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GYNECOLOGIC CANCERS (NS REED, SECTION EDITOR)

New Directions in Vulvar Cancer Pathology

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Abstract

Purpose of Review The aim of this article is to provide clinicians and pathologists with an understanding of the aetiopathology, pathogenesis and classification of vulval neoplasia and their molecular correlates.

Recent Findings There is an increased understanding of subcellular changes in vulvar malignancies. These provide the direction for further research and aid personalised treatment for patients.

Summary The article explores concepts of the aetiology of vulvar cancer and updates the reader with the equivalence of terminology of preneoplastic vulval disease. The differential diagnosis of squamous neoplasia and their clinicopathological correlation is detailed. The salient findings from recent literature into the understanding of the disease of squamous cell neoplasia and rare vulvar malignancies are summarised.

Keywords Vulvar cancer · Vulvar intraepithelial neoplasia (VIN) · Lichen sclerosus · Human papilloma virus (HPV) · Sentinel node biopsy

Introduction

Vulval neoplasms account for 3–5% of all gynaecological malignancies, with an annual incidence of 1–2 per 100,000 women [1]. Around 80–90% of these tumours are vulval squamous cell carcinomas (VSCCs). Malignant melanomas, Bartholin gland carcinomas, invasive Paget disease and basal cell carcinoma are less frequently encountered malignancies. Other tumour types, such as sarcomas and lymphomas, are extremely rare [1, 2].

The incidence of VSCC has remained relatively stable over the last three decades whilst the reporting of VIN has increased. Analysis of the SEER (Surveillance Epidemiology and End Results) database between 1973 and 2000 found that increased recognition, by clinicians and pathologists, resulted in 411% increase in the diagnosis of VIN whilst diagnosis of VSCC increased by 20% [3].

Pathogenesis

VSCCs are believed to have two predominant aetiopathogenetic pathways. Numerous classification schemes have been used over the years, and the present classification is based on these pathways (Table 1).

HPV-positive VSCC that develops from usual-type vulval intraepithelial neoplasia (uVIN), which is also referred to as classical VIN, is defined by infection with high-risk HPV. This pathogenetic pathway which accounts for 20–40% of VSCC is commoner in younger women, more often located in the perineum [7•] and is associated with smoking and immunosuppression. The risk of the progression of uVIN to VSCC seems low, occurring in 9–16% of patients who do not receive treatment and in approximately 3% of patients who have been treated [8]. Introduction of HPV vaccination in several parts of the globe is expected to result in the reduction of HPV-related neoplasia [9]. The other pathway for the development of VSCCs is not associated with HPV infection and is referred to as the HPV-negative pathway. The preneoplastic condition that is most associated with this type

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Table 1 Classification of squamous precursor lesions of the vulva incorporating LAST (2012), WHO (2014) and ISSVD (2015) terminologies

Contemporary terminology [4–6]	Terminology in previous classification schemes
Low-grade squamous intraepithelial lesion (LSIL)	Condyloma, flat condyloma
VIN1	Mild dysplasia
	Koilocytic atypia; HPV effect
High-grade squamous intraepithelial lesion (HSIL)	Moderate and severe dysplasia
uVIN2, uVIN3	Bowen disease
	Bowenoid dysplasia
	Carcinoma in situ
Differentiated-type vulval intra-epithelial neoplasia (dVIN)	

is differentiated vulval intraepithelial neoplasia (dVIN). It occurs in older women and associated with inflammatory dermatoses, mostly lichen sclerosus. Up to 3–5% of women with lichen sclerosis appear to develop VSCC. This aetiology-based classification appears to be clinically significant, and HPV-related carcinomas have a better overall outcome than non-HPV-associated tumours [10, 11]. dVIN comprises less than a third of vulval preneoplasia whilst most VSCCs are non-HPV dependent [12•]. This seeming paradox is explained by the greater likelihood of progression of dVIN to invasion [13]. Histologically, dVIN is often seen adjacent to squamous cell carcinoma. dVIN can occur in a field change of lichen sclerosus and is associated more often with synchronous or metachronous VSCC [14]. VSCC occurring in a background of dVIN tends to recur [15, 16•].

Genetic and Epigenetic Changes

Few studies have looked at genetic alterations in VIN and VSCCs beyond HPV status. This area of research is in evolution. At this time, perhaps due to different methods of testing, the results are varied. Using next-generation sequencing (NGS), in a study of 118 VIN and VSCCs with a follow-up cohort of 236 patients, Nooij and colleagues [17] showed that the mutation frequency in HPV-positive cancers is significantly lower than that in HPV-negative cancers. The latter can be broadly divided into p53 mutant and p53 wild-type profiles with p53 wild-type identified as a distinct clinicopathologic subgroup with frequent *NOTCH1* mutations. A more recent study [18] showed that pathogenic mutations were detected at comparable frequencies in hrHPV(+) and hrHPV(−) tumours in 13/50 genes examined. They suggest that genetic mechanisms of the two routes of VSCC pathogenesis may be similar. A limited number of studies are available on epigenetic changes in vulval lesions, with hypermethylation of CDKN2A being the most frequently investigated change. DNA methylation is regulated by three enzymes known as DNA methyltransferases (DNMT). It has been shown that DNMT3A overexpression is associated with HPV-negative VSCC and an increased risk of local vulval recurrence [19]. Hedgehog (Hh) signalling pathway dysregulation has been described in HPV-associated cancers. In a study of 91 cases, the key components of Hh signalling pathway were overexpressed and underexpression of one of the components (PTCH1) was associated with a greater risk of local recurrence [20].

Preneoplastic Lesions

HPV-Related Squamous Lesions

uVIN is associated with HPV infection. Additional risk factors for the development of uVIN are tobacco smoking and immunosuppression, including HIV infection. The most common HPV types in high-grade squamous intraepithelial lesion (HSIL)-VIN2/3 and vulval carcinoma described in a meta-analysis of published literature encompassing a variety of detection methods were HPV-16 (71.9%), HPV-33 (8.0%) and HPV-18 (5.0%). Low-grade squamous intraepithelial lesion (LSIL)-VIN1 is associated with infection with a broader range of both low- and high-risk HPV types. The most common HPV types in VIN1 were HPV-6 (22.4%), HPV-16 (9.8%) and HPV-11 (9.0%) [21]. Prophylactic vaccination against HPV6, 11, 16 and 18 has been shown to result in a decrease in the development of vulval lesions [22], and it should be anticipated that the relative proportions of HPV- and non-HPV-associated disease will alter as vaccine coverage increases and with the use of vaccines protecting against additional HPV types.

Histology of uVIN

VIN1/LSIL

These are squamoproliferative lesions that show features of productive HPV infection. The lesions are characterised by hyperkeratosis, parakeratosis, anisonucleosis and koilocytosis. Although therapeutic strategies have variable success, the

lesions usually regress with a very low risk of progression to cancer.

VIN2,3/HSIL

VIN2,3 is usually seen as discrete macular, papular or condylomatous lesions which may be pigmented and erythematous. They show acetowhite change. Up to two-thirds are multifocal. VIN2 and VIN3 are characterised histologically by variable hyper and parakeratosis overlying crowded epithelial cells with hyperchromasia and anisonucleosis with suprabasal and atypical mitoses. The lesions are graded by the extent of maturation: VIN2 showing lack of maturation up to the middle third of the epidermis whilst in VIN3 the changes extend into the upper third. Basaloid and warty morphologic subtypes are described and both represent high-grade lesions. In hair-bearing skin, colonisation of hair follicles is common and there is often an inflammatory infiltrate in the superficial underlying stroma [12•].

Ancillary Studies in uVIN

The distinction between uVIN and dVIN is mostly straightforward; however, the description of a basaloid variant of dVIN (lacking maturation and potentially mimicking uVIN) [23] or the co-existence of the changes of lichen simplex chronicus supervening on uVIN (with increased apparent maturation) [24] may create diagnostic difficulty. Since the distinction is consequential with regard to the risk of recurrence and progression, ancillary diagnostic studies may be useful. Immunohistochemistry for p16, a cyclin-dependent kinase inhibitor that accumulates in transforming HPV infection, correlates strongly with HR HPV infection. The positive predictive value for progression of low-grade lesions is poor, limiting its use as a prognostic marker. Stathmin 1 (oncoprotein 18) is a microtubule-destabilising protein with normal function in mitoses and cell motility, which is overexpressed in a range of malignant neoplasms. This has been shown to have increased sensitivity, relative to p16, in distinguishing LSIL from HSIL [25].

Non-HPV-Related Squamous Lesions

Differentiated-type VIN (dVIN) typically occurs in postmenopausal women in the sixth to eighth decades of life but can occur in younger patients. dVIN tends to be unicentric at presentation and is often associated with adjacent lichen sclerosus (LS) and/or chronic inflammatory dermatoses [26].

Histology of dVIN

dVIN is characterised by non-uniform elongation of rete ridges, cytological atypia of the basal layer and basal and suprabasal mitoses including atypical mitotic forms [16•]. Altered maturation is characterised by individual cell keratinisation, abnormal low keratinisation and deep squamous eddies or keratin pearls. The histological changes in dVIN can be subtle. Although good agreement is obtained by pathologists who are experienced in reporting vulval pathology, interobserver variation can be poor [27, 28].

Ancillary Studies dVIN

There are no definitive markers for dVIN. When considering a diagnosis of dVIN, staining for p53, Ki67 and p16 may be helpful [29]. Diffuse strong p53 staining of the basal layer with suprabasilar extension has been described in about 85% of cases [30]. Complete loss of staining (null pattern) has also been described [31]. In contrast, non-VIN epithelium shows wild-type staining, which is identified as staining of variable intensity in the nuclei of basal cells. Whilst aberrant p53 expression can be appreciated in resection specimens where adjacent normal epithelium is available, it can be difficult to interpret in small biopsy specimens where 'normal' epithelium is not available for assessment. p53 and Ki67 lack specificity and sensitivity, and they are most strikingly abnormal in those cases that are obvious on conventional histology. Increased p53 staining can also be seen in LS and LSC [32].

Recent studies support the use of CK17 (a cytokeratin that marks the basal layer) as a useful marker in the diagnosis of dVIN. In a study of 29 cases [33] of dVIN, 28 cases (97%) showed strong, diffuse expression, which sharply terminated at the junction with normal epithelium. UVIN and LSC showed staining only of superficial layers. CK17 may be useful in conjunction with morphology especially in distinguishing dVIN from LSC. Complete absence of another cytokeratin, CK13, is seen in less than 20% of dVIN but is fairly diagnostic [34].

Differential Diagnosis of dVIN

dVIN presents clinically as foci of grey-white discoloration with a rough surface, vaguely defined as thick white plaques, or elevated nodules [26]. The histological changes of dVIN can be subtle. The differential diagnoses associated with this clinical appearance and need to be distinguished from dVIN are the following:

Lichen simplex chronicus

This is not a specific diagnosis but a description of thickening of the skin due to rubbing and scratching; it may occur in any pruritic dermatoses. It typically shows hyperplasia of the squamous epithelium with a prominent granular layer and hyperkeratosis. LSC lacks basal atypia and shows normal maturation—features that assist in differentiation from dVIN.

Squamous cell hyperplasia

This is characterised by acanthosis, 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 or parakeratosis [16•].

Psoriasis with prominent acanthosis

The acanthosis noted in psoriasis is uniform and associated with the presence of neutrophils in the parakeratosis. Basal atypia and abnormal keratinisation are not seen [16•].

Verrucous carcinoma

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. They lack infiltrative invasion thus resulting in confusion with an in situ disease.

Vulvar acanthosis with altered differentiation

It is characterised by marked acanthosis with verruciform architecture, loss of the granular cell layer with superficial epithelial cell pallor and plaque-like layers of parakeratosis [35]. Lack of atypia distinguishes VAAD from neoplastic and preneoplastic conditions. The verruciform outline of VAAD is not a feature of psoriasis.

Atypical verruciform lesions

There are lesions that are associated with acanthosis or verruciform growth pattern with abnormal keratinocyte differentiation but do not show conspicuous nuclear atypia. Such lesions are seen adjacent to nearly 30% of non-HPV-associated VSCC. These lesions do not fit neatly into the categories of differentiated VIN, verruciform carcinoma, vulvar acanthosis with altered differentiation or lichen simplex chronicus. They do not show the significant basal atypia that is seen with dVIN. They lack p53 mutations. On next-generation sequencing, 73% show PIK3CA mutation. They are collectively referred to as differentiated exophytic vulvar intraepithelial lesion [36].

Warty uVIN

A variant of uVIN, this is characterised by a papillary surface with hyperkeratosis and parakeratosis. This appearance mimics dVIN. Most of these cases appear to be associated with HPV 16. Accentuated p53 wild-type staining shown by intense but intermittent nuclear staining can be seen and mistaken for p53 overexpression and confusion with dVIN [37].

Lichen Sclerosus

End-stage lichen sclerosus shows squamous epithelium that is typically thinned and a sub epithelial layer of homogenised eosinophilic stroma with a band of lymphocytes deep to this. Diagnosis of early lichen sclerosus can be a challenge to histopathologists. The squamous epithelium may show mild irregular, occasionally psoriasiform acanthosis and focal basement membrane thickening. The squamous epithelium may even be thickened and hyperkeratotic. Wide ectatic capillaries can be seen in dermal papillae. The lymphocytic infiltrate can be sparse or dense. Superadded eczematous changes and changes of lichen simplex chronicus may complicate the morphological picture. Good clinical information, high index of suspicion, knowledge of differentials and performing serial sections are needed to arrive at the diagnosis.

Squamous cell carcinoma (SCC) of usual and verrucous appearance has been described in genital LS. SCC is not associated with extra-genital LS. In the literature, the incidence of SCC in vulval LS is reported to be between 0.3 and 4.9% [38, 39]. More recent literature suggests that the risk is lowered in well-controlled disease [40, 41]. Patients with an overlap of LS and lichen planus appear to be at increased risk of the development of VSCC [42].

P53 and cell cycle regulation markers have been studied in lichen sclerosis. The results show an overlap between normal vulva, LS and dVIN [43]. There is no robust relation to outcome. Recently, a study of microRNA (miRNA) has shown results that may provide new insights into the disease. The researchers showed that miR-155-5p is notably upregulated in LS and promotes cell proliferation by targeting both FOXO3 and CDKN1B [44]. At this time, it appears that LS itself may act either as initiator or promoter of carcinogenesis through HPV-independent mechanisms.

Prognostic Features in Invasive Squamous Cell Carcinoma

Conventional histological findings that predict poor outcome are stage of disease and lymph node status. More recently, it

has been shown that even after adjustment for stage, patients with perineural invasion have a greater risk for death and progression [45]. The biological pathways that underlie the development of vulval squamous cell carcinoma are increasingly well described, offering the opportunity for targeted therapy. Dysregulation of cell cycle control via p53- and pRb-mediated pathways may be common to both HPV-dependent and independent lesions. Aberrant cyclin D1 expression is described in both VIN and VSCC and has been associated with depth of invasion of tumour and the presence of regional metastases [46]. The role of tyrosine kinases is described in the development and progression of VSCC. cKit expression is associated with longer overall survival [47]. EGFR is commonly expressed in vulval squamous cell carcinoma, but the relationship between HPV and the EGFR pathway is not completely clear since the evidence relating expression to tumour grade stage and survival is variable [48]. VEGF expression may be a crucial factor in the evolution of VIN to VSCC, with the reduction of VEGF mRNA expression within invasive lesions. Tumour vascularity is associated with size and depth of invasion and hypoxia-inducible factor (HIF)1a expression has been shown to be an independent prognostic factor. Comparative genome hybridisation may also reveal novel prognostic markers [49]. A study based on comparative genome hybridisation (aCGH) and genome-wide expression array identified two novel prognostic markers in vulval cancer, one with favourable prognosis (GNB3) and the other with unfavourable prognosis (PLXDC2) [50].

Paget Disease

Paget disease is an uncommon, intraepithelial adenocarcinoma, which arises most commonly on the vulva, usually in postmenopausal Caucasian women. Most lesions arise from a pluripotent epidermal stem cell within the interfollicular epidermis or folliculo-apocrine-sebaceous units. Occasionally, origin from an underling skin appendage adenocarcinoma or carcinoma of anorectal or urothelial origin is seen. In the majority of cases, disease is confined to the epithelium but in up to 20% of cases there is an invasion into the underlying stroma although the risk of progression to invasive disease or metastasis following treatment for noninvasive PD is low. The lesion is characterised by an apparently well-demarcated, painful and erythematous eczematoid lesion, usually on the labia majora. Histologically, there is a population of large round cells with pale cytoplasm and nuclei with prominent nucleoli distributed throughout the epithelium as single cells or clusters. The tumour cells express cytokeratin 7, carcinoembryonic antigen and apocrine cell marker GCDFP15, investigations which are of use in distinction from other intraepidermal neoplasms such as malignant melanoma in situ [51].

The borders of the lesions seen clinically correlate poorly with the histological extent of the disease, which may account for the high rate of recurrence after primary surgery (up to c 35%), which remains the mainstay of treatment. Margin controlled surgical procedures such as Moh's micrographic surgery show a lower incidence of recurrence [52].

A variety of topical therapeutic approaches to treatment include 5 FU, imiquimod and chemotherapeutic strategies include trastuzumab, paclitaxel, vincristine, cisplatin, carboplatin, etoposide and docetaxel. Data on the pathogenesis and potential specific targetable driver lesions within PD are, however, limited. Androgen receptor may be detected in over half of vulval PD cases and represents a potential therapeutic target. Overexpression of HER2/neu (ERBB2) is present in at least one-third of PD lesions and potentially in a subgroup with a worse prognosis with respect to invasion, recurrence and nodal metastasis, but further clarity is needed to establish precise biological significance [53]. Alterations in MMR genes may also be common in a subset of patients with PD and may be involved in its pathogenesis (with germline mutations in 34% and somatic mutations in 13%) [54].

Malignant Melanoma

Melanoma is the second most common malignancy arising in the vulva, accounting for up to 10% of primary vulval malignancies [1]. Primary vulval melanoma is uncommon compared with those at UV-exposed sites (with a ratio of sun-exposed skin to vulva melanoma of 71:1) and is diagnosed at an older age. Up to 40% of women present with regional or distant metastasis. Compared with cutaneous and non-gynaecological mucosal melanomas, prognosis is relatively poor (5-year survival of 58% for vulval melanoma compared with up to 81% for cutaneous disease) [55]. Lesions are typically asymmetric, with irregular borders and uneven pigmentation and there may be surface ulceration. Up to 25% may be amelanotic. Adverse prognostic factors are advanced clinical stage, Breslow thickness greater than 1 mm, vertical growth phase, ulceration and mitotic index over 1 per mm sq. Microsatellite lesions and perineural invasion are associated with increased local recurrence.

Understanding of molecular alterations within melanoma has led to the expansion of treatment options and increased survival. Mucosal melanomas are genetically distinct from those in the skin, more commonly showing KIT and NRAS mutations [56]. Vulvovaginal melanoma appears to be different, from both cutaneous melanoma and that from other mucosal origins. BRAF mutations are described at different rates within the literature, perhaps reflecting detection techniques or the evolution of lesions with the presence of metastatic

disease. One recent paper demonstrated BRAF mutation in 26% of vulvovaginal melanoma, only half of which had BRAF V600E mutations. PDL1 (56%) and PD1 (75%) were among the most frequent markers expressed, highlighting the potential use of immunotherapy [57]. Low level of mutations in BRAF, KIT and NRAS has been noted in vulval and vaginal melanomas in comparison with their cutaneous counterparts, confirming these melanomas are genetically distinct from cutaneous melanoma [58].

Rarer Vulval Malignancies

Rarer vulval malignancies include tumours arising from Bartholin and other specialised anogenital glands, neuroendocrine tumours including Merkel cell tumour, soft tissue tumours including rhabdomyosarcomas and leiomyosarcomas as well as lymphomas and metastatic. Vulval cancer registry data are useful for tracking broad diagnostic categories but are less reliable for vulval cancer subtypes. It is difficult to provide an accurate estimate of the occurrence of different subtypes [59].

Bartholin gland carcinomas constitute fewer than 5% of all vulval cancers. Nearly 85% are squamous cell carcinomas with adenocarcinomas being the second commonest [60]. Rarer tumour types include adenoid cystic carcinomas [61]. When compared with VSCC, patients with Bartholin gland carcinomas tended to be younger and more likely to receive radiation. Most Bartholin gland carcinomas have an aetiological association with high-risk HPV infection [62].

Sentinel Lymph Node Biopsy in Vulval Cancer

The current standard of practice in vulval carcinoma cases higher than FIGO (International Federation of Gynecology and Obstetrics) stage IB involves radical surgery that comprises the excision of the primary malignant lesion and unilateral or bilateral superficial and deep inguinofemoral node dissection [63]. Reported groin recurrence rates after negative inguinofemoral lymphadenectomy (IFL) vary from 1.6 to 5% and are associated with lymphovascular space invasion, poor tumour differentiation, depth of invasion and a low lymph node count after IFL [64–66]. However, because only 25 to 35% of patients with early-stage disease have lymph node metastases, a significant 65 to 75% possibly do not benefit from elective IFL and the associated morbidities [67].

Lymph node involvement has been shown to represent the most important prognostic factor for recurrence and survival [68]. Although the 5-year disease-specific survival with negative inguinofemoral lymph nodes ranges from 70 to 93%, it significantly decreases to 25 to 41% in patients with involved nodes [69, 70]. Because unrecognised disease in the inguinofemoral nodes is nearly always fatal, sentinel node testing that assesses the first lymph node draining the field of malignancy is used. Indications for a sentinel node procedure [71, 72] are unifocal tumours confined to the vulva, tumours less than 4 cm in diameter, stromal invasion more than 1 mm and clinically negative groin nodes. In the laboratory, the node is entirely sampled [73]. If more than one lymph node is retrieved, then each lymph node is clearly labelled and submitted separately. If metastasis is confirmed, the largest deposit is measured and no further action is taken [63]. If there is no evidence of nodal metastasis on routine examination, ultrastaging is done. Ultrastaging is the term used to indicate extensive or complete microscopic examination of the lymph node by routine and immunohistochemical stains. This is a labour-intensive procedure, both in terms of laboratory and pathologist workload and time. The current evidence indicates it is more effective at identifying micrometastases than routine sampling. The British Association of Gynaecological Pathologists (BAGP) is currently working with the British Gynaecological Cancer Society (BGCS) to produce a protocol for handling sentinel lymph nodes that will be available on their websites.

Conclusion

Vulval neoplasia is an uncommon disease. The classification of preneoplastic changes in vulval squamous cell carcinoma, their differential diagnoses and the importance of clinicopathological correlation have been discussed. Comprehending the molecular basis of the recent advances in delineation of pathogenetic mechanisms will aid clinicians in providing stratified therapy. Rare vulval malignancies and the sentinel node technique have also been reviewed.

Compliance with Ethical Standards

Conflict of Interest Anthony Williams declares that he has no conflict of interest.

Sheeba Syed declares that she has no conflict of interest.

Shireen Velangi has received honoraria for the preparation of vulval dermatology lectures delivered during dermatology courses throughout the UK. These courses are run by Guy's and St. Thomas' NHS Foundation Trust, in association with the British Association of Dermatologists, University Hospitals Birmingham and Birmingham Children's Hospital.

Raji Ganesan declares that she has no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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