Update on Intraepithelial Neoplasia of the Vulva: Proceedings of a Workshop at the 2009 World Congress of the International Society for the Study of Vulvovaginal Diseases, Edinburgh, Scotland, September 2009

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■ **Abstract:** A workshop on updates on intraepithelial neoplasia of the vulva was held at the 2009 World Congress of the International Society for the Study of Vulvovaginal Diseases in Edinburgh, Scotland, September 2009. This is a review of the information presented. ■

Key Words: vulvar neoplasms, intraepithelial neoplasia, carcinoma in situ

Intraepithelial neoplasia of the vulva continues to be an area of diagnostic and therapeutic difficulty, for clinicians and pathologists alike. The goal is to provide optimum therapy while causing the least tissue destruction. The recent change in the classification of vulvar intraepithelial neoplasia (VIN) put forth by the International Society for the Study of Vulvovaginal Diseases (ISSVD; Table 1) and the newer therapeutic modalities have been areas where there is a role for education and discussion. A workshop on updates on intraepithelial neoplasia of the vulva was held at the 2009 World Congress of the ISSVD in Edinburgh, Scotland, September 2009. This is a review of the information presented.

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VULVAR INTRAEPITHELIAL NEOPLASIA (USUAL/HUMAN PAPILLOMAVIRUS–RELATED) AND HUMAN IMMUNODEFICIENCY VIRUS

It has been well recognized that there is a high frequency of vulvar intraepithelial neoplasia (VIN), vaginal intraepithelial neoplasia, and anal intraepithelial neoplasia as well as condyloma in human immunodeficiency virus (HIV)–positive women [1]. Vulvar cancer is a potential

Table 1. Classification of Squamous Intraepithelial Lesions of the Vulva (ISSVD, 2004)

Older terminology	Current terminology
VIN 1	Reactive changes/HPV effect/condyloma
VIN 2	VIN, usual type[a]
VIN 3	VIN, usual type[a]
Differentiated VIN	VIN, differentiated type

VIN, vulvar intraepithelial neoplasia.
[a]Encompasses VIN, warty type, VIN, basaloid type, and VIN, mixed type, warty, and basaloid.

concern as well based on the known increased risk of invasive vulvar disease in renal transplant patients; however, the exact risk of developing invasive vulvar disease in HIV-positive women is not known. The estimated VIN prevalence in HIV-positive women ranges from 5.6% to 37% in various studies [1]. Patients with acquired immunodeficiency syndrome are at significantly increased risk of all in situ human papillomavirus (HPV)–associated cancers (standardized incidence ratios ranged from 8.9 [95% confidence interval {CI} = 8.0–9.9] for cervical cancer to 68.6 [95% CI = 59.7–78.4] for anal cancer among men) and of invasive HPV-associated cancers (standardized incidence ratios ranged from 1.6 [95% CI = 1.2–2.1] for oropharyngeal cancer to 34.6 [95% CI = 30.8–38.8] for anal cancer in men) compared with the general population. Risks were higher for in situ than for invasive cancer at all sites. Risks of all HPV-associated in situ and invasive cancers of the anus, vagina, or vulva increased significantly from 5 years before to 5 years after the onset of acquired immunodeficiency syndrome [2].

Before the institution of highly active retroviral therapy (HAART), it was recognized that VIN was a particularly problematic condition in women infected with HIV. In 1 study [3] presented at the HIV-Infected Women Conference at Washington, DC, in 1995, 58 women were recruited and underwent colposcopy. Fourteen of them had VIN not associated with the degree of immunosuppression but with a high degree of recurrences after therapy. It has been noted that in various studies, 9% to 37% of women who referred for colposcopy for abnormal Pap test results had biopsy-confirmed VIN in various studies. Because of the 100 times greater risk of developing invasive vulvar cancer as opposed to 13.6 times risk of developing invasive cervical cancer in immunosuppressed renal transplant patients, the concern for development of vulvar invasive cancer in HIV-positive women was raised [1]. This is of particular concern in patients who may have VIN for many years, such as the 8-year-old child with high-grade VIN and anal intraepithelial neoplasia with multiple recurrences presented by De Gois et al. [4]. Korn et al. [5] looked at 28 VIN cases, 8 from HIV-positive women and 20 from HIV-negative women. The relative risk for recurrence or persistence after therapy in HIV-positive women was 3.3. Weaknesses of this study included small numbers, patients being unstratified by immune status, and HIV-negative patients may have been actually HIV-positive because HIV status was self-reported. Of note, this study was published in 1996, the year HAART became available, and usage became more widespread in the ensuing years.

Jamieson et al. [6] followed up 192 HIV-positive women and 88 HIV-negative women who were at high risk for HIV for more than 6 years. At baseline, 3 (1.6%) of the HIV-positive and none of the HIV-negative women had vulvar/vaginal/perianal intraepithelial neoplasia. During their study, 16 (8.5%) of 189 HIV-positive and 1 (1.1%) of 88 HIV-negative women developed intraepithelial neoplasia. The incidences were 1.96 per 100 person years for HIV-positive and 0.026 per 100 person years in HIV-negative women ($p = .03$). Paradoxically, they noted an increased risk in patients who were on antivirals at baseline and postulated that this confounder may have been due to a more advanced HIV disease in these patients. Other associations with the disease in this study were CD4 cell count of less than 200/μL, HPV positivity, and high-risk HPV types.

In an attempt to better understand the mechanism of progression of VIN in HIV-positive women, Taube et al. [7] investigated localized immunity by staining for Langerhans cells (LCs). The density of LCs has been shown to be decreased in the cervix of HIV-positive women, and this is thought to contribute to the progression of cervical intraepithelial neoplasia (CIN). Cases of VIN were immunostained with S-100 protein to highlight LCs in HIV-positive and HIV-negative women. Mean S-100 + LC counts were 5.82/high-power field for HIV-positive women and 9.86/high-power field for HIV-negative women ($p = .0026$), suggesting a role for altered local immunity. There was no significant difference in either group relating to smoking status. Dedes et al. [8] studied anal intraepithelial neoplasia in HIV-positive women and noted a high relapse rate and substantial invasive potential.

A few large studies have been published since the availability of HAART. Massad et al. [9] conducted a multicenter prospective study of women without warts or VIN at baseline, following them with CD4 counts, HIV virus RNA, examination, Pap tests, and biopsies

as indicated at 6 months, with HPV typing at baseline. The wart incidence was 1.31 vs 5.01 per 100 person years in HIV-negative versus HIV-positive women ($p < .001$). The incidence of VIN was 1.31 vs 4.67 per 100 person years in HIV-negative compared with HIV-positive women ($p < .001$). Vulvar intraepithelial neoplasia was found in 359 (23%) of HIV-positive women versus 35 (7%) of HIV-negative women during the course of the study. Vulvar intraepithelial neoplasia was linked to HAART (relative hazard = 0.065), CD4 count (relative hazard = 0.092), abnormal Pap test result (relative hazard = 16.03), and high- or medium-risk HPV (relative hazard = 1.37), and the authors concluded that HAART decreases the incidence of warts and VIN.

Ferenczy et al. [10] conducted a meta-analysis of 50,000 HIV-positive women and found relative risks of 4.6 for VIN and 5.8 for invasive cancer of the vulva or vagina, noting that the long-term effect of HAART on progression and treatment outcomes needs to be determined, and recommending treating and monitoring the patients.

TREATMENT OF VIN (USUAL/HPV-RELATED)

Treatment of VIN is aimed at both relief of symptoms and prevention of progression to invasion. This has become more significant with the increasing incidence of VIN, particularly in younger patients, which introduces additional limits to the desirability of extensive and potentially mutilating surgical procedures. Treatment is aimed at eradicating all visible lesions and must be individualized, with the goal of preserving normal anatomy and genital function. Therapy has traditionally been divided into surgical (cold knife, CO_2 laser) and medical, with topical chemotherapies such as 5-fluorouracil and bleomycin, nucleoside analogs (cidofovir), or immunotherapies such as dinitrochlorobenzene and interferon-α [11]. Cidofovir is an acyclic nucleoside phosphate derivative known to have a broader spectrum of activity against different families of DNA viruses. Efficacy has been shown when treating high-grade CIN. Further work is planned to investigate the efficiency of this compound because recent data may indicate a potential rationale for increased risk of genetic instability and thus transformation due to drug-induced 20-fold increase in high-risk E6-expressing cells [12]. Newer modalities have included photodynamic therapy, imiquimod, and vaccination.

Photodynamic therapy involves using a tumor-localizing photosensitizer (5-ALA) and nonthermal light to generate light-induced oxidative reactions leading to cell death. Response rates have ranged from 0% to 71%, but studies have been small, nonrandomized, and uncontrolled. Multifocal disease has resulted in shorter disease-free interval. Recurrence rates are similar to laser or excision, with minimal tissue destruction, short healing time, and minimal adverse effects [13].

In a review of the use of imiquimod for VIN, Iavazzo et al. [14] reported on 17 studies conducted between 2000 and 2007, for a total of 210 patients. Treatment duration ranged from 3 to 32 weeks, with follow-up ranging from 1 week to more than 30 months. In the different series, regression was complete in 26% to 100% of patients and partial in 0% to 60% of patients and recurrences varied from 0% to 37% of patients. The most common adverse event was burning and soreness. Le et al. [15] conducted a phase 2 study on the use of imiquimod in treating VIN 2/3. Thirty-nine patients were observed for up to 16 months, and recurrence data were compared with a historical cohort of surgically treated patients. The response rate with imiquimod was 77%, with a recurrence of 20.5% compared with a recurrence rate of 53.5% in the historic cohort of surgically treated patients. No patient progressed to invasive disease. Mathiesen et al. [16] conducted a randomized double-blinded controlled trial of escalating dose of imiquimod more than 16 weeks on 31 patients, mostly with unifocal disease ($n = 22$), biopsy confirmed as VIN 2 or 3, in a 2:1 ratio to placebo-treated controls and found an 81% complete response and a 10% partial response to imiquimod with no progression to invasive disease. van Seters et al. [17] conducted a randomized controlled trial of 52 patients with multifocal VIN 2/3 for more than 16 weeks, with 12 months of follow-up and found a 35% complete response and 46% partial response. Treatment was associated significantly with histological regression, viral clearance, and relief of itch and pain, and all complete responders were free of disease at 12 months; however, there were 3 patients who progressed to invasive disease.

A recent article investigated immunogenicity and efficacy of a synthetic long-peptide vaccine in 20 women with HPV-16–positive high-grade VIN [18]. Five women had complete regression of the lesions, and HPV-16 was no longer detectable in 4 of them. At 12 months of follow-up, 15 (79%, 95% CI = 54%–94%) of 19 patients had clinical responses, with a complete response in 9 (47%, 95% CI = 24%–71%) of these 19 patients. The complete response rate was maintained at 24 months of follow-up. Clinical outcome seemed to correlate with induction of HPV-16–specific immunity.

A few early studies are out on vaccination, both therapeutic [19] and prophylactic [20], and it is possible that vaccination may play a role in the future, possibly in combination with imiquimod or photodynamic therapy. Some success has been achieved as well with a combination of photodynamic therapy and imiquimod [21].

PROGNOSIS OF VIN (USUAL/HPV-RELATED)

The natural history of VIN is uncertain. Some authors believe that there is a low but inevitable progression to vulvar carcinoma, although others believe that the risk of progression is low. What little we know is based on 3 types of clinical observations:

(1) Areas of VIN adjacent to vulvar carcinoma: A large percentage of invasive squamous cell carcinomas (SCCs) of the vulva are seen in association with lichen sclerosus (47%), whereas VIN is much less common, seen in only 23% in the study of Rouzier et al. [22].

(2) Occult carcinoma found within VIN: The finding of occult carcinomas confirms the potential of VIN to become invasive. Chafe et al. [23] described 69 patients with biopsy-proven VIN who underwent surgical excision. Unexpected invasive carcinoma was found in 13 patients (18.8%). The invasion was superficial in 61% of these patients. In a review of 3,332 patients with VIN [24], a total of 215 invasive carcinomas were found (6.5%), of which 107 (3.2%) were occult. Of these cases, only 71% had superficial invasion. The difficulties of pathologically distinguishing superficial invasion from tangential sections and involvement of pilosebaceous units was acknowledged. A recent article [25] showed that VIN 2 and 3 were correctly diagnosed by preoperative vulvar biopsy in 55.8% (29/52) and 88.1% (118/134) patients, respectively. Underdiagnosis occurred in 44% (23/52) and 11.9% (16/134) of preoperative biopsies, with an occult cancer rate of 3.8% (2/52) and 11.9% (16/134) for VIN 2 and 3, respectively.

(3) VIN progressing to carcinoma: Most series show a progression rate of less than 5%; however, these are series of treated patients. Information on untreated patients ranges from 0% to 87% in a variety of studies [24, 26–31]. Follow-up in the more recent studies ranged from a mean of 39 months [24] to 20 years, with an invasive carcinoma noted developing at 7.3 years [29].

Spontaneous regression has been observed in women younger than 30 years with multifocal pigmented papular lesions seen more frequently in the perineum and adjacent skin, and pregnancy may be a contributing factor in the regression. Factors that seem to increase the risk of progression include increasing age, immunosuppression, smoking, and previous radiation therapy [24].

Although it has been suggested that unifocal lesions may be more likely to progress, this has not been supported by the studies of Van seters et al. [24] and Jones and Rowan [29]. The squamocolumnar junction in the anal region is an area of increased risk. Of interest, there was no difference between patients who had positive versus negative margins in 1 study [24]. It has been suggested that basaloid histology is of greater risk than warty in likelihood to progress [32]. p53 has been suggested to be a marker of progression of lichen sclerosus [33]. Maclean et al. [34] showed vascular endothelial growth factor expression in 92% of vulvar carcinomas and in only 6% of VIN. It is possible that vascular endothelial growth factor–positive VIN is at greater risk of progression.

DIFFERENTIATED VIN (NOT HPV-RELATED)

Differentiated VIN is a precursor of SCC. As such, it should be detected and treated as soon as possible. However, many questions need to be answered before recommendations can be made. These questions refer to the pathological definition, the use of biochemical hallmarks, the clinical features, the association with other lesions (the lichens), and prevention.

Classification

Vulvar intraepithelial neoplasia is a histological diagnosis based on loss of squamous epithelial maturation associated with enlarged, hyperchromatic, pleomorphic nuclei, and increased, usually atypical, mitoses. According to the ISSVD classification, VIN is divided into 2 categories [35]:

- *Usual VIN* encompasses former VIN 2 and 3 of warty, basaloid, and mixed types; it is characterized by epithelial full-thickness atypia and is associated with high-risk HPV infection.
- *Differentiated VIN* will be described further in this section.

The World Health Organization nomenclature designates differentiated VIN as "carcinoma in situ (simplex type) (VIN 3)." Both types of VIN are precursors of SCC. The specific features of differentiated and usual VIN were recently reviewed [36].

Histology

In differentiated VIN, atypia is strictly confined to the basal and parabasal layers of the epithelium where the cells have abundant cytoplasm and form abortive pearls (Figure 1). The recognition of differentiated VIN is hindered by a high degree of cellular differentiation. For these 2 reasons, differentiated VIN is a more subtle lesion than usual VIN and can be mistaken easily for an epithelial hyperplasia or a benign inflammatory dermatosis [32, 37] with which differentiated VIN is, most of the time (perhaps always), associated (see succeeding paragraphs). Therefore, pathological diagnosis of differentiated VIN may be tricky. This entity may be overlooked because of the presence of very subtle abnormalities or because the abnormalities observed may be not uniformly recognized by pathologists.

Virological and Biochemical Markers

Because of the difficulties establishing the diagnosis of differentiated VIN, biochemical and virological markers have been searched for.

Presence of High-Risk HPV. As opposed to usual VIN, differentiated VIN is generally not related to high-risk HPV [38].

Biochemical Markers

(1) p16 protein

The overexpression of p16 protein supports a dysfunction in the progression of the cell cycle and in the cell proliferation. On the vulva, p16 protein is positive in HPV-associated VIN but negative in VIN not associated with HPV. Similarly, HPV-associated invasive squamous carcinomas are p16-positive, whereas the more common non–HPV-associated neoplasms are largely negative or focally positive [38, 39].

(2) MIB-1 (Ki-67)

Proliferative activity in tissues can be visualized using a proliferation marker such as MIB-1, which is a monoclonal antibody against the Ki-67 antigen, a nuclear antigen present in human proliferating cells in all stages of the cell cycle but not in the G_0 phase. In differentiated VIN, MIB-1 expression is confined to the basal layers, which can help distinguish differentiated VIN from normal epithelium where the basal cell layer is often negative for MIB-1 [38, 40]. In a study by Mulvany and Allen [41], all 6 vulvar specimens of differentiated VIN showed intense immunoreactivity for Ki-67 in the basal and parabasal cells. A study comparing 8 cases of lichen sclerosus evolving to SCC to lichen sclerosus followed for 9 years without such complication showed that the difference of MIB-1 labeling index of evolving or unchanged LS cases was significant ($p = .005$) [42]. This predictive value of MIB-1 should be confirmed by further studies.

(3) p53

p53 protein is involved in apoptosis regulation. Immunostaining for p53 may be of value in distinguishing differentiated VIN from normal squamous epithelium. The p53 labeling index of the basal cell layer in differentiated VIN is often higher than 90%, and p53-positive cells extend from the basal cell layer into higher levels of the epidermis (suprabasilar extension) [43].

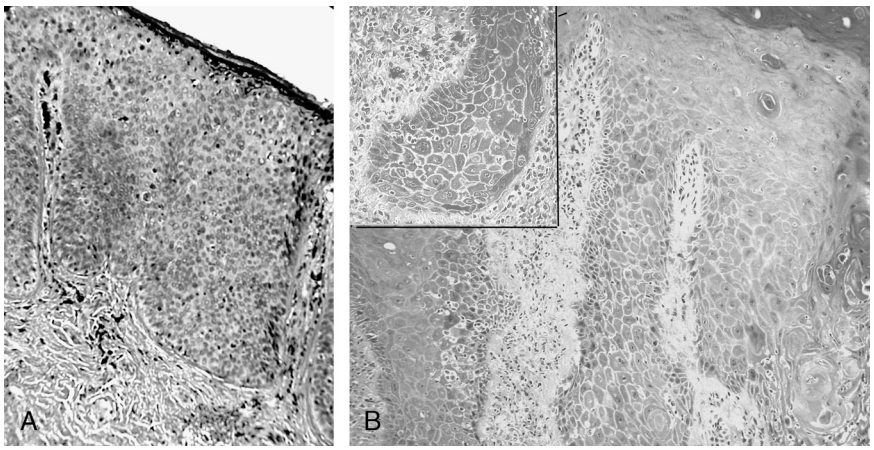

Figure 1. Unlike the clear-cut maturation abnormality seen in usual (HPV-related) VIN (left), the atypia of differentiated (non–HPV-related) VIN is much more subtle (right), with disarray of the cells, mild atypia, exaggerated cell borders, and prominent nucleoli (insert, upper left).

Incidence

During the 1992–2005 period, the incidence of differentiated VIN increased significantly whereas the incidence of vulvar SCC remained stable. This increase is probably related to an increased awareness of this condition related to better-defined histological features [44].

Prevalence

The prevalence of differentiated VIN is much lower than that of usual VIN. However, the rate of differentiated VIN compared with usual VIN greatly varies according to the studies: 2% of 48 VIN [45], 3.5% of 1,893 [44], 18.2% of 164 consecutive VIN [46], and 30% of 241 VIN [47]. The reasons for these discrepancies probably include the difficulty in histologically identifying differentiated VIN owing to the highly differentiated appearance of the neoplastic keratinocytes and the absence of widespread architectural disarray. Less likely, there could be a true difference in the prevalence of differentiated VIN in different countries.

Age

The mean age of patients with differentiated VIN is 67 years [37]. In a recent study, the highest prevalence occurred at ages 75 to 79 years as opposed to usual VIN that showed a bimodal peak incidence at the ages of 40 to 44 and 75 to 79 years [44].

Human Papillomavirus and Chronic Dermatosis

As opposed to usual VIN, differentiated VIN is not related to high-risk HPV infection. Differentiated VIN is associated with inflammatory dermatosis (lichen sclerosus or lichen planus; Figure 2). In a pathological study, lichen sclerosus was associated with 32% of vulvar SCCs, and 56.3% of these cases of lichen sclerosus were associated with differentiated VIN [48]. In another study, lichen sclerosus was found in half of the differentiated VIN associated with SCCs [47]. In clinical practice, however, differentiated VIN seems to be almost always associated with lichen sclerosus or planus, either active or quiescent. The reason for this discrepancy between the clinical and the pathological data could be the deceptive pathological criteria of vulvar lichens (sclerosus or planus) particularly in their quiescent phases [49].

Clinical Features

Clinical features of differentiated VIN have not been fully studied. Differentiated VIN, an assumed precursor of SCC, was diagnosed before SCC in only 7 (18.2%) of 29 cases [32]. This low rate of detection of differentiated VIN before it progresses to SCC could be related either to a lack of awareness of subtle lesions or to the fact that differentiated VIN so rapidly evolves to SCC that it cannot be clinically identified. In practice, differentiated VIN is more frequently unifocal than multifocal (as opposed to usual VIN). It should be suspected with a thick white patch. In most of the cases (if not all), this plaque arises on a background of lichen sclerosus or planus. This lesion may be otherwise quiescent, identifiable only on the basis of the architectural modification, in the absence of the shiny pallor or the other specific features that characterize these conditions. Lichen sclerosus associated with SCC is clinically characterized by hyperplasia that may correspond histologically to differentiated VIN or squamous cell hyperplasia without atypia [50]. Differentiated VIN may be clinically deceptive: subtle white or pink patches. Differentiated VIN frequently recurs after treatment, either on the same site or on other sites of the vulva.

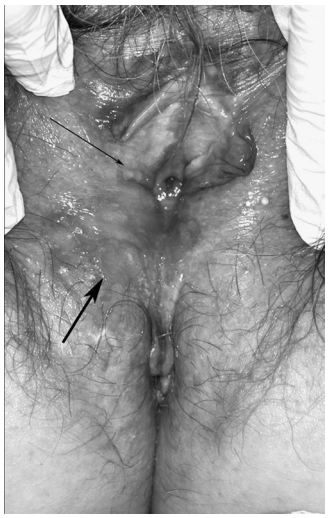

Figure 2. Vulvar intraepithelial neoplasia (upper thin arrow) and invasive SCC (lower heavy arrow) in association with lichen sclerosus. Courtesy of Lynnette Margesson, MD.

In practice, in case of lichen (sclerosus or planus), any thickened area [51] or any other type of lesion resistant to potent topical corticosteroids should be histologically checked and surgically removed.

Prognosis

Differentiated VIN Is More Frequently Associated With Keratinizing SCC Than Usual VIN. Association of SCCs with differentiated versus usual VIN was studied in a series of 44 patients with 48 thin (<5 mm) SCCs that

Table 2. Proposed Classification of Vulvar Paget Disease [54]

Primary (of cutaneous origin) vulvar Paget disease
– Paget disease as a primary intraepithelial neoplasm
– Paget disease as a primary intraepithelial neoplasm with invasion
– Paget disease as a manifestation of an underlying primary adenocarcinoma of skin appendage or subcutaneous vulvar gland
Secondary vulvar Paget disease (involvement of the vulvar skin by a noncutaneous internal neoplasm)
– Paget disease secondary to anal or rectal adenocarcinoma
– Paget disease secondary to urothelial neoplasia
– Paget disease secondary to adenocarcinomas or related tumors of other sites

were keratinizing (78%), warty (13%), or basaloid (8%) [48]. Differentiated VIN occurred in 38% of the 48 invasive tumors, and usual VIN occurred in 43%. However, in 27 (71%) of 38 keratinizing SCC with adjacent VIN, the VIN was of the differentiated type in 67% and of the usual type in 33%. In the same study, there was a striking similarity between the cases associated with differentiated VIN and those without VIN (23%) in age, predominance of keratinizing type of SCC, and presence of lichen sclerosus. The authors suggest that differentiated VIN could be an unrecognized obliterated or unsampled precursor lesion in at least some of the SCCs without demonstrable VIN. Differentiated VIN could have a relatively brief intraepithelial phase before progressing to squamous carcinoma, which could explain some of the cases devoid of adjacent VIN.

Differentiated VIN Is More Likely to Progress to SCC Than Usual VIN. In a study including 580 women with VIN, lichen, or squamous cell hyperplasia, 60 (85.7%) of the 70 women who had biopsies containing differentiated VIN had concurrent, previous, or subsequent vulvar SCC as opposed to only 25% of women with usual VIN [47].

In another recent study, the overall percentage of differentiated VIN patients progressing to vulvar SCC (32.8%) was 5.6 times higher compared with usual VIN patients (5.7%) [44].

Patients With Differentiated VIN Adjacent to SCC Seem to Have a Poorer Prognosis Than Patients With Usual VIN. Of 108 patients with vulvar SCCs, 77 (71%) had an epithelial alteration adjacent to the tumor. Lichen sclerosus and squamous cell hyperplasia "with or without atypia" (in this study, the term differentiated VIN was not used) were identified in 48% (n = 52), and usual VIN was identified in 23% (n =25). The 5-year disease-free and overall survival rates were 39% and 55%, respectively. Patients without associated epithelial alterations (39%) had clinical and prognostic features comparable to those of patients with vulvar lichen sclerosus and squamous cell hyperplasia [22]. Of consideration, patients with SCC adjacent to lichen sclerosus tend to be older than those with VIN, which may possibly contribute to survival differences.

Squamous Cell Carcinomas With Adjacent Differentiated VIN Are More Likely to Recur Than Those With Adjacent Usual VIN. Women with adjacent differentiated VIN are 3 times more likely to experience recurrence of SCC than women with usual VIN [52]. This difference could be because differentiated VIN usually arises on a background of an "at-risk lichen." According to the current recommendations for the treatment of SCC, only the tumor is removed, but vulvar tissues involved by lichen is left, and this lichen could represent a field cancerization.

Treatment

There is no evidence that medical treatment of lichen sclerosus or planus prevents the occurrence of differentiated VIN or SCCs [53]. So far, the best therapeutic option is surgical excision of the lesions. Surgical excision of differentiated VIN allows the pathological examination of the removed specimens and the control of excision margins. Laser or electrocautery does not allow pathological examination. We have no reliable data about the effect of 5-fluorouracil, imiquimod, or dynamic phototherapy.

Table 3. Immunohistochemical Panel to Distinguish Pagetoid Patterns of Neoplasia

	CK 7	CK 20	CEA	GCDFP-15	HMB-45	S-100	Uroplakin-III
Primary Paget	+	+/−	+	+/−	−	−	−
Urothelial	+	+/−	+/−	−	−	−	+
Anorectal	Usually −	+	+	−	−	−	−
Melanoma	−	−	−	−	+	+	−

CK, cytokeratin; CEA, carcinoembryonic antigen; GCDFP, gross cystic disease fluid protein; HMB, human melanoma black.

PAGET DISEASE

Paget disease is usually found in sites with a high density of apocrine glands, such as the vulva, penis, scrotum, anus, perianal region, axilla, eyelids, and external auditory canal. Examination of the vulvar region should also focus on the perianal and periurethral regions because Paget disease of the vulva may represent extension of a noncutaneous neoplasm. Wilkinson and Brown [54] proposed the following classification (Table 2) for vulvar Paget disease and described how a panel of immunohistochemical stains can help make the distinction between the various forms of Paget disease [55], noting that although histologically these different entities may seem similar, clinically they can often be distinguished (Table 3).

Current therapy involves wide local excision; however, recurrences are common, with a 34% recurrence rate in 1 series [56]. Recurrences may relate to the fact that the lesion often extends histologically past the clinically visible lesion. The relationships between disease recurrence and various pathologic parameters were examined in a recent article [57]. Epidermal acantholysis, that is, loss of cohesion between keratinocytes, correlated with increased recurrence rate, thus with decreased disease-free probability. This finding was seen in 72% of patients with recurrence and in 38% of patients without recurrence ($p < .035$) and was associated with a shorter time to recurrence.

In cases of invasive primary Paget disease, recurrence rate may be even higher, with more than 50% recurrence rate in a different series [56] and 24% of the patients dying of disease, highlighting the aggressiveness of the invasive form of the disease. Patients with an underlying adnexal adenocarcinoma or stromal invasion of Paget more than 1 mm should be treated more aggressively, with excision to the fascia in the involved area and inguinofemoral lymphadenectomies bilaterally. Currently, there are no data in the literature regarding the safety of sentinal node dissection or unilateral node dissection in invasive Paget disease. There is currently no recognized concept of microinvasive Paget disease, which is extremely rare, if applying the criteria used for squamous neoplasia, that is, less than 1 mm in depth of invasion. In 1 study [58], sentinal node dissection was negative in 2 of 3 women with superficially invasive Paget disease less than 1 mm in stromal invasion. The third woman with a positive sentinal node underwent additional excision of noninvasive disease as well as bilateral groin node dissections, all negative, without recurrence on follow-up for 12 months. Her-2/*neu* has been suggested as a possible marker of aggressive behavior [59]. Therapy for noncutaneous adenocarcinomas manifesting as Paget disease is directed toward the primary neoplasm, with the vulvar involvement treated as usual intraepithelial Paget disease. There is not much in the literature about approaches to locally advanced, recurrent, or invasive Paget disease. A few studies have described using imiquimod on primary Paget disease [60].

Table 4. In Situ Lesions of the Vulva

Squamous intraepithelial neoplasia (VIN, usual and differentiated types)
Glandular intraepithelial neoplasia (Paget disease, primary and secondary)
Melanocytic intraepithelial neoplasia (melanoma in situ)

Table 5. Differential Diagnosis of the Most Common Pigmented Lesions of the Vulva

Postinflammatory hyperpigmentation
Melanosis
Nevus
Lentigo
VIN
Seborrheic keratosis
Melanoma in situ
Invasive melanoma

MELANOMA IN SITU

Melanoma in situ is a rare form of intraepithelial neoplasia on the vulva (Table 4). It is a form of radial growth where the proliferating malignant melanocytes are confined to the epidermis. An in situ phase exists for 3 of the 4 invasive forms of melanoma, superficial spreading melanoma, lentigo maligna melanoma, and acral lentiginous melanoma. Nodular melanoma does not have an in situ phase. If the diagnosis is considered within the differential diagnosis of pigmented vulvar lesions (Table 5), it is easy to recognize and treat, with excellent prognosis. The ABCDE schema for the recognition of melanoma should be considered in pigmented lesions (*a*symmetry, *b*order irregularities, *c*olor variation, *d*iameter > 6 mm, *e*nlargement or *e*volution of color change, shape, or symptoms) [61, 62]. An excisional biopsy with a 1- to 2-mm rim of normal-appearing skin is the optimal technique for lesions suspected to be melanomas. For large lesions, a punch or incisional biopsy permits the diagnosis of melanoma to be made in most cases. For patients with in situ melanomas, there are no data from randomized trials to define the optimal extent of surgical resection. However,

retrospective data support the routine use of 0.5-cm margins [63, 64].

Studies have shown no worse prognosis if the initial biopsy does not remove the entire lesion, which is later excised. Destruction by cryosurgery or cautery or laser is contraindicated, and all such lesions must undergo histopathological evaluation. Although slow-growing, melanoma in situ of the vulva does have the potential to progress to invasive melanoma over time [65].

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