ACTA OVERVIEW

Premalignant epithelial disorders of the vulva: squamous vulvar intraepithelial neoplasia, vulvar Paget’s disease and melanoma in situ

ANNELINDE TERLOU1, LEEN J. BLOK1, THEO J.M. HELMERHORST1 & MARC VAN BEURDEN2

1Departments of Obstetrics and Gynecology, Erasmus University Medical Center, Rotterdam, The Netherlands, and 2Department of Gynecology, The Netherlands Cancer Institute, Amsterdam, The Netherlands

Abstract

No standard screening programs exist to detect vulvar carcinoma or its precursor lesions, and therefore gynecologists, dermatologists and other healthcare providers in this field should be aware of the clinical features, behavior and management of the different existing premalignant vulvar lesions, squamous vulvar intraepithelial neoplasia (VIN), vulvar Paget’s disease and melanoma in situ. In 2004, a new classification for squamous VIN was introduced by the International Society for the Study of Vulvar Disease, subdividing squamous VIN into the HPV-related usual type, and into differentiated type, which is associated with lichen sclerosus. This review describes the relevant aspects of squamous VIN, vulvar Paget’s disease and melanoma in situ, its epidemiological characteristics, diagnosis, management and malignant potential.

Key words: Vulvar intraepithelial neoplasia, HPV, lichen sclerosus, melanoma in situ, Paget’s disease

Vulvar intraepithelial neoplasia

Terminology and classification

Squamous vulvar intraepithelial neoplasia (VIN) is a premalignant skin disorder that often causes severe and long-lasting pruritus, pain and psychosexual dysfunction. It has a spectrum of clinical and histopathological appearances and can be divided into two subtypes: usual type VIN, which is caused by a persistent infection with high-risk Human papillomavirus (HPV), and differentiated type VIN, which is associated with lichen sclerosus (LS). Squamous intraepithelial lesions were first described in 1912 by Bowen, and since then various terms have been used. In 1965, Kaufman grouped premalignant lesions into three categories: Queyrat’s erythroplasia, Bowenoid carcinoma in situ and carcinoma simplex (1). A new simplified terminology was introduced by the International Society for the Study of Vulvar Disease (ISSVD) in 1976, and all terms were replaced by carcinoma in situ and vulvar atypia (2). Ten years later these terms were replaced by a single term, vulvar intraepithelial neoplasia (3). In addition, VIN was graded, similar to cervical intraepithelial neoplasia (CIN), in three subtypes: VIN 1 (mild dysplasia), VIN 2 (moderate dysplasia) and VIN 3 (severe dysplasia) (3). This grading system suggests a biologic continuum of VIN lesions. However, the presence of such a continuum is not supported by clinicopathological data. Therefore, the ISSVD abolished the grading system in 2004 and introduced a 2-tier classification for squamous VIN: usual type and differentiated type VIN. The two types differ in etiology, morphology, biology, clinical features and malignant potential (4,5). However, the WHO-classification with the three subtypes VIN 1, 2 and 3 is still widely used (6).

Usual type VIN (uVIN) has histologically been divided into warty, basaloid or mixed (warty/basaloid) VIN. UVIN is caused by a persistent infection with high-risk or oncogenic HPV (mostly HPV-type 16, 18).
or 33) (7). It occurs predominantly in younger women and tends to be multifocal (4).

**Differentiated type VIN (dVIN)** is less common, <2–5% of all VIN lesions are of this type, but it has the highest malignant potential (1,8). It is not related to HPV but is associated with LS and is usually found in older women (9). DVIN is mostly uncincentric and strongly associated with past or coincident invasive vulvar squamous cell carcinoma (SCC) (10,11).

Besides modifying the classification of VIN, the ISSVD also modified the grading system. Some studies demonstrated an overlap in the diagnosis of VIN 2 and VIN 3, while it was shown that VIN 1 only occurred in condylomata acuminata (10,12). In addition, it was demonstrated that there is a lack of reproducibility of the pathologic diagnosis of VIN 1, 2 and 3, and that VIN 2 and 3 grouped together is better reproducible (12,13). Nowadays, the term VIN is only applied to histologically ‘high-grade’ squamous lesions (VIN 2 and VIN 3). VIN 1 no longer exists.

**Epidemiology**

Over the last decades, the incidence of VIN has increased, most likely due to a rise in incidence of HPV infections. Overall incidence of vulvar SCC remained the same (8,14–16). However, some studies reported an increase of vulvar SCC in younger women who tend to have a history of HPV and VIN (16–18). A recent study observed the incidence of uVIN, dVIN and vulvar SCC in the Netherlands during a 14-year-period. The incidence of uVIN almost doubled from 1.2/100,000 patients in 1992 to 2.1/100,000 in 2005 and that of dVIN increased nine-fold from 0.013/100,000 patients to 0.121/100,000, while the incidence of vulvar SCC remained stable (8). The incidence of invasive vulvar SCC increases with age (8,19) and a higher rate of vulvar SCC has been observed in white women than in women of other races (19).

**Etiology**

**Usual type vulvar intraepithelial neoplasia.** Lifetime risk to become infected with HPV in western societies is around 80% and approximately 40% of all sexually active, female adolescents are at least once infected with high-risk HPV (hrHPV) (20). When hrHPV persists (in less than 10% of cases), premalignant disorders of the lower anogenital tract, such as uVIN, can develop (21–23).

The majority of VIN (90%) is of the usual type and persistent infection with hrHPV plays an important role in the etiology of uVIN. Reported prevalence of HPV in VIN range from 72 to 100% and in most cases HPV16 is detected (7,24–29).

Immunosuppression and smoking (which reduces local immunity) are important risk factors for VIN (30–34) and it is known that immunosuppressed patients as HIV-positive women have an increased risk of developing uVIN (31,35,36). Several immunological studies demonstrated that the host immune response is of critical importance in determining clearance or persistence of HPV-related VIN (37–41). VIN lesions are characterized by an immunosuppressive state in the epidermis (40,41), while a reduced and insufficient immune response to the hrHPV infection occurs in the dermis of uVIN (38,39,41). Furthermore, spontaneous regression of VIN has been observed with high detectable HPV-specific blood T-cell responses, while patients with persistent disease had no detectable anti-HPV T-cell responses (37).

In the HPV-related pathways, a couple of molecular alterations occur due to infection and subsequent integration of hrHPV. HPV encodes for several viral proteins, of which the oncoproteins E6 and E7 are the most important. HPV E6 can interact with the tumor suppressor gene p53, leading to p53 dysfunction and consequently absence of cell cycle arrest (42). HPV E7 can inactivate the retinoblastoma tumor suppressor gene pRb, which results in overexpression of the cell cycle related biomarkers p16ink4a and p14arf, and hyperproliferation of infected cells (43,44). As a result, most uVIN lesions are positive for p16ink4a and p14arf, but p53 negative (44–48). An increased expression of p16ink4a in combination with low p53 expression was observed in young women when compared to older ones with vulvar SCC (49).

These markers are not conclusive in distinguishing uVIN from dVIN. It was observed that p16ink4a expression is high in all uVIN-associated tumors, but also in 10 of 105 dVIN-associated tumors. Almost all dVIN-associated tumors were HPV negative and none had integration of HPV; this is in contrast to the uVIN-related tumors of which 23 of 25 had integrated HPV (50). Recently, it was shown that in all dVIN lesions gain of chromosome 3q26 was present, while this was only the case in 50% of uVIN lesions. Detection of 3q26 imbalance could be of additional diagnostic value in the diagnosis of VIN lesions together with staining for p16ink4a and p53 (51).

**Differentiated type vulvar intraepithelial neoplasia.** In contrast to uVIN, the presence of HPV in dVIN is very rare and the exact cause of dVIN is still unclear (11,50,52,53). It is assumed that dVIN is related to LS (9) and several reports show a clear relationship between dVIN and LS in adjacent skin of vulvar SCC (54–58). However, it seems that VIN associated with LS without coexisting vulvar SCC is more likely of the undifferentiated type (59). Squamous hyperplasia is
also commonly seen in adjacent epidermis of dVIN patients, and might be a step in carcinogenesis (11,60). dVIN is often HPV-negative and p53 positive (11,45,61,62).

Clinical features and diagnosis

The clinical presentation of VIN is diverse. Lesions can be red, white and pigmented, either flat or raised and erosions or ulcers may be present. Symptoms as pruritus or pain are observed in about 60% of patients (63,64). Since patients can be asymptomatic, accurate vulvar inspection during routine gynecologic examination is important. Clinical features that can help in making the correct diagnosis are color, thickness, surface and focality (1). To confirm the diagnosis, a biopsy of the most suspicious part of the lesion should be performed under local anesthesia (65).

Commonest affected sites are labia majora and minora and the fourchette (32,63). UVIN lesions are often multifocal (9,63,64). In addition, multicentric disease (lesions of cervix, vagina or anus) is common in uVIN patients and is age-related, as it decreases from 59% in women aged 20–34 to 10% in women over 50 years of age (9,29,32,66). Therefore, a careful examination of the lower anogenital tract (vulva, perineum and perianal areas), which also includes the cervix and vagina, is mandatory.

The diagnosis of dVIN is a challenge. dVIN lesions are almost always observed in areas of LS or lichen planus (LP); only one case report describes dVIN as a solitary lesion in a patient without a history of LS or LP (67). Red lesions and areas with hyperkeratosis, ulceration or having a rough and irregular surface are suspicious for dVIN (9,11). Patients are often symptomatic with a long-lasting history of LS or LP-related symptoms of vulvar itching and/or burning (11).

Because of the highly malignant potential of dVIN, any suspicious area in patients affected by LS or LP should be biopsied or excised without delay to obtain a representative histopathological diagnosis.

Histology

Usual type vulvar intraepithelial neoplasia. Histopathologically, uVIN can be classified into different subtypes: warty and basaloid. Both can be easily recognized. Typically, the epidermis is thickened and is accompanied by a surface reaction of hyperkeratosis and/or parakeratosis. There is loss of cell maturation with associated nuclear hyperchromasia, pleomorphism and numerous mitotic figures at all levels of the epidermis (9). The intraepithelial process may also involve the underlying skin appendages (68,69). The epidermis of warty VIN has wide and deep rete ridges, often reaching close to the surface, which gives a characteristic condylomatous appearance. There is striking cellular polymorphism and evidence for abnormal cell maturation. Koilocytosis, corps rounds, multinucleation, (abnormal) mitotic figures and acanthosis are common (9). Basaloid VIN is characterized by a thickened epithelium with a relatively flat and non-papillomatous surface. Large numbers of relative uniform undifferentiated cells with a basaloid appearance are seen in the epidermis. Mitotic figures are numerous, but koilocytic cells and corps rounds are less frequently seen than in warty VIN. Patterns of warty and basaloid VIN are often found in the same lesion, which is referred to as mixed VIN (9).

Differentiated type VIN can be easily mistaken for a benign dermatosis because of the high degree of cellular differentiation and absence of widespread architectural disarray (5,9,11,62). Histological characteristics include a thickened epithelium with parakeratosis, elongated and anastomizing rete ridges and enlarged abnormal keratinocytes with premature eosinophilic cytoplasmic differentiation (9,11). These abnormal keratinocytes are confined to the basal and parabasal layers and are a hallmark of dVIN (9). No studies have been performed to investigate the reproducibility of the histopathological diagnosis of dVIN. Staining with MIB1 (Ki-67), a marker that visualizes proliferating cells, can be helpful in distinguishing dVIN from normal vulvar epithelium. The basal layer of dVIN is positive for MIB1, while normal vulvar epithelium is characterized by an almost MIB1-negative basal layer (70). Another tool that might be helpful is staining for p53 protein. Alteration in the p53 tumor suppressor gene appears to be involved in the development of dVIN and overexpression of p53 has been demonstrated in dVIN (11).

Psychosexual impact

Since the incidence of VIN has increased dramatically – particularly in younger women (16) – attention should be paid to the psychosexual consequences of VIN and vulvar excision. However, the number of studies concerning psychosexual impact of VIN and vulvar surgery is limited. In general, surgical treatment of vulvar lesions may lead to disfigurement, postoperative loss of quality of life (QoL) and impaired sexual function (71–73). In addition, sexual function is correlated to the extent of excision (73,74). Therefore, treatments that preserve the anatomy of the vulva such as imiquimod or CO2-laser vaporization can be important to prevent psychosexual sequelae. However, large studies comparing the effect on QoL of various treatments for VIN are lacking. A recent study evaluated the prevalence of
psychological morbidity in women with VIN. Moderate-to-severe anxiety was observed in 32% of women and moderate-to-severe depression in 18% (75). These psychological factors were strong determinants of QoL, while clinical variables such as duration of disease, presence of symptoms and number of treatments did not have a measurable impact on QoL (75). In conclusion, clinicians should give attention to the psychological and sexual consequences of VIN treatments. Future studies should focus on predictive factors that impair sexual function and QoL after excision for VIN and investigate whether outcomes on QoL and sexual function are better following anatomy- and function-preserving treatments.

Treatment modalities

Usual type vulvar intraepithelial neoplasia. For a long time, choice of therapy for uVIN has been dominated by the premalignant nature of the disease. In the past, extensive surgery such as vulvectomy has been performed to remove the disease. However, surgical margins are often positive, irrespective of the type of surgery, and high recurrence rates are common (64,76,77). In 1995, Kaufman addressed the importance of individualization of treatment. Treatment should be directed towards preservation of the normal anatomy and function of the vulva (78). Therefore, more limited surgery consisting of surgical removal of all visible lesions has been the surgical technique of choice since the last decades (78). Surgical treatment can be performed with different techniques. Cold knife surgery or CO₂-laser vaporization have been used as a single technique or in combination. Laser vaporization can be an effective method in non-hair-bearing areas. But because this technique destroys all tissue, it is recommended to take representative biopsies beforehand (1,76). In conclusion, surgical treatment is effective in removal of premalignant lesions, but recurrence rates are high and one has to be aware of the effect of surgical treatment on QoL and sexual function.

The main advantages of medical treatment are preservation of vulvar anatomy and function. Topical treatment is attractive because it can be applied directly by the patient and is easily monitored for efficacy. However, medical treatment does not provide a specimen for histological evaluation with the risk that early invasion is overlooked. Hence, taking accurate biopsies is important before starting medical treatment.

Imiquimod 5% cream is a topical immune response modifier that acts by binding on Toll-like receptor (TLR) 7 on the cell surface of dendritic cells, thereby inducing secretion of pro-inflammatory cytokines. This results in a profound tumor-directed cellular immune response (79,80). The effectiveness of imiquimod has been assessed in several studies. A systematic review showed the results of 210 patients from 17 studies (1 RCT, 10 case series and 6 case reports). Treatment duration ranged from 3 to 32 weeks and follow-up from 1 week to over 30 months. Complete regression was observed in 26–100% of patients, 0–60% had partial regression and 0–37% experienced recurrence. Most common adverse events were local burning and soreness (81). Recurrence data of imiquimod treatment compared with data from a historical cohort of surgically treated patients showed that the recurrence rate after 16 months’ follow-up was 20.5% for imiquimod treated patients and 53.5% for surgically treated patients (82). Two double-blind RCTs comparing placebo and imiquimod were performed (83,84). In the first, 31 patients were treated with an escalating dose regimen during 16 weeks. Complete regression was observed in 81% and partial regression in 10% of the imiquimod treated patients, while none of the patients treated with placebo showed a response. No progression to invasive disease was observed (83). In the second RCT, a reduction in lesion size was observed in 81% of cases (35% complete responders, 46% partial responders), in comparison with 0% in the placebo group ($p < 0.001$). In addition, no HPV DNA could be detected anymore in 58% of imiquimod treated patients. Two patients treated with placebo and one treated with imiquimod progressed to invasion (<1 mm). Reduction of lesion size was correlated with partial normalization of the numbers of immunocompetent cells (84). It was shown that imiquimod increases the magnitude of the HPV16 specific CD8+ T-cell activity in VIN patients, but magnitude and specificity of the response had no correlation with the clinical response (85). Another study investigated the role of HPV16-specific interferon-γ (IFN-γ) associated CD4+ T-cell immunity in the clinical effect of imiquimod treatment (86). No enhanced HPV16-specific CD4 T-cell response was seen upon imiquimod treatment, but, interestingly, a preexisting HPV-specific type 1 T-cell response was associated with a more favorable clinical outcome upon imiquimod treatment (86). Imiquimod is now recommended as a first-line treatment for VIN. However, long-term follow-up data after imiquimod treatment have not been reported so far, but are expected in the near future.

Topical photodynamic therapy (PDT) uses a tumor-localizing photo sensitizer, 5-aminolevulinic acid (ALA), in combination with non-thermal light of an appropriate wavelength to generate oxygen-induced cell death. Its efficacy has been proved in nonmelanoma skin cancer (87). Several nonrandomized and uncontrolled studies were conducted to assess the efficacy in uVIN. Response rates vary from
As indicated by the number of treatment options for VIN, none is 100% effective and a lot of treatments are a burden for the patient. Consequently, some patients may not want to undergo treatment. A wait-and-see policy, aimed at controlling symptoms and prevention of malignancy, is an option (106). Frequent follow-up visits are advised including careful examination and biopsies in case of suspected malignancy.

Since the host immune response plays an important role in clearance of HPV, prophylactic vaccination could be effective in prevention of HPV-related disease. In order to prevent infection and subsequent development of CIN and cervical cancer, several vaccines have been developed during recent years. There is a quadrivalent vaccine, which acts against HPV 16, 18, 6 and 11, and a bivalent vaccine, which acts against HPV 16 and 18. In a combined analysis of three randomized trials, vaccination with the quadrivalent vaccine was 97% effective in preventing VIN associated with HPV 16 and 18 in a population that was naïve to these viruses at time of first vaccination and 100% effective in a population that was naïve throughout completion of the vaccination regimen. In the intention-to-treat population (which included women who, at day 1, could have been infected with HPV16 or HPV18), vaccine efficacy was 71% (107). There are no data on the prevention of uVIN by the bivalent vaccine.

**Differentiated type vulvar intraepithelial neoplasia.** The preferred treatment for dVIN is radical surgical excision, as it occurs often in correlation with invasive SCC. Follow-up should take place at a specialized vulvar clinic or by a vulvar specialist who has had additional training in managing vulvar disease (108).

**Malignant potential**

**Usual type vulvar intraepithelial neoplasia.** Although dVIN has a much higher malignant potential than uVIN (8,11,57,67), the malignant potential of uVIN should not be underestimated. It has been shown that uVIN is highly proliferative (109). A meta-analysis showed that 8/88 (9%) untreated uVIN patients developed invasive SCC within 1–8 years (64). Others observed a much higher percentage, 10/63 (15.8%) of untreated patients progressed in 1.1–7.3 years (76). In treated patients, rate of progression during follow-up after treatment was 3.3% (208/3322) (64). Progression rate was not affected by the surgical extent (64). A population-based study from Norway showed that free resection margins did not prevent progression to invasive SCC. In this study, 50% of patients who developed vulvar SCC after treatment had free margins.
surgical margins (15). Therefore, one should not enlarge the extent of resection to prevent progression.

The malignant potential of uVIN is also illustrated by the finding of occult carcinomas in VIN. A recent study showed an occult cancer rate in vulvar biopsies of 3.8% for VIN 2 and 11.9% for VIN 3 (the term uVIN was not used) (110). Other studies reported occult carcinoma rates of 3.2–18.8% (64,111). Known risk factors for progression of uVIN are advanced age, raised lesions, immunosuppression and radiotherapy (8,64,112). In addition, it has been suggested that basaloid type uVIN is of greater risk to progress than warty type (5).

Spontaneous regression has also been described in patients with uVIN. It was observed in 1.2% of patients, all were younger than 35 years and often it was related to pregnancy (64), usually presenting with multifocal pigmented lesions (76).

Differentiated type vulvar intraepithelial neoplasia. A recent study showed that the overall percentage of dVIN lesions with subsequent diagnosis of SCC was 32.8% while this was 5.7% for uVIN lesions. Furthermore, median time from dVIN to SCC was 22.8 months, while median time from uVIN towards SCC was 41.4 months (8). The relation between a prior, synchronous or subsequent vulvar carcinoma and dVIN is three times higher than uVIN (85.7 vs. 25.7%) (57). Furthermore, vulvar cancer arising on a background of dVIN appeared more likely to recur than cancers arising from uVIN (58). Another study also noted that the prognosis in terms of 5-year disease-free and overall survival rate were worse in patients with vulvar SCC associated with LS and squamous cell hyperplasia ‘with or without atypia’ (the term dVIN was not used) than in patients with uVIN associated SCC (113).

Conclusion

The incidence of VIN has increased. It is of critical importance to distinguish uVIN from dVIN. Both subtypes have a different clinical appearance, pathology and – most important – malignant potential. Radical surgical excision is treatment of choice for dVIN, while a more conservative approach is recommended for uVIN lesions. UVIN occurs predominantly in younger women and an individual approach, including attention for the psychosexual sequelae, is important. Because of high recurrence rates after various treatments, a thorough follow-up regimen by trained vulvar specialists is recommended.

Recently, most western countries introduced prophylactic vaccination against HPV, which can potentially prevent most HPV-related premalignant lesions and about one-third of all vulvar carcinomas. In the mean time, further studies are needed to improve treatment outcome.

Vulvar Paget’s disease

In 1986 the ISSVD classified vulvar extramammary Paget’s disease (EMPD) as a non-squamous intraepithelial lesion of the vulva (3). Vulvar EMPD is a relatively rare intraepithelial carcinoma. It affects mainly postmenopausal women, with a median age of 72 years. The most common signs and symptoms are itching, burning, moistening and bleeding, for up to 5 years (114–118). Substantial delay between appearance of symptoms and diagnosis can occur in many patients, and this is significantly associated with larger lesions (119). It is usually multifocal and may occur anywhere on the vulva, mons, perineum, perianal area or inner thigh. There are often multiple extensive lesions presenting as moderately well demarcated, scale, moist, eczematoid, erythematous-white plaques often dotted with small, pale islands.

Vulvar EMPD predominantly is an intraepithelial lesion, but has the potential for dermal invasion and on occasion has been associated with an underlying adenocarcinoma. In one large study, 26% of patients had other primary tumors, such as breast, pancreas, endometrium, bladder, stomach and rectum malignancies. Associated vulvar adenocarcinoma (4%) and invasive vulvar EMPD (16%) may frequently coexists and recurrence rate of vulvar EMPD is high (35%) (114). Like in squamous cell carcinoma of the vulva, there is evidence to support the recognition of a category of minimally invasive vulvar EMPD (≤ 1 mm depth of invasion) that has a low risk of distant metastasis and death caused by disease (120,121).

Histopathological examination shows epidermal acanthosis and elongated rete ridges. Paget’s cells are large intraepidermal cells with a large nucleus that often has a prominent nucleolus and abundant usually clear, mucin positive, pale cytoplasm. The cells may occur singly in small clusters or large nests. The squamous epithelium is often hyperplastic with hyper- or parakeratosis. The Paget’s cells may extend into the adnexal duct and pilosebaceous units (114,118,121) and they may be a proliferation of adnexal stem cells residing in the infundibulo-sebaceous unit of hair follicles and adnexal structures (122). It has been suggested that at least a proportion of vulvar EMPD arises multicentrically within the epidermis from pluripotent stem cells (123). There is evidence that vulvar EMPD represents a
heterogeneous group of epithelial neoplasms that can be similar both clinically and histopathologically. Vulvar EMPD can be classified based on the origin of the neoplastic Paget’s cells as either primary (of cutaneous origin), arising within the epithelium of the vulva, or secondary (of noncutaneous origin), resulting from the spread of an internal malignancy, most commonly from an anorectal adenocarcinoma or urothelial carcinoma of the bladder or urethra, to the vulvar epithelium. Primary EMPD can be further subdivided into a primary, intraepithelial cutaneous form with and without invasion and into an intraepithelial cutaneous Paget’s disease as a manifestation of underlying skin appendage adenocarcinoma. Secondary EMPD has an anorectal, urothelial or other origin. These subtypes can present similarly on the skin and may appear similar on routine hematoxylin and eosin-stained slides. Immunohistochemical studies can be used to help differentiate them. Primary vulvar EMPD is immunoreactive for CK 7 and GCDFP-15, but uncommonly for CK 20. Vulvar EMPD secondary to anorectal carcinoma demonstrates CK 20 immunoreactivity but is usually nonreactive for CK 7 and consistently nonimmunoreactive for GCDFP-15. Vulvar EMPD secondary to urothelial carcinoma is immunoreactive for CK 7 and CK 20 but nonimmunoreactive for GCDFP-15. In addition, UP-III, which is specific for urothelium, is immunoreactive in secondary vulvar EMPD of urothelial origin. The distinction is important in that the specific diagnosis has a significant influence on current treatment (124,125).

Wide local excision is the usual treatment of EMPD to a depth of 4–6 mm to include the pilosebaceous units and skin adnexal structures. Patients with an underlying adnexal adenocarcinoma or stromal invasion of EMPD over 1 mm should be treated more aggressively, with excision to the fascia in the involved area, and inguinofoemoral lymphadenectomies bilaterally. Vulvoperineal reconstruction may be necessary by means of skin grafts, local skin flaps, muscle flaps and different fasciocutaneous flaps (126). Based on small series, topical 5% imiquimod cream has been shown as a safe treatment and may induce complete responses in primary or recurrent vulvar EMPD. The therapeutic schedule used varies. A daily application for three weeks, followed by an every other day application for an additional three weeks seems to be effective (127–129). The role of photodynamic therapy (130,131) in the multimodal approach to extensive or recurring vulvar EMPD is still unclear. Radiation therapy in selected cases may be feasible and effective (132). Overexpression of the HER-2/neu protein has been found in about 30% of vulvar EMPD cases. Targeted therapy with trastuzumab may be considered as a possible new therapeutic strategy in selected cases of vulvar EMPD showing overexpression of HER-2/neu. Treatment can result in a significant regression of disease and resolution of symptoms (133). Vulvar EMPD with Her-2/neu expression shows higher recurrence rates suggesting a more aggressive behavior (134).

Recurrences are common (114,135) and may relate to the fact that the extent of histologically demonstrable disease is far greater than that of the visible lesion, the outline of the involved area is highly irregular and multiple foci of disease are present (136). There is no correlation between disease recurrence and margin status, thus disease recurrence is common, regardless of surgical margin status (118,132,137). Disease involving the perineum is suggested to be a significant risk factor for recurrences. Intra-operative frozen section analysis of the margins as well as radical surgery as initial treatment seems not to reduce recurrence rate (132). Long-term monitoring is recommended, as recurrences are common and can be noted many years after the initial treatment and repeat surgical excision is often necessary.

**Melanoma in situ of the vulva**

Melanoma in situ (MIS) is rare on the vulva and appears to have a relatively slow but definite progression to invasive melanoma (138). The ABCDE scheme for recognition of melanoma should be considered in pigmented lesions (Asymmetry, Border irregularities, Color variation, Diameter >6mm, Enlargement or Evolution of color change, shape or symptoms) (139,140). All suspicious pigmented lesions in this region should be biopsied and a punch biopsy is the preferred method because establishing the depth of such lesions is critical. Destruction by cryosurgery, cautery or laser is contraindicated, and all such lesions must undergo histopathological evaluation. Small lesions often can be completely excised, and when sampling hyperpigmented areas, a biopsy of the thickest region is recommended (138,141). If the diagnosis is considered within the differential diagnosis of pigmented vulvar lesions, it is easy to recognize and treat, with excellent prognosis (142). An excisional biopsy of the entire clinically apparent lesion, with a narrow 1- to 2-mm margin of adjacent normal-appearing skin, is the biopsy technique of choice when melanoma is suspected, and shave biopsies should be avoided. An incisional biopsy may be acceptable for larger lesions, since studies have shown no worse prognosis if the initial biopsy does not remove the entire lesion, which is later excised (143,144). In MIS the proliferating malignant melanocytes are confined to the epidermis. Although an
in-situ phase exists for three of the four invasive forms of melanoma, superficial spreading melanoma, lentigo maligna melanoma and acral lentiginous melanoma, for the clinician it is irrelevant, since it should be removed anyway. For patients with MIS, 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 (143,146).

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