ORIGINAL ARTICLE

In the absence of (early) invasive carcinoma, vulvar intraepithelial neoplasia associated with lichen sclerosus is mainly of undifferentiated type: new insights in histology and aetiology

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Background: Differentiated vulvar intraepithelial neoplasia (VIN) is presumed to be the precursor of invasive squamous cell carcinoma (SCC) of the vulva. It is commonly assumed that differentiated VIN is related to lichen sclerosus (LS). However, evidence for this is limited to a small number of studies describing epithelial alterations adjacent to vulvar SCC.

Aim: To study the histology and human papillomavirus (HPV) status in patients with a history of both LS and VIN without coexistent SCC.

Methods: Original biopsy specimens and surgical specimens of patients retrieved from the pathology files were revised for the presence of LS, VIN and (early) invasive SCC, specifically focused on the two different types of VIN: differentiated and undifferentiated. Thereafter, VIN lesions were tested for the presence of HPV DNA.

Results: Twenty-seven patients fulfilled the criteria for LS and VIN without SCC. In all 27 patients, LS was found to be related to undifferentiated VIN. Grading yielded the following results: VIN 1 (n = 10), VIN 2 (n = 11) and VIN 3 (n = 6). Additionally, VIN lesions from 26 patients could be tested for the presence of HPV DNA. HPV DNA, predominantly type 16, was present in 8 (31%) of them. Seven of these eight patients had VIN 2 or 3. During follow-up, three patients progressed to (early) invasive carcinoma. In two of these patients, differentiated VIN was observed overlying early invasive SCC.

Conclusions: VIN related to LS without coexisting SCC is likely to be undifferentiated, in contrast to what was previously thought. HPV DNA was demonstrated in 31% of the lesions, and was strongly related to high-grade VIN.

Invasive squamous cell carcinoma (SCC) of the vulva often arises in association with other vulvar abnormalities.^{1–9} These abnormalities usually fall into two main categories, which can be considered as the main precursor states for invasive carcinoma of the vulva: vulvar intraepithelial neoplasia (VIN) and lichen sclerosus (LS).

According to the International Society for the Study of Vulvovaginal Diseases (ISSVD) and the International Society for Gynecological Pathologists, LS is a non-neoplastic disorder of the vulvar skin and mucosa.¹⁰ Although the presence of LS in the adjacent skin of vulvar SCC is suggestive of a premalignant disease, longitudinal studies report only a slight tendency for LS to evolve into SCC (1–5%).^{11–14}

VIN, on the other hand, is considered a pre-neoplastic disorder of the vulvar skin,¹⁵ although progression to invasive carcinoma remains uncertain. Data on the follow-up of untreated VIN 3 are scarce, and the natural history is mainly based on follow-up after surgery. In the only systematic review on treatment of VIN, with data on 3322 patients, progression to invasive carcinoma was seen in 9% of the untreated patients and in 3% of patients after treatment.¹⁶ VIN can be classified into undifferentiated (classic or bowenoid) and differentiated (simplex) VIN.¹⁷ Undifferentiated VIN is associated with human papillomavirus (HPV), occurs predominantly in younger patients, and tends to be a multifocal and multicentric disease, whereas differentiated VIN is not related to HPV, is usually found in older women, and is commonly unifocal and

unicentric. Differentiated VIN is rather uncommon. It is supposed to be associated with LS,^{17, 18} although evidence for this is limited to a small number of studies describing epithelial alterations adjacent to vulvar SCC.^{4, 7, 19, 20} Since differentiated VIN is often observed adjacent to or overlying superficially invasive SCC, it is presumed to be the precursor of most invasive SCCs of the vulva.¹⁸

Only four studies reported on the coexistence of LS and VIN, either differentiated or undifferentiated, without SCC.^{19–22} A major disadvantage of all the four studies is that the coexistence of LS and VIN was not the main research question, but was a coincident finding. In a series of 86 patients with LS, differentiated VIN was observed twice, as was undifferentiated VIN.²⁰ Three other studies describing the histological features of VIN mentioned the presence of LS in 41 of 437 (9%) cases.^{19, 21, 22} HPV DNA testing was performed in only one of them.¹⁹ Since these three studies involved hardly any differentiated VIN, no conclusions can be drawn regarding the type of VIN and its relationship to LS.

As the relationship between differentiated or undifferentiated VIN and LS was never deliberately investigated, and since the role of HPV is not yet clarified, we studied the histology and

Abbreviations: HPV, human papillomavirus; ISSVD, International Society for the Study of Vulvovaginal Diseases; LS, lichen sclerosus; SCC, squamous cell carcinoma; VIN, vulvar intraepithelial neoplasia

Figure 1 Patient 1: (A) vulvar biopsy with both condylomatous dysplasia and classic lichen sclerosis (LS; H&E, $\times 20$); (B) in more detail, verruciform dysplastic changes that tested positive for human papillomavirus (HPV)-59 DNA, showing mild-to-moderate disturbance of the epithelial architecture with acanthosis and koilocytosis, confirm vulvar intraepithelial neoplasia (VIN) 2 (H&E, $\times 100$); (C) classic LS in more detail, showing oedema and hyalinisation of the dermis, vacuolisation of the dermal-epidermal junction, and hyperkeratosis, with normal architecture of the squamous epithelium (H&E, $\times 100$). Patient 2: (D) local excision of the vulva in a patient with a diagnosis of VIN 3, showing undifferentiated HPV16-positive VIN in the background of LS (H&E, $\times 80$); (E) a higher magnification of (D), showing the transition of normal to dysplastic epithelium (H&E, $\times 200$); (F) histological findings of LS in the same tissue with hyperkeratosis, thinning of the epidermis, and oedema with homogenisation of the dermal stroma (H&E, $\times 125$). Patient 3: (G) coexistence of LS and undifferentiated VIN 2 in the vulva (H&E, $\times 200$); (H) a higher magnification of (G), illustrating dysplastic changes that tested negative for HPV DNA (H&E, $\times 400$).

HPV status in a large group of patients with a history of both LS and VIN without SCC.

MATERIALS AND METHODS

Patients

All cases with both histologically diagnosed LS and VIN were retrieved from the pathology files (1984–2004) of the Academic Medical Centre, the VU University Medical Centre and the Netherlands Cancer Institute in Amsterdam, and the Erasmus University Medical Centre in Rotterdam, The Netherlands. Firstly, a computer search for lichen sclerosis or sclerosis (et atrophicus) was performed (1807 specimens). Secondly, cases of women with anogenital LS were extracted from the list (1207 vulvar specimens). Thirdly, the pathology files of these patients with anogenital LS were searched for VIN without the initial presence of coexistent SCC (46 patients, 137 specimens).

Histology

Slides from the original biopsy specimens as well as those from all subsequent surgical specimens of these 46 patients were collected and revised by an experienced pathologist (FJWtK) for the presence of LS, VIN and (early) invasive SCC.

The diagnosis of LS was based on the presence of dermal hyalinisation, vacuolar alterations of the dermal-epidermal junction and a variable dense lymphocyte infiltrate, whether or not accompanied by epidermal atrophy, progressive loss of rete ridges, hyperplasia and/or acanthosis.²³ VIN terminology was used according to the classification of the ISSVD.¹⁵ We specifically looked for the two different types of VIN: undifferentiated and differentiated. Undifferentiated VIN is characterised by disorientation and loss of squamous epithelial architecture and maturation, together with a variable degree of cellular atypia. Depending on the level of cellular disarray, undifferentiated VIN was graded into VIN 1, 2 or 3. Subsequently, all revised VIN 1 cases were stained with MIB 1, a cell proliferation marker. Differentiated VIN, on the other hand, shows little or no atypia above the basal or parabasal layers, and is therefore a far more subtle lesion than undifferentiated VIN. Enlarged prematurely differentiated keratinocytes with prominent eosinophilic cytoplasm and abnormal nuclei are found deep within the epidermis, frequently near the tips of elongated and branching rete ridges. Deeply located squamous whorls with or without keratin pearls are sometimes seen.^{15 17 24 25} Differentiated VIN should be regarded as VIN 3, owing to its supposed invasive potential.^{15 24}

Figure 2 Patient 4: (A) lichen sclerosus (LS) showing a broad band of hyalinisation underlying an atrophic epidermis with loss of rete ridges (H&E, $\times 50$); (B) undifferentiated human papillomavirus (HPV) 16-positive vulvar intraepithelial neoplasia (VIN) 3 biopsied at the same time in the same patient, but in a different lesion (H&E, $\times 100$). Patient 5: (C) LS with normal epidermal maturation (H&E, $\times 100$); (D) undifferentiated HPV-negative VIN 3 in the same patient, which was diagnosed 6 years after the diagnosis of LS (H&E, $\times 64$). Patient 6: (E) classic LS showing extensive dermal hyalinisation with vacuolisation of the dermal-epidermal junction (H&E, $\times 64$); (F) undifferentiated HPV-negative VIN 1, with focal VIN 2 diagnosed 2 years after the diagnosis of LS (H&E, $\times 160$).

Depth of invasion, if present during follow-up, is described as early invasive or invasive carcinoma.

HPV testing

All confirmed dysplastic lesions were tested for the presence of HPV. To this end, we extracted cellular DNA from corresponding formalin-fixed, paraffin-wax-embedded tissue. Testing for HPV was conducted by using a standard GP5+/6+ PCR enzyme immunoassay, followed by reverse line blot analysis.²⁶ This test is clinically validated.²⁷ We used one assay for the 14 most prevalent high-risk types of HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68), and another for 22 low-risk HPVs (6, 11, 26, 34, 40, 42, 43, 44, 53, 54, 55, 57, 61, 70, 71, 72, 73, 81, 82 (both subtypes MM4 and IS39), 83, 84 and CP6108). In addition, PCR amplification products were analysed for individual types of HPV (reverse line blot). A β -globin test was performed as a control for the presence and quality of DNA in the paraffin-wax-embedded tissue.

RESULTS

Patients

In 31 of the 46 patients who met our computer search criteria, the diagnosis of both LS and VIN could be confirmed after histological revision. Fifteen patients did not fulfil the criteria for VIN ($n = 11$), LS ($n = 2$), or both VIN and LS ($n = 2$). Four

more patients presented with lesions suspected to be malignant at first onset of either LS or VIN, and were therefore excluded. Twenty-seven patients (82 vulvar specimens) remained for evaluation of histology and HPV status. Their mean (range) age at first histological diagnosis was 64 (38–87) years.

Histology

In these 27 patients with both LS and VIN, VIN lesions were all classified as undifferentiated (100%). This was further graded into VIN 1 ($n = 10$), VIN 2 ($n = 11$) and VIN 3 ($n = 6$; table 1).

To confirm the diagnosis, MIB staining in VIN 1 showed increased mitotic activity in all cases. A warty histological pattern was seen in 24 (89%) lesions; basaloid-type VIN was found in one (4%) lesion, and in two (7%) VIN 1 lesions it was not possible to distinguish warty and basaloid VIN. We diagnosed LS and VIN at the same time in the same lesion in 13 patients (fig 1, patients 1–3), mostly contiguous, and at the same time but in a different lesion in three more patients (fig 2, patient 4). In the other 11 cases, a period of 1–9 years was observed between first diagnosis of the two skin disorders (fig 2, patients 5 and 6). VIN arose in pre-existent LS in nine of 11 (82%) cases, whereas LS was diagnosed in pre-existent VIN twice.

Three patients developed (early) invasive SCC 1–4 years after the initial diagnosis of undifferentiated high-grade VIN in

Figure 3 Patient 7: (A) lichen sclerosus (LS; H&E, $\times 50$); (B) undifferentiated vulvar intraepithelial neoplasia (VIN) 3 not related to human papillomavirus (HPV), which was diagnosed 9 years after the diagnosis of LS (H&E, $\times 60$); (C) one year later, this patient developed a lesion suspicious for squamous cell carcinoma, which was surrounded by HPV-negative differentiated VIN (H&E, $\times 50$). Little or no atypia is shown above the (para-) basal layer; (D) pearl-like changes can be distinguished (H&E, $\times 250$).

pre-existing LS. In one case the epithelium adjacent to invasive SCC showed undifferentiated VIN 3, whereas in the other two cases differentiated VIN was found in the epithelium surrounding early invasive SCC (fig 3, patient 7).

HPV testing

Paraffin-wax-embedded tissue for HPV testing was available in 26 patients. HPV DNA was detected in eight of 26 (31%) tissue samples, whereas 18 lesions were found to be negative. Seven lesions contained HPV16 DNA, and one contained HPV59. There was a strong correlation between HPV-positive lesions and high-grade VIN. Whereas almost all VIN 1 lesions were HPV negative (89%), a considerable number of VIN 2 and 3

lesions were positive for HPV (41%). One of these patients with high-grade HPV-related VIN developed SCC after 4 years, the epithelium being surrounded by HPV-positive undifferentiated VIN. The other two patients with progressive disease developed SCC with differentiated VIN not related to HPV in the adjacent skin (table 1).

DISCUSSION

This is the first study describing the histology and HPV status of a large series of patients with both LS and VIN without coexistent SCC. In this study, VIN associated with LS was always of undifferentiated type. HPV DNA was detected in eight of 26 (31%) patients, seven of whom had high-grade VIN.

It is commonly thought that there is a relationship between differentiated VIN and LS.^{17 18} There are, however, only few data available on this subject, and almost all these concern the adjacent skin of vulvar SCC (table 2).^{4 5 7 9 19 20 22 28 29} In 153 of 467 (33%) patients whose skin lesions in the surrounding tissue of vulvar SCC were analysed, the existence of both VIN and LS has been observed (range 5–58%). Predominantly, this VIN was of differentiated type (74%). Sporadically, the coexistence of VIN and LS has been described in vulvar skin without invasive carcinoma (table 3).^{19–22} Since these studies were on either LS or VIN, mostly of undifferentiated type, selection bias might have affected the results.

To date, the presence of HPV DNA in VIN related to LS has been described only once.¹⁹ Haefner *et al* reported on three patients with HPV-negative differentiated VIN, all in the adjacent skin of vulvar SCC, and on three patients with HPV-positive undifferentiated VIN 3, in one of whom VIN was adjacent to SCC and in the other two it was not. The detection of HPV DNA in 41% of our high-grade VIN lesions supports the diagnosis of undifferentiated VIN, although, compared with the prevalence of HPV DNA in VIN not related to LS (78–92%), this

Table 1 Human papillomavirus DNA in 27 patients with lichen sclerosus and vulvar intraepithelial neoplasia without vulvar squamous cell carcinoma				
HPV-DNA	VIN			Total
	1	2	3	
HPV-positive	1*	4†	3‡	8
HPV-negative	8	7§	3¶	18
Unknown	1**	—	—	1

HPV, human papillomavirus.
*HPV16.
†HPV16 (n = 3) and HPV59 (n = 1).
‡HPV16 (n = 3), one of these patients developed squamous cell carcinoma (SCC) surrounded by HPV-positive undifferentiated vulvar intraepithelial neoplasia (VIN) after 4 years.
§One of these patients developed early invasive SCC surrounded by differentiated VIN not related to HPV after 4 years.
¶One of these patients developed early invasive SCC surrounded by differentiated VIN not related to HPV after 1 year.
**No material available.

Table 2 Histological findings of vulvar intraepithelial neoplasia and/or lichen sclerosus in the adjacent skin of vulvar squamous cell carcinoma

Study	Population	n	Skin adjacent to SCC					Other*
			uVIN only	dVIN only	LS only	Both VIN and LS		
						uVIN (%)	dVIN (%)	
Leibowitch <i>et al</i> ^k	SCC	78	24†	—	20‡	—	28 (36)	6
Gómez Rueda <i>et al</i> ^f	SCC	64	5	—	18	11 (17)	3 (5)	27
Haefner <i>et al</i> ⁹	SCC and VIN	15	8	3	—	1§ (7)	3¶ (20)	—
Scurry <i>et al</i> ²⁰	SCC	132	31	18	29	1 (1)	33 (25)	20
Carlson <i>et al</i> ²⁸	SCC	60	16	—	14	19 (32)	6 (10)	5
Vilmer <i>et al</i> ⁷	SCC	67	13	—	12	—	39 (58)	3
Carlson <i>et al</i> ²²	SCC	16	1	—	13	1 (6)	—	1
Derrick <i>et al</i> ²⁹	SCC	21	3	—	13	—	1 (5)	4
Poulsen <i>et al</i> ⁸	SCC	14	7	—	—	7 (50)	—	—
Total		467				40 (9)	113 (24)	

dVIN, differentiated VIN; LS, lichen sclerosus; SCC, squamous cell carcinoma; uVIN, undifferentiated VIN; VIN, vulvar intraepithelial neoplasia.

*Normal (n=22), squamous cell hyperplasia (n=31), lichen simplex chronicus (n=5), granulomatous vulvitis (n=1), lichen planus (n=4), or not associated with lichen sclerosus or vulvar intraepithelial neoplasia (n=3).

†7 of 11 samples tested were HPV-positive by in situ hybridisation (ISH): HPV16 (n=6), HPV31 (n=1).

‡No samples tested were HPV-positive (ISH).

§HPV16 DNA positive (PCR and ISH).

¶3 of 3 samples tested were HPV DNA negative (PCR).

percentage is rather low.^{19–32} This lack of similarity between undifferentiated VIN with and without associated LS might point to a different pathogenesis, at least in half of the cases.

It is known that there are some difficulties in the histological diagnosis of VIN. Firstly, it can be very difficult to distinguish VIN 1 from atypical inflammatory reactive changes or (damaged) normal skin, resulting in a high interobserver variability.^{33–35} In our study, we excluded 10 of 46 patients, in whom low-grade VIN diagnosed in the original histology report was not confirmed at revision following the definition given by the ISSVD.¹⁵ The high prevalence of VIN 1 lesions that tested negative for HPV after revision (89%) gives the impression that we still overdiagnosed VIN. However, MIB 1 staining, which has been suggested to be useful in accurate grading of VIN,³⁵ supported the diagnosis of VIN 1 in all cases. Today, this VIN 1–3 classification is the subject of discussion, and a new classification in which the term VIN 1 will no longer be used has been proposed by the ISSVD. Similarly, Medeiros *et al* recently recommended a distinction between low-grade VIN (VIN 1) and high-grade VIN (VIN 2 or 3 and differentiated VIN) to identify those lesions at risk for vulvar carcinoma.³⁶ In our study, such a new classification would affect the number of patients considerably, causing a significant

increase in the number of HPV-related high-grade VIN associated with LS (43%). Secondly, the presence of differentiated VIN has to be considered when there seems to be only a slight degree of squamous atypia or it is limited to the lower epidermis. Because of its highly differentiated features and absence of widespread architectural disarray, the diagnosis can be easily missed. We specifically looked into the subtle but characteristic features of differentiated VIN, as described in Materials and methods. However, we did not observe any of these features, except during follow-up, surrounding early invasive SCC. Furthermore, it may be difficult to agree upon whether early invasive growth has already occurred in differentiated VIN.²⁵ Conversely, differentiated VIN is easily diagnosed when there is an invasive SCC. The fact that none of our revised VIN 1 cases progressed to invasion strengthens our claim of not having missed any differentiated VIN.

As we studied only those cases with both LS and VIN from the start, a selection bias might have been caused by the design of our study. Revising all LS cases over several years could result in a higher prevalence of LS with coexisting VIN, and therefore possibly in a higher number of differentiated VIN. Revising all VIN cases, on the other hand, might result in a higher number of coexistent diseases as well, with VIN most likely to be of undifferentiated type.

In conclusion, it seems from an overview of the literature that the assumed relationship between differentiated VIN and LS can be supported in the case of coexistent vulvar SCC,^{4 7 19 20 29} but has been described in only five patients without SCC.^{20 22} In this study, we found that VIN related to LS without SCC was always of the undifferentiated type. These data suggest that VIN in the background of LS without SCC is a different type of VIN than that was thought previously, with a different aetiology and perhaps a different prognosis. In that case, we might question ourselves whether or not we tend to overtreat patients if we radically excise VIN in the background of LS. In our opinion, treatment of VIN associated with LS should be individualised, based on type and localisation of the lesion, in order to achieve a better conservation of the anatomy and function of the vulva.

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Table 3 Coexistence of both vulvar intraepithelial neoplasia and lichen sclerosus without vulvar squamous cell carcinoma

Study	Population	n	Both VIN and LS		
			uVIN	dVIN	uVIN/dVIN
Haefner <i>et al</i> ¹⁹	VIN*	50	2†	—	—
Italian Study Group ²¹	VIN‡	370	36	—	—
Carlson <i>et al</i> ²²	VIN§	17	—	2	1
Total		437	38	2	1
Scurry <i>et al</i> ²⁰	LS	86	2	2	—

dVIN, differentiated VIN; LS, lichen sclerosus; SCC, squamous cell carcinoma; uVIN, undifferentiated VIN; VIN, vulvar intraepithelial neoplasia.

*48 uVIN III, 2 dVIN.

†Both human papillomavirus 16 DNA positive (PCR and in situ hybridisation).

‡370 uVIN: VIN 1 (n=148), VIN 2 (n=53) and VIN 3 (n=169).

§14 uVIN III, 3 dVIN.

Take-home messages

- There is a relationship between differentiated vulvar intraepithelial neoplasia (VIN) and lichen sclerosus (LS) in the adjacent skin of vulvar squamous cell carcinoma (SCC).
- VIN associated with LS is more likely to be of the undifferentiated type, without the presence of invasive vulvar carcinoma.
- Human papillomavirus (HPV) DNA is detected in 41% of undifferentiated high-grade VIN coexisting with LS.
- This percentage is rather low compared with the high (>90%) prevalence of HPV DNA in VIN not related to LS, which might point to a different pathogenesis.

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REFERENCES

- Buscema J, Stern J, Woodruff JD. The significance of the histologic alterations adjacent to invasive vulvar carcinoma. *Am J Obstet Gynecol* 1980;**137**:902–9.
- Zaino RJ, Husseinadeh N, Nahhas W, et al. Epithelial alterations in proximity to invasive squamous carcinoma of the vulva. *Int J Gynecol Pathol* 1982;**1**:173–84.
- Borgno G, Micheletti L, Barbero M, et al. Epithelial alterations adjacent to 111 vulvar carcinomas. *J Reprod Med* 1988;**33**:500–2.
- Leibowitch M, Neill S, Pelisse M, et al. The epithelial changes associated with squamous cell carcinoma of the vulva: a review of the clinical, histological and viral findings in 78 women. *Br J Obstet Gynaecol* 1990;**97**:1135–9.
- Gómez Rueda N, García A, Vighi S, et al. Epithelial alterations adjacent to invasive squamous carcinoma of the vulva. *J Reprod Med* 1994;**7**:526–30.
- Kagie MJ, Kenter GG, Zomerdijsk-Nooyen Y, et al. Human papillomavirus infection in squamous cell carcinoma of the vulva, in various synchronous epithelial changes and in normal vulvar skin. *Gynecol Oncol* 1997;**67**:178–83.
- Vilmer C, Cavalier-Balloy B, Nogues C, et al. Analysis of alterations adjacent to invasive vulvar carcinoma and their relationship with the associated carcinoma: a study of 67 cases. *Eur J Gynaecol Oncol* 1998;**19**:25–31.
- Rouzier R, Morice Ph, Haie-Meder C, et al. Prognostic significance of epithelial disorders adjacent to invasive vulvar carcinomas. *Gynecol Oncol* 2001;**81**:414–19.
- Poulsen H, Junge J, Vybberg M, et al. Small vulvar squamous cell carcinomas and adjacent tissues. A morphologic study. *APMIS* 2003;**111**:835–42.
- Ridley CM, Frankman O, Jones IS, et al. New nomenclature for vulvar disease: International Society for the Study of Vulvar Disease. *Hum Pathol* 1989;**20**:495–6.
- Wallace HJ. Lichen sclerosus et atrophicus. *Trans St Johns Hosp Dermatol Soc* 1971;**57**:9–30.
- Hart WR, Norris HJ, Helwig EB. Relation of lichen sclerosus et atrophicus of the vulva to development of carcinoma. *Obstet Gynecol* 1975;**45**:369–77.
- Meyrick Thomas RH, Ridley CM, McGibbon DH, et al. Lichen sclerosus et atrophicus and autoimmunity—a study of 350 women. *Br J Dermatol* 1988;**118**:41–6.
- Carli P, Cattaneo A, De Magnis A, et al. Squamous cell carcinoma arising in vulval lichen sclerosus: a longitudinal cohort study. *Eur J Cancer Prev* 1995;**4**:491–5.
- Wilkinson EJ, Kneale B, Lynch PJ. Report of the ISSVD terminology committee. *J Reprod Med* 1986;**31**:973–4.
- van Seters M, van Beurden M, de Craen AJM. Is the assumed natural history of vulvar intraepithelial neoplasia III based on enough evidence? A systematic review of 3322 published patients. *Gynecol Oncol* 2005;**97**:645–51.
- Fox H, Buckley CH. Epithelial tumours of the vulva. In: Ridley CM, Neill SM, eds. *The vulva*. 2nd edn. Oxford: Blackwell Science, 1999:239–42.
- Hart WR. Vulvar intraepithelial neoplasia: historical aspects and current status. *Int J Gynecol Pathol* 2001;**20**:16–30.
- Haefner HK, Tate JE, McLachlin CM, et al. Vulvar intraepithelial neoplasia: age, morphological phenotype, papillomavirus DNA, and coexisting invasive carcinoma. *Hum Pathol* 1995;**26**:147–54.
- Scurry J, Vanin K, Östör A. Comparison of histological features of vulvar lichen sclerosis with and without adjacent squamous cell carcinoma. *Int J Gynecol Cancer* 1997;**7**:392–9.
- Italian Study Group on Vulvar Disease. Clinicopathologic analysis of 370 cases of vulvar intraepithelial neoplasia. *J Reprod Med* 1996;**41**:665–70.
- Carlson JA, Healy K, Tran TA, et al. Chromosome 17 aneusomy detected by fluorescence in situ hybridization in vulvar squamous cell carcinomas and synchronous vulvar skin. *Am J Pathol* 2000;**157**:973–83.
- Carlson JA, Lamb P, Malfetano J, et al. Clinicopathologic comparison of vulvar and extragenital lichen sclerosus: histologic variants, evolving lesions, and etiology of 141 cases. *Mod Pathol* 2005;**12**:844–54.
- Wilkinson EJ. Normal histology and nomenclature of the vulva, and malignant neoplasms, including VIN. *Dermatol Clin* 1992;**10**:283–96.
- Yang B, Hart WR. Vulvar intraepithelial neoplasia of the simplex (differentiated) type: a clinicopathologic study including analysis of HPV and p53 expression. *Am J Surg Pathol* 2000;**24**:429–41.
- van den Brule AJC, Pol R, Fransen-Daalmeijer N, et al. GP5+/6+ PCR followed by reverse line blot analysis enables rapid and high-throughput identification of human papillomavirus genotypes. *J Clin Microbiol* 2002;**40**:779–87.
- Jacobs MV, Zielinski D, Meijer CJ, et al. A simplified and reliable HPV testing of archival Papanicolaou-stained cervical smears: application to cervical smears from cancer patients starting with cytologically normal smears. *Br J Cancer* 2000;**82**:1421–6.
- Carlson JA, Ambros R, Malfetano J, et al. Vulvar lichen sclerosus and squamous cell carcinoma: a cohort, case control, and investigation study with historical perspective; implications for chronic inflammation and sclerosis in the development of neoplasia. *Hum Pathol* 1998;**29**:932–48.
- Derrick EK, Ridley CM, Kobza-Black A, et al. A clinical study of 23 cases of female anogenital carcinoma. *Br J Dermatol* 2000;**143**:1217–23.
- van Beurden M, ten Kate FJW, Smits HL, et al. Multifocal vulvar intraepithelial neoplasia grade III and multicentric lower genital tract neoplasia is associated with transcriptionally active human papillomavirus. *Cancer* 1995;**75**:2879–84.
- Hørding U, Dagaard S, Junge J, et al. Human papillomaviruses and multifocal genital neoplasia. *Int J Gynecol Pathol* 1996;**15**:230–4.
- Trimble CL, Hildesheim A, Brinton LA, et al. Heterogeneous etiology of squamous carcinoma of the vulva. *Obstet Gynecol* 1996;**87**:59–64.
- Micheletti L, Barbero M, Preti M, et al. Vulvar intraepithelial neoplasia of low grade: a challenging diagnosis. *Eur J Gynaecol Oncol* 1994;**15**:70–4.
- Preti M, Mezetti M, Robertson C, et al. Inter-observer variation in histopathological diagnosis and grading of vulvar intraepithelial neoplasia: results of an European collaborative study. *Br J Obstet Gynaecol* 2000;**107**:594–9.
- van Beurden M, de Craen AJM, de Vet HCW, et al. The contribution of MIB 1 in the accurate grading of vulvar intraepithelial neoplasia. *J Clin Pathol* 1999;**52**:820–4.
- Medeiros F, Nascimento AF, Crum CP. Early vulvar squamous neoplasia: advances in classification, diagnosis, and differential diagnosis. *Adv Anat Pathol* 2005;**12**:20–6.