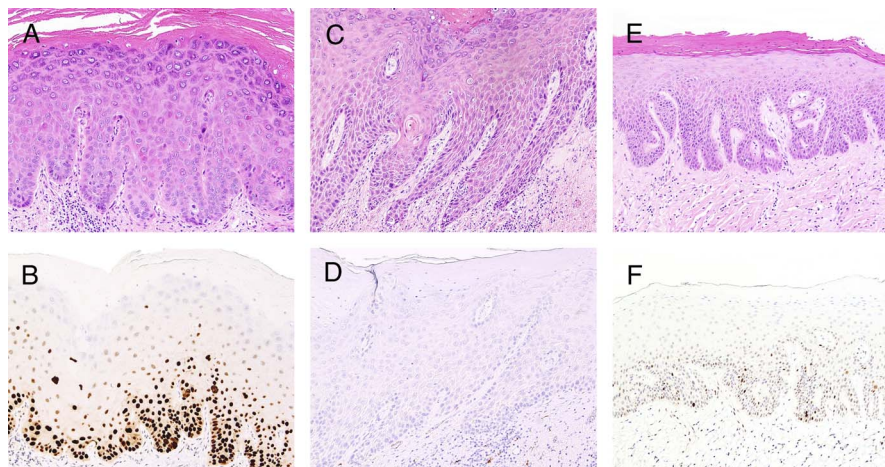


FIGURE 1. Traditional keratinizing dVIN shows moderate acanthosis, often with (A) bulbous or (C, E) elongated, branching rete ridges. Signs of aberrant maturation include (E) PK, a pale band in the upper prickle cell layer, and/or (A, C, E) enlarged suprabasilar cells with eosinophilic cytoplasm. Poor cellular cohesion manifests as (C, E) extracellular fluid with prominent desmosomes. Basal nuclei show pleomorphism, enlargement, abnormal chromatin, and increased mitoses. There are 3 patterns of p53 staining: (B) basal overexpression with dark enlarged nuclei, (D) null, and (F) wild type. Hematoxylin and eosin (H&E), p53 $\times 100$.

Clinical notes were available for 105 women; 87% (91) had dVIN alone or in combination with another diagnosis, and 13% (14) had VAM without dVIN. Previous or synchronous SCC occurred in 66% (69), 13% (14) had dVIN alone, 4% (4) had dVIN followed by SCC, 11% (12) had VAM alone, and 3% (3) had dVIN and VAM without SCC. In addition, 3 cases of VAM progressed to dVIN over 6, 15, and 36 months; 2 received potent steroids for LS and 1 was untreated. Diagnosis of dVIN or VAM without cancer was more likely in metropolitan than regional areas (29/69 [42%] vs 7/36 [19%]; $p = .03$).

Clinical Features

Women were asymptomatic in 9.5% (10/105) of cases, with detection occurring during LS surveillance or as an incidental finding. Immune dysfunction and DM were common (see Table 1); the former comprised thyroid disease in 5, chronic prednisone in 3, 2 with nongynecologic cancer treatment, and 1 each with Addison disease, systemic lupus erythematosus, rheumatoid arthritis, ankylosing spondylitis, polymyalgia rheumatica, and renal failure. Notation of BMI occurred in 79% (83/105), when documented BMI was 30 or greater in 51% (42/83). Three women with advanced SCC lacked a clinical or histopathologic diagnosis of LS; 2 are now deceased.

Clinicians used nonspecific terms like “tumor” or “lesion” to describe 31% (28/91) of dVIN cases. Among the remainder, dVIN appeared as a white plaque in 70% (44/63), a pink-red plaque in 29% (18/63), and a red patch or erosion in 11% (7/63). Simultaneous morphologies occurred in 10% (6/63). Clinicians provided descriptions of VAM in 93% (13/14), noting white nodules or plaques often with rough or verruciform texture.

During follow-up, clinicians prescribed topical steroids of varied potency and frequency in 65% (68/105). Half of women with VAM (7/14) did not require excision because the lesion resolved with super-potent topical steroids. Two women with dVIN avoided excision—one refused and the other used daily clobetasol propionate 0.05% ointment with subsequent biopsies showing VAM.

Among women with concurrent SCC, 15% (10/69) recurred within a year and 33% (23/69) had another cancer after a 1-year disease-free interval. Among those without cancer, 3% (1/36) progressed within a year and 14% (5/36) later developed SCC. Rate of subsequent SCC was lower in women with identification and treatment of preinvasive lesions ($p = .003$).

TABLE 1. Characteristics of Women With dVIN and VAM

	Total (n = 105)	dVIN (n = 91)	VAM ^a (n = 14)
Age at diagnosis, median (range), y ^b	71 (37–93)	71 (37–93)	71.5 (46–89)
SCC at initial diagnosis, n (%)	68 (65)	67 (74)	1 (7)
Remoteness area, n (%) ^b			
City	82/120 (68)	65/100 (65)	17/20 (85)
Inner regional	26 (22)	24 (25)	2 (14)
Outer regional	12 (10)	11 (12)	1 (7)
Primary symptom, n (%)			
Itch	73 (69.5)	64 (70)	9 (64)
Pain	19 (18)	16 (18)	3 (21)
None	10 (9.5)	8 (9)	2 (14)
Tobacco use, n (%)	6 (6)	3 (3)	3 (21)
BMI > 30, n (%)	42 (40)	39 (43)	3 (21)
Immune dysfunction, n (%)			
DM	25 (24)	22 (24)	3 (21)
Other	16 (15)	15 (16)	1 (7)
Focality of initial disease, n (%)			
Unifocal	66 (67)	58 (64)	11 (79)
2 lesions	17 (16)	17 (19)	0
Multifocal	19 (18)	16 (18)	3 (21)
Clinical description, n (%) ^c			
White plaque	57 (54)	44 (48)	13 (93)
Red-pink plaque	17 (15.5)	18 (20)	0
Red patch or erosion	7 (7)	7 (8)	0
Lesion or tumor	29 (28)	28 (31)	1 (7)
Anatomic locations, n (%) ^c			
Periclitoral	58 (55)	52 (57)	6 (43)
Labia minora	52 (49.5)	44 (48)	8 (57)
Labia majora	15 (14)	14 (15)	1 (7)
Introitus and posterior fourchette	18 (17)	16 (18)	2 (14)
Perineum and perianal	23 (22)	19 (21)	4 (29)
Clinical diagnosis of LS, n (%)	76 (72)	62 (68)	14 (100)
Histologic diagnosis of LS, n (%)	89 (85)	76 (84)	13 (93)
Lichenoid biopsy	6 (6)	6 (7)	0
Topical corticosteroids prescribed, n (%)	68 (65)	55 (60)	13 (93)
Specialist providing of follow-up, n (%)			
Gynecologic oncologist	69 (66)	67 (74)	2 (14)
Vulvar clinic	24 (23)	15 (16)	9 (60.4)
General gynecologist	11 (10)	9 (10)	2 (14)
Follow-up, median (range), mo	36 (2–240)	42 (2–240)	22 (3–120)
Outcome, n (%)			
Death or palliation due to vulvar cancer	25 (24)	25 (27)	0 (0)
Death from other causes	9 (9)	8 (9)	1 (7)
Treatments for recurrent disease	13 (12)	13 (14)	0
Surveillance without recurrence	55 (56)	43 (48)	12 (78.5)
Lost to follow-up	3 (3)	2 (2)	1 (7)

^aCases of VAM without comorbid dVIN.^bIncludes 15 cases with unavailable clinical notes (9 dVIN, 6 VAM).^cEach case may have more than 1 occurrence.

Histopathologic Features

Nonkeratinizing dVIN was more likely to arise in squamous mucosa or mucocutaneous junction than keratinizing dVIN (8/28 [29%] vs 5/125 [4%]; $p = .003$). Vulvar aberrant maturation arose in nonkeratinized epithelium in 8% (2/25). Hairless skin was the site of 38% (47/125) of keratinizing dVIN, 32% (9/28) of nonkeratinizing dVIN, and 28% (7/25) of VAM. Hair-bearing skin accounted for 51% (64/125) of keratinizing dVIN, 32% (9/28) of nonkeratinizing dVIN, and 32% (8/25) of VAM. Site could not be determined in the remainder.

Hyperchromatic basal nuclei in dVIN took 2 forms: ovoid enlargement producing a basaloid appearance, or elongated spindle shape as if compressed by extracellular fluid (see Table 2; Figures 1, 2). The latter occurred in 30% (37/125) of keratinizing dVIN and 11% (3/28) of nonkeratinizing dVIN. Vesicular nuclei were round, pale, and contained large or multiple visible nucleoli. All VAM and 17% of keratinizing dVIN had predominantly vesicular nuclei. Compared with dVIN, the nuclei in VAM were smaller, more uniform, and showed fewer and less bizarre nucleoli (see Figure 3).

Epithelial thickness of dVIN ranged over 2 mm, presenting a spectrum from atrophic to hypertrophic (Supplemental Figure 2, <http://links.lww.com/LGT/A178>). Mean thickness was lower in nonkeratinizing than keratinizing dVIN, whereas mean thickness of VAM was higher than dVIN (0.28 vs 0.46; $p = .01$; 0.97 vs 0.43; $p < .0001$). Abnormal SC occurred in 89.5% (137/153) of dVIN and all VAM. When SC was normal, morphology was compact in 69% (11/16) and basket weave in the remainder. The appearance of thick PK was variable; 58% (22/38) of dVIN and 57% (8/14) of VAM had an underlying pale layer and 6% (3/52) showed scale crust. Of 44 cases with HK, 41% (18) alternated with PK (see Figures 2, 3). Erosion was restricted to nonkeratinizing dVIN.

Rete ridge shape was abnormal in all VAM and more than 90% of dVIN. Compared with keratinizing dVIN, rete ridges in nonkeratinizing dVIN were more likely to be clubbed/bulbous (10/28 [35%] vs 19/125 [15%]; $p = .02$) and less likely to be anastomosed/elongated (6/28 [21%] vs 51/125 [41%]; $p = .02$). When reduced in size, rete ridges were blunted in 82% (50/61), clubbed/bulbous in 15% (9/61), and spiky in 3% (2/61; Supplemental Figure 3, <http://links.lww.com/LGT/A179>). Twelve percent (3/25) of VAM showed flat acanthosis between 0.35 and 0.7 mm. Spiky rete ridges of any size were uncommon in dVIN (7%, 11/153) and not seen in VAM.

Evidence of altered cohesion, seen as prominent intercellular prickles and/or intercellular vacuoles, occurred in 53% (94/178; see Figures 1C, E, 2A). These coexisted in 15% (19/125) of keratinizing dVIN and 1 case each of VAM and nonkeratinizing dVIN. Intracellular vacuoles, a manifestation of dyskeratosis, accompanied poor cohesion in 18% (27/153) of dVIN, producing appearances ranging from grouped bubbles to diffuse acantholysis.

Keratinizing dVIN and VAM were more likely to show abnormal collagen than nonkeratinizing dVIN (98/125 [78%] and 22/25 [88%] vs 16/28 [57%]; $p = .03$; $p = .02$), and more likely to have scant infiltrate (67/150 [45%] vs 6/28 [21%]; $p = .02$). There was no relationship between collagen abnormality and lymphocytic infiltrate.

p16 staining was negative in 69% (105/153) of dVIN. Nonblock-positive cases (29%, 45/153) showed focal (83%) or diffuse (17%) uptake across nuclei and cytoplasm. Nonblock-positive p16 occurred in 60% (15/25) of VAM, all focal staining. Two percent (3/105) of keratinizing dVIN had block-positive p16 in combination with null or basal overexpressed p53 (see Figure 4). Basal overexpression was the most common p53 pattern in dVIN (see Figure 1B, Supplemental Figure 1F, <http://links.lww.com/LGT/A177>). Mean persistence of p53

TABLE 2. Histopathologic Features of dVIN and VAM

	Total (n = 178)	Keratinizing dVIN (n = 125)	Nonkeratinizing dVIN (n = 28)	VAM (n = 25)
Hyperchromatic chromatin, n (%)	132 (74)	104 (83)	28 (100)	0
Vesicular chromatin, n (%)	46 (26)	21 (17)	0	25 (100)
Nuclear pleomorphism, n (%)	160 (90)	123 (98)	27 (98)	10 (40)
Nuclear enlargement, n (%)	173 (97)	122 (98)	28 (100)	23 (92)
Basal mitoses, n (%)				
<1	58 (33)	37 (30)	5 (18)	16 (64)
1–2	86 (48)	63 (50)	14 (50)	9 (36)
≥3	34 (19)	25 (20)	9 (32)	0
Epithelial thickness, mean (range, SD), mm	0.50 (0.02–2.5, 0.42)	0.46 (0.02–2.2, 0.37)	0.28 (0.08–0.6, 0.02)	0.97 (0.35–2.5, 0.53)
Epithelial maturation, n (%)				
≥71%	89 (50)	64 (51)	0	25 (100)
41%–70%	61 (34)	61 (49)	0	0
11%–40%	14 (8)	0	14 (50)	0
≤10% = no maturation	14 (9)	0	14 (50)	0
Premature maturation, n (%)	122 (68.5)	97 (78)	0	25 (100)
Intracellular vacuoles, n (%)	25 (14)	20 (16)	3 (11)	2 (8)
Abnormal cohesion, n (%)	94 (53)	79 (63)	7 (25)	8 (32)
Spongiosis	91 (51)	77 (62)	7 (25)	7 (28)
Intercellular vacuoles	24 (13)	21 (17)	1 (4)	2 (8)
Abnormal SC, n (%)	162 (91)	110 (88)	27 (96)	25 (100)
Thin PK	61 (38)	38 (34.5)	20 (74)	3 (12)
Thick PK ± pale layer	52 (32)	36 (29)	2 (7)	14 (56)
HK ± PK	44 (27)	36 (29)	0 (0)	8 (32)
Erosion	5 (3)	0	5 (18)	0
Abnormal rete ridge size, n (%)	165 (93)	113 (90)	27 (96)	25 (100)
Absent/reduced	64 (36)	43 (34)	18 (64)	3 (12)
Enlarged	101 (57)	70 (56)	9 (32)	22 (88)
Abnormal stromal collagen, n (%)	136 (76)	98 (78)	16 (57)	22 (88)
Sclerosis	65 (36)	51 (41)	6 (21)	8 (32)
Fibrosis	35 (20)	22 (18)	8 (29)	5 (20)
Both fibrosis and sclerosis	36 (20)	25 (20)	2 (7)	9 (36)
Lymphocytic infiltrate, n (%)				
Nil or scant	73 (41)	53 (42)	6 (21)	14 (56)
Moderate	64 (36)	48 (38)	10 (36)	6 (24)
Dense	41 (23)	24 (19)	12 (43)	5 (20)
p16, n (%)				
Negative	115 (65)	82 (66)	23 (82)	10 (49)
Nonblock-positive	60 (34)	40 (32)	5 (18)	15 (60)
Block-positive	3 (2)	3 (2)	0	0
p53, n (%)				
Basal overexpressed	114 (64)	82 (66)	18 (64)	14 (56)
Wild-type	39 (22)	26 (21)	2 (7)	11 (44)
Null	25 (14)	17 (14)	8 (29)	0

overexpression was 60% in nonkeratinizing dVIN (range = 30%–100%) and 30% (10%–100%) in keratinizing dVIN. Null p53 was nonsignificantly more common in nonkeratinizing dVIN than keratinizing dVIN (see Figure 1D, Supplemental Figures 1C, 4C, <http://links.lww.com/LGT/A177>, <http://links.lww.com/LGT/A180>). Wild-type p53 was more likely in VAM than dVIN (11/25 [44%] vs 28/153 [18%]; $p = .008$; see Figure 1F).

Morphology of dVIN

Half of nonkeratinizing dVIN was basaloid, displaying full-thickness atypia reminiscent of HSIL. The other half showed

an intermediate appearance with a thin band of maturation at upper epithelium (Supplemental Figure 1, <http://links.lww.com/LGT/A177>). Mean epithelial thickness was 0.23 mm (range = 0.1–0.5) in basaloid versus 0.32 mm (0.08–0.6 mm) in intermediate morphologies. In 18 cases with p53 overexpression, 22% (4) had staining restricted to the lower half of epithelium, 61% (11) had persistence into upper epithelium, and 17% (3) stained full thickness.

Yang and Hart's traditional description represented the most common morphology of keratinizing dVIN—acanthotic with irregular rete ridges, premature maturation above the basal layer, often associated with diminished cellular cohesion (see Figure 1). This

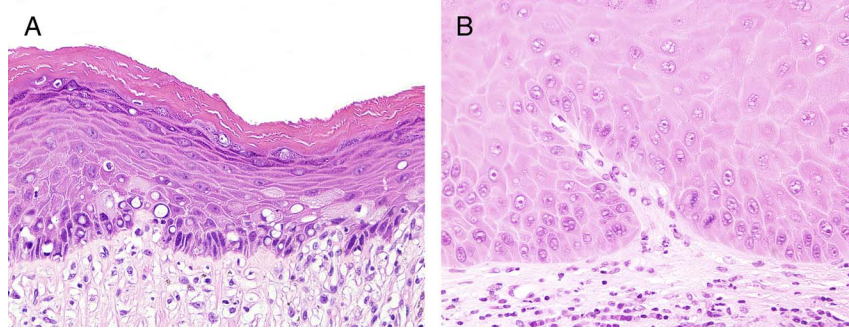


FIGURE 2. There are 2 abnormal chromatin patterns in dVIN: (A) hyperchromatic and (B) vesicular. Nuclei in both types are enlarged, pleomorphic, and may contain intranuclear vacuoles. Hyperchromatic nuclei may be spindle shaped or ovoid with a basaloid appearance and nucleoli are not seen. Vesicular nuclei are pale with prominent, multiple, and/or bizarre eosinophilic nucleoli. H&E $\times 200$.

occurred in 62% (77/125) with a mean thickness of 0.37 mm (range = 0.1–0.9 mm). Acantholysis was the salient feature in an additional 8% (10/125) with a mean thickness of 0.67 mm (range = 0.14–1.5 mm).

Extremes of epithelial thickness demarcated 2 additional categories. Atrophic and hypertrophic dVIN each occurred in 13% (16/125; Supplemental Figure 2, <http://links.lww.com/LGT/A178>). Atrophic dVIN had a mean thickness of 0.08 mm (0.02–0.2 mm) with PK or compact SC, reduced rete ridges, and basal atypia replacing up to 50% of epithelium. Stroma was variable, but when sclerotic or fibrotic, these cases resembled atrophic LS. Mean thickness of hypertrophic dVIN was 1.1 mm (0.6–2.2 mm) with thick SC and complex rete ridges. Borderline epithelial thickness with morphologic similarity to hypertrophic LP was classified as hypertrophic dVIN.

In 5% (6/125), dVIN showed subtle nuclear features and minimal architectural abnormality with a mean thickness of 0.3 mm (0.18–0.35 mm; Supplemental Figure 4, <http://links.lww.com/LGT/A180>). Sclerosis or fibrosis prompted confusion with LS, whereas spiky rete ridges and band-like infiltrate resembled classic LP (Supplemental Figure 3, <http://links.lww.com/LGT/A179>). p53 distinguished between neoplasia and dermatosis and demarcated the margin when overexpression highlighted the size and persistence of abnormal nuclei or when aberrant negative juxtaposed with another pattern (Supplemental Figure 4, <http://links.lww.com/LGT/A180>).

DISCUSSION

Emerging evidence suggests that there are 5 steps to prevention of HPV-independent vulvar SCC: (1) prompt diagnosis of LS with implementation of effective chronic treatment, (2) long-term surveillance to identify and biopsy lesions worrisome for dVIN

and VAM, (3) reliable histopathologic diagnosis, (4) excision of dVIN and treatment-resistant VAM, and (5) optimization of medical therapy to prevent future neoplasia.^{1,4,7,24–27} This study documents diverse and sometimes subtle features of dVIN that make diagnosis difficult in absence of concurrent or previous SCC, highlighting the need for collaboration between clinicians and pathologists to achieve steps 2 through 4. It proposes a new term, VAM, to describe white plaques occurring in LS and associated with increased risk of dVIN and SCC. Finally, this work assists in overturning long-held concepts that impede cancer prevention.

Although characterized as a disease of the elderly, 20% (21/105) of women had initial diagnosis of dVIN or VAM younger than 60 years. As in LS, 10% of women with VAM and dVIN were asymptomatic, arguing against dependence on symptoms to guide treatment. One third of dVIN cases manifested as pink-red patches or plaques, whereas VAM presented as white plaques with surface irregularity. Differentiated vulvar intraepithelial neoplasia and VAM were more often multifocal and located at perineum/perianus than previously reported, countering the belief that these features suggest HSIL.² This study aligns with previous findings that HPV-independent SCC arises from LS, and LS treatment is often neglected in the setting of vulvar neoplasia.^{1,4,16,28}

Adding to previous descriptions of basaloid, LS-, and LP-like dVIN, this work verifies that reliance on abnormal epithelial architecture and dyskeratosis may result in missing one third of dVIN diagnoses. Morphology of dVIN ranges from atrophic to hypertrophic with variable rete ridge size and shape. Keratinizing forms resemble LS, classic or hypertrophic LP, or lichen simplex chronicus (LSC), whereas nonkeratinizing types mimic erosive LP and HPV-related neoplasia. Prominent acantholysis supports dVIN but occurs infrequently. Premature maturation is nonspecific, seen in dVIN, VAM, and sometimes lichenified LS or severe

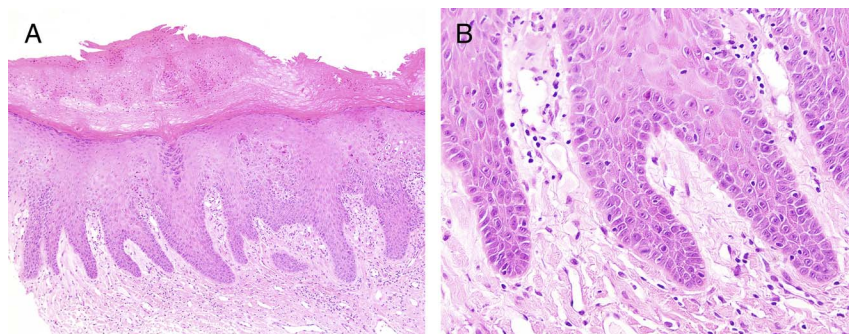


FIGURE 3. Vulvar aberrant maturation shows (A) acanthosis with abnormal maturation, usually seen as thick PK and/or HK and premature maturation, H&E $\times 40$. B, Basal nuclei in VAM are slightly enlarged and vesicular, without significant pleomorphism or mitotic activity. H&E $\times 200$.

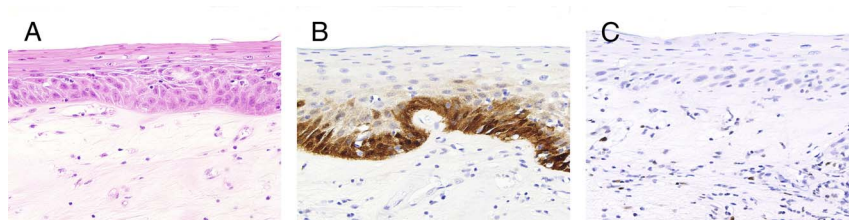


FIGURE 4. Dual-etiology dVIN is rare, arises out of lichen sclerosus, and shows (A) keratinizing morphology, (B) a block-positive p16 consistent with integration of high-risk HPV, and (C) aberrant negative p53. The predominant carcinogenic pathway and clinical behavior of these lesions remains unclear. H&E, p16, p53 $\times 200$.

LSC. Although architectural and dyskeratotic abnormalities raise suspicion, diagnosis of dVIN rests on appearance of basal nuclei in combination with IHC.

Assessment of 4 atypical nuclear features provides structure to a subjective process associated with high intraobserver and interobserver variation.^{22,29,30} Each case of dVIN and VAM represents a point on a biologic spectrum, so there is no formula that reliably identifies or excludes dVIN. Hyperchromasia and multiple mitotic figures support dVIN and make VAM unlikely. However, dVIN may have vesicular chromatin and 80% have 2 or less mitoses per 1 mm of basal layer. The combination and severity of features drive the decision for dVIN versus VAM versus dermatosis. Although recognition of dVIN is important, it is also vital to avoid overdiagnosis and unnecessary partial vulvectomy.

In contrast to variable architecture in dVIN, VAM is uniformly well keratinized and acanthotic with mean epithelial thickness double that of keratinizing dVIN. Disordered maturation is the key feature of VAM, manifesting as premature maturation and abnormal, thick SC, accompanied by insufficient basilar atypia to qualify as dVIN. Vulvar aberrant maturation replaces the obsolete term “squamous cell hyperplasia” (SCH), introduced in 1989 by the International Society for the Study of Vulvovaginal Disease as “epidermal hyperplasia not due to any known cause” that is somewhere on the path to malignancy.^{30–32} Never adequately described or imaged, it remained unclear how to distinguish SCH from lichenified LS or LSC.³¹ As was the original intent of SCH, VAM encompasses a more worrisome appearance than LSC or acanthotic LS, while falling short of dVIN. A decision for VAM versus dVIN has important clinical implications. Women with VAM receive potent topical and sometimes intralesional steroids with short-interval follow-up, whereas those with dVIN undergo wide local excision or vulvectomy dictated by lesion size. Clinicians usually repeat biopsies when VAM persists despite maximal medical therapy and comparison of consecutive specimens aids in detection of neoplastic progression. Further characterization of the clinical trajectory, histopathology, and molecular biology of VAM is necessary to better understand the behavior of these lesions. Until then, careful clinicopathologic surveillance allows for surgical intervention prior to invasion.

p16 and p53 are fundamental to diagnosis of dVIN and exclusion of HSIL. p53 basal overexpression facilitates recognition of dVIN by highlighting enlarged pleomorphic nuclei and persistence of atypia into upper epithelium. It also delineates the junction when adjacent to examples of wild-type staining in dermatosis or VAM. Null p53 likewise demarcates margins, but also confirms dVIN in subtle cases, and provides evidence for rare dual-etiology p16-positive lesions.^{15,16} Wild-type p53 does not distinguish between dVIN, VAM, and dermatoses but contrasts with the suprabasilar-dominant pattern of HSIL.^{16–18}

Recognition of dVIN and VAM relies on partnership between clinicians and pathologists. Clinicians undertaking LS management must inspect for and biopsy treatment-resistant white nodules/plaques and red-pink patches/plaques. Pathology request forms should describe the (1) underlying dermatosis, (2) lesion

location and appearance, and (3) concern for neoplasia. This focuses pathologists' attention to basal nuclear atypia and architectural and dyskeratotic features. p16 and p53 are essential for recalcitrant lesions in LS and presumed LP, suspected HSIL in women with LS or LP, nonresponse to imiquimod/LASER, or older than 45 years. The diagnostic challenge of dVIN and VAM presents an opportunity for robust collaboration between vulvar specialists, pathologists, and gynecologist oncologists in an effort to eradicate HPV-independent SCC through optimal long-term LS treatment and surveillance, identification and excision of precursors, and regular specialist-led review of women with previous VAM, dVIN, or SCC.

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