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Squamous precursor lesions of the vulva: current classification and diagnostic challenges

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Summary

Growing evidence has established two major types of vulvar intraepithelial neoplasia (VIN), which correspond to two distinct oncogenic pathways to vulvar squamous cell carcinoma (VSCC). While the incidence of VSCC has remained relatively stable over the last three decades, the incidence of VIN has increased. VIN of usual type (uVIN) is human papillomavirus (HPV)-driven, affects younger women and is a multicentric disease. In contrast, VIN of differentiated type (dVIN) occurs in post-menopausal women and develops independent of HPV infection. dVIN often arises in a background of lichen sclerosus and chronic inflammatory dermatoses. Although isolated dVIN is significantly less common than uVIN, dVIN bears a greater risk for malignant transformation to VSCC and progresses over a shorter time interval. On histological examination, uVIN displays conspicuous architectural and cytological abnormalities, while the morphological features that characterise dVIN are much more subtle and raise a wide differential diagnosis. On the molecular level, dVIN is characterised by a higher number of somatic mutations, particularly in *TP53*. Here we review the classification, epidemiology, clinical features, histomorphology, ancillary markers and molecular genetics of both types of VIN, and discuss the morphological challenges faced by pathologists in interpreting these lesions.

Keywords

Diagnosis; pathology; precursor; squamous carcinoma *in situ*; squamous intraepithelial lesion; squamous dysplasia; vulva; vulvar intraepithelial neoplasia

INTRODUCTION

Squamous cell carcinomas account for 83% of all malignancies in the vulva.[1] Although the incidence of vulvar squamous cell carcinoma (VSCC) has remained relatively stable over the last three decades, the incidence of vulvar intraepithelial neoplasia (VIN), the putative precursor lesion to VSCC, has increased over time.[2,3] There are two distinct aetiopathogenic pathways leading to VSCC, associated with either: (1) VIN of usual type (uVIN) which is

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human papillomavirus (HPV)-driven, or (2) VIN of differentiated type (dVIN) which develops independently of HPV. The major features characterising these oncogenic pathways are summarised in Fig. 1.

EVOLUTION OF NOMENCLATURE AND CURRENT CLASSIFICATION

Squamous precursor lesions of the vulva were first recognised a century ago, and since the initial description, numerous terms and classification schemes have been proposed (Table 1).[4–6]

Bowen's disease was first described by the dermatologist J. T. Bowen in 1912. He noted extreme hyperplasia of the epidermis, absence of the stratum granulosum, and numerous mitoses as well as clumping and crowding of the nuclei. At the time, Bowen denied the features of 'distinct carcinomatous formation' due to the absence of dermal invasion, but did speculate on the premalignant nature of the lesions.[7] In 1922, Hudelo *et al.* were the first to recognise the histological features of Bowen's disease in the vulva and termed the disease 'erythroplasiform dyskeratosis of the vulvar mucosa'.[5,8] Twenty years later, Knight reported six cases of Bowen's disease of the vulva, of which one was associated with VSCC. In a review of the literature, he identified an additional 26 cases.[9]

In 1958, Woodruff and Hildebrant recognised the variability in terminology used to describe squamous precursor lesions of the vulva and proposed a unifying term 'carcinoma *in situ*' (CIS).[10] Several groups then noticed that a proportion of lesions that were morphologically identical to CIS demonstrated spontaneous regression, particularly in young, pregnant patients with multicentric disease.[5,11,12] In order to distinguish these lesions from those which progressed to invasive carcinoma, Wade, Kopf and Ackerman in 1979 coined the term 'Bowenoid papulosis'.[13]

In 1961, Abell and Gosling reviewed 150 VSCC and reported two types of squamous precursor lesions: (1) intraepithelial carcinoma of Bowen's type, and (2) intraepithelial carcinoma of simplex type.[14] In 1977, the term 'differentiated' was used to highlight the highly differentiated histological features of the simplex type.[6]

The 1976 International Society for the Study of Vulvovaginal Disease (ISSVD) endorsed the term 'squamous cell carcinoma *in situ*' and 'hyperplastic dystrophy'. The latter was further qualified by mild, moderate or severe atypia. The initial appeal of this change in terminology was that it would replace the confusing array of terms in use at the time, including Bowen disease, erythroplasia of Queyrat, carcinoma simplex, squamous cell hyperplasia with atypia, atypical squamous dystrophy and leukoplakic vulvitis.[15]

The term 'intraepithelial neoplasia' was first proposed by Richart in 1967 and subsequently by Crum in 1982, initially for lesions of the cervix and later, the vulva.[16,17] In 1986, the ISSVD adopted the term VIN which was graded as VIN I, II and III. By definition, the dysplasia was confined to the lower one-third of the epithelial thickness in VIN I, to the lower two-thirds in VIN II, and involved two-thirds of the epithelial thickness or more in VIN III. The additional category, 'VIN III, differentiated type' was also introduced.[18]

Over the ensuing years, evidence accrued showing that VIN 1, 2 and 3 did not exist on a biological continuum, as the classification implied. VIN 1 consisted almost entirely of condyloma acuminatum and was associated with low-risk HPV types 6 and 11. In contrast, VIN 2 and 3 were associated with high-risk HPV types and carried a risk of progression to VSCC.[5,19] Recognising the aetiological and prognostic differences, the ISSVD in 2004 removed VIN 1 due to its negligible risk for cancer progression. It proposed a 2-tier classification scheme: (1) uVIN (including lesions previously classified as VIN 2 and VIN 3), and (2) dVIN. uVIN was subdivided into warty, basaloid, and mixed types. The 2003 World Health Organization (WHO) continued to use the VIN 1 designation, due to the small proportion of VIN 1 cases that were associated with high-risk HPV.[4]

In 2005, Medeiros *et al.* proposed a classification scheme similar to the Bethesda system for cervical precursor lesions. They proposed a low-grade vulvar intraepithelial lesion (LG-VIL) category which encompassed several variants of condyloma, and a high-grade VIL category (HG-VIL) which included uVIN and dVIN.[20]

After almost 100 years of evolution, the terminology has finally reached some consensus amongst multiple committees, all supporting the terminology ‘squamous intraepithelial lesion’. The College of American Pathologists (CAP) and American Society for Colposcopy and Cervical Pathology (ASCCP) jointly published the Lower Anogenital Squamous Terminology (LAST) guidelines in 2012, unifying the terminology applied to all HPV lesions involving the cervix, vulva, vagina, anus, perineum and penis, under two headings: (1) low-grade squamous intraepithelial lesion (LSIL), and (2) high-grade squamous intraepithelial lesion (HSIL). LSIL is equivalent to uVIN 1 and HSIL encompasses uVIN 2 and uVIN 3. The intraepithelial neoplasia (−IN) grade could be included in parentheses, if so desired. The two-tier system was advocated as being more reproducible and biologically meaningful than prior schemes. The 2014 WHO and 2015 ISSVD classifications also accept the SIL terminology, but in addition include dVIN as separate category.[21,22]

EPIDEMIOLOGY

The incidence of both uVIN and dVIN has increased over the last 30 years, while the incidence of VSCC has remained relatively unaltered.[2,23] An analysis of 13,176 patients in the SEER (Surveillance Epidemiology and End Results) database between 1973 and 2000 found that VIN increased by 411% while VSCC only increased by 20%.[2] A report of 2935 patients from the Netherlands nationwide database showed that the incidence of uVIN almost doubled from 1.2/100,000 in 1992 to 2.1/100,000 patients in 2005. The incidence of dVIN increased nine-fold, from 0.013/100,000 to 0.121/100,000 patients. Concurrently, the incidence of VSCC remained relatively unchanged.[24] Trends of increasing VIN have also been reported in New Zealand, Norway, Austria and Greece.[23] These trends may reflect a combination of increased detection of VIN and more effective treatment before the development of VSCC.

The recent introduction of universal HPV vaccination in multiple countries is expected to result in a significant reduction of HPV-associated neoplasms in the near future.[25] Factors including the anticipated drop in the incidence of uVIN, growing age of the population and

increased awareness of dVIN will likely lead to increased relative and absolute rates of dVIN. This emphasises the importance of accurate recognition of this entity.

AETIOLOGY

Prior to Harald zur Hausen's proposal of HPV as the agent responsible for cervical carcinoma,[26] multiple aetiological agents were suggested including herpes simplex virus (HSV), arsenic and even granulomas.[9,10,26] Subsequently, HPV was found to be responsible for the vast majority of anogenital squamous carcinomas, and was also detected in VIN.[26,27]

HPV infection is strongly associated with uVIN, with the majority of studies reporting HPV positivity rates of >80%.[19,24,28–34] In a study of 587 VIN from 39 countries, HPV was identified in 509 (86.7%) cases. HPV16 was the most common type (77.2%), followed by HPV33 (10.6%), and HPV18 (2.6%). Over 90% of LSIL were attributed to low-risk HPV types 6 and 11.[35] Maniar *et al.* investigated 11 patients with concurrent LSIL and HSIL. All patients had a history of immunosuppression. All HSIL harboured high-risk HPV types, while all LSIL were positive for low-risk HPV 6 or 11.[36] Hence, the presence of concurrent LSIL and HSIL was due to concurrent infections with low-risk and high-risk HPV. The findings reiterate that LSIL and HSIL are biologically distinct entities.

Although the majority of uVIN are associated with high-risk HPV, the rate of HPV positivity in VSCC is considerably lower. In a study of 1709 VSCC, only 28.6% of cases harboured HPV,[37] and the reported rates in the literature vary from 15% to 79%.[34] This discrepancy led investigators to explore alternative HPV independent pathways to VSCC, leading to the identification of dVIN as a separate oncogenic pathway to VSCC. A cumulative 134 cases of dVIN have been tested for HPV in the literature, of which only two (1.5%) were positive.[28,29,38–41]

CLINICAL FEATURES

VIN, usual type

uVIN tends to occur in young women, in the third to fifth decades of life.[28,37,42,43] Multiple studies have also shown a trend towards younger ages at first diagnosis.[28,43–45] Risk factors include smoking, number of sexual partners and immunosuppression.[38,41,43,46–48] HPV infection is strongly associated with uVIN.[19,24,28–34] Concurrent infection with HSV has been reported in up to 30%.[30,48]

Most patients complain of pruritus or dysuria. Twenty percent of patients are asymptomatic and present with an abnormal self-exam.[9,49] uVIN presents as white or erythematous macules or papules, which can coalesce to create verrucous plaques. Approximately 10% of lesions are pigmented.[41] Over half of patients have multifocal lesions in the vulva,[28,41,49–52] and 18–52% have squamous dysplasia at other anogenital sites, particularly the cervix.[28,32,43,49,51–53] Thus the finding of VIN on clinical examination should prompt thorough examination of the anogenital region and performance of cervicovaginal pap smears.

VIN, differentiated type

dVIN typically occurs in post-menopausal women in the sixth to eighth decades of life, but can occur in younger patients.[6,37] In a survey of 21 VSCC occurring in women <40 years of age, three patients had associated dVIN.[44] dVIN is often associated with adjacent lichen sclerosus (LS) and/or chronic inflammatory dermatoses.[6] In comparison to uVIN, dVIN tends to be unicentric at presentation and produces less bulky lesions. Clinically, the lesions may appear as focal grey-white discolourations with a rough surface, vaguely defined thick white plaques, or elevated nodules.[6]

CLINICAL BEHAVIOUR

VIN, usual type

The rate of progression of uVIN to VSCC has been reported to be less than 5%.[43,45,48,49,52] In an early study, seven of eight (87.5%) patients with untreated VIN 3 progressed to invasive VSCC within 8 years while only four of 105 (3.8%) treated patients developed VSCC after 7–18 years.[43] Another large study reported that eight of 88 (9%) of untreated VIN patients and 108 of 3322 (3.3%) of treated VIN patients developed VSCC.[52] Risk factors for malignant progression included advanced age, radiotherapy and immunocompromised status.[45,50]

VIN recurs in approximately 13–36% of patients.[42,43,54,55] It is unclear whether the lesions represent true recurrences or acquisition of new lesions, given the ‘field effect’ of HPV infection. The relationship between margin status and recurrence risk is yet to be established unequivocally.[45,49,52]

A small proportion of patients exhibit spontaneous regression of disease. A systematic review of 3322 patients with VIN 3 found that spontaneous regression occurred in 1.2%. All patients were <35 years of age and 40% were related to pregnancy. Most lesions regressed 10 months after diagnosis.[52]

VIN, differentiated type

Differentiated VIN comprises 2–29% of all VIN,[3,19,28,56] but studies characterising *in situ* lesions adjacent to VSCC found that dVIN was present in approximately 40%. These findings implied that dVIN was more likely to progress to VSCC than uVIN.[57] This was confirmed by a study of 1826 uVIN and 67 dVIN which found that dVIN had a higher risk of progression to VSCC (32.8% versus 5.7%) and over a shortened timeframe (22.8 months versus 41.4 months) than uVIN.[3] dVIN has also been shown to be more often associated with a history of prior, synchronous or subsequent VSCC (85.7%, versus 25.7% for uVIN).[56]

TREATMENT

Aggressive full or deep vulvectomies were performed for VIN until the mid 1960s. Based on the fact that not all VIN progressed to VSCC and on the recognition of the psychological and sexual morbidity of vulvectomies, less aggressive therapies became available by the late

1970s.[58] Treatment options include local excision, topical imiquimod, cidofovir or 5-fluorouracil, photodynamic therapy and laser ablation.[59]

HISTOLOGY

VIN, usual type

Due to its conspicuous architectural and cytological abnormalities, uVIN is often appreciated on low power examination. Knight's description in 1943 highlights the major features of uVIN, which have stood the test of time: (1) hyperkeratosis and parakeratosis; (2) acanthosis with clubshaped rete ridges; (3) disorientation of the individual cells commencing above the basal cell layer with variable extension to the surface; (4) nuclear clumping with mitotic figures; and (5) an intact basement membrane.[9] The architectural disarray has been referred to as a 'wind-blown' pattern. High nuclear-to-cytoplasmic ratios, hyperchromasia, pleomorphism, mitoses and apoptotic bodies are also common (Fig. 2). In one-third of cases, uVIN extends into the follicular epithelium or sebaceous glands, but seldom involves the acrosyringium.[6,60,61] Rare cases are associated with dermal amyloid deposition.[6]

uVIN has been subdivided into warty and basaloid types, although many cases exhibit mixed morphologies. The warty (condylomatous) type has a spiked or papillary surface with deep and wide rete ridges. Koilocytes, dyskeratotic cells and multinucleated cells are conspicuous. The basaloid (or undifferentiated) type of uVIN is flat and shows basaloid cells typically replacing the full thickness epithelium. Some studies suggest that basaloid uVIN has a worse prognosis than the warty type, but this has been variable in the literature.[4,6]

VIN, differentiated type

The recognition of dVIN is a challenge, even for experienced gynaecological pathologists. On low power examination, there is acanthosis, occasional parakeratosis, and irregular elongation and anastomoses of the rete ridges (Fig. 3). The architectural disarray seen in uVIN is not seen in dVIN. On high power, nuclear atypia is often confined to the basal and parabasal layers. The nuclei are enlarged, uniform in size, contain coarse chromatin or open vesicular nuclei, prominent nucleoli and scattered mitoses.[6,41] One of the most helpful features relates to the phenomenon of 'premature differentiation or keratinisation'. The cells have ample eosinophilic cytoplasm due to the accumulation of intracellular keratin, and the eosinophilic cells can be juxtaposed to the epidermal-dermal junction, which imparts a hypereosinophilic appearance to the lesion. Dyskeratosis, extracellular keratin and abortive squamous pearls may be seen within the lower layers of the epidermis.[62] Prominent intercellular bridges are seen in the absence of inflammation, a feature which is thought to be due to loss of cellular cohesion rather than spongiosis.[6] Extension into the skin appendages, in contrast to uVIN, is rare.[41]

dVIN often lacks full thickness atypia, with normal maturation in the superficial layers and retention of keratohyaline granules. Chronic inflammation can also complicate the diagnosis, as the subtle atypia seen in dVIN becomes very difficult to distinguish from reactive atypia. The optimal biopsy should include the interface between the lesion and

normal skin because dVIN often has an abrupt edge.[63] Approximately 83% of cases have adjacent squamous cell hyperplasia (SCH) or LS.[41]

Vulvar squamous cell carcinoma

Kurman *et al.* made the observation that VSCC arising from uVIN displayed warty or basaloid morphology compared to VSCC arising from dVIN which was more likely to be keratinising VSCC.[54,64,65] Subsequent studies have revealed that this distinction is not always clear-cut. Approximately 9–21% of cases have shown a discrepancy between histology and HPV or p16 detection.[37,66,67] Santos *et al.* found that 37.5% of HPV-positive tumours were keratinising VSCC and 9.2% of HPV-negative carcinomas had basaloid or warty features.[40] Therefore, the presence of keratinisation, basaloid or warty features alone do not necessarily indicate the HPV status of VSCC.

IMMUNOHISTOCHEMISTRY

The major immunochemical patterns seen in VIN are depicted in Fig. 4.

p16

Immunohistochemical positivity for p16 correlates extremely well with high-risk HPV status (>90%) and is commonly used as a surrogate marker for high-risk HPV infection.[39,40,66,68] The staining pattern should be diffuse, strong and continuous (nuclear and cytoplasmic), referred to as a ‘block-like’ pattern. Staining should be present in the basal layer with extension upwards to involve at least one-third of the epithelial thickness.[58]

Almost 100% of HSIL are strongly p16 positive, LSIL is less intense and patchy, and only 0–17% of dVIN show p16 staining, which is generally weak.[39,40,68–71] LS and SCH tend to be negative for p16.[40,68,71]

p53

Yang and Hart reported p53 positivity in 10 of 12 (83%) cases of dVIN with positive staining in the basal layer and suprabasilar extension (ranging from lower one-third to full thickness), with staining in >90% of basal cells. The adjacent normal epidermis showed patchy positivity in <10% of basal cells with no suprabasilar extension.[41] Subsequent studies have reiterated these findings, reporting positive p53 in 66–100% of dVIN.[39,40,69,71,72] Occasional cases of dVIN display strong suprabasilar staining and minimal basal staining (unpublished observations).

However, as a diagnostic adjunct to help distinguish dVIN from SCH and reactive changes, p53 has its limitations. Increased p53 staining can be seen in 5–61% of LS and up to 40% of SCH, and is thought to be due to increased oxidative stress.[71–75]

There are two patterns of aberrant immunostaining due to p53 mutations: strong and diffuse staining due to missense mutations, and completely negative staining (null-pattern) due to nonsense mutations. In a survey by Singh *et al.*, six of 22 (27%) dVIN had null-pattern staining.[63] The null-pattern presents another diagnostic challenge, because normal epithelium can show weak focal p53 making the distinction between the two very difficult.

Ki-67/MIB-1

Ki-67 can be another helpful marker to distinguish dVIN from SCH and normal epithelium. A Ki-67-negative basal cell layer is a distinct feature of normal epithelium; uVIN and dVIN show positive staining for Ki-67 in the basal and suprabasilar layers. In dVIN, Ki-67 staining is positive in the basal layer and a thin parabasal layer[38] which is in contrast to the basal expression seen in LS, which can be a helpful distinguishing feature.[76] Other studies have reiterated these findings.[39,69,77] The staining for Ki-67 in uVIN is much more conspicuous, and usually stains the full thickness of the epithelium.[38,39,77]

Other markers

Increased immunohistochemical expression of ProEx C, telomerase, β-catenin and osteopontin as well as abnormal loss of E-cadherin have been reported in uVIN in a limited number of studies, but their diagnostic utility does not exceed that already offered by p16.[78–81]

Single studies have suggested SOX2, phosphorylated-S6 and cyclin-D1 are helpful in diagnosing dVIN, but further studies with larger numbers are needed to confirm the utility of these markers.[74,77,82–84]

MOLECULAR PATHOGENESIS

The pathogenesis of HPV-induced malignancy has been well described. The HPV protein E6 degrades the tumour suppressor p53, resulting in deregulation of cell cycle arrest. E7 inactivates the tumour suppressor RB and releases E2F transcription factors, causing cellular hyperproliferation. The physiological function of RB is to inhibit the transcription of cyclin-dependent kinase inhibitors p16 and p14. Therefore HPV-associated neoplasms typically show increased p16 expression and minimal to no expression of p53.[34]

The clonality of uVIN has been demonstrated by X-chromosome inactivation and loss of heterozygosity.[85–88]

The pathogenesis of HPV-independent VSCC, on the other hand, is not well understood. When dVIN was first described, many pathologists remained skeptical of the entity. They debated whether dVIN was a true precursor to HPV-negative VSCC or merely a reactive squamous change adjacent to a growing tumour. Others likened dVIN to well-differentiated squamous lesions seen in the oral cavity.[84] Pinto *et al.* studied 11 cases of dVIN with six associated VSCC and sequenced exons 2–11 of *TP53*. Six of 10 cases harboured at least one *TP53* mutation. Two cases had identical *TP53* mutations in the VSCC and adjacent dVIN, confirming their clonal relationship. In addition, disparate foci of dVIN showed different *TP53* mutations, highlighting the presence of multiple neoplastic clones.[89] Some studies have demonstrated clonality and allelic imbalances in LS and SCH, while others have not.[85,87,88,90]

A review by Trietsch *et al.* stated that *TP53* mutations were found in two of 66 (3%) HPV-positive VIN and 10 of 47 (21%) HPV-negative VIN.[91] It is clear that not all HPV-independent VSCC follow the *TP53* pathway, and other pathways of oncogenesis remain

elusive. HPV-negative VSCC have mutations in *CDKN2A* (14.8%), *HRAS* (11.2%), *PIK3CA* (7.9%), *PPP2R1A* (3.3%) and *EGFR*, but these mutations have not been investigated or confirmed in VIN.[67,91] Additional molecular alterations in HPV-negative VSCC include gains in chromosome 3q26 and hyper-methylation of *RASSF2A*, *MGMT* and *TSP1*.[91,92]

DIAGNOSTIC CHALLENGES AND DIFFERENTIAL DIAGNOSIS

There are several diagnostic and differential diagnostic considerations that come into play in the pathological evaluation of vulvar lesions in general, and VIN in particular (Fig. 5). Some of these issues are summarised below.

Assessment of coexistent invasion

Thorough sampling of VIN lesions is important because 3.2–18.8% of biopsies can have unsuspected stromal invasion.[50,52] Early invasion usually presents as single cells or nests of eosinophilic keratinocytes with irregular or angulated contours, invading from the basilar epidermis or from the elongated rete ridges.[34] A desmoplastic stromal reaction is a helpful feature, if present. Tangential sectioning of the rete ridges can mimic invasion; in this situation, the nests are evenly spaced, have rounded or bulbous contours and are not associated with stromal desmoplasia.

The depth of invasion is measured from the epithelialstromal junction of the adjacent most superficial dermal papilla to the deepest point of the tumour. The International Federation of Gynecology and Obstetrics (FIGO) defines a stage IA vulvar carcinoma as having a diameter ≤2 cm and stromal invasion ≤ 1 mm. A stage IA tumour has <1% risk of lymph node metastases, compared to tumours invading 1.1–5.0 mm having lymph node metastasis in up to 20%.[93] Lymphadenectomy is recommended for any tumour beyond stage IA in most institutions and hence accurate assessment of invasion is critically important for guiding patient care. Caution should be exercised when assessing invasion in hair-bearing skin, as one-third of cases can involve the skin appendages as deep as 2.7 mm.[5] However, the interobserver variability amongst pathologists for assessing invasion is suboptimal. Abdel-Mesih *et al.* reports a kappa of 0.24 for diagnosing invasion and 0.51 for measuring depth of invasion.[94] van den Einden *et al.* suggested an alternative method of measuring invasion, from the basement membrane of the adjacent tumour-free rete ridge to the deepest point of invasion. This allowed for 19% of patients with stage IB disease to be downstaged to stage IA. All these patients had fewer recurrences and higher disease-specific survival than the remaining stage IB tumours.[95]

Lichen sclerosus

LS is frequently seen in association with dVIN. The classic appearance of LS is a thinned epidermis with loss of the rete ridges, basal vacuolar change and a wide band of homogenised collagen within the dermis. A band-like lymphocytic infiltrate and variable oedema may also be present, especially in the early phase of development.[96] Long term studies have shown that LS has a very low risk (1–3%) of progression to VSCC, and is not considered a premalignant lesion by most authors.[4,97,98]

The finding of basal nuclear atypia in otherwise ordinary LS has been referred to as atypical LS. Atypical LS can show increased p53 staining, and some authors suspect this may represent a very early form of dVIN.[57,97] LS with hyperplasia, dyskeratosis and parakeratosis (usually as columns above the dermal papillae), has also been referred to as hypertrophic LS. There is minimal basal cytological atypia, no crowding and minimal to no mitoses. While some authors have found an increased progression of hypertrophic LS to VSCC, others have not.[99,100] Further studies with long-term follow-up are needed to clarify the natural history of LS, atypical LS and hypertrophic LS.

Squamous cell hyperplasia

SCH is considered a diagnosis of exclusion, where the cause of hyperplasia is not attributable to a more specific dermatological condition. SCH lacks atypia and the 1976 ISSVD termed this lesion 'hyperplastic dystrophy without atypia'[6]. SCH has an organised proliferation of mildly enlarged but non-atypical keratinocytes and absent or minimal mitoses restricted to the basal layer. Unlike dVIN, SCH does not exhibit features of premature keratinisation, expanded rete ridges nor parakeratosis.[6]

Pseudoepitheliomatous hyperplasia

Pseudoepitheliomatous hyperplasia is a well-documented phenomenon in the skin outside of the vulva. It is a benign proliferative reaction, usually incited by adjacent ulceration, infections and neoplasms. It is characterised by acanthosis, papillomatosis, dyskeratotic cells, and may show some nuclear pleomorphism. Atypical mitotic figures and increased p53 expression are not seen.[96] Patients with HIV have been found to have striking pseudoepitheliomatous hyperplasia due to HSV infection in the vulva, mimicking squamous cell carcinoma.[101–103]

Inflammatory, infectious and other dermatological disorders

The vulva can be affected by a range of dermatological disorders including seborrheic keratosis, psoriasis, lichen planus, eczematous/spongiotic dermatitis, Zoon's vulvitis, hidradenitis suppurativa, Behçet's disease, radiation dermatitis and infections.[104] Lichen simplex chronicus is the most common chronic inflammatory disorder affecting the vulva. It manifests as acanthosis, hyperkeratosis and inflammation. The cells have open chromatin and lack interface atypia.[6]

dVIN with basaloid pattern

Ordi *et al*. described four cases of dVIN with a basaloid pattern, mimicking uVIN. All four cases had conspicuous architectural disorganisation and homogeneous populations of basaloid undifferentiated keratinocytes. They were negative for p16 and HPV, and positive for p53.[105]

Extramammary Paget's disease

Extramammary Paget's disease should always be considered in the differential diagnosis of VIN. Classically, extramammary Paget's disease is positive for CK7, CAM5.2, CEA, PAS, mucin stains, GCDFP-15 and HER2, whereas VIN is positive for CK5/6 and p63. Sah and

McCluggage report two cases of Paget's disease where the cells were confluent and sheet-like, rather than exhibiting the usual nested pattern. Interestingly, both cases were strongly positive for p16. They were also positive for CK7 and CEA. The authors report this lesion as potential mimic of uVIN.[106]

Pagetoid VIN

An estimated 5% of vulvar HSIL has a nested pattern, also known as pagetoid squamous cell carcinoma *in situ* or pagetoid Bowen's disease.[107,108] A challenge in distinguishing it from Paget's disease is that pagetoid VIN is positive for cytokeratin 7. However, it is also positive for cytokeratin 5/6, p63, and negative for CEA, mucin and GCDFP-15, which are helpful in arriving at the correct diagnosis.[107–110]

VIN with mucinous differentiation

Two cases of VIN with mucinous differentiation have been reported. The mucinous cells were positive for CK7, CAM5.2, CEA, EMA, p16 and tested positive for HPV. The authors suggest the mucinous cells were metaplastic and due to aberrant differentiation.[111]

Melanoma

Melanoma should always be in the differential diagnosis of VIN. Neoplastic melanocytes may colonise the epidermis in the form of single cells, nests or more confluent groups, potentially mimicking VIN. Unlike VIN, melanoma is positive for S100, SOX10, HMB45 and MelanA. SOX2 is responsible for self-renewal in neural crest melanocytes and has also been used to confirm a diagnosis of melanoma.[112] A recent study showed that SOX2 was positive in five of 16 (31%) uVIN and eight of 18 (44%) dVIN. Thus, caution should be taken with the use of SOX2 in the differential diagnosis with melanoma.[83]

Verrucous carcinoma

Verrucous carcinoma (VC) is well-known in the oral cavity and is less common in the lower anogenital tract. VC is a non-HPV-related and well-differentiated form of VSCC, displaying acanthosis, parakeratosis, orthokeratosis, organised keratinocytes, minimal cellular atypia and most characteristically bulbous rete ridges termed 'baggy trousers' by Scurry and Wilkinson.[4,113,114] They may be associated with local recurrences but rarely exhibit distant metastases or fatal outcomes.[113,115]

Vulvar acanthosis with altered differentiation (VAAD)

This entity was first described by Nascimento and Crum in a study of nine VC in 2004. Seven cases had a distinct lesion adjacent to VC, termed VAAD. The triad of features that characterised VAAD included: (1) acanthosis with variable verruciform architecture, (2) loss of the granular layer with superficial epithelial pallor, and (3) multilayered plaque-like parakeratosis. All cases of VAAD were HPV negative. VAAD was proposed as the precursor lesion to VC. It was thought to be distinct from dVIN due to its presence of plaque-like parakeratosis, lack of atypia and lack of premature keratinisation as seen in dVIN.[114] Only two other reports of VAAD have been published in the literature since 2004, and both question whether VAAD is truly a distinct entity or whether it represents a morphological

variant of hypertrophic LS or dVIN.[116,117] Further studies are needed to answer these questions.

CONCLUSIONS

Vulvar squamous precursor lesions, uVIN and dVIN, are distinct entities. The histological features of uVIN are more readily recognised than dVIN, which is a particularly challenging diagnosis. Further ancillary markers with greater specificity (likely based on studies that better characterise the genetic and epigenetic alterations in VSCC carcinogenesis) are needed to help pathologists more accurately diagnose these entities.

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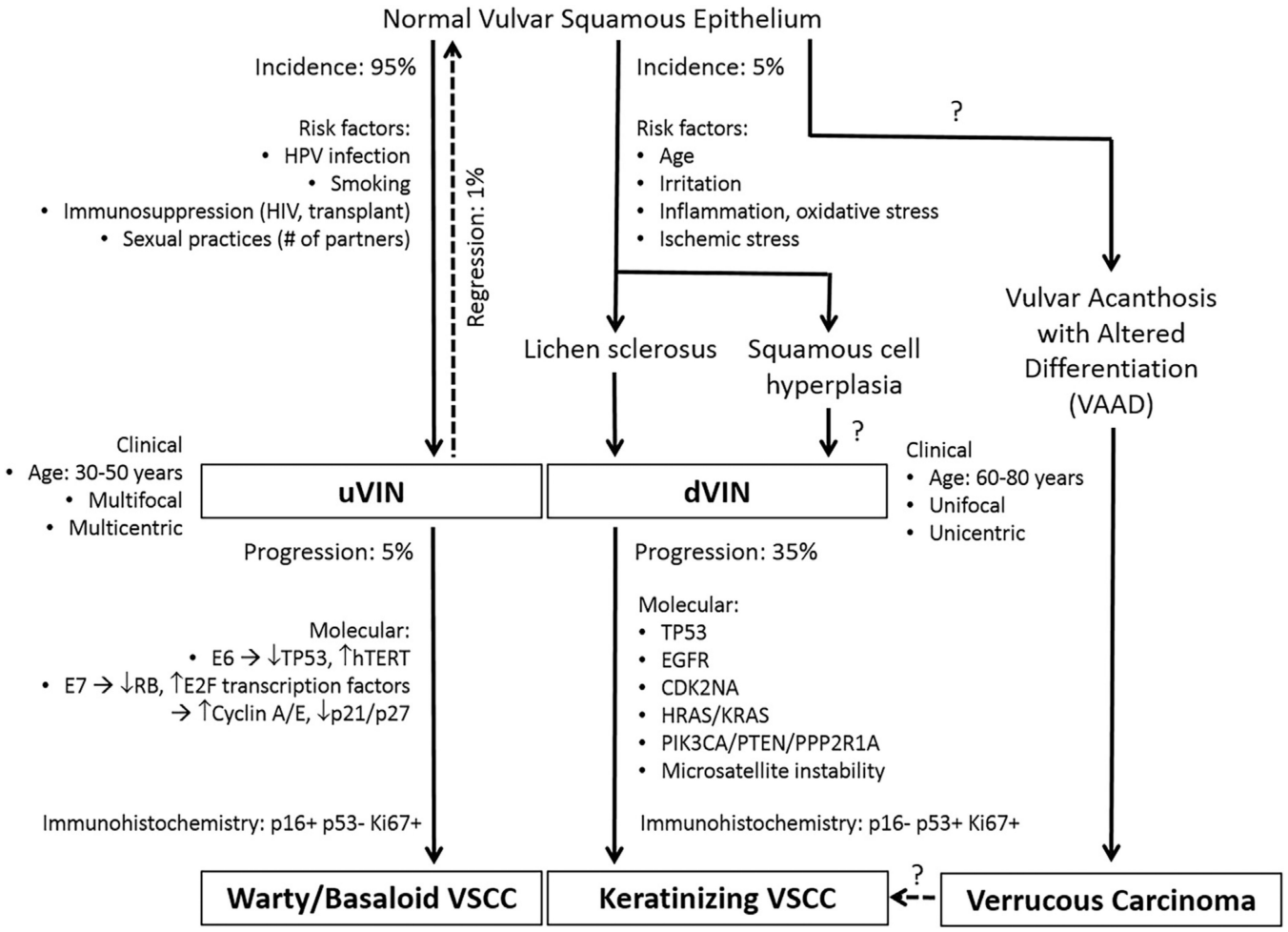

Fig. 1.

Pathways of oncogenesis in vulvar squamous cell carcinoma. Modified from Nascimento *et al.*[114]

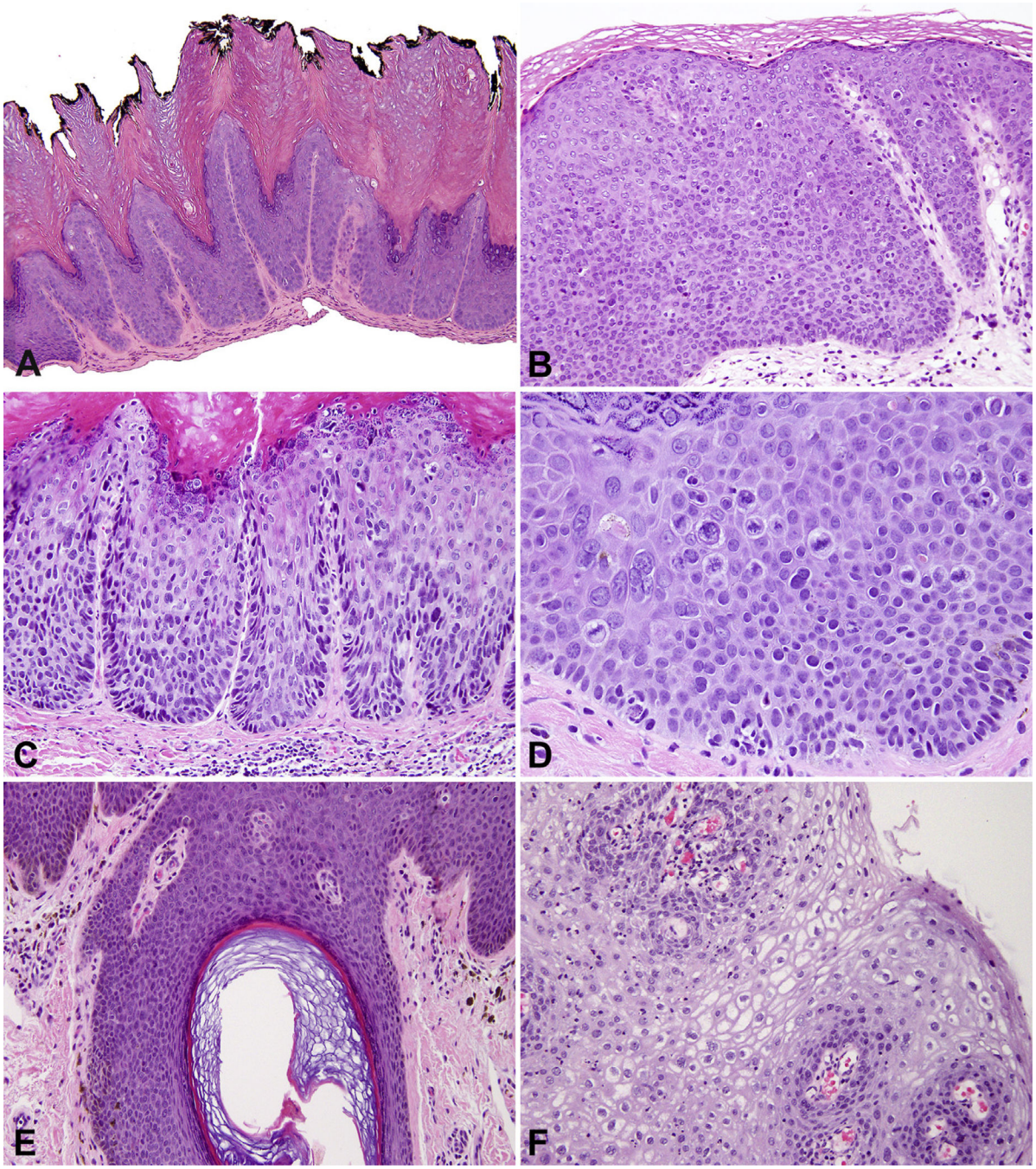

Fig. 2.

Vulvar intraepithelial neoplasia, usual type (uVIN). (A) Warty type; (B) basaloid type; (C) expansion of the rete ridges; (D) loss of nuclear organisation, hyperchromasia and mid-level mitoses; (E) VIN extending into hair follicle; (F) condyloma acuminatum or low-grade intraepithelial lesion with abundant koilocytes.

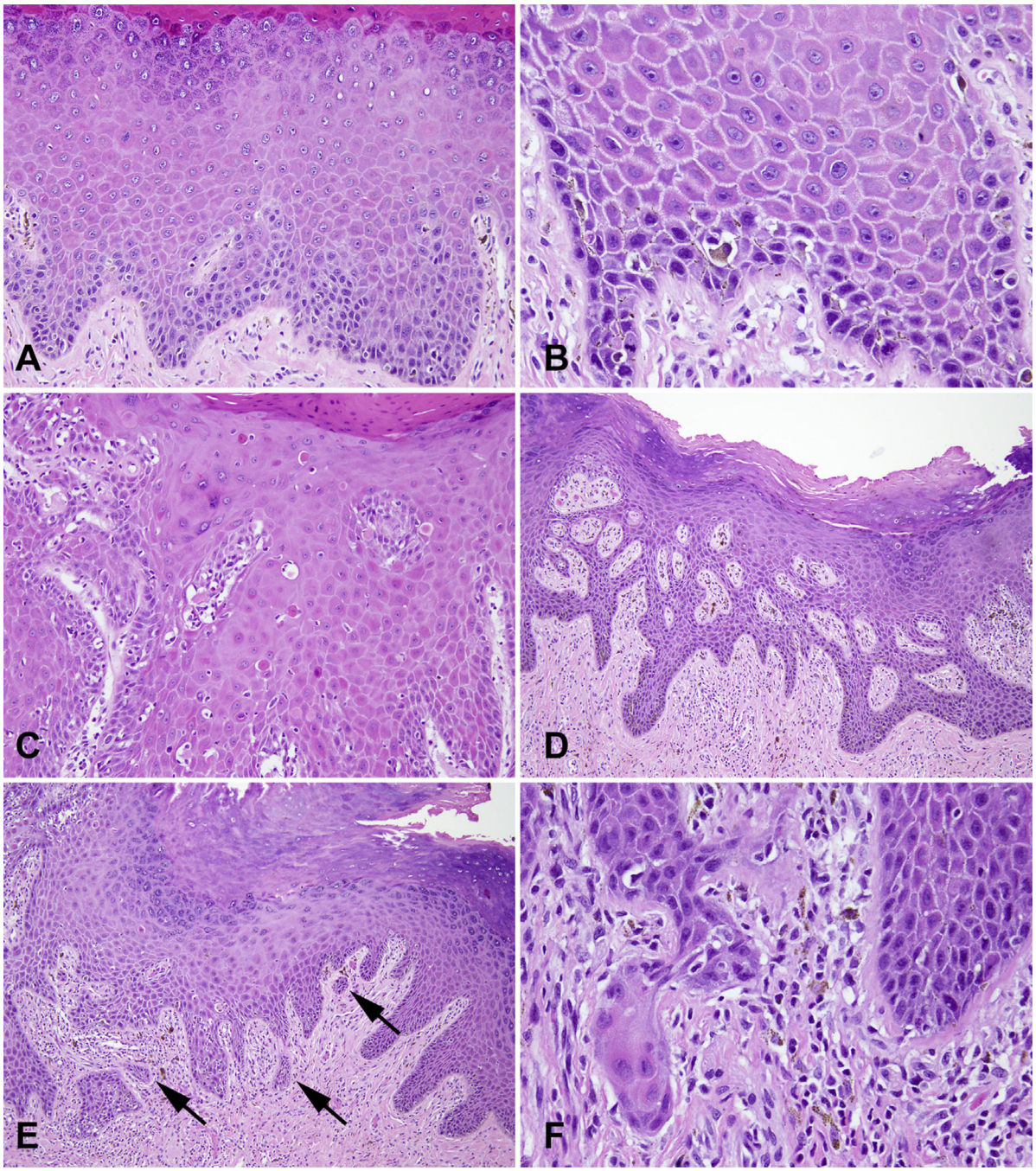

Fig. 3.

Vulvar intraepithelial neoplasia, differentiated type (dVIN). (A) Partial thickness dysplasia with retention of keratohyaline granules; (B) basal atypia, nuclei with prominent nucleoli and intercellular bridges; (C) hypereosinophilia and premature keratinisation; (D) irregular branching and anastomoses of rete ridges; (E) pseudoinvasion, regular spacing of nests with rounded contours (arrows); (F) paradoxical maturation suggestive of early invasion.

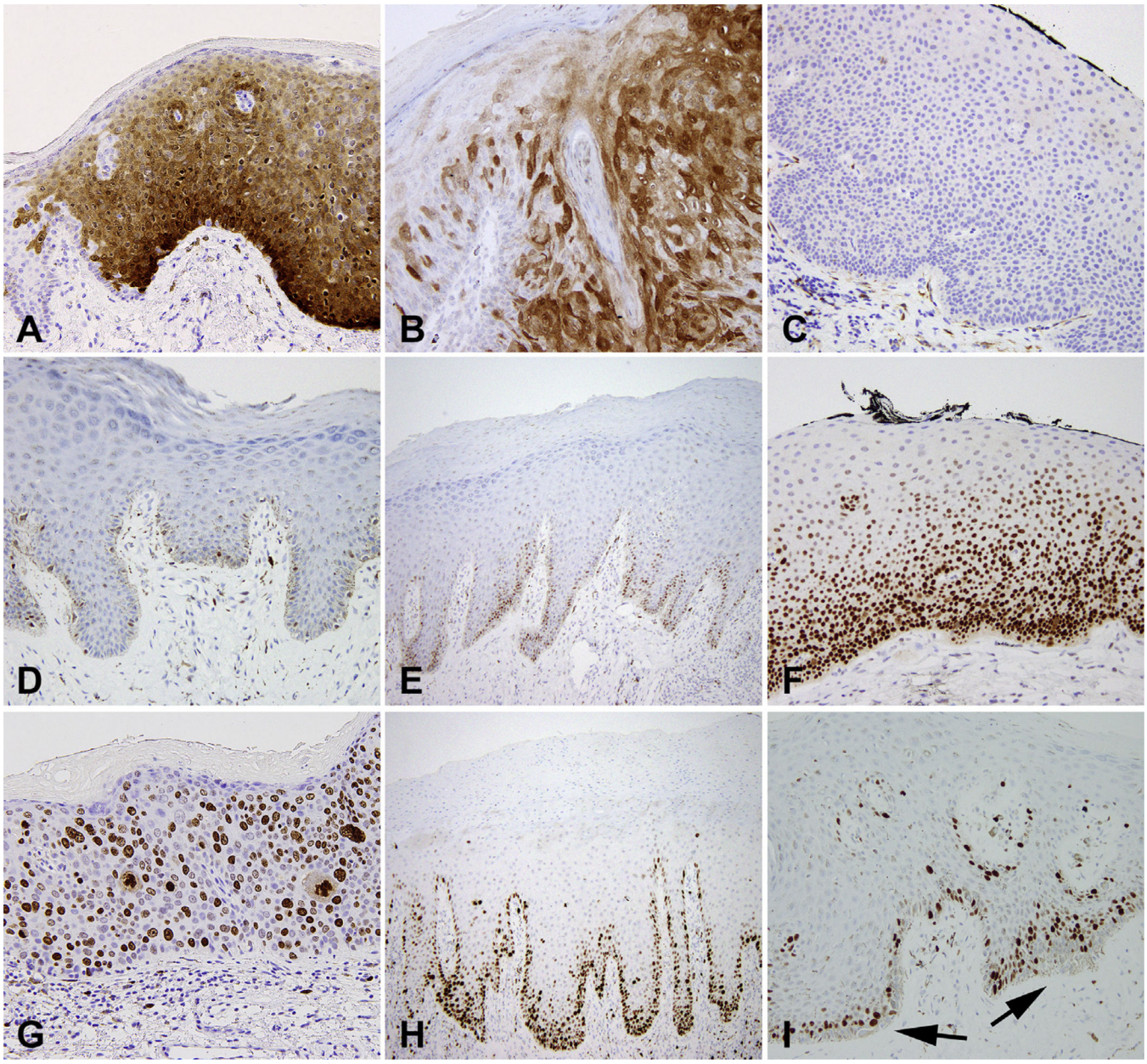

Fig. 4.

Vulvar intraepithelial neoplasia (VIN), immunohistochemical features. p16 with (A) diffuse strong block-like staining in VIN 3; (B) patchy staining in condyloma acuminatum; (C) negative in dVIN. p53 shows (D) weak patchy staining in normal skin, (E) increased basal staining in squamous cell hyperplasia, and (F) increased basal and parabasal staining in dVIN. Ki-67 shows (G) full thickness staining in VIN 3, and (H) increased basal and parabasal staining in dVIN. (I) normal skin shows no Ki-67 staining in the basal layer (arrows).

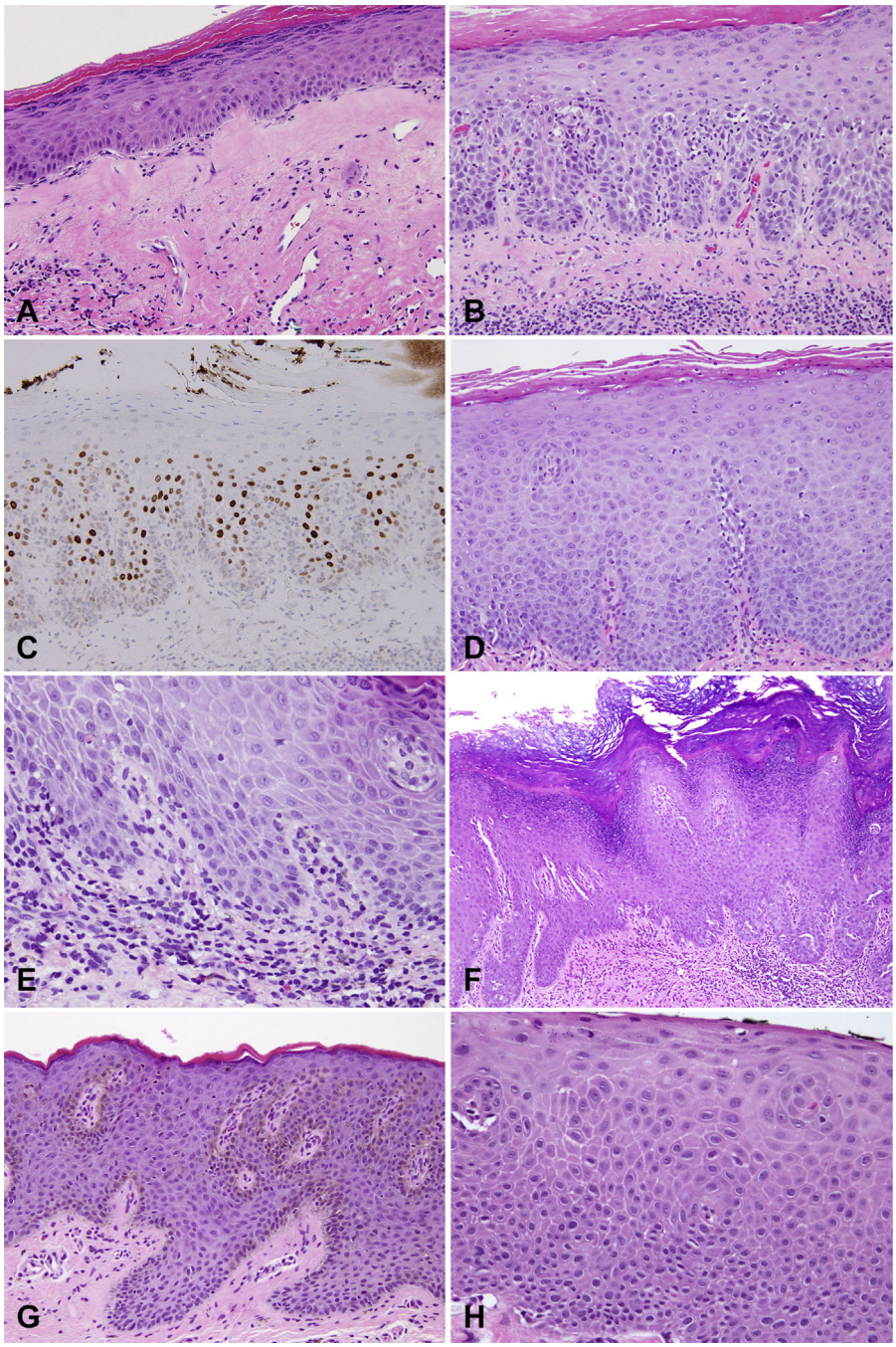

Fig. 5.

Differential diagnosis for vulvar intraepithelial neoplasia (VIN). (A) Lichen sclerosus; (B) hypertrophic lichen sclerosus with (C) increased p53 staining; (D) squamous hyperplasia due to candida; (E) spongiotic dermatitis with rare eosinophils; (F) pseudoepitheliomatous hyperplasia due to extramammary Paget's disease; (G) squamous hyperplasia due to melanoma *in situ*; (H) basaloid variant of dVIN mimicking uVIN.

Table 1

Major classification schemes proposed for squamous precursor lesions of the vulva over time.

1958	1976 ISSVD	1986 ISSVD	2004 ISSVD[a] 2003 WHO[a]	2005 Bethesda-like	2012 LAST 2014 WHO 2015 ISSVD
CIS	Mild atypia	VIN I	_[a]	LG-VIL • Condyloma • VIN 1	LSIL • VIN 1 • Condyloma • Mild dysplasia • Koilocytic atypia
	Moderate atypia	VIN II	uVIN	HG-VIL	HSIL
	Severe atypia or CIS	VIN III, severe atypia or VIN III, CIS	• VIN 2 • VIN 3	• VIN 2–3 • dVIN	• VIN 2–3 • Moderate/severe dysplasia • Bowen disease • Bowenoid dysplasia • CIS
–	–	VIN III, differentiated type	dVIN		dVIN[b]

CIS, carcinoma *in situ*; dVIN, differentiated type VIN; HG-VIL, high-grade vulvar intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; ISSVD, International Society for the Study of Vulvovaginal Disease; LAST, Lower Anogenital Squamous Terminology; LG-VIL, low-grade vulvar intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; uVIN, usual type VIN; VIN, vulvar intraepithelial neoplasia; WHO, World Health Organization.

[a]The 2004 ISSVD no longer recognised VIN 1 but the 2003 WHO retained the designation.

[b]dVIN not included in the LAST guidelines.