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Improving care for women with vulvar squamous (pre)malignancies

Loes van den Einden
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Loes Cornelia Gertruda van den Einden
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geboren op 5 augustus 1982
te Deurne

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Introduction and outline of the thesis

Partly based on:
Prevention, identification and treatment of vulvar squamous (pre)malignancies: a review focusing on quality of care
Loes C.G. van den Einden, Irene A. van der Avoort, Joanne A. de Hullu
Chapter 1
Introduction and outline of the thesis

Introduction

Vulvar squamous cell carcinoma, its precursor lesions (usual and differentiated vulvar intraepithelial neoplasia) and lichen sclerosus are rare diseases that may have large impact on the lives of affected women and their partners. This introduction provides an overview of current knowledge of these vulvar diseases.

1. Vulvar squamous cell carcinoma

1.1 Epidemiology

Vulvar malignancies are rare with a worldwide yearly incidence of one to two per 100,000 women. In The Netherlands vulvar cancer accounts for 6–8% of all gynaecological malignancies and 406 new cases of vulvar cancer were diagnosed in 2011. A rise in absolute numbers is expected because of aging of the population. Although no population-based analyses are available, studies show a tendency towards an increasing incidence of vulvar cancer. Typically, vulvar cancer occurs in the seventh decade. The majority of patients with vulvar cancer have a vulvar squamous cell carcinoma (VSCC); only a minority of patients suffer from basal cell carcinoma, melanoma or adenocarcinoma.

1.2 Etiology

There are two different types of VSCCs with their own associated premalignant lesions. The majority of VSCCs are human papilloma virus (HPV)-unrelated and the oncogenesis is not exactly known. The minority of VSCCs are HPV-related: a meta-analysis showed that 40% of all VSCCs are caused by HPV, but recently our group showed that HPV seems to play an etiological role in only 19% of all VSCCs. See Figure 1 for an overview of the oncogenesis of VSCC and Figure 2 for clinical pictures of HPV and non-HPV related VSCC.

HPV negative pathway

The most common type of VSCC is HPV negative, occurs in elderly women and leads to differentiated keratinizing VSCC. Differentiated vulvar intraepithelial neoplasia (dVIN) is presumed to be the precursor lesion and often occurs in a background of lichen sclerosus (LS). LS is a chronic inflammatory skin disease with a lifetime risk of developing a VSCC of 4-5%. The oncogenesis of LS to VSCC, probably through the development of dVIN, is not exactly known. First of all, the aetiology of LS is unknown; based upon epidemiologic data, hormonal factors, genetic factors, infectious agents, the Köbner phenomenon and autoimmune factors have been suggested to play a role. Up until now, it is considered an autoimmune phenomenon, with an association with other autoimmune diseases, like hypothyroidism and vitiligo in 21-34% of the patients. Squamous cell hyperplasia with atypia might represent a step in the carcinogenesis of the LS/dVIN pathway, but it has
cervical (pre)malignancies. It is primarily affecting younger women, and usual vulvar intraepithelial neoplasia (VIN) is the precursor lesion. In the presence of high-risk HPV, the entire ano-genital tract is at risk for the development of premalignant lesions. A higher prevalence of the latter is seen in HIV infected women and women after organ transplantation.

### 1.3 Clinical characteristics

Most patients with VSCC present with a vulvar mass, although there is often a long history of pruritis or discomfort. Less common presenting symptoms include vulvar bleeding, pain or dysuria. On physical examination an ulcer, a red macule or papule or a white hyperkeratotic (warty) plaque can be seen. Clinically it may be difficult to distinguish early stage VSCC from VIN.

### 1.4 Diagnostics

Biopsy is the gold standard to establish a diagnosis. A punch biopsy can be taken from the most suspicious part of the lesion. In case of an erosion or ulcer, the biopsy should be taken preferably from the edge of the lesion. When there are multiple lesions, vulvar mapping should be performed. A (digital) photo should be taken to note localizations of the biopsied areas, and to document changes during follow-up.

Several studies have tried to explore the role of vulvar cytology as a less invasive replacement option for histology or a triage instrument to determine whether a biopsy of a clinically (pre)malignant lesion is necessary. This could be of special value in patients after treatment of VIN/VSCC with a high risk of recurrence and when vulvar examination is difficult because of scarring due to previous vulvectomy. Various techniques for vulvar cytology have been described with varying results. Until now, cytology is not used in daily practice.

### 1.5 Treatment

Over the past years, efforts have been made to individualise treatment of patients with VSCC and define subgroups of patients that may be treated by less radical procedures. Until 20 years ago radical vulvectomy with ‘en bloc’ bilateral inguinofemoral lymphadenectomy (IL) was the standard treatment for almost all patients with VSCC. Currently, standard treatment entails a wide local excision (WLE) with uni- or bilateral IL via separate incisions. This change in treatment modality has significantly improved the quality of care because of its less mutilating effect. Nowadays, radical vulvectomy with en bloc IL is only performed in case of advanced nodal involvement. More recently, sentinel lymph node (SLN) procedure has been introduced in early stage VSCC and has shown excellent results with a very high negative predictive value of a negative SLN, and is considered as a standard treatment since 2008. Strict criteria (tumour <4 cm, only unifocal lesions) and...
Centralisation of treatment
In many developed countries, centralisation of rare tumours such as VSCC is advised to increase survival and to decrease treatment-related morbidity. There is only scarce literature concerning centralisation of care for women with VSCC. It is believed there is a role for centralisation of the treatment of VSCC, because of the low incidence, the high complex surgery and the introduction of new techniques such as SLN procedure where learning curves are important. Therefore, centralising treatment has been advocated by the national guidelines of the Dutch Society of Obstetrics and Gynaecology in 2000. However, it remains to be proven if this policy indeed improves outcome with respect to prognosis and morbidity of patients.

1.6 Prognosis
Inguinofemoral lymph node status at initial diagnosis is of critical prognostic importance for patients with VSCC. Overall 5-year survival in VSCC is around 70% and decreases by the number of positive lymph nodes and/or FIGO stage. More recent studies on prognosis in VSCC suggest that uVIN-related VSCC has a more favourable prognosis compared with dVIN/LS related HPV-negative vulvar cancer, although other studies on HPV as a prognostic factor have not been able to demonstrate a difference between the two pathways.

2. LS and squamous premalignancies - nomenclature
Various terms have been used to define VSCC precursors. For a long time, VIN lesions were graded similar to CIN: VIN1, VIN2 and VIN3. In 2004, the International Society for the Study of Vulvovaginal Disease (ISSVD) decided to abolish the 3-grade system of VIN because clinicopathological data did not appear to support the concept of a continuous spectrum that we know from CIN and cervical carcinoma. The abandonment of VIN1 and the consolidation of VIN2 and VIN3 into one category simply termed (high-grade) VIN, best fitted the studies that have been performed on grading of VIN so far. In the light of the two different types of VSCC the ISSVD clearly distinguishes: VIN usual type and VIN differentiated type. uVIN can be subclassified into basaloid and warty subtypes, and cases with mixed features are common. In clinical practice, no difference is made between the two types of uVIN. Recently, the College of American Pathologists and American Society for Colposcopy and Cervical Pathology suggested a two-tier classification for all HPV related squamous lesions of the anogenital tract: low-grade squamous intraepithelial lesions and high-grade intraepithelial lesions, paralleling the terminology of the Bethesda System cytological reports. So far, no further classification for dVIN is suggested.
3. Lichen sclerosus

3.1 Epidemiology
The true incidence of LS is unknown and difficult to establish as different specialists are providing care for LS patients. No recent studies of the incidence of LS in the general population are available. In 1971, Wallace calculated incidences of 1:300 to 1:1000 in new patients, referred to a general hospital. The rate of biopsy proven vulvar LS in one general gynaecology private practice was approximately 1.7% (one in 60 women). LS is diagnosed at different ages and has a bimodal peak incidence in prepubertal girls (5–15%) and women aged 50–70 years.

3.2 Clinical characteristics
Typically, the lesions are white plaques and papules, often with areas of erythema, ecchymosis, hyperkeratosis, pallor, fissuring, telangiectasia, hyperpigmentation, bullae, excoriation, oedema and/or ulceration. The clinical appearance can vary from subtle (Figure 3A) to advanced (Figure 3B). Presenting symptoms of LS may include intense pruritus, soreness, pain, burning, dyspareunia, dryness, irritation, urinary complaints, constipation/bowel pain, bleeding and blistering. Painful skin fissures can occur with or after sexual intercourse and defecation.

3.3 Histology
LS can be recognised by a thinned epidermis with loss of normal rete ridges, hyperkeratosis and basal layer vacuolar changes (Figure 4A). Furthermore, a wide band of homogenised collagen below the dermo-epidermal junction and a band-like lymphocytic infiltrate below the homogenised area are present. The dermis can show oedema or hyalinization and sometimes bullae are present.
3.4 Treatment
In general, LS has no cure and follow-up is required to treat symptomatic flare-ups of the disease and possibly diminish anatomical changes. In some cases, especially prepubertal girls, spontaneous resolution of LS has been described. It is not known whether successful control of the disease reduces the long-term risk of malignancy, although a protective effect from malignant evolution has been suggested. The symptomatic treatment with the best evidence of efficacy is the use of topical potent corticosteroid ointments. Corticosteroids have anti-inflammatory, antipruritic and vasoconstrictive effects. Various studies show good clinical response and even histological improvement with the use of these drugs. British Association of Dermatologists guidelines advocate their use, and give specific guidance on length of initial treatment and on maintenance treatment. There is no convincing evidence that corticosteroid treatment of LS influences its prognosis. Maintenance therapy is often advised, as symptoms can recur in women who terminate therapy.

3.5 Malignant potential
There are different reasons to link LS to the development of VSCC. First, the majority of VSCCs has LS, squamous cell hyperplasia or dVIN in the adjacent epithelium. Second, in series of LS patients that underwent long-time follow-up, 4.5% has been reported to develop VSCC. Until now, it is not clear which LS patients are at risk of developing dVIN and eventually SCC. In a retrospective case review, Jones compared clinical parameters of 46 women with LS and VSCC with 213 women with LS but without VSCC. The women with VSCC were significantly older, had significantly more hyperplastic skin changes and showed squamous hyperplasia and cellular atypia more often. There were no differences in the presence and duration of symptoms or loss of vulvar architecture. Although the term 'leukoplakia' was rejected by the ISSVD because of inconsistency of clinical and histological features, several studies described leukoplakia in preceding 60–100% of the cases with vulvar cancer. Therefore, areas of LS that become thickened and do not respond to frequent application of topical steroids should be biopsied in order to exclude malignancy. Several studies have tried to find molecular markers in LS, which are an indication of a higher risk of developing SCC. High expression of or mutations in the tumour suppressor genes p53 has been postulated as a marker for increased likelihood in LS to progress to VSCC, but has also been attributed to ischemic stress. Furthermore, parakeratosis, dyskeratosis, hyperplasia and basal cellular atypia are significantly more often seen in patients with LS that progressed to VSCC compared with LS without progression. Interestingly, these are all characteristics of dVIN, but in the days of most studies, dVIN was not regarded as a separate entity. Finally, little is known about the genetic alterations found in LS with and without progression into dVIN and finally SCC, these might act as markers for progression in patients with LS.

4. Differentiated VIN

4.1 Epidemiology
dVIN accounts for a small proportion (<2–5%) of all VIN lesions. Because of the difficult clinical and histological diagnosis, it is probably considerably underdiagnosed. In a retrospective study by van de Nieuwenhof et al, it was shown that after histopathologic revision of LS lesions that progressed to VSCC, 42% were reclassified as dVIN. dVIN characteristically occurs in postmenopausal women and is associated with LS. As dVIN is seldom found in an isolated form, some authors believe it is actually part of the adjacent VSCC.

4.2 Clinical characteristics
dVIN can present as an area of grey-white discoloration with a roughened surface, an ulcerative red lesion, an erythematous red lesion, or as an ill-defined white plaque (Figure 5A & B). It may be difficult to distinguish dVIN from LS. Patients are, due to the underlying LS, often symptomatic with a long-lasting history of itching and other LS-related symptoms.

4.3 Histology
The histological diagnoses of dVIN is difficult as atypia is only confined to the basal epidermal cell layers. Therefore it is often confused with squamous hyperplasia or lichen sclerosus, though no data on reproducibility of the diagnosis exist. dVIN is characterised by a thickened epithelium that is typically associated with parakeratosis, dyskeratosis and elongation and anastomosis of rete ridges (Figure 4B and 4C). Dyskeratosis is characterised by disturbed maturation and premature keratinisation of squamous cells that are located deeper in the epithelium. In the parabasal layers of the epithelium, individual and clusters of cells show premature maturation, with large cells that show eosinophilia of the cytoplasm and even formation of keratin pearls. The nuclei have prominent nucleoli, usually predominantly in the (para)basal keratinocytes. Atypical mitotic figures may be seen mainly in the lower layers. The most superficial layers show normal maturation without atypical cells.

4.4 Treatment
dVIN is treated by surgical excision preferably with a few millimetre margins of healthy tissue. dVIN lesions are usually confined to non-hair-bearing areas or the external sides of atrophic labia minora. Recurrent lesions are common and so far, there is no place for medical therapy.
5. Usual VIN

5.1 Epidemiology

The incidence of uVIN is approximately five per 100,000 women per year and is increasing worldwide. It is more common in young women, often between the age of 30 and 40 years. Risk factors are smoking and an immune compromised state. A reason for a recently described increased incidence in uVIN might be the more liberal use of vulvar biopsy, which contributed to earlier diagnosis of uVIN lesions that might have been missed in the past. The malignant progression in patients who have been treated for uVIN is estimated to be <5%, so only a limited number of uVIN lesions progress to invasive VSCC.

5.2 Clinical characteristics

UVIN lesions can have a variety of clinical appearances. They often produce large whitish or erythematous plaques, while some lesions are pigmented (Figure 5C and 5D). The most frequently affected sites are the labia majora, the labia minora and posterior fourchette, but the entire vulva can be affected with >40% multifocal involvement. Multicentric intra-epithelial or invasive squamous neoplasia (of the cervix, vagina or anus) is also common, occurring in approximately 35% of the uVIN patients. Therefore a cervical smear and careful inspection of the entire anogenital area should always be performed, also when patients are not in a cervical screening program because of their age. The most common presenting complaint of uVIN is pruritus, present in about 60% of the patients. Other presenting symptoms can be pain, ulceration and dysuria. Approximately a fifth of the patients have no specific complaints, apart from the finding of an abnormal vulvar area by (self) examination.

5.3 Histology

In uVIN the epidermis is thickened and contains atypical cells from the basal membrane up to the surface (Figure 4D). Acanthosis, dyskeratosis, hyper- and parakeratosis, a high nuclear-to-cytoplasmic ratio and koilocytes may be present.

5.4 Treatment

There are several options for treatment of uVIN like cold knife surgery, laser surgery/ vaporization and the use of imiquimod (Aldara®). In the past, extensive surgery has been performed for uVIN. Nowadays, local excision, consisting of removal of all visible lesions, is the surgical technique of choice, especially since studies have shown that surgical margins are no predictor for the risk of invasive disease. Surgery can be performed either by cold knife or laser. Before treatment with laser vaporization, invasive disease must have been excluded.

Figure 5 Clinical pictures of patients with vulvar intraepithelial neoplasia. (A) Patient with differentiated vulvar intraepithelial neoplasia (dVIN) in the background of lichen sclerosus: on the left labium minora two red and erosive lesions can be seen. (B) Patient with dVIN in the background of lichen sclerosus, on the left labium minora a hyperkeratotic plaque can be seen, histopathologically confirmed as being dVIN. (C) Patient with multifocal usual vulvar intraepithelial neoplasia. (D) Detail of the patient with usual vulvar intraepithelial neoplasia.
Imiquimod (Aldara, 3M Pharmaceuticals, MN, USA), an imidazoquinoline amine, is classified as an immune response modifier. It is widely used in the treatment of genital warts with proven efficacy in terms of clearance of the lesions, and a lower recurrence rate compared with conventional surgical treatments.\(^{19}\) By inhibiting viral replication, imiquimod directly treats the cause of uVIN and preserves the anatomy and function of the vulva.\(^{20}\) Several studies evaluated the effect of imiquimod on uVIN. Responses were reached after 6–30 weeks of treatment.\(^{21}\) Follow-up of patients treated with imiquimod is still relatively short; long-term effects cannot yet be established. The results of imiquimod are promising, especially in smaller lesions\(^ {22}\) but large randomized controlled trials are needed to obtain data on the long-term effects\(^ {23}\) and to compare with other treatment modalities like surgery in small lesions. Considering the side effects of imiquimod, its use should be restricted to motivated patients.

5.5 Malignant potential and risk of recurrence

Recurrence rates of uVIN lesions, even after extensive surgical procedures, are common. A number of studies have reported the influence of surgical margins status on recurrence rates. Surgical margins are often positive, irrespective of the type of operation performed\(^ {24}\) and the evidence whether free surgical margins prevent recurrence is lacking\(^ {25}\) although a recent study showed recurrence was associated with positive margins. Furthermore, high rates of recurrences were found to be associated with smoking. The chance of malignant progression in uVIN has long been debated. The rate of invasion after various primary treatments ranges from 3.3 to 5.7\%, as shown in three large studies.\(^ {26, 61, 62}\) Untreated patients have a significantly higher risk of malignant progression.\(^ {27, 62}\)

6. Impact, prevention and identification

6.1 Impact of vulvar LS and (pre)malignancies

In general, both LS as well as VIN/VSCC are often long-term conditions causing significant morbidity and psychological distress. Many women feel embarrassed and have sexual problems. Patients may feel uncomfortable with their complaints and the disfiguring changes that may occur, which might result in avoiding sexual intimacy. Studies show that the majority of patients report an impact on Quality of life (QoL), especially with sexual functioning which causes significant sexual distress.\(^ {63-66}\)

6.2 Diagnostic delay

In patients with LS, there was found to be a self-reported diagnostic delay (age at first symptoms compared with age at diagnosis) of 4.9 years.\(^ {67}\) Furthermore, in these patients the number of symptoms was higher in the group with a worse QoL, which suggests that resolving one or more symptoms by treating the patient, leads to a better QoL. The cohort study of Vandborg et al, in which they looked for reasons for diagnostic delay in different gynaecological malignancies, showed that vulvar cancer had the longest delay of 170 days, mainly caused by patient delay.\(^ {68}\) Furthermore, a GP will only see a few patients with vulvar LS and very few patients with VIN/VSCC during his/her carrier which may also cause delay in diagnosis. Besides, these conditions are not or only little addressed during their medical (specialist) training. Nunns and Mandal showed that only 56\% of the GPs they questioned carried out genital examinations on patients with recurrent vulvar symptoms in their daily practice, because of lack of time or at the patients’ request.\(^ {29}\) As a consequence, only 59\% of patients with recurrent symptoms were investigated prior to treatment. Therefore, it is desirable to perform a gynaecological examination in all women with vulvar complaints.

6.3 Self examination

Because of the increased risk of developing a (pre)malignancy in patients with LS and uVIN, instructions should be given for self-examination. When there are signs of a VSCC (nonhealing erosions, development of tumour(s) or ulcers, itching that changes towards pain), a patient should consult her doctor.\(^ {30}\) It can be helpful to explain the signs of a VSCC to the patient by using a mirror, so patients can get familiar with their own anatomy and colour of their skin.

6.4 A multidisciplinary approach

Multidisciplinary vulvar clinics in which specialist expertise is combined are of great value\(^ {31}\) and are operating in many European countries and the USA. A specialist is defined as a consultant dermatologist and/or gynaecologist who has had additional specialised training in managing vulvar disease.\(^ {32}\) Furthermore, there is an important role for supporting specialists: Collaboration with a sexologist and pelvic floor physiotherapist is vital. Also, there is a need for a trained (gynaeco-)pathologist as the diagnosis of dVIN in LS patients is often difficult to establish.

6.5 Vaccination

Prophylactic

HPV vaccines have been introduced from 2007 onwards with the main goal to reduce the incidence of cervical (pre)malignancies and further to reduce the incidence of other HPV-related lesions like genital warts, oropharyngeal, anal, vaginal and vulvar (pre) malignancies. Two prophylactic vaccines have been introduced (a quadrivalent vaccine against HPV 6, 11, 16 and 18 and a bivalent vaccine against HPV 16 and 18). As a result of the prophylactic vaccines, the estimated reduction of VSCC is 20\% of all patients, while in patients with VIN the reduction will be larger, as the large majority is HPV-related.
Despite earlier studies providing some evidence that dVIN is the true precursor lesion of HPV-negative VSCC, studying the genetic profile may increase the evidence. Therefore, in chapter 2 we studied the corresponding genetic alterations that can be found in dVIN and VSCC with the use of a molecular inversion probe single-nucleotide polymorphism assay.

Second, the (histopathological) identification of dVIN lesions is difficult due to a lack of knowledge and criteria. In order to optimise the identification of VSCC and its premalignancies, we investigated the possibility of improving the methods of diagnosing lesions. In chapter 3 we examined the role of cytology as a triage instrument that may determine whether subsequent biopsy is necessary. In chapter 4 we evaluated the reproducibility of diagnosing dVIN by pathologists and investigated the possible improvement of the reproducibility after providing guidelines with criteria for recognition of dVIN. Finally, in order to find the most optimal method of measuring the depth of invasion in relation to the individual outcome in patients with VSCC, we compared the current and an alternative measuring method in chapter 5.

Third, VSCC is a rare tumour and centralisation of care in oncology centres is advised. Furthermore, the treatment has become less radical. In order to evaluate the effect of these treatment changes and implementation of the advice to centralise care of women with VSCC, two large population-based studies in the Netherlands were performed. In chapter 6 we determined whether the advice to centralise has been adapted and has led to improved survival in the Eastern part of the Netherlands. In chapter 7 trends of incidence and survival of women with VSCC in the Netherlands are described.

In chapter 8, we propose hypotheses and future studies based on the results of the abovementioned research, to gain future insight in the development of HPV-negative VSCC and to further improve care for women with vulvar squamous (pre)malignancies.
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Genome-wide copy number analysis suggests a clonal relationship between differentiated vulvar intraepithelial neoplasia and papillomavirus-negative vulvar squamous cell carcinoma

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Submitted
Abstract

Objective
Differentiated vulvar intraepithelial neoplasia (dVIN) is assumed to be the precursor lesion of vulvar squamous cell carcinoma (VSCC), but genetic evidence is currently lacking. To study the genetic relationship between dVIN and VSCC, we compared the copy number abnormalities between paired dVIN and VSCC lesions.

Methods
Specimen of six patients with dVIN and VSCC were included in this study. High-resolution genome-wide copy number analysis was performed using a molecular inversion probe single-nucleotide polymorphism array on isolated DNA.

Results
Copy number alterations (CNA) were identified in all six VSCC samples, including loss of 8p (present in all cases), gain of 8q (present in 5/6 VSCCs), gain of 7p and loss of 18q (present in 4/6 VSCCs). On average, we found 6 gains and 9 losses per VSCC sample. Copy number profiles of three dVIN lesions passed quality thresholds, and CNAs were identified in one dVIN lesion. In these patients at least three out of the 33 CNAs identified in the VSCC sample were also detected in the paired dVIN sample, including a high-level amplification on chromosome 11q23. These findings suggest that the two lesions originate from a single precursor in which additional alterations may have resulted in the development of VSCC.

Conclusion
Our study revealed several candidate genes and genomic regions that may be associated with VSCC pathogenesis. We have found the first genetic evidence for the clonal relationship between dVIN and VSCC in one patient, which supports the hypothesis VSCC can originate from dVIN precursor lesion.

Introduction
Vulvar cancer is the fourth most common cancer affecting the female genital tract and has an incidence of 1-2/100,000 women per year. The median age of diagnosis is 70 years and, due to the ageing of the Western population, the incidence is increasing. Approximately 80% of all vulvar cancers are of squamous origin and can develop following two different pathways. The minority of vulvar squamous cell carcinomas (VSCCs) is caused by a persistent infection with high-risk human papillomavirus (HPV); its oncogenesis resembles the development of cervical cancer. The oncogenesis of the most common VSCC is still unclear. These HPV-negative tumours arise in a background of the chronic inflammatory vulvar skin disease Lichen Sclerosis (LS) and/or the premalignancy differentiated vulvar intraepithelial neoplasia (dVIN). The exact role of LS and dVIN in the development of VSCC is not yet known, but patients with LS have a lifetime risk of 4-5% to develop VSCC.

In one of our earlier studies we showed that of all VIN lesions diagnosed in the Netherlands between 1992 and 2005, only a minority were dVIN in comparison to the HPV-induced usual VIN. Furthermore, dVIN is rarely found as a solitary lesion. Interestingly, in VSCC about 80% of the cases is HPV unrelated. This discrepancy can be explained by dVIN being a difficult clinical and histopathological diagnosis and the assumed short intraepithelial phase which suggests that dVIN is possibly suffering from underdiagnosis. On the other hand, it has been hypothesised that dVIN is a border phenomenon of VSCC. Though the carcinogenesis has not been fully clarified, there are strong indications that dVIN is a precursor lesion; in recent years the incidence of solitary dVIN is increasing and dVIN is more often found in revised biopsies previously diagnosed as LS in patients that later developed VSCC.

The upcoming techniques to search for genetic changes are promising to provide more information on the aetiology of (pre)malignant vulvar lesions. However, the literature on this topic is limited. In theory, tumours arise from a multistep process of accumulated genetic alterations. The identification of chromosomal regions most frequently affected by copy number alterations (CNA) may be relevant for determining the relationship between dVIN and VSCC. We hypothesise that dVIN is a precursor lesion already showing early neoplastic alterations which are also present in VSCC together with other accumulating events. In order to provide information on the mutations that can be found in VSCC and to provide more evidence for a clonal relationship between dVIN and VSCC, we aimed to determine the genetic abnormalities that can be found in VSCC en dVIN tissues in the same patients with the use of single-nucleotide polymorphism-based copy number analysis.
Chapter 2

Clonal relationship between dVIN and VSCC

32

and B-allele frequency plots. To allow comparison of VSCC copy number profiles with those of the dVIN samples, which were of poorer quality, we performed smoothening of the dVIN probe intensity values using a 15x running smoothing of the median (R package).

Copy number plots of VSCC and smoothened dVIN data per chromosome were made using GenomeGraphs library

1.28.0

Results

Patient samples

In total 12 samples of 6 patients (6 VSCC, 6 dVIN) were hybridised to Oncoscan arrays. The median age of the patients was 52 years (range 47-83), four of six patients (67%) had a FIGO stage IB VSCC and two of six patients (33%) had a FIGO stage III VSCC (Table 1). Nine of the 12 samples generated copy number profiles that passed quality control and could be further analysed, including all six VSCC samples and three dVIN samples, although the latter three were of poorer quality and showed higher levels of noise.

Copy number profiling

Genome-wide copy number profiling was performed using FFPE-compatible Affymetrix Oncoscan arrays OncoScan FFPE Express v.2 (Affymetrix, Santa Clara, CA, USA), according to the protocol provided by the manufacturer[12]. The data that passed quality control (MAPD value ≤ 0.6) were then analysed using Nexus Copy Number software Edition 7 (Biodiscovery, El Segundo, CA, USA) with NCBI build 37 of the human genome. The SNP-FASST2 Segmentation Algorithm was used. The significance threshold was set at 5.0E-7 with a minimum of 250 probes and a maximum contiguous spacing of 1000 kb to define a segment. Copy number gains and losses were set to 0.3 and -0.3, respectively. High-level amplifications and homozygous losses indicating greater than a single copy number change were set at 1.2 and -1.2 respectively. The homozygous frequency threshold was set at 0.85 and the homozygous value threshold was set at 0.8 with a minimum loss of heterozygosity (LOH) requirement of 500 kb. The heterozygous imbalance threshold was set at 0.4. Allelic imbalance and LOH was only scored when larger than 15 Mb, and adjacent sequential calls of allelic imbalance and LOH were merged as one region of uniparental disomy (UPD) upon visual inspection of the copy number and allelic imbalance.

Chromosomal alterations in VSCC

An overview of the distribution and number of gains and losses per patient per sample are displayed in Figure 1 and Table 2. In VSCCs we identified a total of 94 copy number alterations (Table 2 and supplementary Table S1). These alterations consisted of 55 losses and 39 gains, including 7 high-level amplifications. Overall, eleven genomic regions were affected by CNAs in three or more tumours (supplementary Table S2). The most frequently
found alteration was loss of chromosome 8p, present in all tumours, followed by gain of chromosome 8q in five of six tumours, gain of chromosome 7p and loss of chromosome 18q, both present in four of six tumours (Figure 1 and supplementary Table S2). Seven high-level amplifications were seen in three of six VSCCs (Table 3). Five of these encompassed large genomic regions (37-45 Mb), affecting many genes. The others were 1-8 Mb in size and contained known oncogenes including \textit{EGFR} (7p21.1), \textit{FGFR1} (8p11.23), \textit{IL11RA} (9p13), and \textit{YAP1} (11q22.1). Furthermore, 12 regions of allelic imbalance were identified representing copy neutral homozygosity. These regions are known as acquired uniparental disomy (aUPD), and frequently contain mutations in tumour suppressor genes that have become homozygous as a result of mitotic recombination. The regions were not completely homozygous, suggesting it to be present in a subset of the tumour (supplementary Table S3). Chromosome 17p encompassing, among other genes, the tumour suppressor gene TP53, was affected by aUPD in three samples (supplementary Table S3).

Table 2 Overview of gains, losses and aUPD per patient per sample vulvar squamous cell carcinoma.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Total CNAs</th>
<th>Gains(^1)</th>
<th>Losses</th>
<th>High-level amplifications</th>
<th>aUPD</th>
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\(^1\) Including high-level amplifications. Abbreviations: CNA = Copy number alteration. aUPD = acquired uniparental disomy.

Clonal relationship between VSCC and dVIN

Next we analysed whether the chromosomal abnormalities detected in VSCC could already be detected in the paired dVIN samples. Three dVIN samples from patients 1, 3, and 6, passed quality control and could be further analysed. Due to the limited quality compared to VSCC, we first applied smoothing of the samples before detecting copy number alterations. Whereas the dVIN samples of patients 1 and 6 did not show any copy number alterations, three CNAs could be detected in the dVIN sample of patient 3.
The patient was a 54-year-old female with a unifocal lesion of 2.5 cm on the left labium minus localised 1.5 cm left from the clitoris. Histopathological examination showed that the dVIN was located in the immediate surroundings of a moderately differentiated VSCC with presence of lymphovascular invasion, an invasion depth of 9.5 mm and a sentinel node with some tumour cells (Figure 2). The copy number abnormalities in dVIN involved an amplification of 1q22 and a deletion at the telomeric region of 12q. Furthermore, chromosome 14 showed a higher median probe level, suggesting a gain of this entire chromosome. All three copy number alterations were also detected in the VSCC sample (Table 1 and Supplementary Table S1). Direct comparison between the dVIN and VSCC showed that these lesions were very similar, suggesting that they are indeed preserved from VSCC. All other copy number alterations detected in VSCC (five gains and 25 deletions) could not be detected in the dVIN sample. This could be partly due to the poorer quality of the dVIN copy number data, hampering the detection of low-intensity copy number changes. However, chromosome 8 in the dVIN sample also showed no signs of copy number abnormalities, whereas in the VSCC sample a high level

![Figure 2](image)

**Figure 2** Histological overview of the epithelium of patient 3; the area on the left side shows VSCC, the area on the right side dVIN. Micro-dissection was performed in these areas, there is a distance of 2 mm (normal epithelium) between both areas (original magnification x50).
8q22-q24.3 amplification was observed in the VSCC sample (Figure 3D and Table 3). These findings demonstrate that the dVIN of patient 3 is clonally related to the adjacent VSCC lesion, but contains lesser chromosomal abnormalities, illustrating a further progression towards malignancy in the VSCC compared to the dVIN.

Figure 3 Shared and different copy number abnormalities in differentiated VIN (dVIN) and vulvar squamous cell carcinoma (VSCC) of patient 3. Shown are the high-level amplification on chromosome 11 (A and B), and the deletion of 12p-ter (C), which is shared between the two samples, and the copy number gains on chromosome 8, which is absent in dVIN (D). Detail of the amplification on chromosome 11 (B) shows that its boundaries are identical between the two samples.

Figure 3 Continued.
Discussion

We present the first study which investigates the genome-wide copy number alterations of HPV-negative VSCCs and patient-matched dVIN lesions using high-resolution SNP array analysis. Gains of 7p and 8q and losses of 6p and 18q were found in most VSCC lesions, as well as aLOH of chromosome 17p. In one patient, three of the 33 CNAs found in VSCC were also detected in dVIN, including a high-level amplification on 11q22 and a deletion of 12qter. These findings suggest that the two lesions originate from a single precursor cell in which a subset of the genetic alterations, possibly driving premalignant events, were present in an early stage, and additional alterations may have resulted in the progression towards VSCC.

In patient 3 a clonal relationship was found between VSCC and dVIN. Whereas the majority of CNAs, of which one high-level amplification, were found only in VSCC, three CNAs appeared to be present also in dVIN. The most pronounced aberration was a high gain of chromosome 11, which encompassed the BIRC and MMP gene clusters as well as the YES-associated protein 1 (YAP1) as a possible candidate gene. This latter gene is known to play a role in the development and progression of multiple cancers and may function as a potential target for cancer treatment.26 YAP1 expression seems to indicate a poor outcome in several cancer types.27–29 Future studies should reveal whether these aberrations are more common in dVIN lesions and are involved in the progression from dVIN towards VSCC.

One could question whether the CNAs we found in the dVIN lesions were actually CNAs present in a subset of VSCC cells that were present in the dVIN biopsy. However, although the samples were taken from the same surgical excision specimen (vulvectomy), cells for DNA were collected from a different site of the specimen. Furthermore, histological examination of the H&E stained slides showed there was a distance of 2 mm with normal epithelium between the dVIN lesion and VSCC lesion (Figure 2). It is important to note that, due to the low copy number intensities in the dVIN sample of patient 3, and the relatively low signal-to-noise ratio, it might be possible that other aberrations detected in the VSCC sample were simply missed in the dVIN sample. Nevertheless, it is unlikely that the high-level amplification at 8q has been missed in dVIN (Figure 3), which indicates that the dVIN and VSCC samples share genomic alterations, but are not identical, thereby making it less likely that VSCC-derived cells were present in the dVIN sample.

Although we found CNAs in one patient that were shared between the dVIN and VSCC lesions, suggesting a clonal relationship, two other dVIN lesions did not show any CNAs. This, however, does not exclude that the dVIN lesions in these patients are clonally related to the VSCC, since the Oncoscan array identifies only a limited set of possible abnormalities.

Next generation sequencing approaches are expected to reveal this matter in much greater detail.

Liegler and Regauer14,30 suggested that the rare identification of dVIN without VSCC in their patient group raised the question of whether dVIN should be considered a true precursor lesion of VSCC or whether it represents an in situ carcinoma component adjacent to an invasive SCC. They suggested that the interpretation of dVIN as a precursor lesion needs to be carefully reconsidered. In general, epithelial disorders are found adjacent to VSCC in 70-80% of patients. However, evidence that some of these disorders are precursors of VSCC is circumstantial.31 Reason to question dVIN as being the precursor lesion is the low incidence of solitary dVIN compared to uVIN, while the majority of VSCCs is not HPV associated. This low incidence can be explained by the difficulty of diagnosing dVIN which might result in an underdiagnosis4 or the existence of a shorter intra-epithelial phase compared to uVIN.13,32 Though there are no recent studies concerning the incidence of dVIN, in daily practice we experience a higher incidence of solitary dVIN since clinicians and pathologists are more aware of the diagnosis. Molecular evidence in favour of dVIN as a precursor lesion can be found in expression profiles of immunohistochemical markers like p53 and MIB1. The typical staining pattern for p53 reported in dVIN is strong staining in the basal layer extending to nuclei in suprabasal layers of the epithelium,32–34 whereas in reactive conditions, the staining trends to vary in intensity and remains confined to the basal epithelium. Furthermore, this is different from the pattern seen in VSCC in which the entire epithelium shows p53 expression.

The number of studies that did try to find genetic similarities between VIN and VSCC are small and mainly involve a low number of loci investigated. Furthermore, these studies do not differentiate between uVIN and dVIN-related VSCCs. Lin et al11 compared patterns of LOH between different locations of the tumour of one patient with a HPV-negative VSCC, the tumour itself scored positive for LOH in four of seven loci. Furthermore, one site of dVIN shared its locus with the invasive tumour whereas the other dVIN shared one of two loci with the cancer. Normal epithelium and stroma showed no abnormalities. These results are suggestive for dVIN being a precursor lesion, though this conclusion is based on a single case and only seven genomic loci. Pisto et al17 compared 11 identified loci which scored positive for AI in greater than 50% of cases from a prior study of VSCC (n=16)4 to pre-invasive lesions (LS, uVIN, dVIN and hyperplasia). This comparison showed a lower percentage of AI in pre-invasive lesions, though in this comparison no distinction was made between HPV-positive and negative lesions. The advantage of our study is the high number of loci investigated, which allows us to compare the whole genome of dVIN and VSCC which provides more detailed information.
Several studies have reported different patterns of chromosomal alterations with the use of lower resolution techniques in VSCC. Recently, Trietsch et al. published a review of all studies concerning epigenetic alterations. Detection of somatic mutations was mostly focussed on TP53. Furthermore, they showed that HPV-negative tumours harboured more mutations than HPV-positive tumours, and the percentage of mutated samples increased with higher stages of (pre)cancerous lesions. Thomas et al. showed in their review that the CNAs frequently observed with the use of comparative genetic hybridization (CGH) in high risk HPV-positive VSCC (n=33) were gain of 3q and loss of 3p and 11q. These CNAs were also most frequent observed in cervical SCC. In the HPV-negative VSCC (n=14) the picture is different, CGH analysis showed frequent gains at 8q (12/14) and less frequent on 3q (4/14) and 11q (4/14), while most frequent losses were seen at 5q (4/14) and 11q (4/14). Gain of 8q was also frequently found in our study (5/6). Loss of 8p that was found in all cases in our study, is present in other studies though less frequent (3/14). Studies on VIN are scarce and mainly based on usual type VIN. Only Aulmann et al addressed uVIN and dVIN separately. They analysed 3q26 gains using fluorescence in situ hybridisation and showed gains were present in most dVINs and in the minority of uVINs.

In order to provide more evidence for our hypothesis that dVIN is the precursor lesion of VSCC by determining the genetic profile of these lesions, we method we used showed a clonal relationship in one patient. Nevertheless, retrieving enough DNA for analysis has shown to be challenging as only three of six dVIN samples contained enough DNA. Although the Oncoscan FFPE Expres v.2 (Affymetrix, Santa Clara, CA, USA) is especially focussed on TP53, fluorescence in situ hybridisation (CGH) in high risk HPV-positive VSCC (n=33) were gain of 3q and loss of 3p and 11q. These CNAs were also most frequent observed in cervical SCC. In the HPV-negative VSCC (n=14) the picture is different, CGH analysis showed frequent gains at 8q (12/14) and less frequent on 3q (4/14) and 11q (4/14), while most frequent losses were seen at 5q (4/14) and 11q (4/14). Gain of 8q was also frequently found in our study (5/6). Loss of 8p that was found in all cases in our study, is present in other studies though less frequent (3/14). Studies on VIN are scarce and mainly based on usual type VIN. Only Aulmann et al addressed uVIN and dVIN separately. They analysed 3q26 gains using fluorescence in situ hybridisation and showed gains were present in most dVINs and in the minority of uVINs.

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In summary, we have used high-resolution SNP array analysis to investigate the genome-wide alterations in VSCC and its possible genetic relationship with dVIN. One patient showed accumulating events which points in the direction of our hypothesis that dVIN is the precursor lesion of VSCC. In order to collect more evidence for this hypotheses, a proof of concept study will be performed in a larger patient group using whole exome sequencing.


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Table S2 Recurrently affected copy number regions.

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*Samples indicated in bold and underlined carry high level amplifications at these positions.

Table S3 Regions of copy-neutral loss-of-heterozygosity (LOH).

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Cytology of the vulva: feasibility and preliminary results of a new brush

Loes C.G. van den Einden, Johanna M.M. Grefte, Irene A. van der Avoort, Judith E. Vedder, Leon C.L.T. van Kempen, Leon F.A.G. Massuger, Joanne A. de Hullu

British Journal of Cancer. 2012;106:269-73
Abstract

Objective
Taking a biopsy is a standard procedure to make the correct diagnosis in patients with suspicious premalignant vulvar lesions. The use of a less invasive diagnostic tool as triage instrument to determine whether biopsy is necessary may improve patient comfort especially in patients with chronic vulvar disorders that may warrant consecutive biopsies. This study was conducted to investigate whether vulvar brush cytology is feasible and may be used to detect (pre)malignant vulvar lesions.

Methods
A pilot study was performed with patients having clinically normal vulvar skin, lichen sclerosus (LS), usual or differentiated vulvar intraepithelial neoplasia or squamous cell carcinoma. A total of 65 smears were taken with the use of a vulvar brush and biopsies were performed for histopathological analysis.

Results
Out of 65 smears, 17 (26%) were discarded because of poor cellularity. A total of 28 of 29 (97%) smears with a histological proven (pre)malignancy had a smear classified as ‘suspicious’ or ‘uncertain’. Cytology classified 11 smears as ‘non-suspicious’, of which 10 (91%) were indeed normal skin or LS. The accuracy, based on the presence of a lesion, for (pre)malignant lesions with the use of the brush showed a sensitivity of 97% and a negative predictive value of 88%.

Conclusion
Vulvar brush cytology is feasible and may be a first step in the development of a triage instrument to determine whether subsequent biopsy of a clinically (pre)malignant lesion is necessary.

Introduction

Vulvar squamous cell carcinoma (SCC) is a multifactorial disease following two separate and independent pathways. Each pathway has its own precursor lesion; usual VIN (uVIN) is the first precursor and is caused by the human papilloma virus (HPV). Differentiated vulvar intraepithelial neoplasia (dVIN) is the second and most common precursor and often occurs in a background of lichen sclerosus (LS). On the basis of our earlier studies we conclude that dVIN and not LS is the true precursor of SCC.

Patients with LS have a lifetime risk of 4–6% to develop vulvar SCC. Therefore, life-long follow-up is advised. In case of suspicion of a vulvar (pre)-malignancy, histopathological examination is required and considered to be the gold standard. Usually punch biopsies are conducted under local or general anaesthesia. After treating vulvar (pre)malignancies there is often residual disease and/or a high risk of recurrent lesions. In these patients, the vulvar examination can be difficult because of scarring due to a previous vulvectomy. Besides, the majority of these patients fear repeated biopsies making the development of a less invasive, accurate, diagnostic tool desirable to improve patient comfort. Brush cytology has been proven to be a reliable patient-friendly method to diagnose cervical (pre)malignancies. The accuracy of cytology largely depends on the presence of enough cells and the ability to recognise cellular and nuclear atypia. Various techniques for vulvar cytology have been described with disappointing results because of scarce cellularity so vulvar cytology is now far from being common practice.

For the present pilot study, a new vulva brush (Rovers Medical Devices BV, Oss, The Netherlands; Figure 1) for obtaining vulvar cytology was introduced for a feasibility study at our department. A non-invasive tool was designed, resembling the cervex-brush, but with a brushing surface suitable for the vulvar skin and the ability to collect enough cells for cytology. Though histology still remains the gold standard, with this brush we want to make a first step in the development of a triage instrument that can determine whether subsequent biopsy of a clinically (pre)malignant lesion is necessary. This study was conducted to investigate whether vulvar cytology obtained by this brush is feasible and may be used in distinguishing benign from (pre)malignant vulvar lesions.

Patients and methods

The pilot study was performed in patients from the vulvar clinic of the Department of Obstetrics and Gynaecology at the Radboud University Nijmegen Medical Centre, The Netherlands. Over a period of several months, 37 women were recruited having clinically LS, lesions suspicious of uVIN (raised, well-demarcated and asymmetrical lesions, varying
from white and condylomatouslike to brown lesions) or dVIN (raised white plaque, ulcerative or erythematous red lesion) or SCC (ulcerative lesions). All patients underwent cytological brushing and one or more vulvar punch or excisional biopsies were performed. A biopsy was not performed at the site of the brushing of the normal skin.

Brushing was performed only at the site of the lesion; in patients with LS the most affected site was chosen to brush. Saline moistening was used before brushing, in order to remove debris, ointment and/or keratinised squamous cells as much as possible. All smears were prepared following our local standard Thin Prep protocol, using the Thin Prep 3000 Processor (Cytyc Europe Benelux, Almere, The Netherlands), Papanicolaou stained and subsequently (blindly) assessed by both an experienced cytotechnologist (JV) and an expert cytopathologist (JG).

All smears were evaluated and scored for cellularity, presence of hyper and parakeratosis, presence of koilocytes, atypia and squamous cell dysplasia. Regarding cellularity, a slight modification of the Bethesda 2001 guidelines for cervical cytology was followed. In short, <5000 squamous cells and/or anucleate squamous cells per slide were considered inadequate, >5000 but <8000 were suboptimal and <8000 were considered sufficient.

Histological features of uVIN are well recognisable. Atypical cells with increased nucleo-cytoplasmic (N/C) ratio are present at all levels of the epidermis and koilocytes may be numerous. In addition, the chromatin pattern is coarse and mitoses may be numerous (Figure 2A). Histological features of dVIN are more difficult to recognise. Atypia is confined to the (para)basal layers of the epithelium, in which the cells have abundant cytoplasm and may form abortive pearls. Prominent nucleoli are often present. Mitoses may be frequent, but are confined to the (para)basal cell layers. The superficial layers of the epithelium show a normal maturation and do not contain koilocytes. However, individual dyskeratotic cells may be seen. Furthermore, a thick hyper and sometimes parakeratotic layer is often present (Figure 2B). Invasive carcinoma will show a disrupted basement membrane with infiltrating nests of atypical squamous cells surrounded by a desmoplastic stroma reaction. Therefore, the following criteria for cytology were scored: if koilocytes, dyskeratotic squamous cells and cells with increased N/C ratio were present, smears were categorised as suspicious for (pre)malignancy, favour uVIN (Figure 3A and B). If large atypical epithelial cells with prominent nucleoli, eccentric nuclei and abundant non-keratinising cytoplasm were present, in the absence of the above described characteristics, smears were categorised as suspicious for (pre)malignancy, favour dVIN (Figure 3C and D). If only a few atypical cells were present, or if cells showed only slight aberrations, smears were categorised as ‘uncertain’. No attempt was made to specifically diagnose invasive SCC, as this cannot be differentiated reliably from the precursor lesions dVIN and uVIN on cytological brush material.

### Table 1 Classification of cytological smears.

<table>
<thead>
<tr>
<th>Normal</th>
<th>Uncertain</th>
<th>Suspicous for (pre)malignancy:</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Favour uVIN</td>
</tr>
<tr>
<td>No atypical or dysplastic cells</td>
<td>Some atypical cells</td>
<td>Evident dyskaryotic cells and cell groups. Increased N/C ratio, irregular coarse chromatin, irregular nuclear membrane. Koilocytes present.</td>
</tr>
</tbody>
</table>

Abbreviation: N/C ratio= nucleo-cytoplasmic ratio.
All biopsies and excision specimens were routinely fixed (4% buffered formalin) and paraffin embedded. Standard 4-µm thick haematoxylin and eosin-stained sections were used for the classification of the lesions according to current WHO criteria and the recent modification of the ISSVD.\(^1\) Finally, cytological and histological findings were correlated. The study was conducted after obtaining local ethics committee approval from the Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands, and informed consent of all participants.

Statistics
Calculations were performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were used to reproduce study results as percentages, means and medians.

Results
A total of 65 smears with the vulva brush were taken from 37 patients; in 40 of 65 smears a vulvar punch or excisional biopsy was taken immediately adjacent to the brushed area and in 13 of 65 smears a biopsy was taken a median of 5 months before or after the smear. Additionally, in 12 of 37 patients a smear was taken of clinically normal vulvar skin far from the lesion. Brushing was feasible in the outpatient clinic and well tolerated by the patients.

A total of 17 smears (26%) were inadequate because of poor cellularity and excluded from further analysis. Of these excluded smears the diagnosis was LS in 7 of 17 smears (histologically confirmed; 41%) and clinically normal in 7 of 17 smears (41%); an overview of the exact diagnoses can be seen from Table 2. Besides, 18 smears (28%) had a suboptimal cellularity and 30 (46%) had sufficient cellularity.

The correlation between the cytological and histological diagnoses is shown in Table 2. Among the 48 smears when suboptimal or sufficient cellular smears were obtained, 29 were biopsy proven (pre)malignancies; uVIN (n=14), dVIN (n=3) or vulvar carcinoma (n=12). A total number of 28 of 29 (97%) biopsy proven (pre)malignancies had a smear classified as ‘uncertain’ or ‘suspicious for (pre)malignancy’. However, thecellularity of this one false-negative smear was suboptimal and predominantly anucleated squamous cells were present.

Diagnosis of 19 of the remaining 48 smears were biopsy-proven LS (n=14) or samples of normal skin (n=5). In 10 of these 19 cases (53%) the corresponding smear was correctly classified as ‘nonsuspicious’. Seven smears were classified ‘uncertain’, of which six were
histologically diagnosed as LS. Two smears were ‘suspicious for (pre)malignancy’ and were diagnosed as LS and normal.

Although we are aware of the effect the small sample size, we calculated the accuracy of cytology to diagnose a malignancy and/or premalignancy based on the presence of a lesion. Smears of normal skin were excluded for this calculation as it was our purpose to use vulvar cytology to distinguish between benign and (pre)malignant vulvar lesions. Accuracy is shown in Table 3. Cytology has a 100% sensitivity and negative predictive value of 100% in case of a malignancy. In case of premalignancies (uVIN and dVIN), sensitivity of 94% and a negative predictive value of 88% was obtained. For malignant and premalignant samples together 97% sensitivity and a negative predictive value of 88% was calculated. Specificity was 50% for both premalignancy and malignancy. The accuracy of only the smears taken immediately adjacent to the place of biopsy (n=52) showed comparable results.

**Discussion**

Obtaining a rapid and accurate diagnosis in women suspected of VIN or vulvar cancer generally leads to (repeated) punch biopsies and consequent patient discomfort. Though histology remains important as it is currently the gold standard, especially for the primary diagnosis of LS, our results indicate that cytology obtained by the new vulva brush is feasible. Moreover, it may be a possible first step in the development of a triaging instrument to determine which patients with the suspicion of a (pre)malignancy especially in the follow-up, should have a subsequent punch biopsy and in which patients a biopsy might be safely omitted.

In this study, 17 of 53 smears (26%) did not carry enough cells for interpretation. This difficulty in obtaining sufficient material has also been discussed in the previous literature. Whether our brushing technique results in higher cellularity compared with other techniques with a spatula or blade is not clear because studies with different techniques do not use comparable analysing methods. Moreover, the cellularity in vulvar smears is much lower compared with cervical smears. This can be explained by the presence of a thick keratin layer that covers the vulvar epithelium, which requires vigorous brushing to obtain the underlying diagnostic cells for adequate sampling. Additionally, debris and/or keratinous squamous cells may confuse the cytological appearance of the sample; in our study we tried to prevent this by cleaning the surface with saline before brushing. Low cellularity may also be explained by the type of lesion that was brushed; when looking more in detail to the diagnoses of the 17 smears with poor cellularity (Table 2), it is striking and reassuring that the histological findings of these smears are benign. The small proportion of (pre)malignancies with low cellularity (n=3), supports the hypothesis that (pre)malignant lesions may dissociate more easily compared with benign lesions. In this study, brushing was not performed according to a strict protocol, which may have led to a variation in cell collection. For optimising the brushing method a more standardised approach should be followed. This approach should first contain repeatedly firm brushing of the surface. In some patients this may be painful. Probably the use of local anaesthetics such as Xylocaine spray can be helpful in these cases. Second, in cases with remaining low cellularity the use of immunohistochemistry should be considered. There might be an important role for HPV testing and the use of markers such as p53 or p16 to make a distinction between benign, HPV-related (usual) VIN/SCC or HPV-non-related (differentiated)

<table>
<thead>
<tr>
<th>Cytology</th>
<th>Histology</th>
<th>SCC</th>
<th>uVIN</th>
<th>dVIN</th>
<th>Lichen Sclerosus</th>
<th>Normal skin*</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspicious for (pre)malignancy†</td>
<td>Favour uVIN</td>
<td>8</td>
<td>9</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>19</td>
</tr>
<tr>
<td>Favour dVIN</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Uncertain^</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Non suspicious</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>7</td>
<td>3</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Poor cellularity</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>7</td>
<td>7</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>16</td>
<td>3</td>
<td>21</td>
<td>12</td>
<td>65</td>
<td></td>
</tr>
</tbody>
</table>

*Based on clinical appearance, no histologic confirmation. †Atypical cells present, indicative of a (pre)malignancy. ^Presence of atypical cells not conclusive.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Negative predictive value (%)</th>
<th>Positive predictive value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Pre)malignancy*</td>
<td>97</td>
<td>50</td>
<td>88</td>
<td>80</td>
</tr>
<tr>
<td>Malignancy†</td>
<td>100</td>
<td>50</td>
<td>100</td>
<td>63</td>
</tr>
<tr>
<td>Premalignancy^</td>
<td>94</td>
<td>50</td>
<td>88</td>
<td>70</td>
</tr>
</tbody>
</table>

*Including uVIN, dVIN and SCC. †Including uVIN and dVIN.
VIN/SCC. Recently, an attempt was made to identify a new marker of specifically dVIN. Van de Nieuwenhof et al showed localization of mast cells in dVIN, which could be a potential marker for this entity. We hypothesize that the use of markers may lead to adequately differentiation, also in the samples with poor cellularity. Whether this marker will be useful in liquid-based cytology, can be subject of future studies.

Only three prior studies with a limited number of samples, have evaluated whether results from cytology correlate with histological findings of VIN or SCC. Bae-Jump et al concluded that with the use of a spatula end for cytology collection, only 7 of 22 patients (32%) with biopsy proven (pre)malignancies had a vulvar Pap smear significant for VIN or vulvar carcinoma. They concluded that a negative Pap smear was not necessarily indicative for the absence of disease. Likewise, we found one smear with normal cytology, but with a histologically proven dVIN. This cytological misdiagnosis might be explained by suboptimal cellularity and the presence of predominantly anucleated squamous cells in the preparation. As it is known that the atypical cells in dVIN reside predominantly in the (para)basal cell layers, probably in this case brushing was not performed vigorously enough to obtain diagnostic atypical cells. Furthermore, vulvar brush material often contains many anucleated squamous cells. Therefore, in the future a cytological specimen should not only be analysed for cellularity before making a diagnosis, but also additionally be assessed for the presence of enough nucleated squamous cells.

Jimenez-Ayala and Jimenez-Ayala collected cells for cytology (n=563) by scraping with a scalpel blade. They reported that cytology can be used for the diagnosis of malignancy with a sensitivity of 98%, which is comparable to our results, although they also reported a high specificity of 99%. Their better results compared with ours may be due to a more vigorous scraping method in which they probably collected more cells from the deeper tissue layers, underscoring the remarks above. However, patient discomfort was not scored and the accuracy of detecting premalignancies was not investigated in the study.

The accuracy obtained with our vulvar brush is different from the accuracy that can be obtained with the cervical brush to diagnose cervical (pre)malignancies. The accuracy of routine cytology is highly variable in different studies. An overview of Cuzick et al showed that the overall sensitivity (53%, range 18.6–76.7%) is lower compared with the overall specificity (96.3%, range 84.2–99.6%) in detecting high-grade cervical intraepithelial neoplasia (CIN). The primary aim of the coordinated screening programs for cervical cancer is early detection of (pre)malignancies of the cervix in healthy women. This aim is completely different from our study where brushing is used for patients with a suspicious lesion. In our study the sensitivity (97%) was higher compared with the specificity (50%), which is acceptable concerning the aim of triaging patients with suspicious vulvar lesions.

Levine et al used the cytobrush for cell collection and analysed 28 cytological samples of histologically benign or premalignant lesions. With dyskeratosis as the sole cytological criterion for VIN or anal intraepithelial neoplasia (AIN), all samples were consistent with histology except for one (4%) that was cytologically diagnosed as VIN, but no VIN was identified in the biopsy. Presumably, these were all HPV-related VIN or AIN cases; no attempt was made to differentiate between uVIN and dVIN. In contrast with Levine’s percentage of false positive smears (1 out of 28 smears, 4%), in our study 9 smears (19%) were classified as ‘uncertain’ (n=7) or ‘suspicious for (pre)malignancy’ (n=2). Cytological recognition of uVIN generally is easier because koilocytosis and dyskeratotic cells are present in the entire epithelium, whereas in dVIN atypical cells are only present in the basal layer. Because the aim was to diagnose uVIN and dVIN, the threshold of suspicion was lower with consequently more false positives in our study and therefore lower positive predictive value. However, compared with standard management of suspicious vulvar lesions, the amount of biopsies may decrease. We hypothesise that especially those patients, visiting the clinic on a regular basis with recurring premalignancies, may benefit from cytology as a triaging instrument. The experience in our vulvar clinic is in that patients with premalignancies such as uVIN, and in particular dVIN, lesions are difficult to distinguish from benign on the clinical findings. This implies that biopsies may be taken despite the absence of disease. Not seldom we performed a vulvar mapping where no (pre)malignant lesions can be found. In these patients we want to use cytology to safely exclude premalignancies. The next step is to perform a study in a larger group of patients to investigate whether cytology with our vulva brush indeed can function as a triage instrument.

This study shows that cytology obtained by the new vulva brush is promising as a possible first step in obtaining a diagnosis in patients with suspicious (pre)malignant vulvar lesions. By classifying the smear based on the presence or absence of a few parameters, it is possible to detect a (pre)malignancy. However, histology still remains the gold standard and the brushing technique needs to be improved and addressed in future studies to increase the cellularity, which is obligatory for the right diagnosis.
References

5. Van de Nieuwenhof HP, Bulten J, Holsma H, et al. Differentiated vulvar intraepithelial neoplasia is often found in lesions, previously diagnosed as lichen sclerosus, which have progressed to vulvar squamous cell carcinoma. Modern pathology. 2011;24(2):297-305.
Interobserver variability 
and the effect of education in 
the histopathological diagnosis 
of differentiated vulvar 
 intraepithelial neoplasia

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Johanna M.M. Grefte, Peter Bult, Anne Wiersma, Adriana C.H. van Engen-van Grunsven, 
Bart Sturm, Steven L. Bosch, Harry Hollema, Johan Bulten

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Abstract

Objective
No published data concerning intraobserver and interobserver variability in the histopathological diagnosis of differentiated vulvar intraepithelial neoplasia (dVIN) are available, although it is widely accepted to be a subtle and difficult histopathological diagnosis. In this study, the reproducibility of the histopathological diagnosis of dVIN is evaluated. Furthermore, we investigated the possible improvement of the reproducibility after providing guidelines with histological characteristics and tried to identify histological characteristics that are most important in the recognition of dVIN.

Methods
A total number of 34 haematoxylin and eosin-stained slides were included in this study and were analysed by six pathologists each with a different level of education. Slides were reviewed before and after studying a guideline with histological characteristics of dVIN. Kappa statistics were used to compare the interobserver variability. Pathologists with a substantial agreement were asked to rank items by usefulness in the recognition of dVIN.

Results
The interobserver agreement during the first session varied between 0.08 and 0.54, which slightly increased during the second session toward an agreement between -0.01 and 0.75. Pathologists specialised in gynaecopathology reached a substantial agreement (kappa 0.75). The top five of criteria indicated to be the most useful in the diagnosis of dVIN included: atypical mitosis in the basal layer, basal cellular atypia, dyskeratosis, prominent nucleoli and elongation and anastomosis of rete ridges.

Conclusion
In conclusion, the histopathological diagnosis of dVIN is difficult, which is expressed by low interobserver agreement. Only in experienced pathologists with training in gynaecopathology, kappa values reached a substantial agreement after providing strict guidelines. Therefore, it should be considered that specimens with an unclear diagnosis and/or clinical suspicion for dVIN should be revised by a pathologist specialised in gynaecopathology. When adhering to suggested criteria the diagnosis of dVIN can be made easier.

Introduction
Vulvar carcinoma is rare with squamous cell carcinoma as the most common histopathological subtype. Nowadays, we can distinguish two types of squamous cell carcinoma with their own premalignant lesions.1-3 The first type of vulvar squamous cell carcinoma consists of mainly non-keratinizing carcinomas and is caused by an infection with high-risk human papilloma virus (HPV). This type of carcinoma is associated with warty and/or basaloid vulvar intraepithelial neoplasia (VIN, together grouped as usual VIN (uVIN)). The second and most common type of carcinoma is differentiated keratinising squamous cell carcinoma, often occurring in the background of lichen sclerosus. Differentiated VIN (dVIN), which is an entity that has no relation with HPV, is believed to be the precursor lesion associated with this type of vulvar squamous cell carcinoma.3-5

Until 2003, a three grade system for premalignant VIN (VIN grades 1–3, Table 1) was used. As clinicopathological data did not appear to support the concept of a continuous spectrum of VIN lesions leading to vulvar carcinoma that does exist for cervical intraepithelial neoplasia and cervical carcinoma,6-8 this grading system was abolished. The abandonment of VIN 1 and the consolidation of VIN 2 and 3 into one category simply termed (highgrade) VIN, best fitted the studies that have been performed on grading of VIN so far. Nowadays, the concept of usual VIN and differentiated VIN has been accepted more and more by clinical pathologists around the world.

<table>
<thead>
<tr>
<th>Old nomenclature</th>
<th>New nomenclature</th>
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<tbody>
<tr>
<td>VIN 1</td>
<td>No cancer precursor</td>
</tr>
<tr>
<td>Classic (VIN 2/3)</td>
<td>Usual VIN (uVIN)</td>
</tr>
<tr>
<td>(Well-)differentiated VIN 3</td>
<td>Differentiated VIN (dVIN)</td>
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</table>

VIN terminology (ISSVD 2004).
with prominent intercellular bridges. Dyskeratosis is characterised by disturbed maturation and premature keratinisation of squamous cells that are located deeper in the epithelium. In the parabasal layers of the epithelium, individual and clusters of cells show premature maturation, with large cells that show eosinophilia of the cytoplasm and even formation of keratin pearls (Figure 2). The nuclei have prominent nucleoli (Figure 1c), usually predominantly in the (para)basal keratinocytes (Figure 1e). Atypical mitotic figures (Figure 1d) may be seen mainly in the lower layers.9 The most superficial layers show normal maturation without atypical cells, although dyskeratosis may be present above the (para)basal layers with cells that have vesicular nuclei, prominent nucleoli and abundant eosinophilic cytoplasm. As the cytological atypia in dVIN is confined to the basal epidermal cell layers, it is often confused with squamous hyperplasia or lichen sclerosus. The recognition of dVIN is hindered by a high degree of cellular differentiation combined with an absence of widespread architectural disarray, nuclear pleomorphism and diffuse nuclear atypia.9 Probably, this has led to a considerable underdiagnosis of dVIN. Although, it is of great importance to recognise this lesion properly because of its high malignant potential and rapid progression toward vulvar squamous cell carcinoma.9 Making the right pathological diagnosis is of utmost importance to assure proper treatment and follow-up.

Until now, there are no published data on intra and interobserver variability in the histopathological diagnosis of dVIN, although it is widely accepted to be a subtle and difficult histopathological diagnosis.1 In this study, the reproducibility of the histopathological diagnosis of dVIN is evaluated in a group of six Dutch pathologists with different level of experience. Furthermore, we investigated the possible improvement in diagnosing dVIN.
after providing guidelines with histological characteristics of dVIN. Finally, we tried to identify histological characteristics that are most important in the recognition of dVIN.

**Patients and methods**

Haematoxylin and eosin (H&E)-stained slides of vulvar biopsies taken before the diagnosis of vulvar squamous cell carcinoma of 60 patients were collected, all patients subsequently developed vulvar squamous cell carcinoma. All patients were treated at the Department of Obstetrics and Gynaecology of the Radboud University Nijmegen Medical Centre (RUNMC) or the University Medical Centre Groningen between 1990 and 2008. The slides were reviewed by two experts in the field of gynaecopathology from these two hospitals (JB and HH), independently and unaware of the course of the disease. Discrepancies in diagnoses were resolved in a consensus meeting with these two expert gynaecologic pathologists (further on named ‘consensus pathologists’). Consensus diagnoses were based on published criteria, which are shown in Table 2 and considered to be the golden standard.

Of 60 slides, 46 were diagnosed as lichen sclerosus or dVIN during the consensus meeting. Thirty-five corresponding formalin fixed paraffin embedded specimens could be retrieved, re-cut at 4 mm, and H&E stained. To quicken the process of analysis, for each specimen a total number of three slides were re-cut. One consensus pathologist (JB) compared the slides from each specimen to confirm that these were comparable. As one specimen lost quality after re-cutting, it was excluded from further analysis. Finally, three identical sets of 34 slides were assessed for this study.

Six pathologists (consensus pathologists not included) were asked to classify all 34 slides in two separate sessions. The group of pathologists consisted of two gynaecologic pathologists (pathologists that had special training in gynaecopathology), two general pathologists and two pathologists in training. During the first session, the pathologists were asked to diagnose the lesions as lichen sclerosus, dVIN, high-grade dysplasia and/or other. When squamous cell carcinoma (n=4) was present next to lichen sclerosus or dVIN, the pathologists were asked to score both. No information about age, clinical aspect of the lesions or original diagnosis was provided. In between the first and second session, the pathologists were asked to study a guideline. This guideline was developed by the consensus pathologists (HH and JB) and consisted of the descriptive categories (lichen sclerosus, uVIN and dVIN) with extensive description of the pathological features (Table 2), illustrated by low- and high-power field photographs. After a washing out period of at least 3 months, the six pathologists were asked to study the guideline and diagnose the lesions as lichen sclerosus, dVIN and/or high-grade dysplasia and score the histological characteristics (Table 2). To evaluate the reproducibility of the histopathological diagnosis of dVIN, interobserver variability was calculated. Furthermore, the effect of education in each individual pathologist was assessed. To identify histological characteristics that were most important in the recognition of dVIN, one of the consensus pathologists on behalf of the consensus pathologists (JB) and pathologists with a high level of agreement were asked to put criteria in order of usefulness for the diagnosis of dVIN.

**Statistical Methods**

All slides diagnosed with dVIN by each pathologist were compared with the golden standard to give a level of agreement among pathologists. The kappa statistic, often used in studies in order to test the interobserver variability, was used. Values of 0.4–0.6, 0.6–0.8 and 0.8 were taken to reflect moderate, substantial or excellent correspondence, respectively. Analyses were performed using SPSS 16.0 (SPSS, Chicago, IL, USA).

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**Table 2** Histological characteristics of dVIN.

<table>
<thead>
<tr>
<th>Characteristic</th>
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<tbody>
<tr>
<td>Hyperplasia/acanthosis</td>
</tr>
<tr>
<td>Hyperkeratosis</td>
</tr>
<tr>
<td>Parakeratosis</td>
</tr>
<tr>
<td>Elongation and anastomosis of rete ridges</td>
</tr>
<tr>
<td>Basal cellular atypia (including disarray of the basal cellular layers, large pleomorphic keratinocytes, enlarged vesicular nuclei)</td>
</tr>
<tr>
<td>Prominent nucleoli</td>
</tr>
<tr>
<td>Atypical mitosis in the basal layer</td>
</tr>
<tr>
<td>Dyskeratosis (keratin pearl formation)*</td>
</tr>
<tr>
<td>Hypermaturation of rete ridges</td>
</tr>
</tbody>
</table>

Based on published criteria (4, 7, 12–13) *Dyskeratosis = disturbed maturation and premature keratinisation of squamous cells that are located deeper in the epithelium. In the parabasal layers of the epithelium, individual cells and clusters of cells show premature maturation, with large cells that show eosinophilia of the cytoplasm and even formation of keratin pearls.
Results

Of the 34 slides assessed in this study, 20 (59%) were diagnosed as lichen sclerosus, 13 (38%) as dVIN and 1 (3%) as normal skin during the consensus meeting of the consensus pathologists. The median time to development of vulvar squamous cell carcinoma was 44 months (range 9–200); in patients with lichen sclerosus/normal skin this was 92 (range 9–200) months and with a prior biopsy of dVIN 24 (range 8–64) months. The agreement between the consensus pathologists (HH and JB) was 88%; 30 of 34 slides were scored concordant, with a kappa value of 0.73.

The distribution of collected data is shown in Table 3. The median time between the first and second session was 4 months (range 3–5 months). Pathologists D, E and F scored dVIN more often during the first session in comparison with the consensus, while pathologists A, B and C were less likely to score dVIN. The latter more often scored a lesion as high-grade dysplasia and/or other diagnosis, like aspecific dermatitis (n=13) or lichen plans (n=5).

During the second session, the over- and underdiagnosis of dVIN remained present, although less clear. Pathologists were less likely to score high-grade dysplasia during the second session.

The interobserver agreement between pathologists during the first session varied between 0.08 and 0.54 (data not shown). The overall kappa that summarises in a single coefficient the k values relative to the different pairs of pathologists, could not be calculated because of the heterogeneous k values. The interobserver agreement between pathologists after studying the guidelines during the second session slightly increased with values between -0.01 and 0.75.

Table 4 shows the kappa value of the diagnosis dVIN of individual pathologists vs consensus of the first and second session, categorised according to experience of the pathologist. The values range between 0.27 and 0.54 in the first session and between 0.15 and 0.75 in the second session. All pathologists increased in kappa value, except for pathologists B and C. Pathologists with training in the field of gynaecopathology (gynaecologic pathologists), could reach higher kappa values after studying the guidelines compared with general pathologists and pathologists in training.

The most important criteria in order of usefulness for the diagnosis of dVIN were scored by one of the consensus pathologists (JB) and pathologists with a high level of agreement (pathologist E and F) and are displayed in Table 5. The top five of these criteria included atypical mitoses in the basal layer, basal cellular atypia, dyskeratosis, prominent nucleoli, and elongation and anastomosis of rete ridges. A schematic overview of these criteria is

---

Table 3 Diagnosis made by pathologists during the first and second session.

<table>
<thead>
<tr>
<th>Pathologist</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>Consensus</th>
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<tr>
<td>Diagnosis session 1:</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>dVIN¹</td>
<td>7</td>
<td>7</td>
<td>3</td>
<td>24</td>
<td>19</td>
<td>16</td>
<td>13</td>
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<tr>
<td>High-grade dysplasia NOS²</td>
<td>3</td>
<td>5</td>
<td>4</td>
<td>1</td>
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<td>0</td>
<td>0</td>
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<td>Lichen Sclerosus</td>
<td>17</td>
<td>6</td>
<td>12</td>
<td>9</td>
<td>9</td>
<td>12</td>
<td>20</td>
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<tr>
<td>Others~</td>
<td>7</td>
<td>16</td>
<td>15</td>
<td>0</td>
<td>6</td>
<td>6</td>
<td>1</td>
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<td>Diagnosis session 2:</td>
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<td>dVIN</td>
<td>10</td>
<td>15</td>
<td>4</td>
<td>21</td>
<td>13</td>
<td>13</td>
<td>-</td>
</tr>
<tr>
<td>High-grade dysplasia NOS</td>
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<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Lichen Sclerosus</td>
<td>18</td>
<td>7</td>
<td>9</td>
<td>13</td>
<td>16</td>
<td>16</td>
<td>-</td>
</tr>
<tr>
<td>None of the above</td>
<td>5</td>
<td>9</td>
<td>21</td>
<td>0</td>
<td>5</td>
<td>3</td>
<td>-</td>
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</tbody>
</table>

Values are given as N. ¹Differentiated vulvar intraepithelial neoplasia. ²Not otherwise specified. *Pathologist in training. ^General pathologist. #Gynaecologic pathologist. ~Including aspecific dermatitis (n=13), lichen plans (n=5), acanthosis (n=4), squamous cell carcinoma (n=4), polyp (n=2), condylomata (n=2), lichen simplex chronicus (n=1), hyperkeratosis not otherwise specified (n=1), epidermal cyst (n=1), radiation effect (n=1) and not otherwise specified (n=17).

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Table 4 Kappa value of the diagnosis of dVIN of individual pathologists versus consensus dVIN (N=34).

<table>
<thead>
<tr>
<th>Pathologist</th>
<th>Session 1</th>
<th></th>
<th></th>
<th></th>
<th>Session 2</th>
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<th></th>
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<tr>
<td></td>
<td>Agreement with consensus¹</td>
<td>Kappa (95% CI)</td>
<td>Agreement with consensus¹</td>
<td>Kappa (95% CI)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pathologist in training</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>26/34 (77)</td>
<td>0.45 (0.16-0.75)</td>
<td>29/34 (85)</td>
<td>0.67 (0.42-0.93)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>B</td>
<td>24/34 (71)</td>
<td>0.32 (0.00-0.63)</td>
<td>20/34 (59)</td>
<td>0.15 (-0.18-0.48)</td>
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</tr>
<tr>
<td>General pathologist</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>24/34 (71)</td>
<td>0.27 (0.01-0.53)</td>
<td>23/34 (68)</td>
<td>0.21 (-0.07-0.49)</td>
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<tr>
<td>D</td>
<td>21/34 (62)</td>
<td>0.30 (0.06-0.55)</td>
<td>24/34 (71)</td>
<td>0.44 (0.18-0.70)</td>
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</tr>
<tr>
<td>Gynaecologic pathologist</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>26/34 (77)</td>
<td>0.54 (0.28-0.80)</td>
<td>30/34 (88)</td>
<td>0.75 (0.52-0.98)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>23/34 (68)</td>
<td>0.34 (0.03-0.66)</td>
<td>30/34 (88)</td>
<td>0.75 (0.52-0.98)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹All slides were scored as ‘dVIN=yes’ or ‘dVIN=no’ and compared with consensus.

Most important criteria in order of usefulness for the diagnosis of dVIN were scored by one of the consensus pathologists (JB) and pathologists with a high level of agreement (pathologist E and F) and are displayed in Table 5. The top five of these criteria included atypical mitoses in the basal layer, basal cellular atypia, dyskeratosis, prominent nucleoli, and elongation and anastomosis of rete ridges. A schematic overview of these criteria is...
displayed in Figure 3. All histological characteristics scored in slides with consensus of the diagnosis dVIN (n=22) of the pathologists with a substantial agreement (kappa value 0.7 or higher; pathologist E and F) were scored. The top five histological characteristics were identified in nearly all slides.

Discussion

With this study, we show that the agreement on the histopathological diagnosis of dVIN is low and diagnosing dVIN is difficult. After providing guidelines with histological characteristics to diagnose dVIN, agreement did improve but mainly in gynaecologic pathologists, probably due to their long learning curves in the past. Therefore, it should be considered that specimens with an unclear diagnosis and/or clinical suspicion for dVIN should be revised by an experienced gynaecologic pathologist.

Making the right diagnosis is of utmost importance to assure proper treatment and follow-up, as dVIN is known for its rapid progression toward squamous cell carcinoma. Various studies highlighted the difficulty in making the clinical and histopathological diagnosis of dVIN. Unfortunately, this study shows that the pathological reproducibility is low in patients that subsequently developed a vulvar squamous cell carcinoma, which corresponds with the difficulties in diagnosing dVIN by the clinician.

Little is known about how pathologists differ in their interpretation of dVIN. It is widely accepted to be a subtle and difficult histopathological diagnosis as it can easily be mistaken for a benign dermatitis or epithelial hyperplasia and may be difficult to distinguish from the often present background of lichen sclerosus. To our knowledge, only three prior studies have addressed the interobserver variability of VIN1–3 of which all used the abandoned nomenclature of VIN 1–3 to classify the specimens. Trimble et al demonstrated moderate to good agreement among experienced gynaecological pathologists in making the distinction between those lesions related to HPV and those that are not. Unfortunately, these lesions included squamous cell carcinoma and VIN 1–3; no agreement was calculated for the non-HPV VIN3. Preti et al showed an overall agreement of 73.9% for VIN 2/3 lesions, although no cases of differentiated VIN were included. Van Beurden showed a good agreement of 40 specimens with normal skin and VIN 1–3, of which possibly some of the VIN cases may be of the non-HPV type although this is not further clarified. Apparently, there is no literature that focuses on the histopathological diagnosis of dVIN.

The results of this study show that the diagnosis of different pathologists after providing guidelines with histological characteristics to diagnose dVIN, could only reach a substantial agreement of 0.75 (CI 0.52–0.98) in gynaecologic pathologists. Obviously, in diagnosing dVIN, more practice leads to better skills as the highest agreement could be reached in pathologists with more experience. Likely, also continuous exposure of cases with differentiated VIN in daily practice is important to keep experience.

Figure 3 Five most important histological characteristics in the diagnosis of differentiated vulvar intraepithelial neoplasia (dVIN) according to the pathologists with κ>0.7 and the consensus pathologists. Schematic overview of: (A) normal epithelium and (B) dVIN with atypical mitosis in the basal layer, basal cell atypia, dyskeratosis, prominent nucleoli, and elongation and anastomosis of the rete ridges.
Chapter 4 Interobserver variability dVIN

Apparently, studying the guidelines developed by the consensus pathologists (JB and HH) based on literature was not sufficient enough to increase the level of agreement among pathologists during the second session. A different approach in teaching general pathologists in the recognition of dVIN, for example, with an interactive session with an experienced gynaecologic pathologist, may probably reach higher agreement. An interactive session, like a virtual workshop where clinically and pathologically doubtful dVIN lesions are being discussed may be helpful.

The low interobserver agreement indicates the need for some clarity in making the diagnosis dVIN properly. Therefore, we tried to identify the most important histopathological features by asking the consensus pathologists and pathologists with a substantial agreement, to rank the items that they thought were the most important in making the diagnosis of dVIN, which is shown in Table 5. We agree with Hart and Scurry that pathologists should not be focused on nuclear atypia alone, in the diagnosis of non-HPV premalignancy, but should also look for other supporting features. When some of the top five features listed in Table 5 are present, there has to be a concern and the diagnosis dVIN should be considered.

Besides the use of these histopathological criteria in making the right diagnosis, there may also be a role for immunohistochemistry. The use of MIB1 can be helpful to distinguish between normal vulvar epithelium and dVIN as the basal cell layer in dVIN shows a higher proliferation index (percentage of MIB1-positive cells), than normal vulvar epithelium, where the basal cell layer often is negative for MIB1. Furthermore, in dVIN a strong positive staining of the (sub)basal cell layers with p53 can be seen. Strong staining of all cell layers with p16 is suggestive for usual VIN and not for differentiated VIN.

An important and difficult problem, which we did not address in this study, is to decide whether one is looking at dVIN or invasive squamous cell carcinoma. Separated small nests of highly differentiated squamous cells may be seen in the dermis, raising the question of whether early invasion has taken place. In superficial biopsies, distinction of differentiated VIN from early invasive squamous cell carcinoma may be very difficult to make. Therefore, it is important to keep to the classic criteria of invasiveness: small irregular nests of highly differentiated (atypical) squamous cells or individual strongly atypical cells with prominent nucleoli or and a desmoplastic reaction around the invasive nests.

As most specimens with a suspicion of dVIN are seen by a general pathologist in daily practice, it is worthwhile considering revision of specimens with an unclear diagnosis and/or clinical suspicion for dVIN by an experienced gynaecologic pathologist. Probably, interaction between the clinician and pathologist will also be helpful.

In conclusion, the histopathological diagnosis of dVIN is difficult as the interobserver agreement is low. Only among gynaecologic pathologists, kappa values showed a substantial agreement after providing guidelines. To increase agreement among general pathologists, a more extensive instruction like (virtual) workshops with dVIN cases and doubtful lesions may be helpful. The histopathological features: atypical mitosis in the basal layer, basal cellular atypia, dyskeratosis, prominent nucleoli and elongation and anastomosis of the rete ridges that are ranked by pathologists with a substantial agreement, may be helpful in the diagnosis of dVIN.

Table 5 Histological characteristics of dVIN

<table>
<thead>
<tr>
<th>Histological characteristics of dVIN~</th>
<th>Order of usefulness*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atypical mitosis in the basal layer</td>
<td>1</td>
</tr>
<tr>
<td>Basal cellular atypia</td>
<td>2</td>
</tr>
<tr>
<td>Dyskeratosis</td>
<td>3</td>
</tr>
<tr>
<td>Prominent nucleoli</td>
<td>4</td>
</tr>
<tr>
<td>Elongation and anastomosis of rete ridges</td>
<td>5</td>
</tr>
<tr>
<td>Enlarged vesicular nuclei</td>
<td>6</td>
</tr>
<tr>
<td>Keratin pearl formation</td>
<td>7</td>
</tr>
<tr>
<td>Hypermaturation of rete ridges</td>
<td>8</td>
</tr>
<tr>
<td>Prominent intracellular bridges</td>
<td>9</td>
</tr>
<tr>
<td>Epidermal thickening (hyperplasia/acanthosis)</td>
<td>10</td>
</tr>
<tr>
<td>Parakeratosis</td>
<td>11</td>
</tr>
</tbody>
</table>

~Differentiated vulvar intraepithelial neoplasia. *According to the pathologists with K >0.7 (pathologists E and F) and one pathologist on behalf of the consensus pathologists (JB)
References


4. van de Nieuwenhof HP, Bulten J, Holliema H, et al. Differentiated vulvar intraepithelial neoplasia is often found in lesions previously diagnosed as lichen sclerosus, which have progressed to vulvar squamous cell carcinoma. *Modern pathology*. 2012;24(2):297-305.


An alternative way to measure the depth of invasion of vulvar squamous cell carcinoma in relation to prognosis

Loes C.G. van den Einden, Leon F.A.G. Massuger, Johanna K. Jonkman, Peter Bult, Joanne A. de Hullu, Johan Bulten

*Modern pathology. 2015;28:295-302*
Abstract

Objective
Depth of invasion is an important prognostic factor for patients with vulvar squamous cell carcinoma. The aim of this study was to identify the most optimal method of measuring the depth of invasion in relation to the individual outcome in patients with vulvar squamous cell carcinoma.

Methods
Data of 175 consecutive patients with a primary vulvar squamous cell carcinoma with known lymph node status, treated in the Radboud University Medical Centre, the Netherlands (2000–2010), were stored in a database. At pathology review of 148 (85%) cases, depth of invasion was measured using the conventional and alternative methods.

Results
In 148 vulvar squamous cell carcinoma patients, the median depth of invasion was shown to be decreased from 5.5mm (range 1.1–20) using the conventional method to 3.6mm (range 0.2–20) using the alternative method (P<0.05). This led to a change in the FIGO stage in 13 of the 148 (9%) patients and a change in depth of invasion from 3.5 to 0.2mm in one patient (1%) with FIGO stage IIIA. Of all 69 stage 1B patients, 13 (19%) were downstaged to stage IA. The downstaged patients developed less recurrences (15% vs 39%) and had a higher disease-specific survival (100% vs 84%) compared with the patients who remained FIGO stage IB.

Conclusion
Using the alternative method for measuring the depth of invasion in tumours of vulvar squamous cell carcinoma patients, 19% of the patients with a FIGO stage IB tumour might be treated without groin surgery resulting in less treatment-related morbidity. The results are promising but more prospective data on a higher number of patients are necessary.

Introduction

Vulvar squamous cell carcinoma is a rare disease that mainly affects elderly women. For decades, radical surgery (radical vulvectomy with bilateral inguinofemoral lymphadenectomy) has been the standard treatment for the early-stage disease with a favourable prognosis but with the consequence of impressive morbidity such as wound-healing problems, lymph oedema, and psychosexual impact.

Over the past years, efforts have been made to individualise the treatment of patients with vulvar squamous cell carcinoma and define subgroups of patients who may be treated by less radical procedures. Until now, there are some generally accepted modifications for patients with macroinvasive tumours (>1-mm invasion): separate incisions instead of ‘en bloc’ approach; wide local excision instead of radical vulvectomy; unilateral lymphadenectomy in case of a lateralised tumour; and a sentinel lymph node procedure in patients with a unifocal tumour <4 cm without abnormal groin nodes at imaging. In case of microinvasive tumours (<1-mm invasion and a maximum diameter of 2 cm), patients are treated with a wide local excision only, and treatment of the groins can be safely omitted because only <1% of these superficially invasive vulvar squamous cell carcinoma metastasise to the groins. This is in contrast to tumours with >1-mm invasion, which have a risk of nodal metastases of up to 34%. So far, patients with a stage IA tumour are the only group of vulvar squamous cell carcinoma patients who will not need to undergo treatment of the groins.

As the depth of invasion guides the mode of treatment of vulvar squamous cell carcinoma patients, it is important that pathologists use a uniform measuring method that reflects the best clinical outcome. In the past, several methods have been described by Wilkinson, which are shown in Figure 1.

In 1984 the International Society for the Study of Vulvo-Vaginal Disease (ISSVD) and the International Society of Gynaecological Pathologists (ISGYP) recommended to define the depth of invasion as follows: from the epithelial junction of the most superficial adjacent dermal papilla to the deepest point of invasion (Method A in Figure 1). Reasons for choosing this method are not scientific but are mainly based on the following practical issues: (1) the adjacent dermal papilla can be found in all sites of the vulva, (2) it is not altered by variations in the depth of rete ridges, and (3) the measurement is not significantly influenced by hyperkeratosis, tumour surface ulceration, or adjacent epithelial hyperplasia. Only Kurzl et al compared several methods of measurement, searching for the clearest prognostic differentiation between groups of patients. They found that depending on the cutoff point used for the depth of invasion (2, 3, or 5mm), different measuring methods led to different (disease-free) survival. Unfortunately, tumours were not classified
up and over 1mm, and the lymph node status was not available in this study as all patients had undergone groin irradiation.

On the basis of our experience with the clinical outcome of patients with vulvar squamous cell carcinoma and on the basis of the fact that there is no scientific basis for choosing the current measuring method for the depth of invasion, it can be argued whether an alternative method of measuring depth of invasion may give a better reflection of the prognosis. Therefore, the aim of this study was to identify the most optimal method of measuring the depth of invasion in relation to the individual outcome in patients with vulvar squamous cell carcinoma.

**Materials and methods**

**Patients**

All patients with a primary vulvar squamous cell carcinoma and known lymph node status (based on results of inguinofemoral lymphadenectomy or sentinel lymph node procedure, FIGO stage ≥IB) diagnosed between 2000 and 2010 in the Radboud University Medical Centre (RUMC) were included in this study. Data of the patients were collected from medical charts, electronic patient files, and pathology reports, and were stored in a database. Patient characteristics included age, date of diagnosis, FIGO stage (2009), treatment modality, and recurrences. Pathological characteristics included tumour size, depth of invasion of the tumour, multifocality, nodal status, and presence of lymphovascular invasion. Furthermore, information on vital status, date, and the cause of death was obtained until the 1st September 2013.

**Pathologic assessment**

Available haematoxylin and eosin (H&E) slides of surgical resections of all patients were retrieved and revised by an expert gynaecopathologist (JB), independently and unaware of the clinical course of the patients. The depth of invasion was measured using a measuring ocular and two different methods were addressed: the conventional method (measures from the epithelial junction of the most superficial dermal papilla to the deepest point of tumour invasion, method A Figure 1) and the alternative method (measures from the basement membrane of the deepest adjacent (dysplastic) tumour-free rete ridge to the deepest point of invasion, method B Figure 1). Reasons for choosing this particular alternative method were as follows: (1) it is also used in measuring invasion depth in other cancer sites (such as in cervical and larynx/trachea), (2) logically, tumour cells will originate from the nearest rete ridges instead of the most superficial one, (3) besides the conventional method, it is the only method described by Wilkinson that is not altered by variation of rete ridges or influenced by hyperkeratosis, ulceration, or epithelial hyperplasia. In cases where the deepest rete ridge was deeper than the tumour, the most adjacent basement membrane of the rete ridge was used to measure. Besides depth of invasion, grade of differentiation and lymphovascular invasion were determined. In case the tumour was multifocal, the deepest lesion was selected and in case the depth of invasion was ≤1mm, all lesions were assessed. In cases where the depth of invasion was >1mm using the conventional measuring method but ≤1mm using the alternative method, the slides of the biopsies taken before primary surgery were retrieved and assessed using both measuring methods. On the basis of the depth of invasion measured with the alternative method and the characteristics known before (tumour of ≤2 cm diameter) and after groin surgery (groin metastasis yes/no), patients were given a new FIGO stage.

**Figure 1** Schematic drawing of the skin with different methods of measuring the depth of invasion of vulvar squamous cell carcinoma. Method A (conventional method in this study): measurement from the epithelial junction of the most superficial adjacent dermal papillae to the deepest point of invasion, method recommended by the ISSVD and the ISGYP. Method B (alternative method in this study): measurement from the most adjacent dysplastic abnormal rete ridge to the deepest point of invasion, modified from one of the measuring methods described by Wilkinson et al. Method C: measurement from the surface to the deepest point of invasion=tumour thickness. Method D: measurement from the granular layer to the deepest point of invasion (modified from Wilkinson et al).
Patients who were included in this study underwent a sentinel lymph node procedure in case the tumour diameter was <4 cm and the inguinoferoral lymph nodes were clinically nonsuspicious. The pathologic assessment of the sentinel node(s) was performed according to the standard protocol used in the GROINSS-V study. In short, ultrastaging was performed by sectioning the lymph nodes at 2- to 3-mm intervals. In addition, pairs of sections were cut at 350-mm intervals and stained with H&E and immunostained with cytokeratin 1% AE 1/3 antikeratin solution (Boehringer Mannheim, Mannheim, Germany).

Statistical analysis
The median invasion depths measured using the conventional and alternative methods were compared using the Wilkinson-signed rank test. Clinical and pathological characteristics of patients with a change in the FIGO stage were compared with those without a change in the FIGO stage, using the Fisher’s exact test and the χ² test. Furthermore, for these patient groups the overall survival and disease-specific survival were calculated using the Kaplan–Meier method. All analyses were performed using the SPSS software, version 20 (SPSS Inc, Chicago, IL, USA). P-values less than 0.05 were considered to be statistically significant.

Results
Population description
For this study, 175 patients met the inclusion criteria: 27 cases (15%) were excluded because the H&E slides could not be retrieved (n=26) or because of a low quality of the slides for review (n=1). Finally, data from the 148 patients were enrolled in the study. The median age of the study population was 72 years (range 36–91). The median time of follow-up was 54 months (range 2 weeks to 156 months). Tumour-related characteristics are listed in Table 1.

Comparative measurement of tumour invasion
Of all the 148 patients, the depth of invasion was measured using the conventional and the alternative method. The invasion depth of 13 patients (9%) did not change; of 80 patients (54%) the depth decreased 0.1–1 mm; of 20 patients (14%) the depth decreased 1.1–2 mm; and of 35 patients (24%) the depth decreased 2.1–8.1 mm. The median depth of invasion using the conventional method was 5.5 mm (range 1.1–20); using the alternative method the median depth of invasion was 3.6 mm (range 0.2–20), which is significantly lower (Wilcoxon-signed rank test, P<0.05).

Table 1 Characteristics of 148 women diagnosed with vulvar squamous cell carcinoma treated in the Radboud University Medical Centre (2000-2010).

<table>
<thead>
<tr>
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<th>%</th>
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<tr>
<td>I</td>
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</tr>
<tr>
<td>II</td>
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<td>III</td>
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</tr>
<tr>
<td>IV</td>
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<tr>
<td>Tumour grade</td>
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<td>I</td>
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<td>16</td>
</tr>
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<td>II</td>
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<td>53</td>
</tr>
<tr>
<td>III</td>
<td>46</td>
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<td>Lymphovascular invasion</td>
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<td>49</td>
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<tr>
<td>Sentinel lymph node procedure + dissection</td>
<td>24</td>
<td>16</td>
</tr>
<tr>
<td>Adjuvant radiotherapy</td>
<td>47</td>
<td>32</td>
</tr>
<tr>
<td>Recurrence during follow-up</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>92</td>
<td>62</td>
</tr>
<tr>
<td>Local</td>
<td>47</td>
<td>32</td>
</tr>
<tr>
<td>Local + groin</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Groin</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Died during follow-up</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>83</td>
<td>56</td>
</tr>
<tr>
<td>Yes</td>
<td>65</td>
<td>44</td>
</tr>
<tr>
<td>of intercurrent disease</td>
<td>21</td>
<td>14</td>
</tr>
<tr>
<td>of disease</td>
<td>31</td>
<td>21</td>
</tr>
<tr>
<td>of unknown cause</td>
<td>13</td>
<td>9</td>
</tr>
</tbody>
</table>

N= number of cases
On the basis of the depth of invasion measured with the alternative method and the tumour characteristics known before (tumour diameter) and after groin surgery (groin metastasis yes/no), patients were given a new FIGO stage. Table 2 displays the changes in the FIGO stage. Of 134 patients (91%), the FIGO stage did not change and of 14 (9%) it did change. Thirteen of sixty-nine (19%) patients with FIGO stage IB (lesions >2 cm in size or with invasion >1.0mm, confined to the vulva/perineum, with negative nodes) were downstaged to FIGO stage IA. In 1 of 44 (2%) patients with FIGO stage IIIA (tumour confined to the vulva or adjacent spread to the lower urethra, the vagina, or the anus, with one lymph node metastasis ≥5mm or one or two lymph node metastases <5mm) the depth of invasion changed from 3.5mm to <1mm (0.2mm). This single case showed isolated tumour cells in the sentinel lymph node.

<table>
<thead>
<tr>
<th>FIGO stage – conventional method</th>
<th>IA</th>
<th>IB</th>
<th>II</th>
<th>IIIA</th>
<th>IIIB</th>
<th>IIIC</th>
<th>IV</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIGO stage alternative method</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IA</td>
<td>13*</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>IB</td>
<td>0</td>
<td>56</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>56</td>
</tr>
<tr>
<td>II</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>IIIA</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>43</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>43</td>
</tr>
<tr>
<td>IIIB</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>IIIC</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>23</td>
<td>0</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td>IV</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>69</td>
<td>34</td>
<td>6</td>
<td>23</td>
<td>3</td>
<td>148</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*down-staged

Therefore, the patient underwent an unilateral inguinofermal lymphadenectomy in which eight lymph nodes were removed that showed no metastases. After a follow-up of 120 months, the patient is alive and there is no evidence of disease.

**Table 2** Comparison of FIGO stages of 148 vulvar squamous cell carcinoma patients according to the depth of invasion measured with the conventional and the alternative method.

**Tumour and clinical characteristics of the patient with FIGO stage IIIA VSCC that showed a change in the depth of invasion**

The patient was a 49-year-old female with a history of lichen sclerosus and a unifocal lesion on the right labium minus of 2 cm. Microscopic evaluation showed a well-differentiated tumour without presence of lymphovascular invasion. An H&E-stained slide of the tumour is displayed in Figure 2. The patient was treated with a wide local excision and a sentinel lymph node procedure of the right groin. Three lymph nodes were removed; in the immunohistochemical slides of one node two isolated tumour cells were found (Figure 3).

**Figure 2** Microscopical slide of the case with FIGO stage IIIA vulvar squamous cell carcinoma in which the depth of invasion changed from 3.5 to 0.2mm because of the alternative measuring method. A: measurement of depth of invasion with conventional method; B: measurement of depth of invasion with alternative method.

**Tumour and clinical characteristics of patients who changed from FIGO stage IB to IA and those who remained FIGO stage IB**

The median ages of the patients who remained FIGO stage IB (N=56) and who were downstaged (N=13) were 75 and 69 years, respectively. Other characteristics are displayed in Table 3; the patients who were downstaged had more often a well-differentiated tumour (62% vs 18%, P=0.003), less lymphovascular invasion (0% vs 16%), less recurrences (15% vs 39%), and died less frequently because of the vulvar squamous cell carcinoma (0% vs...
The median diameter of the tumours in downstaged patients was 6 mm (range 2–19 mm), in contrast to the patients who were not downstaged with a median diameter of 25 mm (range 5–63 mm). The median depth of invasion in patients who were downstaged changed from 2.4 mm (conventional method, range 1.1–5 mm) to 0.7 mm (alternative method, range 0.2–1.0 mm), and from 5.6 mm (conventional method, range 1.7–14 mm) to 2.6 mm (alternative method, range 0.3–13 mm) in patients who remained FIGO stage IB. An example of the measurements in a tumour that was downstaged is displayed in Figure 4.

Overall survival and disease-specific survival of patients with the conventional FIGO stage IB at 5 years was 75% (95% confidence interval (CI): 64–86%) and 88% (95% CI: 79–96%), respectively. In the group of patients with the alternative FIGO stage IA, the 5-year disease-

### Table 3
Comparison of characteristics between 13 women who were downstaged from FIGO stage IB to IA and 56 women who remained FIGO stage IB with the use of the alternative measuring method for the depth of invasion of vulvar squamous cell carcinoma.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>IB→IA (N=13)</th>
<th>IB=IB (N=56)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>8 (61)</td>
<td>10 (18)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>4 (31)</td>
<td>33 (59)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>1 (8)</td>
<td>13 (23)</td>
<td></td>
</tr>
<tr>
<td>Lymphovascular invasion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0 (0)</td>
<td>9 (16)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>13 (100)</td>
<td>47 (84)</td>
<td></td>
</tr>
<tr>
<td>VIN adjacent to the tumour</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3 (23)</td>
<td>11 (20)</td>
<td></td>
</tr>
<tr>
<td>uVIN</td>
<td>4 (31)</td>
<td>6 (10)</td>
<td>0.52#</td>
</tr>
<tr>
<td>dVIN</td>
<td>6 (46)</td>
<td>39 (70)</td>
<td></td>
</tr>
<tr>
<td>Multifocality</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>10 (77)</td>
<td>39 (70)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3 (23)</td>
<td>17 (30)</td>
<td>0.44#</td>
</tr>
<tr>
<td>Location</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central</td>
<td>11 (85)</td>
<td>45 (80)</td>
<td></td>
</tr>
<tr>
<td>Lateral</td>
<td>2 (15)</td>
<td>11 (20)</td>
<td>0.54#</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Local surgery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wide local excision</td>
<td>12 (92)</td>
<td>41 (73)</td>
<td></td>
</tr>
<tr>
<td>Radical vulvectomy</td>
<td>1 (8)</td>
<td>15 (27)</td>
<td></td>
</tr>
<tr>
<td>Groin surgery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sentinel lymph node procedure</td>
<td>9 (69)</td>
<td>24 (43)</td>
<td></td>
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<tr>
<td>Dissection</td>
<td>4 (31)</td>
<td>26 (46)</td>
<td></td>
</tr>
<tr>
<td>Sentinel lymph node procedure +dissection</td>
<td>0 (0)</td>
<td>6 (11)</td>
<td></td>
</tr>
<tr>
<td>Adjuvant radiotherapy</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>NA</td>
</tr>
<tr>
<td>Recurrences</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>11 (85)</td>
<td>34 (61)</td>
<td>0.09#</td>
</tr>
<tr>
<td>Yes</td>
<td>2 (15)</td>
<td>22 (39)</td>
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</tr>
<tr>
<td>Local</td>
<td>2 (15)</td>
<td>17 (30)</td>
<td></td>
</tr>
<tr>
<td>Groin</td>
<td>0 (0)</td>
<td>4 (7)</td>
<td></td>
</tr>
<tr>
<td>Local + Groin</td>
<td>0 (0)</td>
<td>1 (2)</td>
<td></td>
</tr>
</tbody>
</table>
specific survival was 100% (95% Cl 75–100%), which is higher compared with the 5-year disease-specific survival of the group of patients with the alternative FIGO stage 1B (84%; 95% Cl 73–95%; P=0.15). The disease-specific survival is shown in Figure 5.

Table 3 Continued.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>IB=IA (N=13)</th>
<th>IB=IB (N=56)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Died during follow-up</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>9</td>
<td>69</td>
<td>37</td>
</tr>
<tr>
<td>Yes</td>
<td>4</td>
<td>31</td>
<td>19</td>
</tr>
<tr>
<td>of intercurrent disease</td>
<td>4</td>
<td>31</td>
<td>5</td>
</tr>
<tr>
<td>of disease</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>unknown cause of disease</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
</tbody>
</table>

Abbreviations: FIGO= International Federation of Gynaecology and Obstetrics. NA= not applicable.
*χ² test. #Fisher’s exact test. ^More than 1 cm of the midline.

Figure 4 Microscopical slide of a case which was downstaged because of the alternative measuring method of the depth of invasion in vulvar squamous cell carcinoma. A: measurement of depth of invasion with conventional method; B: measurement of depth of invasion with alternative method.

Figure 5 Disease-specific survival of vulvar squamous cell carcinoma patients* with FIGO stage IA vs stage IB after using the alternative measuring method for the depth of invasion, in months. *Five patients were excluded from analysis because the cause of death was unknown.

Discussion

This is the first study that focused on comparing the conventional with an alternative method of measuring the depth of invasion in order to find the method corresponding with the most optimal individual outcome in patients with vulvar squamous cell carcinoma. The results of this study show that with the use of the alternative measuring method, from the basement membrane of the deepest adjacent tumour-free rete ridge to the deepest point of invasion, 19% of the patients with a FIGO stage IB tumour were...
downstaged to FIGO stage IA. These patients showed less recurrences and a higher disease specific survival compared with the remaining FIGO stage IB tumours. This downstaged group might be treated without groin surgery. A change in the depth of invasion from 3.5 to 0.2mm occurred in one patient (1%) with FIGO stage IIIA who is alive without any evidence of disease 10 years after treatment. The alternative method of measuring the depth of invasion of tumours in vulvar squamous cell carcinoma patients is a promising alternative that needs to be a subject of more extensive research before implementation.

The conventional measuring method was chosen mainly based on practical issues\(^{16}\); the adjacent dermal papilla can be found in all sites of the vulva, is not altered by variations in the depth of rete ridges, and the measurement is not significantly influenced by hyperkeratosis, tumour surface ulceration, or adjacent epithelial hyperplasia. Wilkinson et al\(^{17}\) considered different measuring methods in one of his first studies about microinvasive carcinomas of the vulva and states that the difficulty in measuring from the deepest rete ridge (the alternative method used in this study) is that the overlying epithelium at the site of the neoplasm may itself be neoplastic and would be a variable site of measurement. The problem of variability can be argued; in our study no cases with the invasive overlying epithelium were seen. No disadvantages are described about the conventional measuring method by Wilkinson; however, often it turns out to be difficult to choose the most adjacent superficial rete ridge. In some cases the rete ridge is far from the tumour, and logically it is not likely that the tumour will originate from a point that is far away. Maybe, this difficulty is one of the reasons for the recent findings of Abdel-Mesih et al\(^{18}\); they showed that the interobserver reliability between 11 gynaecologic-orientated pathologists for diagnosing vulvar squamous cell carcinoma as invasive is fair (Kappa=0.24) and for measuring depth of invasion moderate (Kappa=0.51). When using the conventional method, interpretation of the location of the most superficial dermal papilla varied among pathologists. Pathologists used a different measuring method, similar to those displayed in Figure 1, other than the conventional one in 0–39% of the cases for reasons not mentioned. The question arises that how reliable is the use of the alternative method in our study. The recognition of differentiated VIN, which was in the surrounding of the tumour in 50–58% of the cases, is another difficulty in this study. We showed in one of our earlier studies that the interobserver agreement on the diagnosis of differentiated VIN is not high (Kappa 0.08–0.54);\(^{18}\) therefore, it may be more difficult to recognise the most adjacent rete ridge.

When using the alternative measuring method, 19% of the patients were downstaged. This subgroup of patients showed more often a well-differentiated tumour, had less lymphovascular invasion, less recurrences, and a disease-specific survival of 100%. In this group no groin recurrences were identified. Probably this subgroup of patients may be treated without groin surgery, with less morbidity as a result. In our study population, four inguinofemoral lymphadenectomy procedures and nine sentinel lymph node procedures could have been prevented. Although the morbidity of the sentinel lymph node procedure is limited, it might be of great advantage to prevent any surgical procedure of the groins to save the sentinel lymph node procedure for a possible future de novo tumour at follow-up. One should keep in mind that \(\pm 25\%\) of the vulvar squamous cell carcinoma patients will develop local recurrences, requiring complete lymphadenectomy in case of an earlier sentinel lymph node procedure as part of the primary treatment.

There is a chance of <1% of positive lymph nodes in patients with a depth of invasion of <1mm measured with the conventional measuring method.\(^{1,5,19}\) This is substantially lower compared with the 7% when the case with FIGO stage IIIA, which had a depth of invasion of 0.2mm (instead of 3.5mm) with the alternative measuring method, would be included within the group of 13 cases that were downstaged from FIGO stage IB to IA. However, this single case showed only two solitary immunohistochemically detected tumour cells in the sentinel lymph node, and the patient is alive without any evidence of disease 10 years after treatment. Although the finding of the isolated tumour cells in the sentinel lymph node seems to be an important disadvantage of using the alternative measuring method, the value of this finding is questionable. In 2000, the sentinel lymph node procedure was introduced with ultrastaging and the use of immunohistochemistry as a routine procedure. This technique allows a more extensive pathological examination of the lymph nodes compared with that of the conventional lymph node dissection, which will result in an increase in the detection of small tumour deposits such as isolated tumour cells and micrometastases.\(^{20,21}\) In a study of van der Zee et al\(^{20}\) sentinel lymph nodes were examined by routine pathological examination, and only when no metastases were found, ultrastaging was performed. A total number of 163 positive nodes were detected, of which 95 (58%) were detected by routine pathological examination and 68 (42%) by ultrastaging. Oonk et al\(^{22}\) showed that the risk of nonsentinel node metastases increases with the size of the sentinel lymph node metastases; one of 24 patients (4%) with isolated tumour cells had non-sentinel lymph node metastases. Furthermore, they showed that the prognosis of patients with a positive sentinel lymph node based on isolated tumour cells is similar to patients with a negative sentinel lymph node.\(^{5,21}\) However, firm conclusions are difficult to draw because of the lack of power, and therefore Oonk et al\(^{22}\) recommend additional groin treatment for all patients with vulvar SCC with sentinel lymph node metastases, regardless of the size of the lymph node metastases. Besides, the therapeutic effect of this procedure, which was part of the routine treatment, is undefined. More is known about the role of isolated tumour cells in patients with breast cancer. De Boer et al\(^{22}\) showed that isolated tumour cells or micrometastases in the sentinel lymph node were associated with a reduced 5-year disease-specific survival for women with favourable early-stage breast cancer who did not receive adjuvant hormonal therapy or chemotherapy. In patients with...
isolated tumour cells or micrometastases who did receive adjuvant therapy, disease-specific survival was improved. In the study of Pepels et al\(^\text{a}\) the relevance of isolated tumour cells with respect to the risk of regional recurrence is considered to be of uncertain significance, not supporting the routine use of axillary treatment (in contrast to recommendations in sentinel lymph node micrometastases). However, other large studies on isolated tumour cells in patients with breast cancer have not shown any effect on disease-specific survival.\(^\text{b}\) An important issue when comparing vulvar squamous cell carcinoma patients with breast cancer patients is the difference of receiving adjuvant therapy. In vulvar squamous cell carcinoma patients, no adjuvant therapy is given (only radiotherapy in case of more than one intranodal groin lymph node metastasis or extranodal growth), whereas in breast cancer patients (neo)adjuvant systemic therapy and/or locoregional radiation therapy is given in a substantial number of patients. This may result in the eradication of possible (micro)metastases. Another difference is the observation that a recurrence in the groin in a patient with vulvar squamous cell carcinoma is nearly always fatal and is an important reason to be reluctant in omitting treatment of the groin. Further study is needed to establish the prognostic significance of isolated tumour cells in sentinel lymph nodes of patients with vulvar squamous cell carcinoma.

In conclusion, using the alternative method for measuring the depth of invasion in vulvar squamous cell carcinoma, 19% of patients with a FIGO stage IB tumours might be treated less radically, resulting in less treatment-related morbidity. Only one patient with FIGO stage IIIa with microinvasive vulvar squamous cell carcinoma based on the alternative measuring method had isolated tumour cells in the sentinel lymph node. On the basis of our result, it seems reasonable to further explore the introduction of the alternative measuring method in a prospective study with a higher number of patients before implementation in daily clinical practice.

References

Successful centralisation of patients with vulvar carcinoma: a population-based study in the Netherlands

Loes C.G. van den Einden, Katja K. Aben, Leon F.A.G. Massuger, Dick Johan van Spronsen, Joanne A. de Hullu

Abstract

Introduction
In general, centralisation of care for patients with rare malignancies is advised in order to improve outcome with respect to prognosis and treatment related morbidity. Therefore, centralisation of women with vulvar squamous cell carcinoma (SCC), which is an extremely rare tumour, has been advocated by the national guidelines of the Dutch Society of Obstetrics and Gynaecology in 2000. The objective of this study was to determine whether this advice has been adapted and has led to improved survival.

Methods
All patients diagnosed with vulvar malignancies between 1989 and 2008 in the Eastern part of the Netherlands were retrieved from the population-based cancer registry held by the Comprehensive Cancer Centre, The Netherlands. Patient- and tumour characteristics and vital status until January 2011 were retrieved. Data of patients diagnosed in two periods (before and after release of the guideline; 1989–1999 and 2000–2008) were compared. Relative survival rates were calculated as a good approximation of cause-specific survival.

Results
A total number of 382 patients with vulvar SCC with invasion >1 mm, who had an indication for groin surgery, were included in the analysis. In the first decade 62% (123 of 198 patients) were treated in a specialised oncology centre, which increased to 93% (172 of 184 patients) in the more recent period. Overall, the 5 year relative survival improved slightly from 68% (95% confidence interval (CI) 59–76%) to 72% (95% CI 63–81%). After adjustment for age and stage, being treated in a specialised oncology centre was an independent prognostic factor for survival.

Conclusion
Centralisation of care for vulvar SCC patients has been well adopted in the Eastern part of the Netherlands. Being treated in a specialised oncology centre was associated with a better survival after adjustment for age and stage.

Introduction
Vulvar carcinoma is a very rare gynaecologic malignancy with a yearly incidence of 1–2 per 100,000 women. In the Netherlands 320 new cases of vulvar carcinoma are diagnosed each year. The majority of patients have a squamous cell carcinoma (SCC); only a minority of patients suffers from basal cell carcinoma, melanoma or adenocarcinoma. Vulvar SCC spreads mainly via lymphatics, primarily to the inguinal lymph nodes.

The cornerstone of the treatment of vulvar SCC is surgery which offers an excellent chance of cure. Current standard treatment entails a wide local excision (WLE) with uni- or bilateral inguinal lymphadenectomy via separate incisions. To decrease treatment related morbidity such as wound healing problems and lymphoedema, patients with an early stage disease may be managed by a WLE and a sentinel lymph node dissection (SLND) preferably within the setting of a clinical trial. Radiotherapy is only indicated when a patient is not eligible for surgery or postoperatively when lymph node metastases and/or narrow local resection margins are present. The most important prognostic factor is the inguinal lymph node status. In patients without groin node metastases the 5-year disease specific survival (DSS) is 80–90% whilst in patients with nodal disease the DSS approaches only 50%. The recurrence rate is high, up to 30%. Whilst local recurrences can mostly be cured with surgery, groin recurrences are nearly always fatal.

In other rare malignancies, such as oesophageal and pancreatic carcinoma, an association exists between volume and/or specialisation of a hospital on the one hand and better survival on the other hand. Studies in these malignancies showed that the outcome of patients treated in a high-volume specialised centre was significantly better compared to outcome of patients treated in low volume hospitals. Benefits of centralisation of care are the development of expertise, the opportunity to give patients an appropriate treatment from experienced clinicians using new techniques that may improve prognosis and/or lower the treatment related morbidity, and the facilitation of training and research.

Because vulvar carcinoma is a rare tumour and the technical skills to perform surgery are no part of the training for general gynaecologists in the Netherlands, centralisation to specialised oncology centres has been advocated by national guidelines of the Dutch Society of Obstetrics and Gynaecology in 2000. However, it remains to be proven if this policy indeed improves outcome with respect to prognosis and morbidity of unselected patients in the general health care environment.

To our knowledge, this is the first population-based study in the Netherlands evaluating the centralisation of vulvar cancer patients as recommended by the Dutch guidelines.
Chapter 6

Patients and methods

Patient selection
Patients diagnosed with a primary vulvar malignancy in the period 1989–2008 in the Eastern part of the Netherlands were retrieved from the population-based cancer registry held by the Comprehensive Cancer Centre, The Netherlands (IKNL). This region has approximately 1.3 million inhabitants and is served by one specialised oncology centre and seven community hospitals. Eligible patients fulfilled the following criteria: primary invasive vulvar tumour diagnosed between 1st January 1989 and 31st December 2008 and ICD-O-3 topography code C510-512, C518-C519.9

Data
Standard cancer registry data were retrieved from the database. These data were collected by trained registration staff through consulting pathology reports and medical files. Data concerning patients age, date of diagnosis, tumour characteristics (topography, histology, invasiveness, stage and treatment), type of hospital (community hospital or specialised oncology centre) and follow-up (vital status, date of emigration and date of death) were obtained. Two periods were identified: before (between 1989 and 1999) and after (between 2000 and 2008) the introduction of the guideline in 2000. The follow-up of all patients was completed until the 1st January 2011 by the IKNL.

All pathologically confirmed carcinomas were staged according to the FIGO surgicopathological classification system of 1992.7 Tumours diagnosed before 1992 that were staged according to an earlier FIGO classification system were converted to the classification system of 1992. Pathology records of stage I tumours without known differentiation into stage IA (≤1 mm invasion) or IB (>1 mm invasion) (N = 82) were reviewed retrospectively through PALGA, the nationwide network and registry of histo- and cyto-pathology in the Netherlands. When possible, the differentiation into stage IA and IB was made. When insufficient information was available for pathological staging, the clinical stage was used in the analyses.

Statistical analyses
Descriptive analyses were performed to give insight in the differences in patient and tumour characteristics. Furthermore, the number of patients treated in a specialised oncology centre or community hospital in the period 1989–1999 and 2000–2008 was evaluated by using the \( \chi^2 \) test. When addressing histopathological subtypes without taking into account the period of diagnosis, SCC was the most common tumour type found in both periods. The proportion of patients treated in the specialised oncology centre increased over time (p < 0.0001, \( \chi^2 \) test). When addressing histopathological subtypes without taking into account the period of diagnosis, SCC was the most common tumour type found in the specialised centre (n = 318, 84.9%) and community hospitals (n = 102, 59%). The percentage of patients with a basal cell carcinoma was higher in community hospitals (n = 49, 28.3%) compared to the specialised oncology centres (n=17, 4.5%).

Secondly, relative survival analyses were performed to evaluate the effect of being treated in a specialised oncology centre versus community hospital on survival. For these analyses, a subgroup of patients with vulvar SCC stage IB or higher was selected. Reason for this is that especially patients with stage IB or higher are appropriate candidates for treatment in a specialised oncology centre because they need groin surgery (elective inguinofemoral lymphadenectomy or SLND), that preferably should be performed by a fully trained gynaecologic oncologist in a specialised oncology centre. Patients with stage 1A vulvar SCC, basal cell carcinoma, and melanoma were excluded from further analysis, because in these patients elective inguinofemoral lymph node dissection can be safely omitted. Therefore, for these patients no strict need for specialised care with respect to the groins exists. Relative survival rates were calculated as a approximation of cause-specific survival. This method adjusts crude survival rates amongst patients with malignancies for the expected mortality according to annual life tables of the general population matched on age, gender, calendar period and geographic area. Besides computing the 1-, 5-, and 10-year relative survival rates (RSR) before and after introduction of the guideline, 1-, 5-, and 10-year RSRs were calculated by stage, age and type of hospital. The follow-up used in the analyses was calculated as the time between date of diagnosis and date of death or 1st January 2011 (end of follow-up). Univariable and multivariable relative survival models were estimated using a generalised linear model with an assumed Poisson distribution for the observed number of deaths.20 Analyses were performed using the software package SAS version 9.2 (SAS Institute, Cary, North Carolina, United States of America (USA)) and SPSS 16.0 (SPSS Inc, Chicago, IL, USA).

Results
Between the 1st January 1989 and the 31st December 2008 in the Eastern part of the Netherlands a total number of 549 patients were diagnosed with a primary vulvar malignancy. Of all patients, 295 (54%) were diagnosed between 1989 and 1999 and 254 (46%) between 2000 and 2008. See Table 1 for an overview of the patient- and tumour characteristics before and after introduction of the guideline. No difference in age at diagnosis between the two periods was found. SCC was the most common histopathological tumour type found in both periods. The proportion of patients treated in the specialised oncology centre increased over time (p < 0.0001, \( \chi^2 \) test). When addressing histopathological subtypes without taking into account the period of diagnosis, SCC was the most common tumour type found in the specialised centre (n = 318, 84.9%) and community hospitals (n = 102, 59%). The percentage of patients with a basal cell carcinoma was higher in community hospitals (n = 49, 28.3%) compared to the specialised oncology centres (n=17, 4.5%).
After excluding all non-SCC vulvar malignancies and stage IA SCCs of the vulva, data of a total number of 382 patients with a vulvar SCC stage IB or higher were analysed; 198 before and 184 after introduction of the guideline. For 347 patients (91%) the pathologically based FIGO stage was available. In Table 2 the stage distribution by period is presented. The percentage of patients with an advanced stage SCC (stage III and IV) increased over time; 26.7% in the first time period versus 37.0% in the second time period. The proportion of patients treated in a specialised centre increased significantly over time ($p < 0.0001$, $\chi^2$ test); in the first period 123 of 198 patients (62%) were treated in a specialised centre compared to 172 of 184 patients (93.5%) during the second period. This is also displayed in Fig. 1. Since 2000 only 12 of 184 patients (6.5%) with vulvar SCC were treated in a community hospital. The median age of these 12 patients was 80 years of age and 6 of 12 patients did not receive any treatment.

The 5 year RSR of patients with stage IB or higher vulvar SCC ($n = 382$) was 70% (95% CI 64–77%). Table 3 shows the RSRs of patients with SCC stage IB or higher by stage, age, type of hospital, and period of diagnosis. The 5- and 10-year RSR of patients with an early FIGO stage (IB and II, which means absence of lymph node metastases) were significantly better compared to patients with an advanced stage tumour (III and IV). Because the type

### Table 1: Description of patient and tumour characteristics of all patients with vulvar cancer before and after release of the guideline.

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>549</td>
<td>295</td>
<td>254</td>
</tr>
<tr>
<td>Median age at diagnosis (range)</td>
<td>73 (18-99)</td>
<td>73 (18-90)</td>
<td>73.5 (20-99)</td>
</tr>
<tr>
<td>Place of treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specialised oncology centre (%)</td>
<td>376</td>
<td>159 (53)</td>
<td>217 (85)</td>
</tr>
<tr>
<td>Community hospital (%)</td>
<td>173</td>
<td>136 (47)</td>
<td>37 (15)</td>
</tr>
<tr>
<td>Histopathological subtype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>421</td>
<td>220</td>
<td>200</td>
</tr>
<tr>
<td>Basal cell carcinoma</td>
<td>66</td>
<td>38</td>
<td>28</td>
</tr>
<tr>
<td>Melanoma</td>
<td>24</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>15</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>Carcinoma NOS a</td>
<td>8</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Others</td>
<td>15</td>
<td>8 b</td>
<td>2.7</td>
</tr>
</tbody>
</table>

*NOS = not otherwise specified. aIncluding: vulvar sarcoma (n=4, 1.4%), Pagets disease (n=1, 0.3%), lymphoma (n=2, 0.7%) and adenosquamous carcinoma (n=4, 1.6%). bIncluding: vulvar sarcoma (n=1, 0.3%) and Pagets disease (n=1, 0.3%).

### Table 2: Description of patient and tumour characteristics of patients with SCC stage IB or higher; before and after release of the guideline.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>382</td>
<td>198</td>
<td>184</td>
</tr>
<tr>
<td>Median age at diagnosis (range)</td>
<td>73 (17-98)</td>
<td>73 (17-92)</td>
<td>73 (39-98)</td>
</tr>
<tr>
<td>Place of treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specialised oncology centre (%)</td>
<td>295</td>
<td>123 (62)</td>
<td>172 (93.5)</td>
</tr>
<tr>
<td>Community hospital (%)</td>
<td>87</td>
<td>75 (38)</td>
<td>12 (6.5)</td>
</tr>
<tr>
<td>FIGO stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage I</td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>199</td>
<td>25.9</td>
<td>(47)</td>
<td>23.7</td>
</tr>
<tr>
<td>Stage II</td>
<td>116</td>
<td>30.4</td>
<td>28.3</td>
</tr>
<tr>
<td>Stage III</td>
<td>97</td>
<td>25.4</td>
<td>23.9</td>
</tr>
<tr>
<td>Stage IV</td>
<td>24</td>
<td>6.3</td>
<td>8.7</td>
</tr>
<tr>
<td>Stage unknown</td>
<td>12</td>
<td>3.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Clinical stage only</td>
<td>34</td>
<td>8.9</td>
<td>19.0</td>
</tr>
</tbody>
</table>

*aIncluding stage IB (n=41) and I with unknown stage (IA or IB; n=8). bIncluding stage IB (n=19) and I with unknown stage (IA or IB; n=1). c$\chi^2$ test.

Figure 1: Number of patients with squamous cell carcinoma stage IB or higher treated in a specialised oncology centre and community hospital, 1989-2009.
of hospital and the period of diagnosis were calculated as well as which is presented in Table 3. Patients treated in a specialised oncology centre in the period 2000–2008, appeared to have a comparable 5-year RSR compared to patients treated in a specialised centre in the first time period as can be seen in Fig. 2B.

Table 3  Relative survival in all patients with SCC stage IB or higher (n=382) by stage, age, type of hospital and period of diagnosis

<table>
<thead>
<tr>
<th>N</th>
<th>1-Year survival (95% CI)</th>
<th>5-Year survival (95% CI)</th>
<th>10-Year survival (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>382</td>
<td>86.9 (82.6–90.5)</td>
<td>69.6 (63.2–75.6)</td>
</tr>
<tr>
<td>TNM</td>
<td>231</td>
<td>96.2 (91.7–99.1)</td>
<td>82.4 (74.2–89.6)</td>
</tr>
<tr>
<td>Advanced stage (III/IV)</td>
<td>139</td>
<td>74.6 (66.1–81.6)</td>
<td>50.3 (40.3–60.0)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;65</td>
<td>115</td>
<td>90.8 (83.7–95.0)</td>
<td>75.9 (66.5–83.2)</td>
</tr>
<tr>
<td>&gt;65</td>
<td>267</td>
<td>85.2 (79.6–91.7)</td>
<td>66.7 (58.3–74.8)</td>
</tr>
<tr>
<td>Type of hospital</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specialised oncology centre</td>
<td>295</td>
<td>90.3 (85.8–93.8)</td>
<td>74.6 (67.5–81.0)</td>
</tr>
<tr>
<td>Community hospital</td>
<td>87</td>
<td>73.5 (63.9–84.1)</td>
<td>52.1 (38.4–65.7)</td>
</tr>
<tr>
<td>Period of diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1989–1999</td>
<td>198</td>
<td>87.1 (81.0–91.7)</td>
<td>67.5 (58.7–75.7)</td>
</tr>
<tr>
<td>2000–2008</td>
<td>184</td>
<td>86.8 (80.3–91.7)</td>
<td>72.3 (62.8–80.7)</td>
</tr>
<tr>
<td>Type of hospital and calendar period of diagnosis b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specialised centre 1989–1999</td>
<td>123</td>
<td>91.3 (84.0–96.0)</td>
<td>74.0 (63.2–83.3)</td>
</tr>
<tr>
<td>Specialised centre 2000–2008</td>
<td>172</td>
<td>89.6 (83.2–94.1)</td>
<td>75.3 (65.6–83.7)</td>
</tr>
<tr>
<td>Community hospital 1989–1999</td>
<td>75</td>
<td>80.0 (68.1–88.6)</td>
<td>56.4 (41.5–70.7)</td>
</tr>
<tr>
<td>Community hospital 2000–2008</td>
<td>12</td>
<td>45.2 (16.5–72.1)</td>
<td>-</td>
</tr>
</tbody>
</table>

*95% confidence interval. *Items type of hospital and period of diagnosis combined.
In Table 4 the results of the uni- and multivariable survival analyses are presented. Disease stage and being treated in a specialised oncology centre were univariably associated with a better survival. In the multivariable analysis these variables remained independent prognostic factors. Advanced stage and not being treated in a specialised centre both were associated with a dismal prognosis.

### Table 4

<table>
<thead>
<tr>
<th></th>
<th>Univariable RER (95% CI)</th>
<th>Multivariable RER (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Younger than 65 years</td>
<td>1.52 (0.94-2.45)</td>
<td></td>
</tr>
<tr>
<td>65 years and older</td>
<td>1.00 (1.00-1.00)</td>
<td></td>
</tr>
<tr>
<td>Period</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1989-1999</td>
<td>0.87 (0.54-1.40)</td>
<td></td>
</tr>
<tr>
<td>2000-2008</td>
<td>1.00 (1.00-1.00)</td>
<td></td>
</tr>
<tr>
<td>Stage</td>
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<td></td>
</tr>
<tr>
<td>Stage I-II</td>
<td>1.00 (1.00-1.00)</td>
<td></td>
</tr>
<tr>
<td>Stage III-IV</td>
<td>5.17 (2.89-9.28)</td>
<td>6.41 (3.62-11.34)</td>
</tr>
<tr>
<td>Specialised oncology centre</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2.51 (1.57-4.00)</td>
<td>3.63 (2.15-6.11)</td>
</tr>
<tr>
<td>No</td>
<td>1.00 (1.00-1.00)</td>
<td></td>
</tr>
</tbody>
</table>

*RER* = Relative Excess Risk, *Reference category* = 95% CI = 95% Confidence Interval

### Discussion

This population-based study demonstrates that centralisation of care of patients with vulvar malignancies, as recommended by the Dutch guidelines in 2000, has been well adopted in the Eastern part of the Netherlands. Being treated in a specialised oncology centre was associated with better survival even after adjustment for age and stage.

In comparison with the period before introduction of the guideline, when 53% of the patients were treated in a specialised oncology centre, the number of patients treated in a specialised oncology centre increased until 85% in the more recent period. Even more important, amongst patients with vulvar SCC with invasion >1 mm who had an indication for groin surgery (inguinofemoral lymphadenectomy or SLND) the number of patients treated in a specialised oncology centre increased from 62.0% during the first period towards 93.5% in the second period. A group of only 12 (of 184, 6.3%) patients was treated in a community hospital in the second time period. As the median age of these 12 patients was 80 years of age and six patients did not receive any treatment, these patients may have had a poor performance status or were suffering from multiple co-morbidities. It can be assumed that these patients were more likely to stay in a community hospital for palliative care because of patients’ or gynaecologists’ preference. Unfortunately, detailed information about performance status was not available in this study. Apparently, a clear trend towards centralisation is observed in women with the need for groin surgery (SCC with >1 mm invasion). Only a minority (12 patients in 9 years in the whole region) of patients with vulvar SCC was not referred to a specialised oncology centre.

To evaluate whether the policy of centralisation indeed improved outcome of patients with vulvar malignancies, we compared the RSR of patients with vulvar SCC stage IB or higher (>1 mm invasion) before and after introduction of the guideline. Overall, the 5-year RSR increased from 68% before towards 72% after the introduction of the guideline which is not significant possibly due to lack of power. The rise in RSR is not likely to be caused by a difference in age as the age distribution was equal in both time periods. Furthermore, FIGO stage either causes this difference as in the first time period the percentage of patients with a FIGO stage III or IV is lower (26.7%) compared to the second time period (37.0%). However, one needs to realise that this observed difference may be caused by the introduction of the SLND as a result of which pathologic analysis enhanced, such as multiple sectioning and application of specific immunohistochemical staining. This has led to a more sensitive detection of micro metastases in the SLN which resulted in an upstaging of patients with early stage vulvar cancer. Although the overall RSR of patients with vulvar SCC improved over time for the whole region (Fig. 2A), the RSR of patients being treated in a specialised centre was almost the same before and after centralisation (Fig. 2B). This can probably be explained by the fact that patients with a bad prognosis because of advanced disease stage and/or low performance status were treated in community hospitals in the first period (which resulted in a 5-year relative excess risk (RER) of 56%, see Fig. 2B), whereas almost all these patients were treated in a specialised centre during the second time period. Therefore, in this period the case mix in the oncology centre was probably less favourable, but the survival remained similar which indicates an overall improvement of survival. Moreover, we hypothesise there may be an additional role for the absence of improvement by the introduction of the SLN procedure. Although morbidity decreases, it might have a slightly decrease of prognosis, due to the learning curve and missing a positive SLN. Important mentioning is that there are some limitations of the use of survival as an end-point to evaluate the outcome of vulvar malignancies, as different aspects can have an effect on survival (e.g. treatment delay). This implicates that the results should be interpreted carefully.
Besides an improvement in survival, another advantage of more patients being treated in a specialised oncology centre might be less morbidity after the treatment. In recent years, during the second decade of our study, the treatment of patients with early stage vulvar SCC shifted from inguinofemoral lymphadenectomy into the SLND procedure. Patients benefit from less treatment-related morbidity like wound breakdown, cellulitis, lymphoedema and erysipelas after SLND. It is suggested that surgeons should perform this type of surgery at least 5–10 times per year to meet quality standards. In a rare tumour such as vulvar SCC, this will require centralisation.5

In conclusion, the present study showed that centralisation of the treatment of patients with vulvar SCC who need groin surgery has been well adopted in the Eastern part of the Netherlands. Being treated in a specialised oncology centre is associated with a better survival.

References
Trends in incidence and survival of Dutch women with vulvar squamous cell carcinoma


*These authors equally contributed

Abstract

Introduction
Previous studies showed an increase in incidence of vulvar intraepithelial neoplasia (VIN), the premalignant lesion of Vulvar Squamous Cell Carcinoma (VSCC). Furthermore, during the last decades treatment of VSCC became less radical. Considering these changes the aim of this study was to describe trends of incidence and survival of patients with VSCC in the Netherlands.

Methods
All patients with VSCC diagnosed between 1989 and 2010 (n = 4614) were selected from the Netherlands Cancer Registry. Trends in age-adjusted incidence rates were evaluated by calculating the estimated annual percentage change (EAPC). Joinpoint regression analysis was used to detect changes in trends. Five-year relative survival rates were calculated for four time periods.

Results
The incidence of VSCC has increased since 2002 (EAPC 5.0%; 95% confidence interval (CI): 2.7–7.7%). In women aged <60 years incidence rates increased significantly during the whole study period (EAPC 3.5%; 95% CI: 2.0–4.9), while in women aged ≥60 years only an increase has observed from 2004 onwards (EAPC 5.0; 95% CI: 1.5–8.6). Survival rates did not change over time.

Conclusion
The incidence rate of VSCC has increased from 2002 onwards in all women. Over the whole study period the increase was strongest in women aged <60 years. The introduction of less radical surgery did not affect survival.

Introduction
Vulvar carcinoma is a rare malignancy. In the Netherlands it is accounting for six to eight percent of all gynaecological malignancies. Annually, approximately 360 new cases of vulvar carcinoma and over 100 deaths are reported. A two to fourfold increasing incidence of the premalignant lesion of vulvar carcinoma, vulvar intraepithelial neoplasia (VIN), is observed in several countries. Until now, only few studies showed a tendency towards an increasing incidence in vulvar carcinoma.

About 80 percent of all vulvar malignancies are squamous cell carcinomas (SCCs) of which 20 percent is related to the human papilloma virus (HPV) and associated with the precursor lesion usual VIN (uVIN). This type of vulvar carcinoma primarily affects younger women. The majority of SCCs is non HPV related and occurs in elderly women, often in the background of lichen sclerosus (LS) and/or differentiated VIN (dVIN).

The last two decades, treatment of patients with vulvar SCC (VSCC) has changed. Until 20 years ago radical vulvectomy with ‘en bloc’ bilateral inguinalfemoral lymphadenectomy was the standard treatment for almost all patients with VSCC. Since the early nineties the surgical treatment of VSCC has become more individualised. Current treatment entails a wide local excision (WLE) with uni- or bilateral inguinalfemoral lymphadenectomy via separate incisions. More recently, sentinel lymph node dissection (SLND) has been introduced in early stage VSCC as a safe technique with a very low false negative rate. Though it is the standard management of early stage VSCC, all SLND procedures in the Netherlands are performed within the setting of a clinical trial from 2000 onwards. Compared to a complete lymphadenectomy, SLND is associated with less treatment-related morbidity without compromising prognosis. As the technical skills to perform surgery are not part of the training of general gynaecologists in the Netherlands and the incidence of VSCC is low, treatment in a specialised oncology centre has been advocated by national guidelines of the Dutch Society of Obstetrics and Gynaecology in 2000.

With the intention to study the effect of the increase of patients diagnosed with VIN and the given recent changes in treatment modalities, the aim of this population-based study was to determine the incidence and survival of VSCC in the Netherlands in the period between 1989 and 2010.
Patients and methods

Data collection
Patients diagnosed with a primary vulvar malignancy in the period 1989–2010 in the Netherlands were selected from the Netherlands Cancer Registry (NCR). This nationwide registry documents all newly diagnosed patients with cancer and has a nationwide coverage since 1989. The completeness of the NCR is estimated to be at least 95%. Standard cancer registry data were retrieved from the NCR. These data were collected by fully trained registrars from pathology reports and patient files. Data concerning patients’ age, date of diagnosis and tumour characteristics (topography, histology, invasiveness, stage and treatment) were obtained. Information on vital status and date of death was retrieved from municipality registries and from the database of deceased persons of the Central Bureau for Genealogy and the municipal demographic registries (GBA). The follow-up data were completed until the 1st January 2011.

Topography and morphology are coded according to the International Classification of Diseases for Oncology (ICD-O). Tumour-node-metastasis (TNM) classification is used for tumour staging and converted to the classification of the Fédération Internationale de Gynécologie et d’Obstétrique (FIGO). To evaluate the centralisation to specialised oncology centres, place of treatment (community hospital or specialised oncology centre) was retrieved. A specialised oncology centre is defined as a centre that treats at least 20 patients with vulvar cancer per year, in this study eight academic centres and two large public hospitals were classified as specialised oncology centre. As the classification system of FIGO stage was changed in 2009, analyses considering FIGO stage were only performed until 2009.

Statistical analyses
For the analyses concerning incidence and survival, only patients with VSCC were included. The study period was divided into four periods (1989–1994, 1995–1999, 2000–2004 and 2005–2010). Descriptive analyses and Chi-square tests were performed to evaluate differences in patient and tumour characteristics between different time periods. Annual age-standardised incidence rates adjusted to the European standard population (ESR) were calculated. Changes in rates were evaluated by calculating the estimated annual percentage change (EAPC) and the corresponding 95% confidence interval. To calculate this, a regression line was fitted to the natural logarithm of the rates, using the calendar year as regressor variable (i.e. \( y = ax + b \) where \( y = \ln(\text{rate}) \) and \( x = \text{calendar year} \), then \( \text{EAPC} = 100 \times (a-1) \). The Joinpoint Regression Programme (version 3.5.1) from the Surveillance Research Programme of the US National Cancer Institute (http://surveillance.cancer.gov/joinpoint/) was used to identify significant changes in trends.

Results

Between 1989 and 2010, a total number of 5680 women were diagnosed with a vulvar malignancy; 4614 (81%) women were diagnosed with SCC, 8% with basal cell carcinoma, 6% with vulvar melanoma and 5% with other histological subtypes. The distribution of histological subtypes did not differ over the calendar periods (\( p = 0.2 \)).

Further analyses were only performed in patients with VSCC. See Table 1 for an overview of patient and tumour characteristics by period of diagnosis. Between 1989 and 2010 the absolute number of women diagnosed with VSCC increased from 183 to 315, which is an increase of more than 70%. The incidence rate (ESR) of VSCC in 1989 was 2.0 per 100,000 and increased to 2.7 per 100,000 in 2010 (Figure 1). This increase is statistically significant (EAPC: 1.4%; 95% confidence interval (CI): 0.6 to 2.1). Joinpoint analyses revealed a statistically significant annual increase in incidence of VSCC of 5.0% (95% CI: 2.7–7.7) between 2002 and 2010, while during 1989 and 2002 no increase was observed (Table 2). The incidence rate (ESR) of VSCC in women aged <60 increased statistically significantly between 1989 and 2010 (EAPC 3.5; 95% CI: 2.0–4.9). In women aged 60 years and older no increase was observed over the whole study period (EAPC 0.55; 95% CI: -0.1 to 1.2). Nonetheless for this group a statistically significant increase was observed between 2004 and 2010 (EAPC 5.0; 95% CI: 1.5–8.6).

Figure 2 shows somewhat higher age-specific incidence rates (per 100,000 women) in women aged 30–70 years during the years 2000–2010 compared to 1989–1999. For all other age categories incidence rates were nearly equal between the two periods.

Relative survival rates (RSR) were calculated as an estimation of cause-specific survival according to the Ederer II method. In relative survival analyses the ratio of observed survival to the expected survival is calculated. Survival time was defined as date of diagnosis to date of death. Five-year relative survival rates were calculated by period of diagnosis, age-category (<60 and ≥60) and FIGO classification. Multivariable relative survival analyses, using Poisson regression modelling were performed to calculate relative excess risk of dying (RER). Age, period of diagnosis and FIGO classification were entered in the model. To study the effect of place of treatment the variable treated in a specialised centre (yes/no) was added to the model. Analyses were performed using Stata statistical software package (version 12.0). A p-value of less than 0.05 was considered statistically significant.
FIGO stage distribution did change especially between the time periods 1995–1999 and 2000–2004; the percentage of patients with FIGO stage II decreased from 31% to 25% while FIGO stage III increased from 20% to 26%. The percentage of patients with FIGO stage I increased slightly over the years while the percentage of patients with FIGO stage IV remained stable. Joinpoint analyses of ESR per FIGO stage did not reveal significant changes in trends. However, a continuously significant increase was observed for stages I (EAPC 2.1%; 95% CI: 0.9–3.2) and III (EAPC 2.2%; 95% CI: 0.9–3.5), while unknown stage decreased annually with 5.7% (95% CI: -8.5 to -2.7). No statistically significant trends in ESR of FIGO stages II and IV were observed (Table 2).

Over time more women were diagnosed or treated in a specialised centre; the percentage increased from 50% to 79% (p < 0.01) between 1995–1999 and 2005–2010 (Table 1).

Table 3 shows the 5-year relative survival rates (RSR). Survival of VSCC varies between 70% and 72% and did not change over time. Women aged below 60 years had a considerably higher 5-year RSR compared to women aged 60 and older.

Table 1 Patient and tumour characteristics of women diagnosed with VSCC in the Netherlands (1989-2010).

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
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<td>N %</td>
<td>N %</td>
<td>N %</td>
<td>N %</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1037</td>
<td>947</td>
<td>997</td>
<td>1613</td>
<td>4614</td>
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<td>Age at diagnosis</td>
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<td>192</td>
<td>20.3</td>
<td>235</td>
<td>23.6</td>
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<tr>
<td>≥60</td>
<td>874</td>
<td>82.7</td>
<td>755</td>
<td>77.7</td>
<td>762</td>
<td>76.4</td>
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<td>FIGO stage</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>I</td>
<td>335</td>
<td>33.6</td>
<td>310</td>
<td>33.2</td>
<td>356</td>
<td>35.7</td>
</tr>
<tr>
<td>II</td>
<td>327</td>
<td>32.5</td>
<td>293</td>
<td>30.4</td>
<td>246</td>
<td>24.7</td>
</tr>
<tr>
<td>III</td>
<td>213</td>
<td>21.0</td>
<td>188</td>
<td>19.9</td>
<td>256</td>
<td>25.7</td>
</tr>
<tr>
<td>IV</td>
<td>116</td>
<td>11.0</td>
<td>101</td>
<td>10.7</td>
<td>114</td>
<td>11.4</td>
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<tr>
<td>unknown</td>
<td>46</td>
<td>4.4</td>
<td>45</td>
<td>4.5</td>
<td>35</td>
<td>3.7</td>
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<td>Specialised centre</td>
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</tr>
<tr>
<td>Yes</td>
<td>N.A.</td>
<td>N.A.</td>
<td>477</td>
<td>50.4</td>
<td>615</td>
<td>61.7</td>
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<tr>
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<td>N.A.</td>
<td>470</td>
<td>49.6</td>
<td>382</td>
<td>38.3</td>
</tr>
</tbody>
</table>

Abbreviation: N.A= not available.
*Data on FIGO stage are available until 2009. 1 data on centre of treatment is available since 1995. 2 two patients treated outside the Netherlands were excluded from analyses.
Table 2  Observed trends (EAPC and 95% CI) in incidence rates (ESR) of VSCC by age-category and stage in the Netherlands (1989-2010).

<table>
<thead>
<tr>
<th>Period</th>
<th>EAPC</th>
<th>(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1989-2002</td>
<td>-0.4</td>
<td>(-1.5 to 0.7)</td>
</tr>
<tr>
<td>2002-2010</td>
<td>5.0*</td>
<td>(2.7 to 7.7)</td>
</tr>
<tr>
<td>&lt;60</td>
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<td></td>
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<tr>
<td>1989-2010</td>
<td>3.5*</td>
<td>(2.0 to 4.9)</td>
</tr>
<tr>
<td>≥60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1989-2004</td>
<td>-0.6</td>
<td>(-1.4 to 0.3)</td>
</tr>
<tr>
<td>2004-2010</td>
<td>5.0*</td>
<td>(1.4 to 8.6)</td>
</tr>
<tr>
<td>FIGO stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1989-2009</td>
<td>2.1*</td>
<td>(0.9 to 3.2)</td>
</tr>
<tr>
<td>II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1989-2009</td>
<td>-0.2</td>
<td>(-1.3 to 1.0)</td>
</tr>
<tr>
<td>III</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1989-2009</td>
<td>2.2*</td>
<td>(0.9 to 3.2)</td>
</tr>
<tr>
<td>IV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1989-2009</td>
<td>1.3</td>
<td>(-0.6 to 3.3)</td>
</tr>
<tr>
<td>Unknown</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1989-2009</td>
<td>-5.7*</td>
<td>(-8.5 to -2.7)</td>
</tr>
</tbody>
</table>

Abbreviations: EAPC= Estimated annual percentage change. ESR= European age-standardised incidence rate.

a Total EAPC 1989-2010 1.4 (0.6 to 2.1).
b ≥60 EAPC 1989-2010 0.55 (-0.1 to 1.2).
c Data on FIGO stage are available until 2009. *The APC is significantly different from 0 (P <0.05).

Figure 2  Incidence of vulvar squamous cell carcinoma (VSCC) per 100,000 women in the Netherlands by age-category and period of diagnosis (1989-2010).

Table 3  5-year relative survival rate by period of diagnosis.

<table>
<thead>
<tr>
<th>Period of diagnosis</th>
<th>Overall</th>
<th>Age at diagnosis</th>
<th>FIGO stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>70.6 (66.7-74.3)</td>
<td>71.5 (67.4-75.4)</td>
<td>72.1 (68.2-75.8)</td>
</tr>
<tr>
<td>&lt;60</td>
<td>82.3 (75.7-87.4)</td>
<td>80.4 (73.8-85.6)</td>
<td>85.6 (80.2-89.8)</td>
</tr>
<tr>
<td>≥60</td>
<td>67.9 (63.4-72.3)</td>
<td>69.1 (64.2-73.8)</td>
<td>67.5 (62.7-72.2)</td>
</tr>
<tr>
<td>FIGO stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>87.2 (81.0-92.6)</td>
<td>93.1 (87.3-97.9)</td>
<td>93.4 (87.9-97.9)</td>
</tr>
<tr>
<td>II</td>
<td>79.3 (72.2-85.9)</td>
<td>74.7 (66.7-82.2)</td>
<td>78.2 (69.7-85.8)</td>
</tr>
<tr>
<td>III</td>
<td>54.4 (46.0-62.6)</td>
<td>50.8 (42.0-59.4)</td>
<td>54.9 (47.3-62.3)</td>
</tr>
<tr>
<td>IV</td>
<td>25.8 (17.2-35.8)</td>
<td>23.8 (14.8-34.5)</td>
<td>34.0 (24.1-44.7)</td>
</tr>
<tr>
<td>Unknown</td>
<td>66.5 (57.1-83.3)</td>
<td>83.5 (59.5-102)</td>
<td>47.3 (23.9-71.2)</td>
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<tr>
<td>Treated in specialised centre</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>NA</td>
<td>69.6 (63.9-74.9)</td>
<td>72.5 (67.6-77.0)</td>
</tr>
<tr>
<td>No</td>
<td>NA</td>
<td>73.5 (67.6-79.2)</td>
<td>71.5 (64.9-77.6)</td>
</tr>
</tbody>
</table>

Abbreviation: N.A = not available.

*Data on FIGO stage is available until 2009. *Data on centre of treatment available since 1995.
Table 4 presents the results of the multivariable survival analyses, which did not show a significant difference in relative excess rate (RER) of dying between the four time periods. Women aged 60 and older and women diagnosed with higher FIGO stages show statistically significant higher RERs. In univariable survival analyses treatment in a specialised centre did not reveal a statistically significant RER of dying (RER 0.9; 95% CI: 0.7–1.1) (data not shown). After adjustment for several factors treatment in a specialised centre was a favourable prognostic factor for survival; women who were treated in a specialised centre showed a statistically lower RER compared to women who were not (RER 0.6, 95% CI: 0.5–0.8). Furthermore, a higher RER of dying was observed for women diagnosed between 2005 and 2010 compared to women diagnosed between 2000 and 2004 when adjusted for age, stage and treatment in a specialised centre (RER 1.3; 95% CI: 1.1–1.5).

**Discussion**

During the last 20 years the incidence rate of VSCC has increased, especially from 2002 onwards. Over the whole study period the increase was strongest in women aged below 60, while incidence of VSCC in women aged 60 years and older started to increase from 2004 onwards. Despite several changes in surgical treatment, such as the introduction of wide local excision, inguinofemoral lymphadenectomy via separate incisions and the sentinel node procedure, no changes in survival rates are observed during the last 20 years.

Incidence rates of vulvar carcinoma increase with age. More than 50% of the patients are at least 70 years old at time of diagnosis. This study shows that the incidence is rising in patients 60 years and older, but only from 2004 onwards. We also show an increasing incidence in women aged below 60 years during the whole study period. Bodelon et al. and Judson et al. reported a rising trend in age-adjusted incidence of vulvar tumours (SCC and non-SCC) present among women of all ages in the USA, not specifically in younger women. In contrast, an increasing incidence with a trend towards younger women has also been observed in Denmark. Another population-based study in the USA and a study from Norway reported a more stable incidence but these studies consider an earlier or smaller study period. In contrast to the slightly increasing incidence of VSCC, VIN shows a clear increasing trend in most population based studies. The incidence rates increased two- to fourfold in the USA, Norway and Denmark. We did not have data concerning VIN available, however, van de Nieuwenhof et al. showed that the incidence of uVIN in the Netherlands has doubled and the incidence of dVIN increased nine fold from 1992 to 2005. Only Watson et al. reported no trend in incidence between 1973 and 2000 in the US population. The increasing incidence of uVIN might have led to a rise in incidence in VSCC. The question is whether there is a ‘real’ rise of incidence or whether a part of the rise can be explained by increased detection. Only since a few years, there is consensus about the nomenclature; the use of terminology ‘uVIN’ and ‘dVIN’ was introduced by the International Society for the Study of Vulvovaginal Disease (ISSVD) in 2004 and it took some years before the nomenclature was generally accepted, possibly resulting in difficult interpretation of the literature. Moreover, dVIN is clinically and histopathologically a difficult entity to diagnose and may be extremely underreported because it may easily be mistaken for other histopathologic entities. Furthermore, vulvar premalignant lesions have gained more public awareness, and a more liberal use of biopsies may have led to the observed increase.

It is striking that particularly the incidence of VSCC in women below the age of 60 has increased. This increase might be related to human papilloma virus (HPV) infection, that is associated with changing sexual habits and smoking. This may have caused a rise in
HPV-related uVIN resulting in a higher incidence of HPV-related SCCs. Though HPV (especially HPV 16 and 18) is associated with VSCC in only 20% of all cases, this type of VSCC mainly affects younger women.24-26 Possibly, this can explain the rise in incidence of SCC in women below the age of 60. Details about HPV status of VSCCs were not present in the database, though unpublished data of 371 VSCCs at our clinic showed that 21 of 264 (8%) SCCs of patients aged 60 years or older had adjacent uVIN in comparison with 40 of 105 (38%) SCCs of patients younger than the age of 60.

Furthermore, HPV infections are more likely to develop into anogenital (pre)malignancies in women that are immune compromised, like in women after organ transplantation, HIV seropositivity or autoimmune disorders. For example in patients after kidney transplantation there is a 50-fold increased vulvar cancer risk.25-27 Several studies show that 100% of the vulvar (pre)malignancies among renal transplant recipients are HPV-positive, compared to 20% in the general population.28 The group of immunocompromised women is growing, as newer immunosuppressive regimens have extended the life expectancy of allograft recipients and effective antiretroviral therapy prolonged the survival of HIV-infected people.29 Probably, HPV might explain a part of the increase, the recent introduction of HPV vaccination may result in a reduction of HPV-related VSCCs and uVIN, but this will take some decades.

Other risk factors associated with the development of VSCC are smoking, young age at first intercourse, larger number of sexual partners and genital warts.30-32 However, these factors are all related to HPV infection. Looking at these risk factors in more detail, smoking seems to be strongly associated with VSCC and also a synergistic effect between smoking and HPV has been suggested. However, mainly current smoking and not former smoking seems to be associated with VSCC and an increased prevalence of HPV.33-35 In the Netherlands, the percentage of female smokers was highest during the 1960s and 1970s and the percentage of female smokers did not increase during our study period. Therefore, smoking probably cannot explain the observed trends in our study.36 Furthermore, several studies in different countries show a tendency towards earlier sexual debut and more sexual partners during lifetime.34 In the Netherlands age at first intercourse decreased slightly from 17.7 to 17.3 years between 1995 and 2005 and remained stable until 2011.35 The number of women with more than five sexual partners during lifetime increased considerably from 14% in 1991 to 35% in 2006.36 Finally several studies observed a substantial increase in the number of women who were diagnosed with genital warts during the last few decades.37-38 Also in the Netherlands an increase in diagnosis during the last decade has been observed.39 Women with genital warts have a strongly elevated risk for developing HPV-related malignancies.

Increases in incidence rates of FIGO I and FIGO III were observed. An explanation for the increase in FIGO I is not clear, perhaps increased awareness among women and health professionals or changes in help-seeking behaviour have contributed to this increase. The increase in FIGO III might be related to the increasing use of SLND in the treatment of VSCC. Sentinel lymph node dissection with ultrastaging allows a more extensive pathology examination of the lymph nodes compared to lymph nodes removed during a complete lymphadenectomy, which can result in an increase in the detection of micrometastases.40-41 In a study of van der Zee et al.42 sentinel lymph nodes were examined by routine pathological examination and only when no metastatic sentinel nodes were found ultrastaging was performed. In total 163 positive nodes were detected of which 95 (58%) were detected by routine pathological examination and 68 (42%) by ultrastaging. This can also explain the shift from FIGO II to FIGO III seen between 1994–1999 and 2000–2004, as the SLND with ultrastaging was introduced in the Netherlands in 2000. So far, we did not find previous studies evaluating the impact of the implementation of SLDN on stage distribution at population-based level.

Furthermore, we studied the 5-year survival rate of VSCC patients, which was stable during the whole study period and was similar to rates reported in the literature.42 In spite of the changed surgical treatment during the last two decades. This was also observed by Ramanah et al.43 in women with late stage VSCC in the United States between 1988 and 2007. The advice of treating women with SCC in a specialised oncology centre advocated by the Dutch national guidelines, has been followed; more patients have been treated in a specialised oncology centre, which was found to be an independent prognostic factor for survival in this study, which is in line with our earlier study.44

The multivariable survival analysis showed higher RERs for older women and for women with a higher stage at diagnosis, as expected. A remarkable finding is the higher RER for women diagnosed between 2005 and 2010 when compared to women diagnosed between 2000 and 2004 in the model including treatment in a specialised centre. In the model without treatment in a specialised centre which includes two earlier time periods we did not observe statistically significant RERs for period of diagnoses. We do not have an explanation for this higher RER, it would be interesting to evaluate whether a higher RER can still be observed when data of more recent years are available.

In this study we focused on trends in incidence and survival. Adding mortality data to this study could be valuable but unfortunately mortality rates are not available for VSCC specifically but only for all histologic subtypes together. However as the vast majority of all vulvar malignancies are SCCs this might provide an estimation for mortality rates of VSCC. Mortality rates of vulvar cancer in the Netherlands increased annually with 0.9% between 1989 and 2010 (95% CI: 0.0–1.9) but is still less than 1 per 100,000 women (ESR).
Trends in incidence and survival of Dutch women with vulvar squamous cell carcinoma

In conclusion, an increase in incidence in VSCC is observed with the strongest increase in younger patients. This might suggest HPV attribution to the observed increase. It is expected that incidence of vulvar malignancies will slightly decrease in a few decades due to the prophylactic HPV vaccination programme which started in 2009; however, this decrease will only be limited as only a minority of VSCC is caused by HPV. Finally, despite the introduction of more individualised treatment in VSCC with the aim to reduce morbidity, no changes in survival rate are observed during the last 20 years.

References

7. Van de Nieuwenhof HP, Bulten J, Hollema M, et al. Differentiated vulvar intraepithelial neoplasia is often found in lesions, previously diagnosed as kichen ulcers, which have progressed to vulvar squamous cell carcinoma. Modern pathology. 2011;24(2):297-305.


General discussion
It is generally accepted that there are two separate pathways leading to vulvar squamous cell carcinoma (VSCC): the HPV-related pathway with usual vulvar intra-epithelial neoplasia (VIN) as precursor and the more frequent pathway related to lichen sclerosus (LS) and differentiated VIN (dVIN). This thesis focuses on possibilities to improve clinical care for women with vulvar squamous (pre)malignancies in each step from diagnosis to treatment. In this chapter, these steps are discussed and future perspectives are suggested.

The role of dVIN and LS in the oncogenesis of vulvar squamous cell carcinoma

Patients with LS have a lifetime risk of 4-5% to develop VSCC. However, it still has to be clarified how VSCC exactly arises from LS and dVIN in the HPV-negative pathway. Evidence that dVIN is the true precursor lesion of HPV-negative VSCC is growing: although dVIN is rarely found as a solitary lesion, it is often found in revised biopsies previously diagnosed as LS in patients who later developed VSCC. A reason for the low number of published solitary dVIN lesions in studies might be explained by the idea that dVIN has a short intra-epithelial phase that rapidly progresses into VSCC. Difficulties with the clinical and histopathological recognition of dVIN might be a second reason underlying the relative under-reporting of dVIN. Further evidence for dVIN being a precursor lesion can be found in DNA aneuploidy and p53 expression. Both occur in the earliest stages of malignant transformation and were found in dVIN lesions.

The field of (epi)genetics is largely unexplored in linking dVIN to VSCC as true precursor lesion. A first attempt to provide information about the genome-wide changes in vulvar (pre)malignancies has been made in chapter 2 by using a high resolution array. We found identical copy number alterations in dVIN and concurrent VSCC in the same patient, which indicates a clonal relationship. This finding should be further explored by expanding the number of samples. Retrieving enough DNA for analysis has turned out to be challenging. This is a known problem in vulvar lesions, especially when DNA is isolated from formalin-fixed paraffin-embedded blocks. In future research, somatic mutations and possible recurrent (driver) mutations should be identified in VSCC lesions using whole exome sequencing. Afterwards, these somatic mutations may be identified in patient-matched dVIN lesions using backtracking. To prove a clonal relationship, VSCC lesions should be compared with a histologically confirmed dVIN diagnosed several months before VSCC diagnosis, preferably at the same localisation within the vulva. An attempt to identify genetic alterations associated with malignant transformation was made in oral cancer research. Cervigne et al. conducted an array comparative genomic hybridization analysis in 25 samples from five patients; 20 progressive leukoplakia and five same-site carcinomas (with a time-interval of 2 to 9 years between the dysplasia and SCC). Specific genetic alterations were mapped in the progressive dysplasia, and in their corresponding oral SCCs in 70% of patients, indicating that these alterations may be associated with disease progression.
Considering dVIN as the early stage of HPV-negative VSCC on genetic grounds, the next question emerges on how the precise relationships are between LS, dVIN and VSCC. Based on clinical and histopathological characteristics, it is impossible to predict which patients with LS will develop a VSCC and who will not. Although not all women with LS seem to have the same risk of malignancy, nowadays all patients with LS have at least yearly follow-up \(^6, 7\) based on national and international guidelines. However, frequent follow-up results in more discomfort and high health care costs. Therefore, being able to predict the risk of LS to progress to VSCC might help to select patients who do need at least yearly follow-up. For patients at low risk of VSCC, a personalised follow-up schedule can be proposed, consisting of longer time intervals and/or patient-initiated contact in case of any changes. Possibly, results of epigenetic research might help finding differences between LS that progresses towards VSCC and LS that does not. However, there is limited literature on this topic. Furthermore, it might be of additional value to explore epigenetic differences between genital and extra-genital LS, because the latter never progresses to an invasive lesion. Until now, there is no explanation for this difference in progression towards a malignancy. Remarkably, dVIN or a comparable lesion is never found in extra-genital LS, which corresponds with the absence of development of malignancy. Histopathologically, genital and extra-genital LS cannot easily be distinguished as classic histopathological features of LS are seen in both lesions. Only some minor features differ: genital LS contains less atrophy and more often a thin epidermis with a lymphocytic infiltrate in the suprabasal layer.\(^6, 10\)

In conclusion, we have gained genetic evidence which points in the direction that dVIN is the precursor lesion of VSCC, but the exact role still has to be further clarified. Identification of genetic alterations can help clarifying this role and might help predicting which patients with LS will progress towards VSCC and which do not. A challenge in the interpretation of results will be the concept of intra-tumour heterogeneity, i.e. different clones in one tumour.\(^11\) Another issue is the concept of field cancerisation; it is possible that several subtypes of LS with different malignant potentials are present within the vulvar area of one patient. This may explain why VSCC develops in some areas, whereas LS does not change for years in other areas.

**How to improve the histopathological detection of dVIN?**

Diagnosing vulvar (pre)malignancies is challenging with often a delay in diagnosis by patients as well as doctors.\(^12, 13\) As mentioned before, difficulties with the histopathological recognition of the lesion might contribute to the relative under-reporting of dVIN. In chapter 4, we have shown that the interobserver agreement was low, and even written guidelines with histological characteristics to diagnose dVIN did not increase agreement among general pathologists. The question is how the recognition can be improved. First, specimens with an unclear histopathological diagnosis and/or clinical suspicion for dVIN should be revised by a pathologist specialised in gynaecopathology. Second, international agreement on the histopathological criteria to diagnose dVIN is urgently needed. Recently, the ISVD took a survey amongst experts in the field of diagnosing dVIN in order to reach consensus on the usefulness of histopathological criteria. The results of this survey will be presented on the next meeting of the ISVD (2015 World Congress). Third, besides the use of histopathological criteria to diagnose dVIN, there may be a role for immunohistochemical markers like MiB1, p16 and p53.\(^14, 15\) Last, when the clinical impression and histopathological diagnosis disagree, the clinician should discuss the case with the pathologist.

Not only the histopathological diagnosis of dVIN is difficult, but also the clinical diagnosis, in daily practice it is challenging to distinguish dVIN from LS. As a continuation of our research into making the histopathological diagnosis dVIN, it appears meaningful to investigate possibilities to establish criteria for the clinical diagnosis dVIN. DVIN appears mostly as a subtle erode lesion in a background of LS, although there are various presentations. Improvements are already made by taking digital photos of the vulvar and perianal area as a standard routine, facilitating comparison of the anogenital area over time in much more detail. Another point of action to improve clinical recognition, is education of gynaecologists, dermatologists and pathologists in training because the subject ‘vulva’ is receiving very little attention during medical school and in educational programs. Therefore, we developed a digital education program for medical students which was recently introduced in their curriculum. Currently, we are developing an advanced version of the program for general practitioners, gynaecologists and dermatologists in training. A multidisciplinary approach for vulvar pathology is important to improve the understanding of vulvar disease, making correct diagnoses, and prescribing optimal treatment. Attending a multidisciplinary vulvar clinic is associated with improved quality of life of patients.\(^16, 17\) Furthermore, centralisation of care that increases doctors’ exposure may be a factor of influence. Also patient factors may play a role.\(^12, 13, 18\) Improvements can be made by educating women about their vulvar disease in case of LS and/or VIN, and involve patients in the diagnostic and follow-up process. Regular self-examination of the skin should be advised\(^17\) and patients with vulvar (pre)malignancies should be instructed to contact their doctors/nurses in case of complaints.

**What is the role of cytology in clinical care for patients with vulvar (pre)malignancies?**

Cytology might be used as a triage instrument for LS patients with suspicious and unclear vulvar lesions and, as a consequence, may decrease the number of biopsies needed. In chapter 3, we investigated whether vulvar brush cytology was feasible in detecting LS, VIN and VSCC. In this study 26% of the smears were discarded because of poor cellularity. In the samples with sufficient cellularity, a sensitivity of 97% and a negative predictive value of 88% could be achieved when cytology was compared to histology. In the group
of LS without clinical suspicion of a premalignancy, performance of cytology was poor since the specificity was found to be low (50%). Although the results seem promising, histology remains the gold standard until the brushing technique is improved. The main problem of vulvar cytology is that cellularity is often too low. Besides, dVIN lesions mainly show dysplastic cells in the basal layer which might be difficult to reach with cytology. In order to overcome these problems, the most optimal brushing method with respect to cellularity should be addressed. Immunocytochemical markers like p16, mast cells and p53 could improve the usefulness of cytology. Furthermore, there might be an additive role for epigenetic alterations in cytology material in the future. For example, in the cytological detection of CIN2 or worse, DNA methylation marker research recently has been shown to be of value. Combined methylation marker analysis of two genes on HPV positive cervicovaginal lavage material has shown to be non-inferior to cytology triage in the detection of CIN2 or worse. In vulvar lesions a limited number of studies are available on epigenetic changes; the true landscape of epigenetic alterations in VSCC still needs to be determined before the value in the diagnostic process can be set.

Organisation of care for patients with VSCC and individualisation of treatment

The evidence for the relation between high surgeon/hospital volume and improved patient outcome is increasing for certain tumour types like in breast cancer, lung cancer and colorectal cancer. It is well established that centralisation can improve patient outcome and limits health care costs, especially in low incidence cancers that require high complex treatment policies such as vulvar cancer. In chapter 6, we showed that the majority of patients with VSCC from the Eastern part of the Netherlands is treated in the specialised oncology unit of the Radboudumc nowadays. Treatment in a specialised oncology centre was found to be an independent prognostic factor for a favourable outcome. This finding suggests that we should continue centralisation as recommended by the Dutch Inspection of Healthcare (IGZ) and Dutch Society of Obstetrics and Gynaecology (NVOG).

High-volume centres may lead to better opportunities for multidisciplinary cooperation, research and education. In the Netherlands, the IGZ has established a minimum of 20 cases per centre per year for VSCC under the condition that surgeries should be performed by a fixed team of surgeons with supporting staff such as nurses and pathologist. However, caseload/experience of the individual surgeon may be more important than the total caseload per centre. Structured multidisciplinary care may also increase the knowledge of vulvar diseases and improve diagnostic abilities to diagnose and recognise difficult vulvar lesions such as dVIN (chapter 4). A systematic review performed by Chowdhury et al. in which 163 studies of 43 surgical procedures (of high and low incidence cancers) were analysed, showed that surgeon volume and specialisation are associated with improved patient outcome, while high hospital volume is of limited benefit. Currently, the only advice concerning surgeon-threshold values in the treatment of VSCC is that gynaecologic oncologists should perform at least 5-10 sentinel lymph node (SLN) dissections per year to meet the quality standards. However, it needs to be realised that this number of surgeries is chosen experience-based instead of evidence-based. To ensure that each individual gynaecological oncologist retains sufficient expertise for the treatment of vulvar cancer, further reduction in the number of specialised oncology centres for the treatment of vulvar cancer may be necessary.

Effort has been put in individualising treatment of patients with VSCC and in defining subgroups of patients that may be treated less radical. While patients were treated with standard radical vulvectomy with inguinofemoral lymphadenectomy in the past, the wide local excision and SLN procedure have acquired a permanent place in the treatment of early-stage vulvar cancer. Given the high loco-regional recurrence rate of VSCC, it is advantageous to avoid groin surgery as much as possible. In order to reduce the (slight) morbidity of the SLN procedure in cases where it is not necessary and to keep the option of SLN procedure for the future. Currently, the invasion depth of VSCC determines the need for groin surgery. Groin surgery is omitted in VSCCs with less than 1mm invasion depth (FIGO stage IA), based on the negligible risk of lymph node metastases from these tumours. Given our experience with clinical outcome of patients with VSCC and given the lack of scientific basis for the current measuring method, we questioned whether the invasion depth of VSCC measured by this current method is accurate in predicting the risk of lymph node metastases. In chapter 5 we showed that using an alternative measuring method, 19% of patients with originally FIGO stage IB tumour without node metastases were restaged to FIGO stage IA. As a consequence, these patients would have been treated without groin surgery with less treatment related morbidity. In a greater scope, 400 new VSCC patients are diagnosed in the Netherlands each year; approximately 200 of them have a FIGO stage IB tumour (based on the FIGO 2009 classification). Using the alternative measuring method, nearly 40 (19% of 200) of these women will be down staged and will not need any groin surgery. One should keep in mind that missing a groin metastasis is nearly always fatal. Therefore, further studies with a higher number of patients should be performed before this alternative measuring method can be implemented. First, (inter)national consensus should be reached between pathologists on how to determine the depth of invasion. Further research is needed to study the relation between depth of invasion and risk of lymph node metastases. Extra attention is warranted for difficult cases, for example when an ulcer is present. A national prospective trial in which the invasion depth is measured using both the alternative and conventional methods will provide information on the feasibility and accuracy of the alternative measuring method. One point that needs to be critically addressed is the clinical consequences of isolated tumour cells (ITC) in the SLN found after ultrastaging. In chapter 5, we found one patient that was restaged to FIGO stage 1A based on depth of invasion but with ITC in the SLN. Up until now, the consequence of this finding is unknown. Further data are needed to learn about
the clinical significance of these isolated tumour cells and to establish their role in clinical decision making. How often are ITCs present and what is the consequence of ITCs with respect to disease specific survival? Results from GROINS-V-I showed that ITCs were present in SLNs of 24 of 403 patients (6.2%). In an in-depth analysis, it was shown that the proportion of patients with non-SLN metastases increases with the size of the SLN metastasis. Only one of 24 patients with ITCs in the SLN had non-SLN metastases (4%). Furthermore, the prognosis of patients with a positive SLN based on ITC is similar to patients with a negative SLN, although firm conclusions are difficult to draw because of the lack of power. Currently, GROINS-V-II is prospectively evaluating the safety of primary groin radiotherapy instead of full inguinal/femoral lymphadenectomy for women with micrometastases (deposits ≤ 2 mm) detected in SLNs. Moreover, there is a registration of all patients with early-stage (4 cm or smaller lesions) VSCC without SLN metastases. As a consequence of the study design of GROINS-V-II we will not get additional information about the clinical value of ITCs because these patients will undergo additional radiotherapy. Though data from this study might be used to study the relation between depth of invasion and lymph node metastases. In contrast to VSCC, more is known about the role of ITCs in SLNs in breast cancer. Submicrometastases (metastases no larger than 0.2mm) are classified as N0 and these patients are treated the same way as SLN-negative patients. However, results of SLNs of breast cancer patients are difficult to compare with results of VSCC patients. Firstly, because the pattern of metastasizing of breast cancer is completely different from VSCC. Secondly, because an increasing number of breast cancer patients receive (neo)adjuvant systemic therapy and/or locoregional radiation therapy, which may result in the eradication of possible ITCs. In order to further individualise the treatment of VSCC, a next GROINS-V study should be considered with an observational arm for patients with micrometastases (after finishing inclusion of patients in GROINS-V-II at the end of 2015). In theory, omitting treatment for possible ITCs might lead to groin metastases and thereby possibly to worse survival rates in a subset of patients.

**Trends in incidence, prevalence and prognosis of vulvar (pre)malignancies**

Since 1989, all cancer patients in the Netherlands are registered in the database of the Netherlands Cancer Registry (NCR). This gives the opportunity to study trends in incidence, prevalence and survival of patients with various types of cancer in the population. The Dutch system is rather unique, as it has a high coverage rate decreasing the risk of selection bias. For recent years, more detailed data about either surgery/pathology results and co-morbidity are registered, making future population-based studies much more relevant. In chapter 6 and 7, we used the NCR database to study the incidence of VSCC and effect of changes in its treatment during the last two decades. Although earlier studies indicate that the incidence of VSCC is increasing, we were the first to confirm these results with our population-based data. An increase of absolute numbers of VSCC was expected because of the growing group of elderly. However, even after correction for changes in the composition of the population using the European age standardised incidence rates, an increasing incidence was observed especially in patients below the age of 60. Exact reasons for this increase are unclear. We hypothesise that this might be a consequence of an increased number of HPV infections as HPV-associated VSCCs mainly affect younger women. A study of the HPV VAP study group confirmed our hypothesis by showing that the HPV-DNA detection and p16 over-expression in VSCC significantly increased between 1980-1999 and 2000-2011. HPV and p16 positivity was consistently higher amongst younger women. Analyses of antibody seroprevalence of high-risk HPV types between Nationwide Surveillance Studies from 1995-1996 and 2000-2007 in the Netherlands showed an increase in seropositivity in the general population. Besides, an increase in the incidence of genital warts and the proportion of HPV-positive samples is observed in other types of cancer such as anal cancer and oropharyngeal cancer. The increasing contribution of HPV might be due to higher virulence of HPV but the involvement of multiple HPV types makes this hypothesis unlikely. Explanations for increased attribution of HPV in these cancers are probably linked to risk factors such as younger age at first intercourse, a higher number of sexual partners during lifetime, smoking habits and the higher number of immune-compromised patients due to more organ transplantations and broader indication area for the use of immunosuppressive medication for autoimmune disorders such as rheumatic diseases and inflammatory bowel diseases. Two commercial vaccines (Cervarix and Gardasil) are available in many countries worldwide; these have been found to be highly efficient in preventing persistent HPV infections and lesions from the lower genital tract. It is expected that the incidence of HPV-associated VSCC and vulva will decrease in a few decades as a consequence of the Dutch prophylactic HPV-vaccination program with the use of Cervarix (HPV 16 and 18) for girls of 12 years old which started in 2009. With the use of the 9-valent HPV vaccine (includes types 6, 11, 16, 18, 31, 33, 45, 52 and 58), which is investigated at this time, it is expected that even a higher percentage of women can be prevented from infection and subsequent anogenital HPV-related diseases.
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Summary

Vulvar squamous cell carcinoma (VSCC) is a rare gynaecological malignancy. In the Netherlands, 400 new cases are diagnosed yearly. There are two different types of VSCCs, both with their own associated premalignant lesions. The majority of VSCCs develop in elderly women and often arise in the background of lichen sclerosus (LS) or differentiated vulvar intraepithelial neoplasia (dVIN), but the exact oncogenesis is not known. HPV-related VSCCs are mainly seen in younger women and include the minority of VSCCs with usual VIN (uVIN) as precursor lesion. VSCC, VIN and LS have a large impact on quality of life in affected women and their partners. Lesions are sometimes difficult to diagnose by clinical doctors such as GPs, dermatologists, gynaecologists and pathologists because of the rarity of vulvar squamous (pre)malignancies with a variety of symptoms. Proper and timely treatment is important in order to minimise the morbidity and mortality caused by these lesions. In this thesis, we describe opportunities for the further improvement of clinical care of women with vulvar (pre)malignancies with the focus on the diagnostic process and treatment.

In chapter 1 an overview of the oncogenesis, clinical presentation, diagnostics and the treatment of VSCC, VIN and LS is given.

dVIN is assumed to be the precursor lesion of the majority of HPV-unrelated VSCCs, but genetic evidence that supports this theory is currently lacking. In order to study the genetic relation between dVIN and VSCC, in chapter 2 we compared the copy number abnormalities between paired dVIN and VSCC lesions using a molecular inversion probe single-nucleotide polymorphism array on DNA isolated from these lesions. Copy number alterations (CNA) were identified in six VSCC samples, including loss of 8p (present in all cases), gain of 8q (present in 5/6 VSCCs), gain of 7p and loss of 18q (present in 4/6 VSCCs). Copy number profiles of three dVIN lesions passed quality thresholds, and CNAs were identified in one of them. In these patients at least three out of the 33 CNAs identified in the VSCC sample, were also detected in the paired dVIN sample, including a high-level amplification on chromosome 11q23. These findings suggest that the two lesions originate from a single precursor in which additional alterations may have resulted in the development of VSCC. Furthermore, it supports the hypothesis that VSCC originates from dVIN precursor lesions.

Taking a biopsy is a standard procedure to make the diagnosis in patients with suspicious premalignant vulvar lesions. The use of a less invasive diagnostic tool as triage instrument to determine whether biopsy is indeed necessary may improve patient comfort. In chapter 3 we investigated whether vulvar brush cytology is feasible and may be used to detect (pre)malignant vulvar lesions. We took 65 smears of normal skin, LS, uVIN, dVIN and VSCC with a vulvar brush and compared the findings with histology. Out of 65 smears, 17 (26%) were discarded because of poor cellularity. A total number of 28/29 (97%) smears with a histological
proven (pre)malignancy had a smear classified as ‘suspicious’ or ‘uncertain’. Cytology classified 1 1 smears as ‘non-suspicious’, of which 10 (91%) were indeed normal skin or LS. The accuracy for (pre)malignant lesions with the use of the brush showed a sensitivity of 97% and a negative predictive value of 88%. Based on these results we concluded that vulvar brush cytology is feasible and may be a first step in the development of a triage instrument to determine whether subsequent biopsy of a clinically suspicious lesion is necessary.

The histopathological diagnosis of dVIN is difficult, though no published data concerning the reproducibility of the diagnosis are available. In chapter 4, we evaluated the interobserver variability of the histopathological diagnosis of dVIN. Six pathologists, each with a different level of education, analysed haematoxylin and eosin-stained slides with LS and dVIN. The interobserver agreement varied between slight (kappa =0.08) and moderate (kappa =0.54). In order to increase the agreement, guidelines with histological characteristics of dVIN were provided after which the pathologists were asked to analyse the slides again. The interobserver agreement increased slightly toward an agreement between slight (kappa =0.01) and substantial (kappa =0.75). Only pathologists specialised in gynaecopathology reached a substantial agreement (kappa =0.75). Therefore, it should be considered that specimens with an unclear diagnosis and/or clinical suspicion for dVIN should be revised by a pathologist specialised in gynaecopathology. Furthermore, we present five criteria (atypical mitosis, basal cell atypia, prominent nucleoli, dyskeratosis, elongation and anastomosis of rete ridges) that were ranked to be the most useful in diagnosing dVIN. When adhering to these criteria the diagnosis of dVIN might be made easier and earlier.

Depth of invasion is an important prognostic factor for patients with VSCC and guides the treatment plan; in case of a microinvasive tumour (depth of invasion ≤1mm, FIGO stage IA) patients are treated with a wide local excision only, while in case of a macroinvasive tumour (depth of invasion >1mm, FIGO stage IB or higher) patients will undergo treatment of the groins. It would be of advantage to prevent any surgical procedure of the groins to decrease treatment related morbidity, although one should be reluctant in omitting treatment of the groin because a recurrence in the groin is nearly always fatal. Based on the fact that there is no scientific basis for choosing the current measuring method, we hypothesised that an alternative method of measuring invasion may give a better reflection of the prognosis of VSCC. In chapter 5 we aimed to identify the most optimal method of measuring the depth of invasion in relation to the lymph node status and survival in patients with VSCC. Depth of invasion of 149 VSCC cases was measured using the conventional (measures from the epithelial junction of the most superficial dermal papilla to the deepest point of tumour invasion) and an alternative (measures from the basement membrane of the deepest adjacent tumour-free rete ridge to the deepest point of invasion) method. The median depth of invasion decreased significantly with the use of the alternative method. This resulted into a change in FIGO stage from stage IB to IA in 19% of the patients. These down-staged patients developed less frequent recurrences (15.4% versus 39.3%) and had a higher disease specific survival (100% versus 84.2%) compared to the patients who remained FIGO stage IB. These patients might be treated without groin surgery resulting in less treatment-related morbidity. Furthermore, a change in depth of invasion from 3.5 mm to 0.2 mm in one patient with FIGO stage IIIA (based on two isolated tumour cells in the sentinel lymph node (SLN)) was observed. Although the results are promising, the value of isolated tumour cells in a SLN and more prospective data on a higher number of patients using the alternative method of measuring are necessary.

Centralisation of care for women with VSCC has been advocated by the national guidelines of the Dutch Society of Obstetrics and Gynaecology in 2000. In chapter 6, we determined whether this advice has been adapted and has led to improved survival. Therefore, all patient and tumour characteristics of women diagnosed with VSCC between 1989-1999 (n=198) and 2000-2008 (n=184) in the Eastern part of the Netherlands were retrieved from the population-based cancer registry and compared. The percentage of women treated in a specialised oncology centre increased from 62% to 93%. Overall, the 5-year relative survival improved slightly from 68% to 72%. After adjustment for age and stage, being treated in a specialised oncology centre was an independent prognostic factor for survival.

During recent decades the treatment of VSCC became less radical. Furthermore, previous studies showed an increase in the incidence of VIN. In order to study the effect of these changes, in chapter 7 the incidence and survival of patients with VSCC in the Netherlands were studied. Therefore, all patients with VSCC diagnosed between 1989 and 2010 (n=4614) were selected from the Dutch Cancer Registry. Data from these patients showed that the incidence of VSCC has increased significantly since 2002. In women aged <60 years incidence rates increased significantly during the whole study period, while in women aged ≥60 years only an increase was observed from 2004 onwards. Survival rates were not affected by the introduction of less radical surgery such as the introduction of a wide local excision with uni- or bilateral inguinofemoral lymphadenectomy via separate incisions and the SLN dissection, as the survival rates remained stable over time.

In chapter 8, clinical implications and future perspectives based on the results of the abovementioned studies are discussed with the aim to improve the care for women with vulvar squamous (pre)malignant lesions.
Samenvatting

Het vulvacarcinoom is een zeldzame gynaecologische kanker en wordt in Nederland jaarlijks 400 keer gediagnosticeerd. Er zijn twee typen vulvacarcinoom, beide met een ander voorloperstadium. De meerderheid van de vulvacarcinomen komt voor bij oudere vrouwen, vaak patiënten met Lichen Sclerosus (LS) of één gedifferentieerde vulvaire intra-epitheliale neoplasie (dVIN). Hoe deze vorm van kanker exact ontstaat, is echter onbekend. Bij jongere vrouwen worden met name HPV-gerelateerde vulvacarcinomen gezien. Dit betreft een minderheid van de vulvacarcinomen. Het voorloperstadium van dit type vulvacarcinoom wordt usual VIN (uVIN) genoemd. Het vulvacarcinoom, VIN en LS hebben grote invloed op de kwaliteit van leven van patiënten en hun partners. De afwijkingen zijn soms moeilijk te diagnosticeren door de huisarts, dermatoloog, gynaecoloog en de patholoog vanwege hun zeldzaamheid en de variëteit aan symptomen. Om de ernst van de ziekte en de sterfte hiernaar te minimaliseren is het belangrijk dat tijdig een adequate behandeling wordt gestart. In dit proefschrift worden verschillende mogelijkeheden beschreven om de zorg voor vrouwen met (voorloperstadium van het) vulvacarcinoom te verbeteren, met name gericht op de diagnostiek en het behandelproces.

In hoofdstuk 1 wordt een overzicht gegeven van de ontstaanswijze, het klinische beeld, de diagnostiek en behandeling van het vulvacarcinoom, VIN en LS.

Over het algemeen wordt aangenomen dat dVIN de voorloper van het niet-HPV-gerelateerde vulvacarcinoom is, maar genetisch bewijs dat deze theorie ondersteunt is er tot op heden niet. Met het doel de genetische relatie tussen dVIN en het vulvacarcinoom te bestuderen, hebben we in hoofdstuk 2 het DNA van dVIN vergeleken met het DNA van het vulvacarcinoom. Dit hebben we gedaan met behulp van een DNA-array waardoor chromosoomafwijkingen gevoelig kunnen worden geplotypeerd. Het gaat hierbij om numerieke afwijkingen (extra kopieën of verlies van chromosomen), of (kleine) deleties of amplicaties op een chromosoom. Aan de hand van de aan- of afwezigheid van deze afwijkingen konden we het genoem van het vulvacarcinoom van zes patiënten nauwkeurig in kaart brengen, en konden we een vergelijking maken met de afwijkingen die we aantroffen in de dVIN van dezelfde patiënt. Vulvacarcinomen hadden veelal een verlies van chromosoom 8p (de korte arm van chromosoom 8), een extra kopie van chromosoom 8q (de lange arm), en een verlies van 18q. Van drie dVIN-laesies waren de chromosoomprofielen van voldoende kwaliteit voor beoordeling, waarbij in één sample afwijkingen werden gedefinieerd. Hier vonden we het belangrijkste bewijs waar we naar op zoek waren: drie chromosomale afwijkingen van het vulvacarcinoom troffen we ook aan in de dVIN, maar een groot aantal andere afwijkingen was afwezig. Deze bevindingen suggereren dat dVIN en het vulvacarcinoom uit dezelfde voorlopercel voortkomen, waarbij de extra afwijkingen die werden gevonden in het vulvacarcinoom geleid kunnen hebben tot de verdere ontwikkeling van een vulvacarcinoom uit de dVIN. Dit ondersteunt de hypothese dat het vulvacarcinoom voortkomt uit dVIN.

De standaardprocedure om te onderzoeken of vulvaire afwijkingen mogelijk een (voorloper)stadium van het vulvacarcinoom zijn, is het nemen van een biopt. Het patiëntcomfort zou verbeterd worden als eerst een minder invasive methode zou kunnen vaststellen of een biopt noodzakelijk is. In hoofdstuk 3 werd onderzocht of cytologie verkregen met een vulvabrush gebruik zou kunnen worden om een (voorloperstadium van het) vulvacarcinoom te detecteren. We namen 65 cytologische uitstrijkjes van de normale huid, LS, uVIN, dVIN en het vulvacarcinoom met een vulvabrush en vergeleken de resultaten met histologie. Van de 65 uitstrijkjes werden er 17 (26%) afgekeurd vanwege onvoldoende celopbrengst. In totaal werden 28 van de 29 (97%) uitstrijkjes met een histologisch bewezen vulvacarcinoom of voorloperstadium geclassificeerd als ‘verdacht’ of ‘mogelijk verdacht voor een (voorloperstadium van het) vulvacarcinoom’. Eerst uitstrijkjes werden als ‘niet verdacht’ geclassificeerd, waarvan 10 (91%) inderdaad normale huid of LS waren genomen. Met cytologie verkregen via de vulvabrush kon een sensitiviteit van 97% en een negatief voorspellende waarde van 88% worden bereikt voor het diagnostiseren van een (voorloperstadium van het) vulvacarcinoom. Op basis van deze resultaten werd geconcludeerd dat cytologie afgenomen met een vulvabrush mogelijk de eerste stap is in de ontwikkeling van een triage-instrument om te bepalen of een biopt wel of niet nodig is.

Het stellen van de histopathologische diagnose dVIN is lastig en er zijn geen gepubliceerde data over de reproduceerbaarheid van de diagnose. In hoofdstuk 4 werd de variatie in de histopathologische diagnose van dVIN door verschillende pathologen (de interobserver variabiliteit) bepaald. Zeer pathologen (pathologen in opleiding, algemene pathologen en gespecialiseerde pathologen) analyseerden hematoxyline-eosine gekleurde coupes met LS en dVIN. De interobserver overeenkomst varieerde tussen ‘gering’ (kappa (κ)=0.08) en redelijk (κ=0.54). Om de overeenkomst te vergroten werden richtlijnen verstrekt met histologische karakteristieken van dVIN. De pathologen werden na bestudering hiervan gevraagd de coupes opnieuw te beoordelen. De interobserver overeenkomst verbeterde minimaal en varieerde tussen ‘slecht’ (κ=-0.01) en ‘voldoende tot goed’ (κ=0.75). Alleen pathologen gespecialiseerd in gynaecopathologie konden een overeenkomst bereiken die ‘voldoende tot goed’ was (κ=0.75). Derhalve moet overwogen worden of een (voorloper)stadium van het vulvacarcinoom is, het nemen van een biopt. Het patiëntcomfort zou verbeterd worden als eerst een minder invasive methode zou kunnen vaststellen of een biopt noodzakelijk is. In hoofdstuk 3 werd onderzocht of cytologie verkregen met een vulvabrush gebruik zou kunnen worden om een (voorloperstadium van het) vulvacarcinoom te detecteren. We namen 65 cytologische uitstrijkjes van de normale huid, LS, uVIN, dVIN en het vulvacarcinoom met een vulvabrush en vergeleken de resultaten met histologie. Van de 65 uitstrijkjes werden er 17 (26%) afgekeurd vanwege onvoldoende celopbrengst. In totaal werden 28 van de 29 (97%) uitstrijkjes met een histologisch bewezen vulvacarcinoom of voorloperstadium geclassificeerd als ‘verdacht’ of ‘mogelijk verdacht voor een (voorloperstadium van het) vulvacarcinoom’. Eerst uitstrijkjes werden als ‘niet verdacht’ geclassificeerd, waarvan 10 (91%) inderdaad normale huid of LS waren genomen. Met cytologie verkregen via de vulvabrush kon een sensitiviteit van 97% en een negatief voorspellende waarde van 88% worden bereikt voor het diagnostiseren van een (voorloperstadium van het) vulvacarcinoom. Op basis van deze resultaten werd geconcludeerd dat cytologie afgenomen met een vulvabrush mogelijk de eerste stap is in de ontwikkeling van een triage-instrument om te bepalen of een biopt wel of niet nodig is.
Invasiediepte is een belangrijke voorspellende factor voor patiënten met een vulvacarcinoom en is leidend voor het behandelplan in het geval van een microinvasieve tumor (invasiediepte ≤1mm, FIGO stadium IA) worden patiënten alleen behandeld met een ruime lokale excisie, terwijl in het geval van een macroinvasieve tumor (invasiediepte >1mm, FIGO stadium IB of hoger) patiënten tevens een chirurgische ingreep in de liezen moeten ondergaan om lymfeklieren te verwijderen. Deze ingreep veroorzaakt vaak klachten, daarom zou het wenselijk zijn deze ingreep niet uit te hoeven voeren. Anderzijds kan in lymfeklieren die niet verwijderd zijn de kanker terugkeren, wat bijna altijd een fatale afloop kent. De gemeten invasiediepte bepaalt of een patiënt in de liezen geopereerd wordt of niet. Er zijn geen wetenschappelijke gronden voor de keuze van de huidige methode om de invasiediepte te meten. In hoofdstuk 5 onderzochten wij of een alternatieve methode om de invasiediepte te meten een betere weergave geeft van de prognose van vrouwen met een vulvacarcinoom. Het doel was om de optimale meetmethode te identificeren in relatie tot de lymfklierstatus en overleving van patiënten met een vulvacarcinoom. De invasiediepte van 148 vulvacarcinomen werd gemeten met de conventionele methode (gemeten vanaf de meest oppervlakkige dermale papil tot het diepste punt van de tumor) en met de alternatieve methode (gemeten van de diepste en meest dichtbij gelegen, tumorvrije papil tot het diepste punt van de tumor). De gemiddelde invasiediepte was significant minder voor patiënten waarbij het FIGO-stadium veranderde van IB naar IA. Deze patiënten ontwikkelden minder frequent een recidief (39.3% versus 15.4%) en hadden een hogere lymfklierstatus overleving (84.2% versus 100%), vergeleken met patiënten bij wie het FIGO-stadium IB niet veranderde. De groep patiënten waarbij het FIGO-stadium veranderde van IB naar IA zou mogelijk behandeld kunnen worden zonder lymfeklierchirurgie, met minder klachten als gevolg. Echter bij één patiënt met een FIGO stadium IIIA (gebaseerd op twee geïsoleerde tumoren in de schildwachtklier) werd een invasiediepte van 3.5 mm gemeten. Ook al lijken de resultaten veelbelovend, de betekenis van geïsoleerde tumoren in de schildwachtklier moet verder worden uitgezocht. Eveneens is meer prospectieve data nodig van een grotere patiëntengroep waarbij gemeten is met de alternatieve meetmethode.


In hoofdstuk 8 worden klinische implicaties en toekomstige perspectieven gebaseerd op de resultaten van bovengenoemde studies bediscussieerd, met als doel om de zorg voor vrouwen met een vulvacarcinoom nog verder te verbeteren.
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Bedankt!

Loes
Curriculum Vitae