VULVAR PAGET DISEASE

MICHELLE VAN DER LINDEN

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COLOPHON

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For reasons of consistency within this thesis, some terms and abbreviations have been standardised throughout the text, and might therefore slightly differ from the original publications.

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CHAPTER 1

INTRODUCTION
AND
OUTLINE OF THESIS

INTRODUCTION

This thesis focuses on different aspects of vulvar Paget disease (VPD). After a review of the history of this rare disease, the anatomy of the vulva is explained, followed by the pathogenesis and treatment of vulvar carcinomas. Concluded by an outline of this thesis on VPD.

THE HISTORY OF VULVAR PAGET DISEASE

Sir James Paget (1814-1899), was the surgeon of the English royal family. He had a great interest in pathology. Together with Rudolf Virchow, he is considered to be one of the founders of modern scientific medical pathology.

In 1877, he was the first to describe a chronic inflammation of the bone, in which the formation and breakdown of the bones was disturbed, causing multiple deformities.[1] This disease is nowadays well known as Paget's disease of the bone, or osteitis deformans. After osteoporosis, it is the second most common disease of the bones in the elderly.[2]

In 1874, Sir James Paget published a paper on a nipple ulceration.[3] He had seen 15 women, with a chronic, eczema-like nipple ulceration, that all developed a malignancy of the mammary glands afterwards. He raised the question whether this skin eruption was a precursor for an underlying malignancy or invaded into the breast. Nowadays, we call this skin disease Paget's disease of the breast, or mammary Paget disease (MPD), and is diagnosed when Paget cells are seen in the epithelium. Of all patients with MPD, 55% have an underlying invasive ductal carcinoma of the breast, and 35% has the premalignancy called ductal carcinoma in situ (DCIS).[4] DCIS is premalignant but aggressive, and therefore it is treated as a malignancy. MPD, with or without an underlying carcinoma or DCIS, is treated according to guidelines for breast cancer in general.

In response to Sir Paget's paper on MPD, Crocker reported a case of penoscrotal Paget disease in 1889.[5] Two years later the first case of vulvar Paget disease was reported by a French dermatologist, William Dubreuilh.[6] Ever since, when located elsewhere than the breasts, the skin disease is called extramammary Paget disease (EMPD). Alternative names are related to

specific locations of the skin disease: for example VPD, or penoscrotal Paget disease. EMPD, irrespective of location, is also diagnosed when the same Paget cells as in MPD are seen in the epithelium.

Breast cancer, or a premalignancy such as DCIS, are commonly seen in MPD. VPD has comparable signs and symptoms as MPD, and in both diseases Paget cells are present in the epithelium. Therefore, from the first report of VPD, it is thought that these diseases are related. It still remains unclear how these diseases are related, and even if they are related. The tissue of the breast resembles the tissue of the vulva, suggesting a similar pathological mechanism causing both diseases. However, EMPD has also been reported in the hair-bearing skin of the axilla and ear canal. Thus, EMPD can also be seen in different parts of the hair-bearing skin.

Little is known about VPD, as it is extremely rare. The exact incidence is unknown, nor is the natural course of disease. To optimise care for patients with VPD, it is necessary to understand the disease better.

THE VULVA

The vulva is the external part of the female reproductive tract. The basic anatomy of the vulva is presented in figure 1.[7]

The orifices of the urethra and vagina are located in the centre of the vulva; the vestibule. The vestibule is lined by the clitoral frenulum, labia minora, medial labia majora, and the fourchette. The vagina is separated from the vulva by the hymen. The major labia are hair-bearing on the lateral side and fuse into the mons pubis on the anterior side, and into the perineum on the posterior side. The tissue in between the major and minor labia is called the interlabial sulcus. The anus and the perianal skin are strictly not part of the vulva, but may be involved in genital skin disease.

The outer part of the vulva consists mainly of keratinised squamous epithelium. The so-called Hart's line separates the keratinised epithelium of the outer part of the vulva from the inner non-keratinised epithelium. The urethra in females does not contain typical transitional cells as the urethra in males does, but consists of glycogen rich stratified squamous epithelium like the vagina and ectocervix.

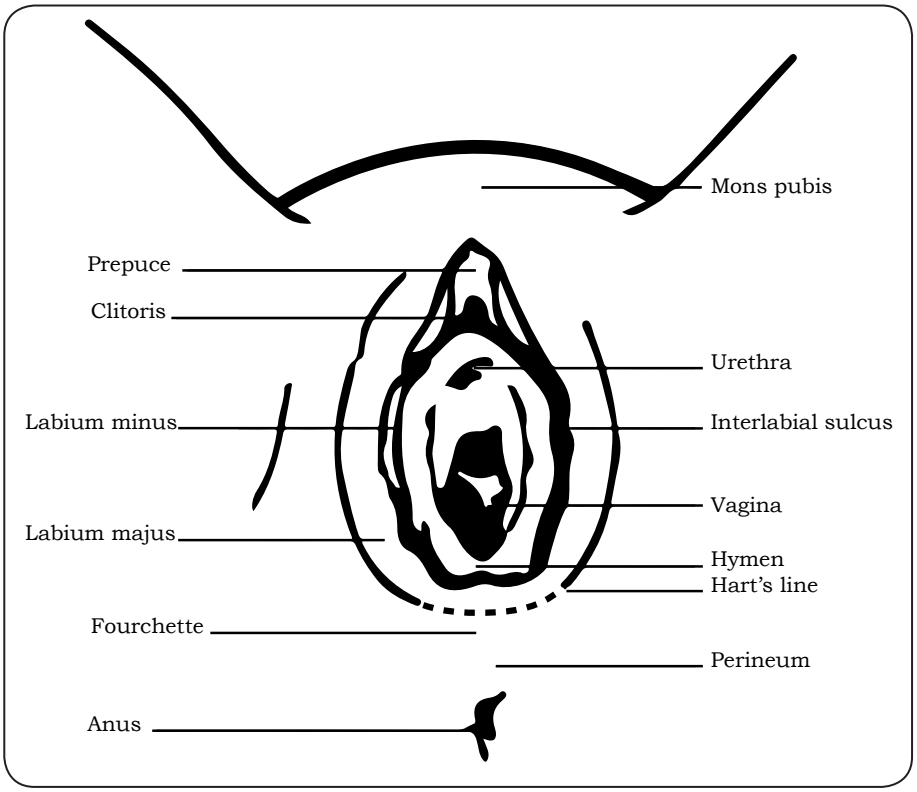

FIGURE 1 Basic anatomy of the vulva

The mons pubis, the lateral part of the labia majora and peri-anal skin are hair-bearing and besides hair follicles, also contain pillosebaceous glands and adnexal glands: eccrine or apocrine sweat glands and sebaceous glands. The medial part of the labia majora does not contain hair follicles, but does contain sebaceous glands along Hart's line.

The vulvar vestibule contains three main types of mucinous glands: Bartholin's glands, also known as the major vestibular glands, the minor vestibular glands, and Skene's ducts. Bartholin's glands are located on either side of the distal vaginal orifice. The epithelium contains mucus-secreting columnar cells, secreting in Bartholin's duct. Bartholin's duct is lined by transitional epithelium, adjacent to columnar epithelium, which is adjacent to the non-keratinised stratified squamous epithelium of the vulvar vestibulum. The minor vestibular glands are multiple simple tu-

bular glands lined with mucus-secreting columnar epithelium, blending into the stratified squamous cells of the vulvar vestibule. Skene ducts are located bilaterally and immediately posterolateral of the urethra. These glands (counter parts of the male prostate) are lined with pseudo-stratified mucus-secreting columnar epithelial cells, and the ducts with transitional type cells adjacent to the stratified squamous cells at the duct's orifice.

VULVAR CANCER

Clinical characteristics

All cells in the human body have a malignant potential, including those of the vulva. Figure 2 presents an overview of the different types of vulvar malignancies. Actual data on the incidence of the different types is unknown. It is thought that about 90% of the vulvar malignancies are epithelial, of which 80% are of the squamous cell type. In the Netherlands,

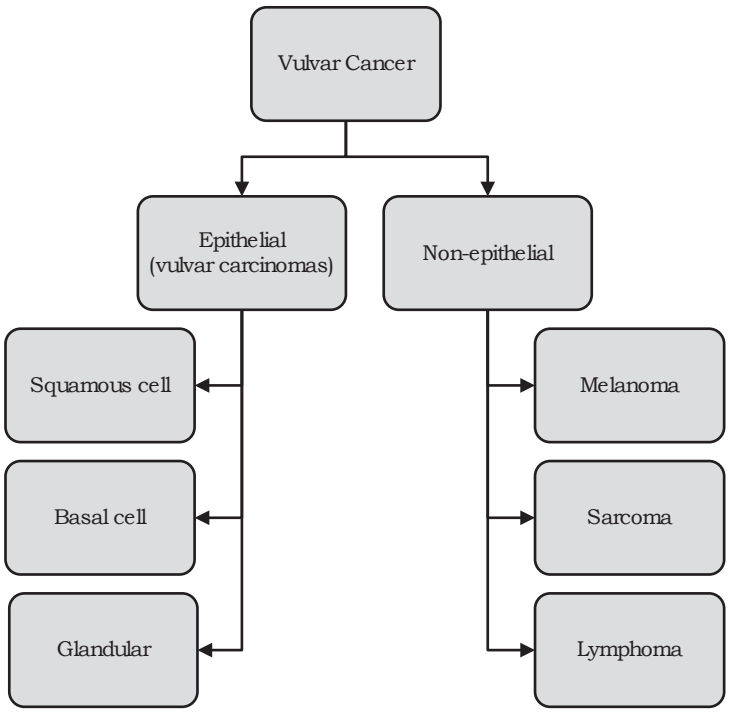

FIGURE 2 Flow-chart of different types of vulvar malignancies

little over 400 women are diagnosed with a vulvar malignancy every year, representing +/- 5% of all gynaecological malignancies.[8]

About 8% of the vulvar malignancies are the basal cell carcinomas (BCC).[9] BCC is a relatively common malignancy with good prognosis and little risks for metastases. BCCs occur over the entire body; about 2% are located on the vulva.

Pathogenesis of vulvar carcinoma

In vulvar squamous cell carcinoma (VSCC) the pathogenesis is quite established. Two different pathways can be recognised: the human papilloma virus (HPV) positive and HPV negative pathway. Most cases, about 80%, are HPV negative and are thought to arise from differentiated vulvar intraepithelial neoplasia (dVIN), a premalignancy often seen in the autoimmune disease Lichen Sclerosus. HPV positive VSCC are often preceded by the premalignancy high grade squamous intraepithelial lesion (HSIL), previously known as usual VIN (uVIN).[10]

The pathogenesis of BCCs is unclear. It is currently thought they originate from pluri-potent cells in the basal layer of the epidermis, or pillosebaceous unit.[11]

Glandular malignancies are a separate entity, and are most commonly called adenocarcinomas. The vulvar adenocarcinoma is rare, so little knowledge is available. 'Adenocarcinomas' originate from the glandular epithelium or mucosal tissues, for example of the vulva, breast or intestine. Adenocarcinomas originating from a specific gland or tissue type may be considered as a separate entity, with a specific name. The most common cause of a vulvar adenocarcinoma is supposedly the rare skin disorder VPD. Other types of primary vulvar glandular malignancies are Bartholin gland tumours, Skene gland tumours, mammary-like-gland tumours and sweat gland tumours.[12-14] These glandular malignancies are rare, and may seldomly be seen by a pathologist. The origin of these glandular malignancies of the vulva is unknown: there are no specific genetic, microbiological or other causes identified for the development of a vulvar glandular malignancy.

Treatment of vulvar carcinoma
In the Netherlands, care for patients with a vulvar carcinoma is centralised since 2009. All cases should be treated in tertiary centres by gynaecologic oncologists, except for BCC. In general, surgery with or without adjuvant treatment is the treatment of choice. Because vulvar glandular malignancies are extremely rare, there is no specific guideline for these types of malignancies. Therefore, these are treated according the (local) guidelines for VSCC.

OUTLINE OF THESIS

This thesis investigates glandular malignancies of the vulva, with specific attention to VPD, a skin disorder which is considered to be an adenocarcinoma in situ with a malignant potential. Little research on this disease has been performed in the last decades. In the recent years, care for patients with vulvar disease has been improved due to specialised vulvar clinics and new treatment methods. This thesis aims to improve care for patients with VPD by evaluating the most evident clinical challenges.

We investigated the incidence and survival of glandular malignancies of the vulva in the Netherlands in **chapter 2**, with the use of pathology reports of all cases in the Netherlands between 2000 and 2015, supplemented with data from the National Cancer Registry.

One of the main origins of vulvar glandular malignancies is VPD. VPD may be of a primary cutaneous origin, or secondary to an intestinal or urological malignancy. In **chapter 3** we reviewed literature on the clinical symptoms, diagnosis, treatment and survival of VPD patients. This raised some additional questions, which we explored in further chapters.

To understand the clinical course of VPD, we reviewed the medical charts of all patients in eight tertiary university medical centres. **Chapter 4** gives an overview of most common clinical aspects of the disease, and the impact of VPD on survival.

In **chapter 5** we investigated whether patients with VPD have a higher risk of developing other malignancies associated with the disease once di-

agnosed with VPD. We used the histology reports of all patients diagnosed with VPD in the Netherlands between 2000 and 2015. We investigated whether the risk was increased and whether routine screening is necessary in the work-up of patients with non-invasive cutaneous VPD.

The origin of VPD remains uncertain. It is speculated that primary VPD originates from the skin adnexa, mammary-like-glands or Toker cells. Non-cutaneous VPD may be secondary to intestinal or urological malignancies, but where the typical Paget cells arise in these malignancies is unknown. In **chapter 6** we reported an extremely rare case of retrograde cervical spread of VPD secondary to an intestinal malignancy. We genetically proved all lesions originate from the same tumour with *p53* analysis.

In skin samples of primary VPD, we often see an immune infiltrate. This may be a component in the development of clinical symptoms, or may play a role in new treatment options. Therefore we investigated which immune cells are present in the immune infiltrate in non-invasive primary VPD in **chapter 7** and compared our findings to those in vulvar HSIL and healthy controls.

In **chapter 8** we present the protocol of a clinical trial investigating topical 5% imiquimod cream for non-invasive VPD.

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CHAPTER 2

INCIDENCE AND SURVIVAL OF GLANDULAR VULVAR MALIGNANCIES IN THE NETHERLANDS

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ABSTRACT

Aim: There is limited knowledge in the field of glandular vulvar malignancies. The aim of this study is to describe the incidence and survival of women with glandular vulvar malignancies.

Methods: We searched PALGA, a nation-wide database registering all histo- and cytopathology in the Netherlands, for all cases of glandular vulvar malignancies between 2000 and 2015. Additional data were retrieved via the Netherlands Cancer Registry. Incidence rates were calculated per 1,000,000 women per year. Five-year net survival rates were calculated.

Results: We identified 197 patients with a glandular vulvar malignancy. Of these patients 55% had a primary malignancy while 45% had secondary malignancies: expansion of another tumour in 17%, and metastases or recurrences of another malignancy in 28%. There is a great variety of different diagnoses of primary vulvar malignancies: 11 different types were identified. We found an overall incidence rate of glandular vulvar malignancies of 0.9 – 2.5 per 1,000,000 women per year. Five-year net survival for patients with a primary malignancy was 68.5%. Most of the secondary vulvar malignancies originated from (ano-)rectal malignancies.

Conclusion: Glandular vulvar malignancies are extremely rare and primary tumours are slightly more common. Overall survival of patients with primary glandular vulvar malignancies is comparable to patients with a vulvar squamous cell carcinoma, with five-year survival around 70%. The great variety in diagnoses combined with the low incidence should lead to routine pathologic revision and treatment in specialised gynaecologic oncology centres.

BACKGROUND

In the Netherlands the incidence of vulvar cancer was 4 per 100,000 women in 2015,[1] representing 7% of all gynaecological malignancies. Primary vulvar malignancies can be divided into epithelial and non-epithelial malignancies, figure 1 presents an overview of the different types. More than 80% of the cases are of the squamous cell type.[2,3] Vulvar melanomas and basocellular carcinomas are less common.[3] Epithelial carcinomas of the extremely rare adenotype are called glandular tumours altogether and consist of several diagnoses.

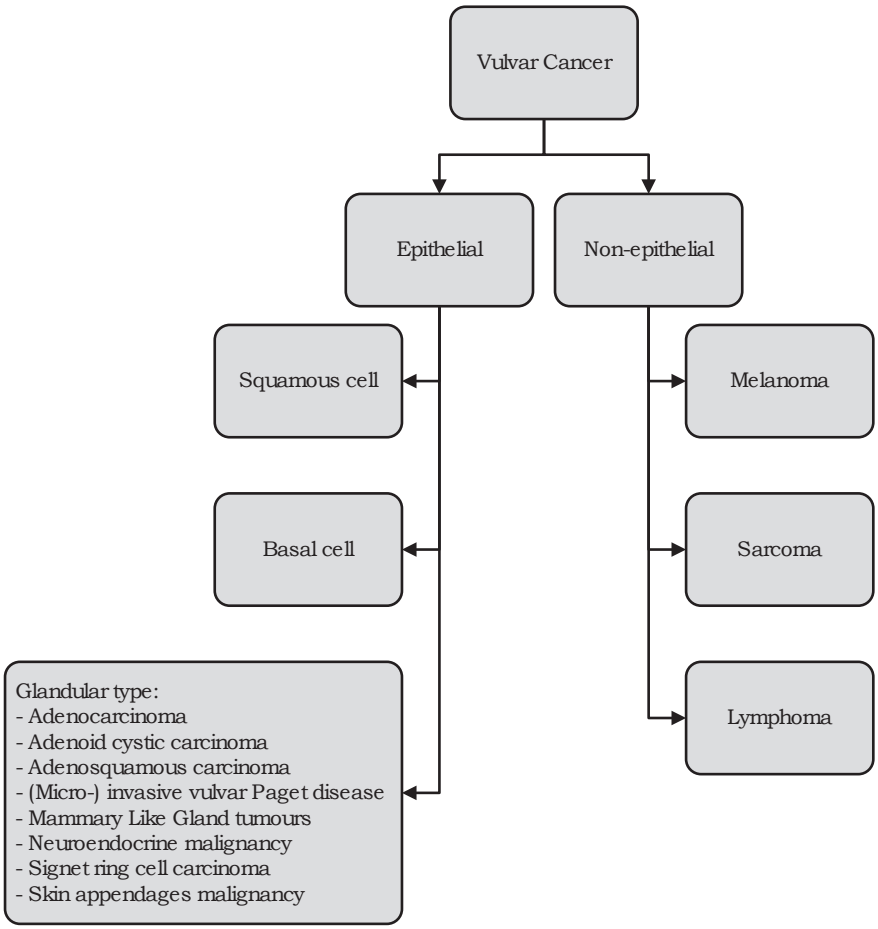

FIGURE 1 Overview of different types of primary vulvar cancer.

The most common and ambiguous term for a glandular vulvar malignancy is vulvar adenocarcinoma. Invasive vulvar Paget disease (VPD) and Bartholin's gland tumours are the most common adenocarcinomas. Tumours of the skin appendages and mammary-like-gland (MLG) tumours are extremely rare.[4,5] Besides these primary adenocarcinomas, several case reports are published about patients with secondary vulvar metastases of other primary malignancies, such as: breast,[6] colon,[7] lungs,[8] or other gynaecological malignancies.[9-11]

In contrast to vulvar squamous cell carcinomas (VSCC), with a human papilloma virus related and a lichen sclerosus related pathway, the oncogenesis of glandular vulvar malignancies is unknown. Furthermore, there are no recent data on the incidence rates, nor on the survival rates of patients with these glandular vulvar malignancies.

In the Netherlands, centralization of care for patients with VSCC in specialised oncology centres was implemented in 2000 with beneficial effects on survival.[12] The national guideline states all vulvar malignancies except vulvar basal cell carcinomas should be treated in specialised oncology centres.[13] Due to its rarity, there is no disease specific guideline for management of glandular malignancies of the vulva in general; patients with glandular vulvar malignancies are treated according to the guidelines for VSCC.

This national study aims to present the incidence rate and survival of patients with various types of glandular vulvar malignancies.

METHODS

PATIENT SELECTION

We performed a search in the PALGA database, a nationwide network and registry of histo- and cytopathology in the Netherlands (~17 million inhabitants). The PALGA network has national coverage since 1991. We selected all women with a diagnosis of an invasive glandular malignancy of the vulva between 2000 and 2015 (ICD-O: C51.0-9). We excluded cases with a benign diagnosis, basal cell carcinoma, Merkel cell carcinoma, car-

cinosarcoma, (myo-)epithelial carcinoma, or spindle cell carcinoma. Cases in which the diagnosis was not specified, e.g.: carcinoma, undifferentiated carcinoma, were also excluded.

We matched the cases of invasive glandular vulvar malignancies to the Netherlands Cancer Registry (NCR) based on age, and date of diagnosis for additional clinical data. We retrieved data on morphology, TNM classification, FIGO stage, differentiation grade, and treatment. The Netherlands Comprehensive Cancer Organization (IKNL) maintains the NCR by documenting all primary malignancies in the Netherlands, and has national coverage since 1989. Information on the vital status and date of death is obtained by annual linkage to the Municipal Personal Records Database, and was available up to 1 February 2016.

Tumour characteristics are reported according to the International Classification of Diseases for Oncology (ICD-O) and the Tumours Node Metastasis (TNM) classification guidelines.[14] The quality of the NCR is ensured by regular consistency checks, and completeness is estimated to be at least 95%.[15,16]

STATISTICAL ANALYSIS

Incidence rates were calculated for the most common diagnoses per 1,000,000 women per year. The number of inhabitants was obtained from Statistics Netherlands, and categorised per year and 5 year age category.[17] To obtain an estimation of the probability of survival of glandular vulvar tumours in the absence of other causes of death, we calculated net survival using the Porhar-Perme estimator, since this has been shown to be an unbiased estimator of net survival. Results of this estimator can be interpreted as survival in the hypothetical world where it is not possible to die from other causes. This allows the data to be compared with data from other countries.[18] Actuarial survival time was calculated from the date of diagnosis to the date of death or February 1st 2016, whichever came first. We analysed survival of two different groups: early stage defined as FIGO Stage I or II, and advanced stage defined as FIGO stages III and IV. Difference in survival rate between groups were analysed visualised with the 95% confidence intervals (CI). All analyses were performed using Microsoft Office Excel 2007, STATA software (STATA Corporation, 2002) and SPSS for Windows, version 20.

RESULTS

POPULATION

We identified 197 patients with a glandular malignancy of the vulva in the Netherlands between 2000 and 2015. Overall, 108 patients (54.8%) had a primary glandular vulvar malignancy and 89 (45.2%) had a vulvar malignancy secondary to another malignancy. Secondary glandular vulvar malignancies can be divided into expansive growth of an internal malignancy, or a metastasis of another malignancy, see figure 2. Additional clinical data for 71 (65.7%) of the patients with a primary tumour was available through the NCR.

See table 1 for an overview of the different primary glandular vulvar malignancies. Each year, an average of seven primary glandular malignan-

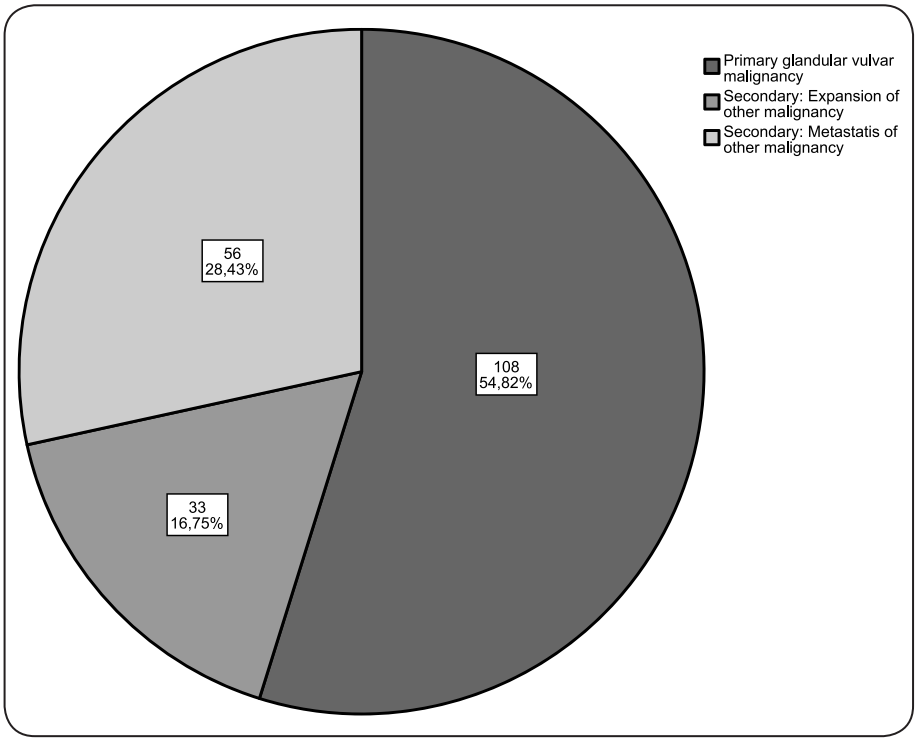

FIGURE 2 Distribution of diagnoses.

Primary malignancies		Secondary metastasis / recurrence		Secondary expansion	
Adenoid cystic carcinoma	11 (10%)	Adenocarcinoma NOS	2 (3.6%)	Anal malignancy	1 (3.0%)
Adenosquamous carcinoma	2 (1.8%)	Breast malignancy	2 (3.6%)	Endometrial malignancy	2 (6.0%)
(Micro-) invasive VPD	33 (30.1%)	Cervical malignancy	3 (5.3%)	PUIN	1 (3.0%)
MLG-tumour	5 (4.6%)	Coecal carcinoma	1 (1.8%)	Rectal carcinoma	17 (51.5%)
Mucinous adenocarcinoma	3 (2.8%)	Endometrial malignancy	9 (16.0%)	Signet ring cell carcinoma	1 (3.0%)
Neuroendocrine tumour	6 (5.5%)	Lung malignancy	3 (5.3%)	Transitional cell carcinoma	1 (3.0%)
Signet ring cell carcinoma	1 (0.9%)	Neuroendocrine carcinoma	1 (1.8%)	Urethral adenocarcinoma	3 (9.1%)
Transitional cell carcinoma	2 (1.8%)	Ovarian carcinoma	3 (5.3%)	Urothelial cell carcinoma	7 (21.2%)
Tumour of the skin appendages	21 (19.3%)	Signet ring cell carcinoma	2 (3.6%)		
Vulvar adenocarcinoma	24 (22.0%)	Rectal carcinoma	22 (39.3%)		
		Renal cell carcinoma	1 (1.8%)		
		Urothelial cell carcinoma	7 (12.5%)		
TOTAL	**108**		**56**		**33**

TABLE 1 Overview of specific diagnosis.

NOS: not otherwise specified, MLG: mammary-like-gland, PUIN: Pagetoid Urothelial Intraepithelial Neoplasia, VPD: vulvar Paget disease.

cies of the vulva were diagnosed. Furthermore, an average of six cases of secondary malignancies were diagnosed each year. These data represent a crude incidence rate of 0.47 to 1.3 per 1,000,000 women per year for primary, and 0.47 to 1.2 for secondary glandular vulvar malignancies. The overall median age at diagnosis was 69 years (range 26-97).

PRIMARY GLANDULAR VULVAR MALIGNANCIES

The median age of patients with a primary glandular malignancy was 71 (range 26-93) at time of diagnosis. FIGO stage was reported for 54 (50%): 39 patients (72.2%) had early stage disease, and 15 had advanced stage disease. Treatment was available for 80 patients (74.1%) with a primary glandular malignancy: 56 underwent surgery (51.9%), 17 (15.7%) received (neo-)adjuvant radiotherapy or chemotherapy besides surgery. Four pa-

	(Micro-) invasive VPD (n=33)	Adenocarcinoma NOS (n=26)	Skin appendages (n=21)	Adenoid cystic carcinoma (n=11)
Median age (range)	74 (50-89)	71.5 (43-89)	65 (26-92)	68 (30-78)
FIGO stage, n (%):				
- Early (I or II)	11 (33.3%)	4 (15.4%)	11 (52.4%)	6 (54.5%)
- Advanced (III or IV)	3 (9%)	4 (15.4%)	2 (9.5%)	2 (18.1%)
- Unknown	19 (57.7%)	18 (69.2%)	8 (38.1%)	3 (27.4%)
Primary treatment, n (%):				
- Surgery	21 (63.6%)	7 (26.9%)	14 (66.7%)	4 (36.4%)
- Surgery + RT/CT	4 (12.1%)	3 (11.5%)	0	4 (36.4%)
- RT	0	2 (7.7%)	0	0
- Other	0	1 (3.8%)	0	1 (9%)
- None	0	1 (3.8%)	0	0
- Unknown	8 (24.3%)	12 (46.3%)	7 (33.3%)	2 (18.2%)

TABLE 2 Clinical characteristics of most common primary glandular vulvar malignancies.
VPD: vulvar Paget disease, NOS: not otherwise specified.

tients (3.7%) received radiotherapy only, two patients (1.9%) received other, not specified, treatment, and one patient (0.9%) did not have any treatment.

Of the 108 patients with a primary vulvar malignancy, 10 (9.3%) had breast cancer before or after the diagnosis of their vulvar malignancy. Ten patients (9.3%) were diagnosed with intestinal malignancies before or after the diagnosis of their vulvar malignancy, and 3 (2.8%) with a urological malignancy. Twenty-two patients were diagnosed with other malignancies, the most frequent tumours were squamous cell carcinoma of the skin (5), endometrial cancer (5), and cervical cancer (3).

Table 2 reports the clinical characteristics of patients diagnosed with the most common primary glandular vulvar malignancies: (micro)invasive VPD, adenocarcinoma NOS (not otherwise specified), malignancy of the skin appendages, or adenoid cystic carcinoma. Invasive VPD was reported in 22 cases, and micro-invasive VPD in 11 cases. In two cases of invasive VPD characteristics of a vulvar squamous cell carcinoma were mentioned in the histology report. Twenty-six patients were diagnosed with an adenocarcinoma NOS, in four cases there was evidence of VPD or characteristics of a VSCC in the tumour. In six cases of adenocarcinoma NOS Bartholin's gland was reported to be the origin of the malignancy. Of the 21 reported malignancies of the skin appendages, five were specified as malignancies

of the sebaceous glands, 10 of the apocrine or eccrine sweat glands, and one of the hair follicle. In eight of the eleven adenoid cystic carcinomas, Bartholin's gland was specifically reported as the origin of the malignancy.

Survival

Five-year net survival for primary glandular vulvar malignancies was 68.5% (95%CI 50.9-80.7%). For early stage disease it was 69.9% (95%CI 44.1-85.5%), whereas it was 36.1% (95%CI 12.1-61.2%) for advanced stage disease, see figure 3. The FIGO stage of 54 (76%) of the patients was available. Most deaths (78.6%) occurred in the first two years after diagnosis.

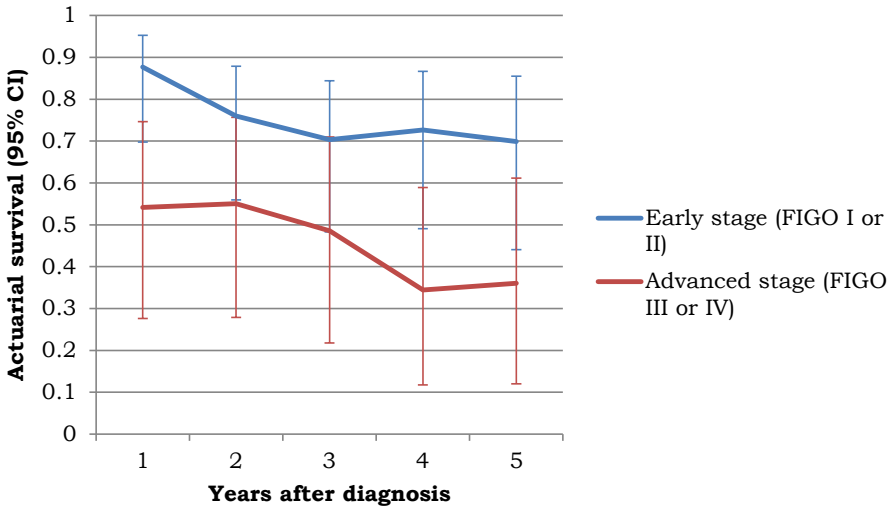

FIGURE 3 Five-year net survival, early versus advanced stage disease.

SECONDARY GLANDULAR VULVAR MALIGNANCIES

Tumour expansion to the vulva

Overall, 33 cases were reported as an expansion of another malignancy to the vulva. The median age of the patients at time of diagnosis was 68 years (range 44-97). The vulvar lesions expanded from an intestinal tumour in 19 patients, a urological malignancy in 12, and another gynaecological malignancy in 2 patients.

Most lesions were reported to be located at the labia: 14 cases. In eight patients the vulvar lesion was located at the perineum and in four at the peri-anal skin, these 12 lesions expanded from an intestinal malignancy. Vulvar expansion of urological malignancies was seen around the urethra in five of the 12 cases (41.7%) that originated from a urological malignancy.

In 13 cases (39.4%), the pathology report suggested the presence of an internal malignancy, or the internal malignancy was diagnosed by histological examination of the vulvar lesion.

In 16 patients (48.5%), the vulvar lesion was diagnosed either before or at the same time as the internal malignancy. In eight cases, the vulvar lesion was diagnosed within half a year (24.2%), and in two cases within a year (6.1%) after diagnosis of the internal malignancy. In four patients (12.1%) the vulvar lesion was diagnosed more than a year after the initial diagnoses, in these cases the vulvar lesion was an expansion of a recurrent urological or intestinal malignancy. Two cases (6.1%) were diagnosed more than two years after the initial diagnosis, and it can be debated whether the vulvar lesion should have been reported as a recurrence or metastasis rather than an expansion.

Tumour metastasis or recurrence to the vulva

Overall, 56 cases were reported as distant metastases of other malignancies to the vulva. The pathology reports lacked standardised terminology to be able to distinguish vulvar metastases from vulvar recurrences of other malignancies. We defined metastasis as a vulvar lesion diagnosed at the same time as the primary malignancy. Vulvar lesions that were diagnosed after the primary malignancy are reported as vulvar recurrences. The median age at time of diagnosis of the vulvar lesion was 66 (range 33-89). Most lesions originated from intestinal malignancies: 25 (44.6%), lesions originated from gynaecological malignancies in 15 (26.8%), and urological malignancies in eight (14.3%) patients. Other malignancies metastasised to, or recurred at the vulva in six (10.7%) patients, and from an unknown primary malignancy in two (3.6%) patients. Most vulvar metastases and recurrences originating from an intestinal malignancy were located at the perineum or peri-anal skin: 21 cases.

In six cases (10.7%) the vulvar lesion was diagnosed at the same time as the internal malignancy, or revealed the presence of another malignancy.

These cases were metastases from endometrial cancer (2), ovarian cancer (1), lung cancer (1), urothelial carcinoma (1), and of an unknown origin (1). Four vulvar recurrences (7.1%) were diagnosed within half a year, and eight (14.3%) within a year. The other 38 patients (67.9%) had a vulvar recurrence more than 12 months after the diagnosis of the primary tumour. The median time between the primary diagnosis and diagnosis of the vulvar recurrence for these patients was 39.5 months (range 14-298 months).

Twelve patients had one or more recurrences, besides the vulvar metastasis, of their internal malignancy. The internal malignancy metastasised only to the genital skin in 38 patients. Others had metastases to the genital skin and the pelvis (3), lung (2), pleura (2), peritoneum (1), retro peritoneum (1), or intestine (3).

DISCUSSION

To our knowledge, this is the first population-based study with national coverage to present the incidence and outcome of rare glandular vulvar malignancies over a significant time period. Most studies on rare diseases entail small single-institute case series or retrospective institutional reviews, lacking power to present accurate incidence rates. Based on national data of 15 years, the incidence of glandular vulvar malignancies can be estimated at 0.9 to 2.5 per 1,000,000 women per year. Primary glandular tumours are slightly more common than secondary glandular vulvar tumours: 54.8% versus 45.2%. The most common primary diagnoses were (micro)invasive VPD, adenocarcinoma NOS, malignancy of the skin appendages, and adenoid cystic carcinoma. Secondary glandular vulvar malignancies were most commonly caused by expansion, metastases, or recurrences of intestinal malignancies.

The five-year net survival of primary glandular vulvar malignancies was 68.5%, which is comparable to the five-year survival in patients with the more common VSCC in the Netherlands.[19-21] In VSCC, prognosis is influenced by stage of disease. We therefore also analysed survival data for FIGO stages ≤ II compared to FIGO stage > II. Five-year net survival in early stage disease was 69.9% and 36.1% in advanced stage, which is also comparable to survival rates in VSCC.[22] However, due to missing data of

the FIGO stage in a quarter of the patients and the small sample size, this analysis should be interpreted with caution. Sufficient data on the cause of death lacked; therefore we were unable to calculate the disease specific survival.

In secondary vulvar malignancies, the pathology report suggested or revealed the presence of another malignancy in about half of the cases. The importance of adequate pathological diagnosis is emphasised.

As glandular vulvar carcinomas are rare, expertise is necessary. The hodgepodge of diagnoses indicates the extreme difficulty of accurately diagnosing vulvar pathology. Because of the rarity of these diagnoses, pathologists seldomly encounter these kind of cases. Therefore it may be assumed that interobserver variability is extremely high. Many publications have paid attention to the difficulty of diagnosing glandular vulvar lesions, and notice many different entities are reported.[23,24] However, there is still little knowledge on the possible oncogenesis of all different lesions. The heterogeneity of glandular skin malignancies, especially of the skin adnexa and the so-called mammary like glands, is pointed out in several previous publications.[25,26] The clinical characteristics of these different diagnoses vary, and influence the treatment options and prognosis of the patient. Therefore, there is high need for a collaboration between pathologists specialised in dermato- and/or gynaecopathology. A standardised, and perhaps simplified, classification of these lesions, might prevent the development of an abundance of pathological diagnoses. Revision of the pathological sample as well as treatment should take place in a specialised gynaecologic oncology centre.

In conclusion, though the overall sample size of our study is relatively small, we present an overview of the population-based incidence of glandular vulvar malignancies. Because of the great variety in diagnoses, it is difficult to define any clinical implications at this moment. Vulvar glandular tumours are rare with an incidence of 0.9 to 2.5 per 1,000,000 women per year. About half of the cases are primary vulvar glandular malignancies, the other half is secondary to other internal malignancies. Survival of patients with a primary glandular vulvar malignancy is comparable to survival of patients with a VSCC. To improve health care for patients with these rare malignancies, more research into the oncogenesis and biological characteristics should be conducted. Revision of histological samples

by an expert (gynaeco-) pathologist should be standard care. Patients with these rare malignancies should be treated at specialised gynaecologic oncology centres.

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CHAPTER 3

PAGET'S DISEASE OF THE VULVA

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ABSTRACT

In this review, we provide an overview of the clinical aspects, histopathology, molecular genetics, and treatment options for Vulvar Paget's Disease (VPD), a rare skin disease, most commonly found in postmenopausal Caucasian women. The underlying cause of VPD remains not well understood. VPD is rarely associated with an underlying urogenital, gastrointestinal or vulvar carcinoma. In approximately 25% of the cases, VPD is invasive; in these cases, the prognosis is worse than in non-invasive cases. Recurrence rates in invasive VPD are high: 33% in cases with clear margins, and even higher when surgical margins are not clear, regardless of invasion. Historically, surgical excision has been the treatment of choice. Recent studies show that imiquimod cream may be an effective and safe alternative.

INTRODUCTION

Sir James Paget (1814–1899) was a surgeon with a special interest in pathology and physiology. In addition to his work as a surgeon for the English royal family and his practice in London, he wrote 'Lectures on Surgical Pathology', a work that established him as one of the founders of modern pathology along with Rudolf Virchow.[1] Sir James Paget is best known for his paper describing a nipple ulceration that was associated with an underlying breast carcinoma.[2] That paper reported a series of 15 cases of chronic skin eruption of the nipple and areola, a condition that is now referred to as mammary Paget disease. In 1889, shortly after James Paget's report, Crocker described the first case of Paget disease on extramammary skin, a case involving penoscrotal Paget disease.[3] In 1901, Paget disease of the vulva was described for the first time by a French dermatologist, William Dubreuilh.[4] Mammary and extramammary Paget disease are characterised by the presence of intraepithelial mucin-producing neoplastic cells known as Paget cells. However, the exact origin of these cells remains unclear.[5] This review presents an overview of the current literature on invasive and non-invasive VPD, including its epidemiology, clinical aspects, histopathology, treatment options, and survival.

METHODS

DATA SOURCES

Relevant publications were identified by a computer search in the PubMed database (date of last search April 9th 2015). We searched the database using combinations of the following terms: 'Paget's disease', 'Paget disease, extramammary' or 'Paget' in title and text. Subsequently, these terms were combined with 'vulva', 'vulvar', 'vulval', 'genitalia', 'perianal' or 'anogenital'. The button 'related articles' in PubMed and reference lists from selected articles were used to identify additional papers. Also, gynaecologic oncology, pathology and dermatology handbooks were used. Overall, 852 studies were found, 324 studies were not available in full-text and after assessment of all titles and abstracts, 298 were considered relevant. Main reasons for exclusion were: extramammary Paget disease in males, or locations other than

the vulva. The remaining publications on VPD were considered for inclusion in this review if they reported one of the topics mentioned in this review. Of these 298 studies 230 reported clinical or histopathological data of patients with VPD, including 79 case-reports and 53 case series with <10 patients. We prepared tables of clinical data based on studies that included 10 patients or more, to ensure the size of the tables remained manageable. Studies that reported on both male and female patients with EMPD were considered for inclusion. However, we only used data of female patients with EMPD located on the (ano)genital skin, unless there was no data on VPD available.

TERMINOLOGY

In this review, the terms mammary Paget disease (MPD) will be used for Paget disease of the breast, and extramammary Paget disease (EMPD) will be used for other locations, including the anogenital skin in males. The term vulvar Paget disease (VPD) will be used for a disease location in the genital area in females, including the perineal and perianal skin.

CLINICAL CHARACTERISTICS

EPIDEMIOLOGY

Only one study presents the occurrence of MPD versus EMPD: 90% of all cases of Paget disease are MPD and 10% of all cases of Paget disease are EMPD.[6] The overall European incidence of EMPD is 0.7 per 100,000 persons per year, and is slightly higher for women than men. A study in 16 European countries reported 871 cases of invasive EMPD in 13 years, including 231 male and 640 female patients.[7] Of the 640 female patients, disease was located at the vulva in 533 patients (83%), 3 cases were reported as 'Paget disease of the female genital tract, not otherwise specified' (0.5%), and 21 cases were reported as 'Paget disease of the anal canal and perianal skin' (3%). VPD is reported to occur most often in postmenopausal Caucasian women. In the Asian population, EMPD is seen mostly in males,[8] but there is considerable literature describing VPD in Asian women.[9-14] However, the exact incidence of VPD is unknown.

EMPD may be associated with underlying vulvar adenocarcinoma. Invasive VPD represents 1%–2% of all vulvar carcinomas.[15] A Dutch epidemiology study including 226 cases of EMPD over a 13-year period found that 178 (79%) cases were invasive and 48 (21%) non-invasive. When the data were categorised by location, invasive VPD (n=59) was reported twice as often as non-invasive VPD (n=32).[16] These data were taken from the Netherlands Cancer Registry, which may have resulted in underreporting of non-invasive disease. Most clinical studies used Wilkinson's classification, and report invasion in 16-19% of the cases, and a vulvar adenocarcinoma in 4-17% of all cases.[17-19] Moreover, VPD might not be recognised and thus be underreported if no skin biopsy is performed to confirm the diagnosis.

ORIGIN OF EXTRAMAMMARY PAGET DISEASE

The origin of EMPD has not been clarified, although there are currently three theories. The first suggests that EMPD has an intraepidermal origin from adnexal structures, like apocrine glands, multipotent stem cells in the epidermal basal layer or infundibular stem cells of the hair follicle.[15,20,21] EMPD is typically located in the hair-bearing skin of the axilla or genital area which supports the disease origins from adnexal structures. Although, VPD can also occur in the modified mucosa of the interlabial sulcus, or, in advanced cases, in the glycogenated mucosa without adnexal structures,[22-24] which supports another theory suggesting that Paget cells originate from mammary-like glands, which are located in the interlabial sulci.[25] A more recent theory is that Toker cells are precursor cells in MPD as well as in EMPD and VPD.[26,27] Toker cells have a single round nucleus and pale cytoplasm and are usually found in the nipple and areola.[28,29]

SIGNS AND SYMPTOMS

In the majority of patients, VPD causes symptoms such as irritation, itching, and burning. VPD can be asymptomatic in some patients: about 5%–15% of patients have no symptoms at the time of diagnosis.[30,31] Upon physical examination, VPD presents as an erythematous plaque with typical white scaling known as "cake-icing scaling". It is a clinical chameleon as it can present with a variety of colours and macular or plaque-like presentation. The plaque may be ulcerated and crusted with a papillomatous

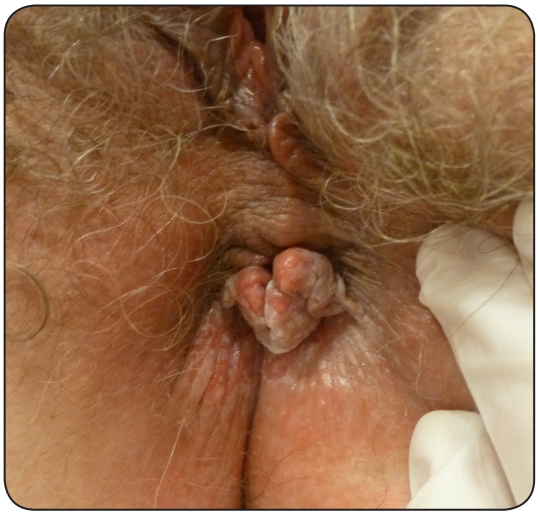

FIGURE 1 Peri-anal Paget's disease.

Poorly demarcerated erythematous perianal plaque with small erosions and white scaling.

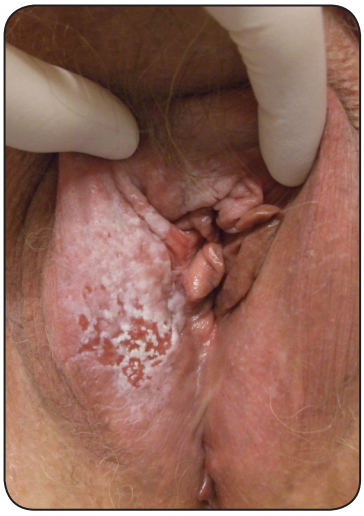

FIGURE 2 Vulvar Paget's disease.

White hyperkeratotic plaque with typical 'cake-icing' scaling with small superficial erosions on the right labium majus.

surface, as shown in Figures 1 and 2. The symptoms experienced by the patient are not always related to the extent of the visible lesion.

Studies have shown that symptoms are typically present for an average of almost 2 years before the diagnosis is made, due to both patient and doctor delays.[31-33] There is limited knowledge regarding the natural course of VPD, as most study reports describe patients who have undergone surgery. The 'Radiumhemmet series' of 28 women describes 4 patients with untreated VPD. Of these four women, two women were inoperable and two women refused surgery. Both inoperable patients died of other causes. One of the two women who refused surgery died of a squamous cell carcinoma (SCC) of the vagina, and the other patient had progressive VPD.[34]

DIAGNOSIS

In cases of suspected VPD, an accurate medical history should be taken, including a history of vulvovaginal complaints and gastrointestinal and urological symptoms. In addition, a full gynaecological examination

should be performed that includes vulvar, vaginal, and rectal examinations. All raised, pigmented, or otherwise suspicious lesions should be addressed appropriately by a thorough report, digital photography, and histological examination. Digital photographs can help monitoring the course of the disease. Invasive disease should be excluded, preferably by vulvar mapping, including multiple biopsies of the involved and surrounding uninvolved skin. In case of a small unifocal lesion, it can be considered to perform a single biopsy, in which the visible lesion is completely excised. The diagnosis is confirmed by the histological presence of Paget cells.

DIFFERENTIAL DIAGNOSIS

In addition to eczema and vulvovaginal candidiasis, the differential diagnosis of VPD consists of psoriasis, lichen simplex chronicus, lichen sclerosus, lichen planus, differentiated vulvar intraepithelial neoplasia (VIN) or usual VIN (synonymous: high grade squamous intraepithelial lesion, or HSIL), SCC, histiocytosis, condylomata acuminata and melanoma. In addition to these clinical diagnoses, a histological differential diagnosis of intraepithelial Pagetoid cells can include the following: melanoma (in situ), pagetoid spitz naevus, sebaceous carcinoma, clear cell papulosis, eccrine porocarcinoma, cutaneous T-cell lymphoma, and Langerhans cell microabscess.[33,35,36]

CLASSIFICATION

The World Health Organization (WHO) defines VPD as 'an intraepithelial neoplasm of epithelial origin expressing apocrine or eccrine glandular-like features and characterised by distinctive large cells with prominent cytoplasm, referred to as Paget cells'.[37] In the International Society for the Study of Vulvovaginal Disease (ISSVD) Terminology and Classification of Vulvar Dermatologic Disorders (2011), VPD is assigned to the morphological group 2, described as 'Red lesions, patches and plaques' and to subgroup B, 'Red patches and plaques (no epithelial disruption)'.[38]

In 2001, Wilkinson et al. proposed a histopathological classification of VPD that distinguishes primary/cutaneous VPD (type 1) from secondary/non-cutaneous VPD.[39,40]

Primary VPD (cutaneous)	Type 1a	Cutaneous vulvar non-invasive Paget's disease
	Type 1b	Cutaneous vulvar invasive disease: dermal invasion of Paget cells
	Type 1c	Cutaneous vulvar disease as a manifestation of an underlying vulvar adenocarcinoma
Secondary VPD (non-cutaneous)	Type 2	VPD originates from rectal or anal adenocarcinoma
	Type 3	VPD originates from urogenital neoplasia

TABLE 1 Classification of vulvar Paget disease by Wilkinson.[39,40]
EMPD: Extramammary Paget's disease.

As shown in table 1, secondary VPD originates from a malignancy of the gastrointestinal tract (type 2) or the urogenital tract (type 3).[41] In cases of pagetoid extension of an urothelial carcinoma, the term pagetoid urothelial intraepithelial neoplasia (PUIN) may be used. The current literature often refers to Wilkinson's classification, which is mainly based on the histopathological features of VPD.

However, this classification is no longer supported by the most recent WHO Classification of Tumours of Female Reproductive Organs (4th edition).[37] Moreover, it is a matter of debate whether non-cutaneous EMPD should be regarded as a form of VPD. Some consider it a direct extension ('pagetoid spread') of an intestinal or urothelial malignancy and use immunohistochemistry to distinguish primary from secondary VPD.[42] There are no accurate data regarding the distribution of types 2 and 3 versus type 1 VPD. Together with Wilkinson's classification, the subdivision of cutaneous and non-cutaneous EMPD is regularly used in current literature.

Cutaneous VPD (type 1) is further subdivided according to the presence or absence of dermal invasion: type 1a (intraepithelial disease), is reported to account for 75%–81% of all primary VPD cases, type 1b in 16%–19% and type 1c in 4%–17% of all cases.[17-19] In contrast, 60% or more of patients with MPD have an underlying breast malignancy.[43] It is hypothesised that Paget cells migrate from the epidermis to the dermis in type 1b and that in type 1c the Paget cells have migrated into the epidermis ("pagetoid spread") from an underlying vulvar adenocarcinoma.

Most studies do not report the definition of 'invasive VPD' or 'vulvar adenocarcinoma' that was used. Some described dermal invasion as 'invasion >1 mm'. Curtin et al. defined vulvar adenocarcinoma as invasive adenocarcinoma of sweat gland origin.[44] Lee et al. defined invasive VPD as

Paget disease with in situ involvement of the underlying sweat glands and defined vulvar adenocarcinoma as an invasive adnexal adenocarcinoma.[45] Because most studies lack clear definitions of invasive VPD and vulvar adenocarcinoma, we are unable to present an overview of the incidence distribution of non-invasive VPD, invasive VPD, and VPD with an underlying adenocarcinoma.

ASSOCIATED MALIGNANCIES

Patients diagnosed with EMPD are reported to have a higher risk of developing a second primary cancer, especially the first year after diagnosis (standardised incidence ratio of 1.39 with a 95% CI of 1.11 to1.73).[7] VPD is reported to be associated with other malignancies in 11%–54% of the cases, including malignancies of the breast, vagina, cervix, uterus, ovary, gallbladder, and liver.[19,45-47] However, some studies consider an underlying vulvar, rectal, or urothelial carcinoma to be an associated malignancy, whereas others reserve this term for distant malignancies. Data need to be interpreted with caution, since most studies reporting on associated malignancies had no adequate age matched control groups.

A total of 15 studies with 10 or more patients, reported intestinal or urological malignancies in patients with VPD.[14,31,34,48-59] Of the 456 included patients 10 (2.2%) had intestinal malignancies, and 18 (3.9%) had urological malignancies. Five patients were reported to have VPD and a simultaneous bladder carcinoma (1.1%) and 3 patients (0.7%) had an anal carcinoma that occurred simultaneously with VPD.[48,52,54,59] Twenty-six studies with 10 patients or more reported that 51 (3.2%) of the included 1598 patients had a history of breast cancer.[14,17-19,31,33,34,45-47,49-53,55,56,58-66] The time relative to the diagnosis of VPD varied greatly. Based on currently available literature it is not proven that there is a clinical relationship between VPD and breast cancer.

EXCLUDING OTHER MALIGNANCIES

There is no current consensus if women with VPD should be screened for associated malignancies, or which additional tests should be performed. Because of the presumed association of VPD with locoregional and distant

malignancies, guidelines advise excluding the presence of other malignancies, although the proposed policies vary. The Royal College of Obstetricians and Gynaecologists states that "the gastrointestinal and urinary tracts and the breasts should be checked",[67] and that "women with VPD should have prolonged follow-up in a multidisciplinary vulvar clinic or by a gynaecological oncologist".[68] The U.S. Department of Health & Human Services advises, "evaluate the breasts, genitourinary, and gastrointestinal tract (level C evidence: consensus and expert opinion)".[69]

HISTOPATHOLOGICAL DIAGNOSIS

HISTOLOGICAL CHARACTERISTICS

Histologically, VPD is characterised by the presence of large oval or polyhedral intraepithelial cells that have pale cytoplasm and large nuclei with prominent nucleoli, these cells are the so-called Paget cells. Paget cells can be visualised using haematoxylin and eosin (HE) staining. They are arranged either singly or in clusters throughout the epithelium to a variable extent, and may form a lumen or gland-like structures. Sometimes reactive changes are seen in the surrounding epithelial surface, such as acanthosis, papillomatosis, and hyperkeratosis; these changes in themselves are not sufficient for diagnosis. A lichenoid inflammatory infiltrate can be seen in the underlying papillary dermis. The scattered Paget cells are diagnostic, but they are interspersed within the normal epithelium and can be difficult to detect at times (figures 3 and 4).

In the pathologic assessment of VPD, it is important to exclude invasive growth. This is challenging because it is not uncommon for VPD to extend into the adnexal structures. An additional problem is the frequent presence of a dense infiltrate that can obscure the epithelial/stromal interface. Invasion is characterised by the presence of dyscohesive neoplastic Paget cells infiltrating the underlying dermis or submucosa (figures 5 and 6). In case of invasion, the pathologist is required to report the depth of invasion, as this has proven prognostic significance and determines the type of treatment.[70,71]

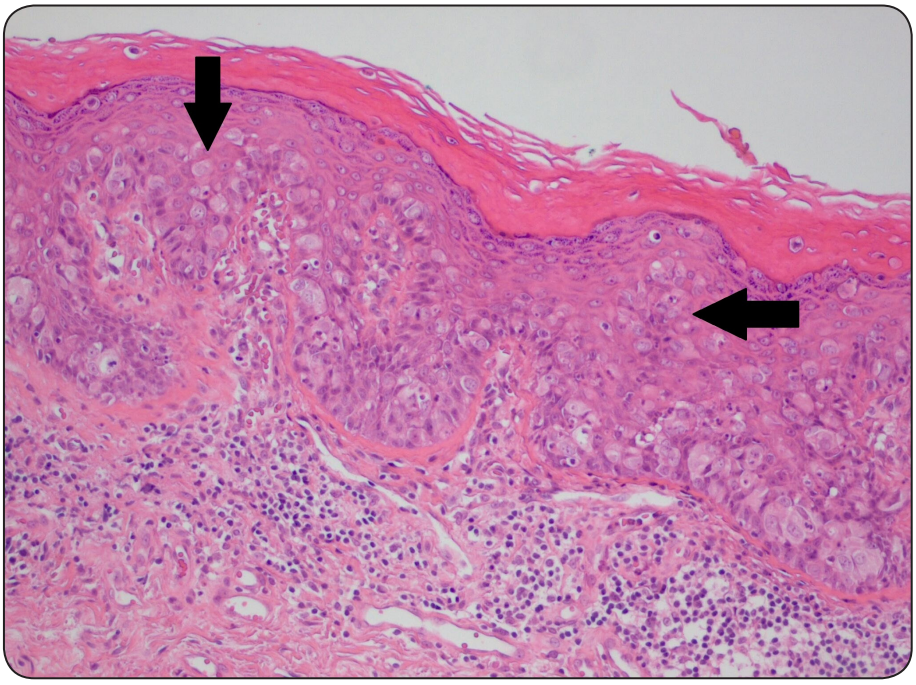

FIGURE 3 Non-invasive vulvar Paget's disease (HE stain, 100x).

Solitary cells and large cell nests are present in the lower parts of the epidermis (arrows). The Paget cells have pale cytoplasm and large rounded atypical nuclei. There is no invasive growth.

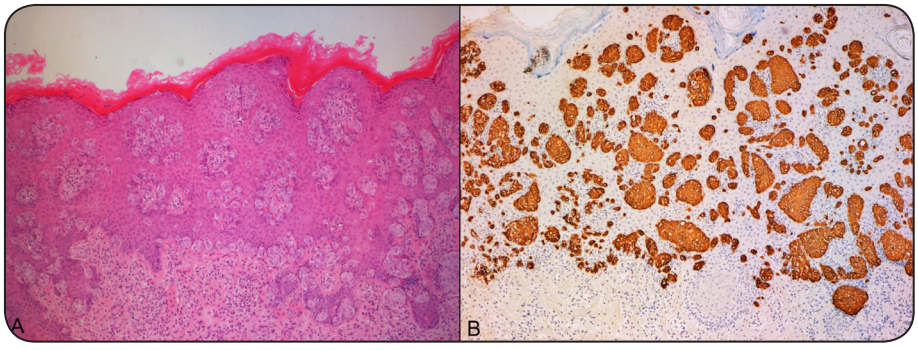

FIGURE 4 **A:** Non-invasive vulvar Paget's disease (HE, 100x), **B:** CK7 stained cells (100x).

Large pale solitary cells and cell nests are present throughout the thickened hyperplastic epidermis. With CK7 it is clearly depicted that there is no invasive growth in the underlying vulvar stroma.

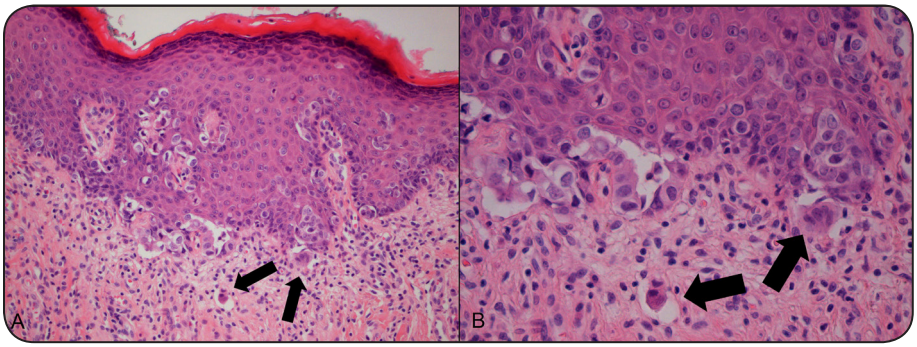

FIGURE 5 **A:** Micro-invasive vulvar Paget's disease (HE, 100x) **B:** Detail (HE, 200x).

Some large atypical pale cells and cell nests are present in the basal layers of the vulvar epithelium. At magnification it is shown that two small cell clusters have invaded the underlying vulvar stroma (arrows). The invasive growth is less than 1 mm.

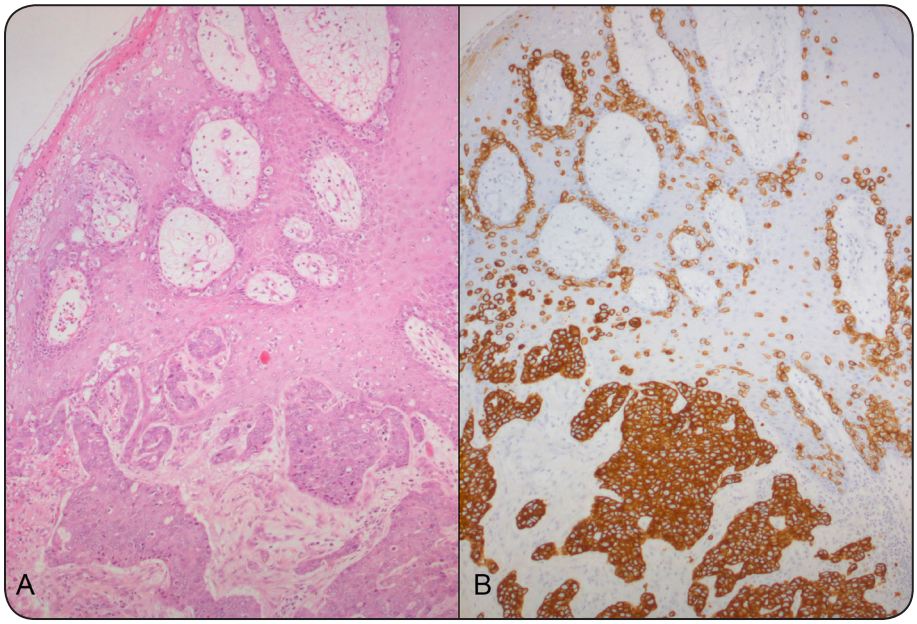

FIGURE 6 **A:** Invasive Paget's disease with underlying anogenital invasive adenocarcinoma (HE, 100x), **B:** CK7 stained cells (100x).

At left a poorly differentiated adenocarcinoma is present directly under the hyperplastic thickened vulvar epithelium. With CK7 immunostaining it is depicted that tumour cells spread alongside the basal parts and rete ridges of the vulvar epithelium.

IMMUNOHISTOCHEMISTRY

A number of immunohistochemical stains can be used to distinguish cutaneous VPD from its histological mimics. Paget cells can be highlighted by PAS reaction and/or by immunohistochemistry, as they are usually positive for cytokeratin (CK) 7 and carcinoembryonic antigen (CEA).[36,72,73] They do not express markers of squamous cell differentiation, such as p63 and p40, and these markers can therefore be used to exclude squamous intraepithelial lesions such as uVIN, also known as HPV-induced HSIL with a pagetoid growth.[74,75] However, VPD may over express p16 and mimic uVIN (or: HPV-induced H-SIL), which strongly over express p16 as well.[76,77] In addition, Paget cells do not express melanocyte markers, such as Mel-A, HMB45 or S100, and this can help distinguish VPD from (in situ) melanoma. Paget cells may express androgen receptors, but in general are negative for estrogen and progesterone receptors.[78-81]

Immunohistochemistry can also be helpful in determining the primary location of an underlying adenocarcinoma. For example, pagetoid extension of urothelial cancer will likely express CK20, uroplakin-III, and GATA-3,[36,81] whereas CK20, CDx2, and MUC2 positivity might indicate an underlying anorectal adenocarcinoma.[36,73,82] It is therefore recommended that a combination of these markers be used in cases in which pagetoid extension from an underlying adenocarcinoma is suspected.[83] Because of the rarity of a co-existent intestinal and/or urological we suggest this may be performed in cases in which the patient has a clinical suspicion of an underlying intestinal and/or urological tumour. See table 2 for an overview of the expression patterns of common markers.

TUMOUR MICROENVIRONMENT

Studies of the local tumour microenvironment of VPD are limited, and have not investigated the types of cells present in the immune infiltrate. Only specific markers have been investigated, such as regulatory T-cells (Tregs) that suppress effector T-cells. Tregs express Foxp3, CD4, and CD25 (or IL-2-Rα), and high numbers of Tregs are associated with adverse clinical outcomes in several types of cancer.[84-86] A study of Tregs in 44 cases of VPD showed that Tregs are frequently found at the epidermal-dermal junction whereas the surrounding healthy skin is negative for Tregs.[59] That study

	CEA	p63	CK7	CK20	Uro-III	GATA-3	CDx2	MUC2	GCDFP-15
Primary cutaneous VPD (type 1)	+	-	+	-	-	-	-	-	+
Secondary to intestinal malignancy (type 2)	+	-	-	+	-	-	+	+	-
Secondary to urological malignancy (type 3)	+	+	+/-	+	+	+	-	-	-

TABLE 2 Overview of common expression patterns in vulvar Paget disease.

CEA: carcinoembryonic antigen, CK: cytokeratin, Uro-III: uroplakin-III, MUC2: mucin 2, GCDFP-15: Gross cystic disease fluid protein.

also described a correlation between the number of FOXP3+ Tregs and positive surgical margins and recurrence.[59] The percentage of Tregs is significantly higher in non-invasive EMPD than in invasive EMPD, while CD163+ macrophages are detected more frequently in invasive EMPD.[84]

GENETIC PROFILE

Given the rarity of VPD, there are limited data on genetic alterations in VPD, and this field is therefore largely unexplored. Her2/Neu amplification is probably the most studied genetic alteration in VPD, likely because of its therapeutic potential and its association with mammary Paget disease. HER-2/Neu overexpression is found in 70%–100% of MPD cases.[87,88] However, the reported frequency of Her2/Neu amplification in EMPD varies significantly.[89,90] Small series and case reports have investigated various genetic abnormalities: mutations in the PIK3CA (Phosphatidylinositol-4,5-Bisphosphate 3-Kinase, Catalytic Subunit Alpha) gene, divergent DLC1 (Deleted in Liver Cancer 1) methylation, single nuclear polymorphism in the XRCC1 (X-ray repair cross-complementing protein 1) gene, chromosome 7 gains and X chromosome losses have been described.[91-93] However, the clinical significance of these abnormalities remains to be determined.

TREATMENT

Traditionally, the treatment of choice for VPD is surgical excision.

SURGERY

Surgical treatment of VPD consists mainly of wide local excision, with or without inguinofemoral lymph node dissection.[31,33,94] A inguinofemoral lymphadenectomy is indicated in cases showing invasive VPD (>1 mm). The main clinical challenge of wide local excision is obtaining clear surgical margins; it is unclear what the surgical margin should be in VPD, as Paget disease spreads microscopically throughout the epidermis. This makes it difficult to determine the complete extent and spread of the lesion.[95] Paget cells may be difficult to recognise on frozen sections: frozen section evaluation in EMPD is reported to have a false negative rate ranging from 10.4% to 13.2%.[96,97] The relationship between surgical margin status and recurrence rates remains unclear.

In Mohs microsurgery (MMS) the vulvar lesion is excised through the epidermis and dermis, and 100% of the peripheral margins are examined immediately.[98] Excision is repeated, enlarging the circumference each time, until the margins are clear. MMS was reported to be used for VPD for the first time in 1991,[99] after 90 years of surgical treatment consisting of wide local excision or (hemi-)vulvectomy.

For large lesions, different plastic surgery methods for reconstruction of large vulvovaginal defects have been reported and include local fasciocutaneous flaps, gluteal fold flaps, pudendal thigh flaps, and gracilis myocutaneous flaps.[100-103]

Sentinel lymph node

There are no studies on the accuracy of sentinel lymph node (SLN) biopsies in invasive VPD. The current literature describes SLN only sporadically in cases with microinvasive VPD,[104] and in cases with suspected lymph node metastases.[105,106] However, based on current knowledge of surgery for vulvar SCC, microinvasion is not an indication for SLN, and uni- or bilateral inguinofemoral lymphadenectomy is indicated in cases of invasive VPD (>1 mm) with clinically suspected lymph node involvement.

Complications of surgery

Vulvar surgery is associated with significant morbidity. Local vulvar complications consist mainly of infection, hematomas, and wound breakdown, with incidence rates for wound breakdown ranging from 9%–45%.[107-109] According to quality of life assessments, extensive surgery, such as radical vulvectomy, tends to cause more discomfort than wide local excision.[110] The incidence of complications after inguinofemoral lymphadenectomy ranges from 17.5%–84%. Early complications (<1 month after surgery) are mainly lymphocyst formation, wound breakdown, and infection of the wound. Late complications (≥1 month after surgery) include lymphedema, leg pain, and erysipelas.[109,111-113]

Psychosexual complications

There are no studies concerning the psychosexual effects of vulvar surgery in VPD. One study showed that women with a history of vulvar excision for VIN more frequently reported sexual function impairment and worse quality of life than healthy women.[114] However, two other studies showed no significant differences in quality of life and sexual function between patients surgically treated for VIN, Bowen's disease, or VPD and a healthy population.[115,116] In patients who underwent vulvar surgery for vulvar carcinoma or carcinoma in situ, there was no correlation between the extent of the surgery, the type of vulvectomy, and sexual dysfunction severity. Elderly women were more likely to stop sexual activity after such surgery, and women with high depression scores more often had sexual aversion disorder, increases in body image disturbance, and sexual dysfunction.[117] Following vulvar surgery for VIN, older women had lower quality of life and lower sexual function according to the Female Sexual Function Index questionnaire compared to younger women.[114]

NON-SURGICAL TREATMENT

Surgery for VPD is not always possible or desirable due to the location or size of the lesion or due to patient factors or preferences. In addition, recurrence rates after surgical treatment are high and morbidity is impressive.[30,33] There is thus a pressing need for alternative treatment options for VPD.

Topical imiquimod cream

Imiquimod is registered for the treatment of condylomata acuminata, superficial BCCs, and actinic keratosis. It has also shown to be effective in the off label treatment of usual VIN in randomised controlled trials.[118,119] Imiquimod, a toll-like receptor (TLR) 7 agonist, is an immune response modifier. It triggers immune cells to produce cytokines, including interferon-α, interleukin 1, 6, and 8, and TNF-α.[120] It also indirectly stimulates the production of pro-inflammatory T helper type 1 cytokines. In the skin, imiquimod activates Langerhans cells, which enhance antigen presentation to T cells.

A few observational studies and case reports have shown imiquimod to be effective for the treatment of perineal, scrotal, and inguinal Paget disease.[121] Topical imiquimod cream for the treatment of recurrent VPD was first described by Wang et al. in 2003,[122] and subsequently around 25 retrospective case series were published on the use of topical imiquimod cream in non-invasive VPD. The treatment schedules differed widely in these studies, ranging from daily application to application three times a week. The duration of the treatment ranged from 5 to 26 weeks, and follow-up ranged from 2 to 55 months. In these studies, a total of 64 women with VPD were treated with imiquimod cream; 56 (88%) had an objective clinical response, 43 (67%) had a complete response, and 13 (21%) had a partial response. Only 8 women were reported to have residual disease after treatment.[57,123-143] Figure 7 shows a patient from our clinic who has obtained a complete response with topical 5% imiquimod treatment.

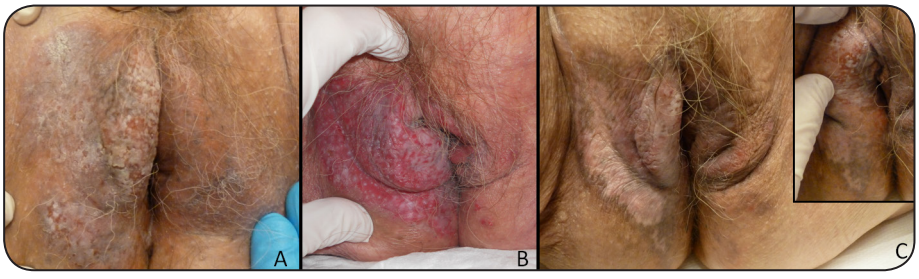

FIGURE 7 Extended vulvar Paget's disease before (A), during (B), after (C) treatment with topical 5% imiquimod cream.

A recent observational study examined the effectiveness of imiquimod cream in 10 patients with non-invasive VPD.[144] The patients were treated for 5 to 7 months. Nine patients had a complete response, and one patient had a partial response. Local skin reactions like pain and ulceration were reported in this cohort without systemic reaction. We found 16 publications reporting adverse events in 59 patients.[57,125-127,129-134,136-138] A local adverse reaction, such as pain, ulceration, or inflammation, was reported in 16 patients.[57,122,129-134,136,137] Five patients reduced the application frequency or stopped for one or more weeks because of these adverse reactions.[122,126,143] In most cases, the severity of adverse effects as reported by the patient was reduced after 4 or 5 weeks of treatment. The recurrence rate after treatment with topical imiquimod cream for VPD is unknown. Overall imiquimod seems to be effective, but treatment schedules differ greatly between the studies, and there may be a high risk of publication bias.

Chemotherapy

Chemotherapy for metastatic EMPD has been reported in one small study (n=7).[145] The clinical response of 4 patients after FECOM therapy (combination therapy with 5-fluorouracil, epirubicin, carboplatin, vincristine, and mitomycin C) at 4-week intervals showed a partial response according to RECIST (Response Evaluation Criteria in Solid Tumors). One case report describes a patient with untreated non-invasive VPD who was treated with radiotherapy and FEC100 (fluorouracil, epirubicin, and cyclophosphamide) followed by 3 courses of docetaxel for a lobular breast carcinoma. The VPD clinically disappeared after chemotherapy, but recurred 2 years later.[146] Another case report describes the clinical response and regression of lymph node metastases in a patient with Her2-Neu-overexpressing VPD after treatment with trastuzumab in combination with paclitaxel.[147]

One study describes the topical use of bleomycin for 2 weeks with a resting period of 4 to 6 weeks in 7 patients with non-invasive VPD. Four patients had a complete response: three after 2 two-week courses and one patient needed 4 courses but had a recurrence after 30 months The other three patients could not be evaluated: one patient died of intercurrent disease, in one patient therapy was stopped due to adverse events, and one patient refused further therapy after a partial response.[148]

Other topical treatment

Topical corticosteroids are not proven to be effective for VPD. The current literature includes case reports of women who were treated with topical corticosteroids without success for an irritative vulvar skin lesion. Treatment failure led to further examination of the initially misdiagnosed lesion and resulted in a VPD diagnosis.[130,131,149,150] Based on clinical experience, topical application of lidocaine (as a cream or ointment) can relieve pain, and emollients with zinc oxide can prevent secondary infection of the lesion. There is no literature available on the symptomatic treatment of VPD.

Radiotherapy

Radiotherapy has been used as a primary treatment option for patients with invasive and non-invasive VDP who were not eligible for surgery or who refused surgery, as a treatment option for patients with recurrence after surgery, and as adjuvant postoperative therapy.[151,152] Son et al. described a case series of 3 patients with VPD that included 1 patient with invasive VPD.[152] All 3 patients had a clinical complete response. Karam et al. performed a large retrospective study of 1,439 patients with EMPD, 781 of whom had VPD. In total, 92 patients received radiotherapy, but these were not analysed by sex or by lesion location.[151] The invasion depth was not reported. Adjuvant postoperative radiotherapy was given to 51 patients (55.4%), and 40 patients received radiotherapy as primary treatment. A dose of 40 to 50 Gy is recommended for intraepithelial EMPD and 55 to 65 Gy is recommended for invasive EMPD or for an associated adenocarcinoma.[153-155] Long-term follow-up shows that recurrence rates after radiotherapy are less than 20%.[152] Lower doses may be less effective and may thus have a higher recurrence rate.[156] One case report described the use of high-dose-rate superficial brachytherapy, also known as plesiotherapy, for VPD. A total dose of 54 Gy was administered in 3 weekly fractions for 4 weeks. The patient had a complete response and was free of disease during 18 months of follow-up.[157]

Photodynamic therapy

Photodynamic therapy (PDT), also known as photochemotherapy, is mainly prescribed by dermatologists.[158] A photosensitizer such as 5-aminolevulinic acid (5-ALA) or methyl 5-amnoplevilunate (MAL) is applied to the lesion. After an incubation period of several hours, the photosensitizer is washed off and the lesion is irradiated with visible red light, destroying prolifer-

ating tumour cells that have absorbed the photosensitizer.[159,160] PDT has shown clinical efficacy for treating superficial cutaneous (pre-) malignancies such as superficial BCCs, actinic keratosis, and usual VIN.[161] PDT can be painful and can cause inflammation.

Several case reports have evaluated PDT for VPD. Three case reports of three patients all reported complete responses to PDT therapy.[159,162,163] The reports described using topical antibiotics, tretinoin 0.05%, and vitamin E ointment at the lesion site. The patients received 2 to 3 sessions of 5-ALA or MAL PDT, and all were free of disease for the follow-up periods of 3 to 6 months.[162,163] As side effects, one case reported minimal pain and mild erythema for several days after a session. She had a partial response and refused surgical treatment. During the 3-year follow-up period, there was no invasion of the lesion.[159] Raspagliesi et al. reported a case series of 7 patients who were treated with PDT; 4 had a complete response, 1 had a partial response, and 2 patients had stable disease after 1 to 5 months of follow-up. The patients, who were premedicated with benzodiazepine and NSAIDs, received 3 sessions of MAL PDT with a 3-week intervals. Two patients reported having pain for several days after the treatment.[160]

Laser therapy

CO_2 laser therapy has been used as a treatment for recurrent VPD after surgery. One case report described the use of 30W CO_2 laser therapy for recurrence after multiple extended surgeries. Invasion was not reported. After the laser treatment, there was no recurrence during the 12 months of follow-up, and the clinical response was satisfactory.[164] Laser therapy has also been used in combination with PDT and surgery. One case series describes additional 10W CO_2 laser vaporization after surgery. One patient had a vulvar adenocarcinoma and was primarily treated with a radical vulvectomy. The other patients underwent wide local excision prior to laser therapy. All patients were free of disease 4 months to 4.5 years after treatment.[165] A trial that included 3 patients with recurrent VPD after surgery subsequently treated the patients with CO_2 laser therapy and 5-ALA PDT. Invasion was not reported. All patients showed complete response during 12 months of follow-up, although 1 died due to other causes.[166]

RECURRENCE RATES

Reported local recurrence rates after surgical treatment of VPD vary from 34%–56%.[30,33] Recurrences have also been reported in reconstructive skin grafts and flaps.[167-169]

Table 3 presents an overview of the local recurrence rates of invasive and non-invasive VPD in surgically treated patients.

Author	Number of patients with local recurrence in non-invasive VPD	Number of patients with local recurrence in invasive VPD
Black 2007[55]	17/28 (60.7%)	0
Cai 2013[61]	7/22 (31.8%)	1/5 (20%)
Crawford 1999[49]	5/10 (50%)	3/10 (30%)
Creasman 1975[170]	0/7 (1†)	0/5 (3†)
Curtin 1990[44]	6/28 (21.4%)	2/5* (2†) (40%)
De Magnis 2013[31]	13/30 (43.3%)	2/4 (50%)
Fanning 1999[33]	30/84 (35.7%)	3/12 (25%)
Feuer 1990[46]	7/14 (50%)	2/3 (1†) (66.7%)
Goldblum 1997[62]	4/13 (30.8%)	1/6 (16.7%)
Gregori 1978[63]	2/13 (15.4%)	0
Jones 1979[171]	7/32 (21.9%)	4/9 (44.4%)
Lee 1977[45]	1/5 (20%)	2/7 (28.7%)
Liu 2014[14]	7/23 (30.4%)	4/8 (50%)
Mendivil 2012[18]	8/13 (61.5%)	1/3 (33.3%)
Scheistrøen 1997[172]	6/15 (40%)	2/4 (50%)
Shaco-Levy 2010[173,174]	15/46 (32.6%)	3/10 (30%)
Tebes 2002[53]	4/14 (28.6%)	2/6 (33.3%)
Zollo 2000[66]	5/15 (33.3%)	2/6 (33.3%)
TOTAL	**144/402 (35.8%)**	**34/103 (33.0%)**

TABLE 3 Local recurrences after surgical treatment.

*VPD: vulvar Paget disease. *1 patient had metastases in LN. †Number of patients that died of Paget disease.*

Some studies report high recurrence rates regardless of the surgical margin status,[53,55,175] whereas others found a significant correlation between negative margins and lower recurrence rates.[52] Table 4 presents an overview of studies that looked at associations between recurrence rates and surgical margin status.

Gunn et al. performed a topographical study of VPD in 1980.[95] Four specimens from affected vulvas were examined, and it was found that the

Author	Number of patients with recurrence in patients with positive surgical margins	Number of patients with recurrence in patients with negative surgical margins
Black 2007[55]	14/20 (70%)	3/8 (37.5%)
Cai 2013*[61]	N/A (38.5%)	N/A (18.8%)
Crawford 1999[49]	7/13 (53.8%)	1/7 (14.3%)
Curtin 1990[44]	2/6 (33.3%)	3/11 (27.2%)
De Magnis 2013[31]	10/15 (66.7%)	5/19 (26.3%)
Lee 1977[45]	0/1 (0%)	3/11 (27.3%)
Liu 2014[14]	9/15 (60%)	11/31 (35.5%)
Mendivil 2012[18]	5/11 (45.5%)	4/5 (80%)
Scheistrøen 1997[172]	1/8 (12.5%)	0/6 (0%)
Shaco-Levy 2010[173,174]	20/50 (40%)	3/17 (17.6%)
Tebes 2002[53]	6/16 (37.5%)	2/7 (28.6%)
Zollo 2000[66]	1/6 (16.7%)	5/12 (41.7%)
TOTAL†	**75/161 (46.6%)**	**40/134 (29.8%)**

TABLE 4 Local recurrences in relation to margin status after surgical treatment of vulvar Paget disease.

*N/A: not available. *Percentages available only. †Excluding Cai 2013.*

histological presence of disease extended far beyond the visible lesion. This may contribute to the difficulties in obtaining a clear margin. Multifocality of the disease may also play a part in recognising the extend of the disease.

The type of surgery may also influence the recurrence rate. It is well known that Paget cells are present in hair follicles and bulbs, which can be found deeper into the adipose tissue of the mons pubis and labia minora. A skinning vulvectomy may leave diseased hair bulbs in the patient, and it is known that laser ablation does not reach this deep as well.

There are no data available on the recurrence rates according to type of VPD, invasion depth or location of the disease. Topical treatment of VPD is relatively new. Therefore, publications on this topic do not report sufficient follow up for recurrence rates. Other treatment modalities have been described in such small sample sizes, that we are unable to explore this topic.

PROGNOSIS AND FOLLOW UP

The overall 5-year survival rate for EMPD in male and female patients is 75%–91%.[7,70,71] Patients with invasive Paget disease or with an associated malignancy seem to have significantly shorter survival. As a comparison, the overall 5-year survival rate for vulvar cancer is reported to be 70%.[176] Hatta et al. reported on 76 male and female patients with EMPD and found that 5-year survival was 100% in the 43 patients with intraepithelial disease and 88.2% in the patients with microinvasion to the papillary dermis. Eleven patients with deep invasion did not survive for 5 years.[70] Ito et al. reported 30 patients with EMPD and showed that survival was 100% for intraepithelial disease and dermal invasion ≤1 mm. The 5-year survival rate for patients with dermal invasion >1 mm was 15%.[71] Table 5 presents an overview of studies that report the number of patients who died from EMPD based on invasion depth.

Based on the currently available literature, the risk of developing invasive VPD disease or metastases after treatment for non-invasive VPD is

Author	**Death of disease in patients with non-invasive EMPD**	**Death of disease in patients with microinvasive EMPD (≤1 mm)**	**Death of disease in patients with invasive EMPD (>1) and/or malignancy**
Crawford 1999[49]	0/11	0/7	1/3 (33.3%)
Creasman 1975[170]	1/10 (10%)	N/A	3/5 (60.0%)
Curtin 1990[44]	0/28	N/A	2/5 (40.0%)
De Magnis 2013[31]	1/29 (3.4%)†	0/3	0/2#
Feuer 1990[46]	0/14	NA	1/3 (33.3%)
Ito 2012[71]	0/18	0/9	5/8 (62.5%)
Jones 2011[51]	0/38	N/A	2/5 (40.0%)
Mendivil 2012[18]	0/15	N/A	0/1
Niikura 2006[17]	0/18	N/A	0/4
Shaco-Levy 2010[173,174]	0/46	N/A	1/10 (10%)
Zollo 2000[66]	0/19	N/A	1/9 (11.1%)
TOTAL	**2/246 (4,9%)**	**0/19**	**16/55 (29.1%)**

Table 5: Overview of literature reporting patients who died of Extramammary Paget's disease.

When studies reported cases with invasion ≤1 mm, they are reported separately in the 'micro-invasion' column. Otherwise we assumed cases with invasion ≤1 mm are classified as 'non-invasive'. EMPD: Extramammary Paget disease. N/A: not available. †Patient that died of disease was also diagnosed with vulvar squamous cell carcinoma. #Both patients had non-invasive VPD with a vulvar adenocarcinoma.

Author	N	Number of patients with invasive VPD after treatment for initial non-invasive VPD	Number of patients with metastases after treatment for initial non-invasive VPD
Baehrendtz 1994[34]	28	3	0
Black 2007[55]	28	1	0
Fanning 1999[33]	88	1	2
Goldblum 1997[62]	19*	1	0
Jones 1979[171]	39	0	2
TOTAL	**202**	**6 (2.9%)**	**4 (1.9%)**

TABLE 6 Overview of number of patients with invasive vulvar Paget disease or metastases after treatment for initial non-invasive vulvar Paget disease.

*N: Total number of patients with non-invasive vulvar Paget disease, VPD: vulvar Paget disease. *Includes 5 cases of micro-invasion (< 1 mm), the patient with an invasive recurrence did not have micro-invasive disease at time of first diagnosis.*

very low (table 6). Fanning et al. describe 2 patients with extra-vulvar adenocarcinoma metastases after initial non-invasive VPD.[33] Jones et al. describe 2 patients with metastases, but do not specify the location and histological type[171]. Lesions with nodules, elevated CEA, deeper invasion depth, and lymph node metastases all correlate with a shorter survival time and patients with intraepidermal disease or microinvasion had significantly longer survival time than patients with invasive EMPD.[70,71]

CONCLUSION AND DISCUSSION

VPD is a rare skin disease that typically occurs in elderly women. Its origin remains unclear. Wilkinson's classification is used most frequently to distinguish primary or cutaneous VPD from secondary VPD; the latter is associated with an intestinal or urological malignancy. This separation, however, has been omitted in the 2014 WHO classification.[37]

The majority of patients with VPD have non-invasive cutaneous disease. About 20% of the cases involve invasive VPD, and 5.7% are associated with an underlying vulvar adenocarcinoma. There is no consensus on how to distinguish between invasive VPD, VPD with an underlying associated intestinal/urological malignancy, or vulvar adenocarcinoma. Intestinal and urological malignancies might spread to the vulva in a pagetoid pattern, or Paget cells might cause an underlying malignancy.

VPD is reported to be associated with other malignancies, in our review we found that 3.2% of patients with VPD were reported to have been diagnosed with breast cancer, 2.2% with an intestinal malignancy, and 3.9% with an urological malignancy. Based on these low figures, we want to raise the question on the association with breast, intestinal, and urological malignancies, as there are no studies with age matched control groups. We especially question the association with concurrent intestinal and urological malignancies, as they are reported in 1.1% of VPD patients and in 0.7% respectively. Therefore screening for all associated malignancies might be superfluous. However, as 12% of women will develop breast cancer during their lifetime, we do think that all women with VPD should undergo mammography, which is an easy and affordable test.[177] More research on this topic should be conducted to support a screening protocol.

A diagnosis of VPD is confirmed by the presence of Paget cells on histological examination. Immunohistochemical markers can be used to differentiate between cutaneous and non-cutaneous VPD, and may serve as a decision aid in the work-up of patients with VPD. Invasive disease should be excluded by accurate histological examination or by vulvar mapping. The risk of progression into invasive VPD or to metastasis after treatment for non-invasive VPD is low (2.8% and 1.9%, respectively), and the prognosis of non-invasive VPD is excellent. We therefore suggest that aggressive surgical treatment can be avoided in cases of non-invasive VPD. There seems to be a place for topical treatment, and sometimes more symptomatic treatment could be considered. Because of the lack of literature on SLN in VPD, there is no place for SLN procedures in VPD. In case of invasive VPD >1 mm, standard treatment of the groin area should consist of uni- or bilateral inguinofemoral lymphadenectomy.

The risk of recurrence after standard surgical treatment is high i.e. about 35% for non-invasive VPD. The use of topical imiquimod cream for the treatment of VPD shows promising results in small case series, but more research is needed before definite conclusions can be drawn. One ongoing study on this topic is registered at clinicaltrials.gov (NCT00504023); which is currently not recruiting. Our group has started an observational trial in 20 patients with non-invasive VPD and is currently recruiting patients (NCT02385188).

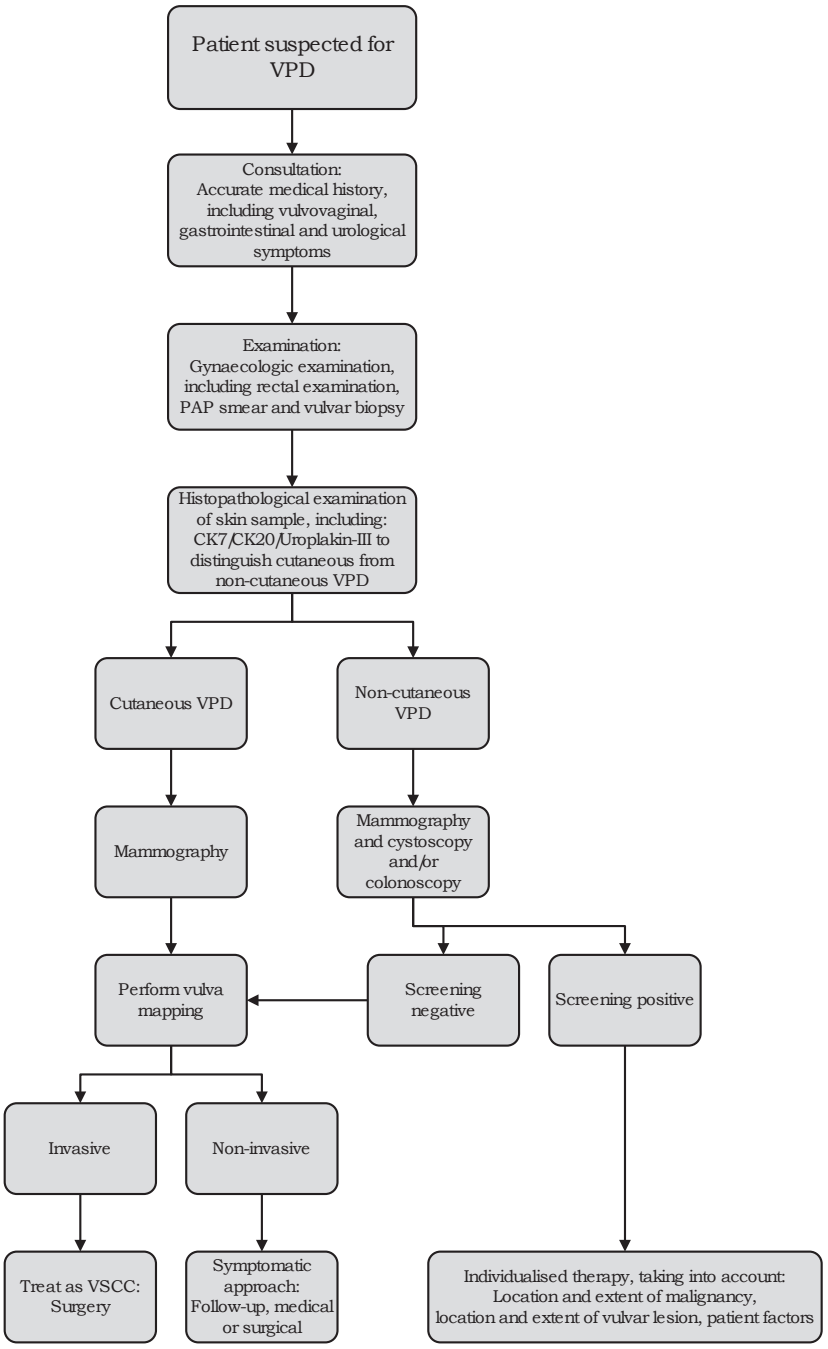

FIGURE 8 Flowchart for diagnostic procedures and treatment of patients with VPD.

Based on the results of this review, we suggest that the work-up of VPD patients should include a consultation that addresses symptoms that could indicate an underlying intestinal or urological malignancy. A full gynaecological examination should be performed, including rectal examination. A pap smear can be performed if no recent results are available, and a vulvar biopsy should be performed to confirm the VPD diagnosis. The immunohistochemical expression pattern can be used to distinguish primary from secondary VPD. Screening for an associated locoregional malignancy should be performed in non-cutaneous VPD, or if the patient has symptoms of a malignancy elsewhere. Even though the risk of progression into invasive VPD is small, invasion should be excluded in all patients by vulvar mapping.

Given its aggressive clinical behaviour, invasive VPD should be treated similarly to vulvar SCC. However, patients with non-invasive VPD can be treated with a symptomatic approach that should be individualised. In case of non-cutaneous VPD with an underlying intestinal or urological malignancy, individualised therapy should be provided. The location and extent of the malignancy and skin lesion should be taken into account along with symptoms and patient factors. We therefore propose a flowchart, based on the information reported in this review, which is intended to function as a supportive decision aid (figure 8). The treatment of patients with VPD should be individualised, taking into account the size and location of the lesion, the symptoms it causes, and individual patient factors.

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CHAPTER 4

VULVAR PAGET DISEASE IN THE NETHERLANDS

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ABSTRACT

Background: Vulvar Paget disease (VPD) is a rare skin disorder, considered premalignant.

Objective: To assess the clinical course, treatment schedules and the effect of invasion and treatment on recurrence and survival in patients with VPD.

Methods: Data on women with VPD were retrieved from the medical files and pathology reports in all Dutch tertiary university medical centres. Disease free survival (DFS) and 5-year disease specific survival (DSS) were estimated using Kaplan-Meier curves.

Results: Data of 113 patients diagnosed between 1991 and 2016 were analysed. Seventy-seven percent had non-invasive VPD. Most women underwent surgery (65%). Recurrences were reported in 40%. Of women with non-invasive VPD 8% developed invasion. There were no disease specific deaths reported in women with non-invasive VPD. The 5 year DSS was over 98% in non-invasive and micro-invasive VPD, but significantly worse in invasive VPD: 50% ($p<0.0005$).

Limitations: The main limitations of this study are its retrospective character and that original pathology samples were not available for reassessment.

Conclusions: VPD is extremely rare and recurrence rates are high. Most patients have non-invasive VPD, which does not affect survival and should be considered a chronic disorder with a limited invasive potential. In case of invasive disease survival decreases significantly.

INTRODUCTION

Vulvar Paget's disease (VPD) is an extremely rare skin disorder. Most women are postmenopausal and experience vulvar or perineal irritation, burning sensations, itching and pain. Clinical examination may reveal an eczematous, papillomatous, scaling or ulcerating erythematous plaque.[1] At the first clinical presentation, VPD is often not recognised and misdiagnosed as eczema or mycosis. Early histological assessment with a biopsy is crucial to make the correct diagnosis.

In literature, about 25% of the VPD cases are associated with invasion or an underlying vulvar adenocarcinoma.[2-4] Non-invasive VPD is considered an adenocarcinoma in situ, and a debate on the malignant potential of non-invasive VPD is ongoing. Treatment of women with invasive VPD is comparable with vulvar squamous cell carcinoma with surgery as the cornerstone of treatment.

Historically, surgical excision was considered the treatment of choice for both invasive and non-invasive VPD. Recurrence rates range from 15 to 70%: the influence of positive surgical margins on the recurrence rate is still debated. Wide local excision often leads to permanent mutilation and functional impairment.[3, 5-8] Recently, there is more attention for other treatment options such as topical treatment with 5% imiquimod cream, but large studies on this topic are lacking.

The influence of VPD on survival is unclear. Retrospective analysis of limited literature (with the largest study in 76 women) shows that death of disease in cases of non-invasive and micro-invasive VPD is rare, and more common in invasive disease.[1,8]

Since two decades, treatment of vulvar malignancies is centralised in eight gynaecologic oncology centres in the Netherlands.[9, 10] Gynaecologic oncologists are involved in multidisciplinary teams of vulvar clinics, due to their experience and surgical skills.

To understand the course of this rare disease, and to optimise the current protocols it is important to gain more insight on VPD. Clinical data of a high number of women may help in recognizing possible areas for improvement in clinical care, the effect of different treatment options and disease

specific outcomes. The objective of this national study is to assess the clinical course of VPD, to analyse current treatment schedules, and to study the effect of invasion and treatment on recurrence and survival rates.

MATERIAL AND METHODS

This retrospective cohort study took place in all eight gynaecologic oncology units in the Netherlands: Amsterdam Medical Centre, Antoni van Leeuwenhoek Hospital Amsterdam, ErasmusMC in Rotterdam, Leiden University Medical Centre, Maastricht University Medical Centre, Radboud university medical centre in Nijmegen, University Medical Centre Groningen, and the University Medical Centre Utrecht.

Local pathology databases were searched for all women reported with a first diagnosis of VPD or a recurrence between January 1st,1991 and January 1st, 2016. The lesion had to be located on the vulva, perineum and/or peri-anal skin. We retrieved medical files of all women. Data on woman characteristics, diagnostic methods and treatment schedule for the first episode as well as recurrences, including follow-up data were collected. All data were entered in a blinded digital database (CastorEDC, the Netherlands) according to Good Clinical Practice guidelines.[11]

Data on the woman characteristics, diagnostic processes and treatment schedules were analysed in a descriptive manner. We estimated the disease-free survival (DFS), defined as the time between the date of diagnosis to the date of the first recurrence, or in case of no recurrence until the last date of follow-up (or death), using a Kaplan-Meier curve. Five-year disease-specific survival (DSS), defined as the time between the date of diagnosis to the date of death of vulvar carcinoma, or in case of no death or death of other cause the date of last follow-up/date of death, was estimated using a Kaplan-Meier curve. Recurrences were defined as histologically confirmed diagnoses after a complete response, or in cases a complete response was not obtained and the residual lesion necessitated treatment. We performed subgroup analyses for three different initial diagnoses: non-invasive VPD versus microinvasive VPD and invasive VPD. We also analysed the main treatment methods: surgery, topical treatment and no treatment (watchful waiting), and surgical margin status in women that underwent surgery.

Differences in DFS and DSS rates between groups were analysed with log-rank tests. A *p*-value < 0.05 was considered statistically significant. All analyses were performed using SPSS for Windows, version 20.

As assessed by the institutional review board of the Radboudumc, the study was not subject to the Dutch 'Medical Research Involving Human Subjects Act', i.e. it was exempt from approval.

RESULTS

STUDY POPULATION

Searches of the local pathology databases in the eight participating centres identified 151 cases of VPD diagnosed before January 1st, 2016. After excluding cases because data was not available (n=27), or they were diagnosed with other diseases (n=11), data of a total number of 113 women were available for the study.

WOMEN CHARACTERISTICS

Overall, 87 women (77.0%) were initially diagnosed with non-invasive VPD, 10 (8.8%) with micro-invasive VPD, seven (6.2%) with invasive VPD, six (5.3%) with VPD with an underlying vulvar adenocarcinoma, and in three (2.7%) cases the VPD was metastasised. The median age of the 113 included women was 73 years (range 41-97 years) at time of diagnosis, and there was no statistically significant difference between the different diagnoses (p >0.3). See table 1.

NON-INVASIVE VPD

At the first consultation with the medical specialist, the duration of symptoms was often reported as "symptoms for several months", or "about a year", or even "a few years". Forty-nine women (56.3%) were diagnosed within four weeks after first consultation. Twenty-nine women (34.5%)

	ni-VPD n = 87	mi-VPD n = 10	i-VPD n = 16
Age	72 (41-97)	73.5 (54-86)	74 (49-89)
Hospital of diagnosis:			
Referring hospital	56 (64.4%)	6 (60%)	10 (62.5%)
University medical centre	30 (34.5%)	4 (40%)	6 (37.5%)
Unknown	1 (1.1%)	0	0
Initial treatment:			
Surgery	51 (58.6%)	10 (100%)	13 (81.3%)
Topical, imiquimod	18 (20.7%)	0	0
Topical, other	3 (3.6%)	0	0
Chemotherapy	1 (1.1%)	0	0
Radiotherapy	1 (1.1%)	0	2 (12.5%)
None	12 (13.8%)	0	1 (6.2%)
Unknown	1 (1.1%)	0	0

TABLE 1 Clinical characteristics of patients with VPD.

Ni-VPD: non-invasive vulvar Paget disease, mi-VPD: micro-invasive vulvar Paget disease, i-VPD: invasive vulvar Paget disease.

had a history of VPD, or were already diagnosed elsewhere when they first presented at the hospitals included in our study. Reports of mammography performed as screening after VPD diagnosis were available for 33 women after diagnosis with VPD and in one case (1.1%) a malignancy was found. Additional diagnostics and screening did not reveal any abnormalities. Vulvar mapping, where several biopsy samples are taken from all suspected areas, was performed in 37 women (42.5%): one woman was suspected to have invasive disease and was treated with a skinning vulvectomy, but invasion was not found in the surgical specimen.

Of the 51 surgically treated women, 33 women (64.7%) underwent a wide local excision, five (9.8%) a hemivulvectomy, and 12 (23.5%) a (skinning) vulvectomy. Data on the type of surgery was missing for one woman. Margin status was available for 47 women (92%) of the surgically treated women: surgical margins were clear in only five women.

Eighteen women were treated with topical 5% imiquimod cream, treatment schedules differed from one to five times per week, and women were treated for three weeks to an entire year. Of all women initially treated with topical 5% imiquimod cream, four (22.2%) had a complete response, seven (38.8%) a partial response and four (22.2%) no response or stable

disease. Data on the treatment response was unavailable for three women. Of the 13 women without a complete response, four (30.7%) underwent additional surgery.

MICRO-INVASIVE VPD

Five out of ten women (50%) with micro-invasive VPD were diagnosed at their first hospital visit. Three women (30%) were already diagnosed at time of referral. Screening for additional malignancies did not reveal other tumours. Vulvar mapping was performed in six cases (60%).

All women with micro-invasive VPD were treated with surgery. None of the women with micro-invasive disease underwent groin surgery. Margin status was available for nine cases: all were positive for Paget cells, but clear of micro-invasion.

INVASIVE VPD

All women with invasive VPD were diagnosed within four weeks of the first hospital visit. Five women (31.3%) had a history of VPD or were already diagnosed at time of referral. Screening for additional malignancies via mammography, colonoscopy, or cystoscopy was performed in five women and did not reveal other tumours. Vulvar mapping was performed and was positive for invasion in nine cases (56.3%). In five women an ultrasound of the groins was performed, in four women a suspected groin lesion was detected.

Most women with invasive VPD were initially treated with surgery: 13 women (81.3%). Six (37.5%) underwent a (skinning) vulvectomy, two (12.5%) a hemivulvectomy, and five (31.3%) a wide local excision. The type of surgery was unknown for one woman. Groin surgery, uni- or bilateral sentinel lymph node procedure or inguinofemoral lymphadenectomy, was performed in seven women (29.2%). Six women had lymph node metastase, see table 2 for details on lymph node status for all patients with invasive disease.

	Groin treatment	Groin metastases	Status at last FU	FU (months)
1	None		DVC	6
2	None		DVC	2
3	None		AWD	42
4	None		NED	118
5	None		AWD	5
6	None		NED	138
7	None		DVC	1
8	None		AWD	21
9	SLN bilateral	Negative for metastasis	DOC	7
10	IFL unilateral	Negative for metastasis	DVC	131
11	SLN unilateral	Positive for metastasis	DVC	18
12	IFL unilateral	Positive for metastasis	DVC	6
13	IFL unilateral	Positive for metastasis	DOC	17
14	IFL bilateral	Positive for metastasis	DVC	19
15	Radiotherapy	Positive for metastasis	DVC	2
16	Radiotherapy	Positive for metastasis	AWD	6

TABLE 2 Groin metastases in patients with invasive vulvar Paget disease

Overview of treatment of the groins in all patients with initial diagnosis of invasive VPD, conclusion of the pathology report, and the status of the patient. SLN: sentinel lymph node. FU: follow-up. AWD: Alive with disease. DOC: death of other cause. DVC: death of vulvar carcinoma. NED: no evidence of disease.

Margin status was available for 11 women, margins were clear of Paget cells in only one patient.

Two women with invasive VPD were initially treated with radiotherapy, of whom one received chemotherapy in advance. Limited data on the details of treatment were available.

RECURRENCE RATES

Overall, 46 women (37.4%) had 87 recurrences: ranging from one to nine recurrences per woman.

Of the 87 women with non-invasive VPD, 32 women (36.8%) had one or more recurrences. Nineteen (59.4%) had only one recurrence: in one case the recurrence was invasive, and in one case the disease metastasised, in all other women the recurrences were non-invasive. See figure 1 for an overview of all recurrences in women with non-invasive VPD. One woman had a clinical recurrence and was also diagnosed with a metastasised cer-

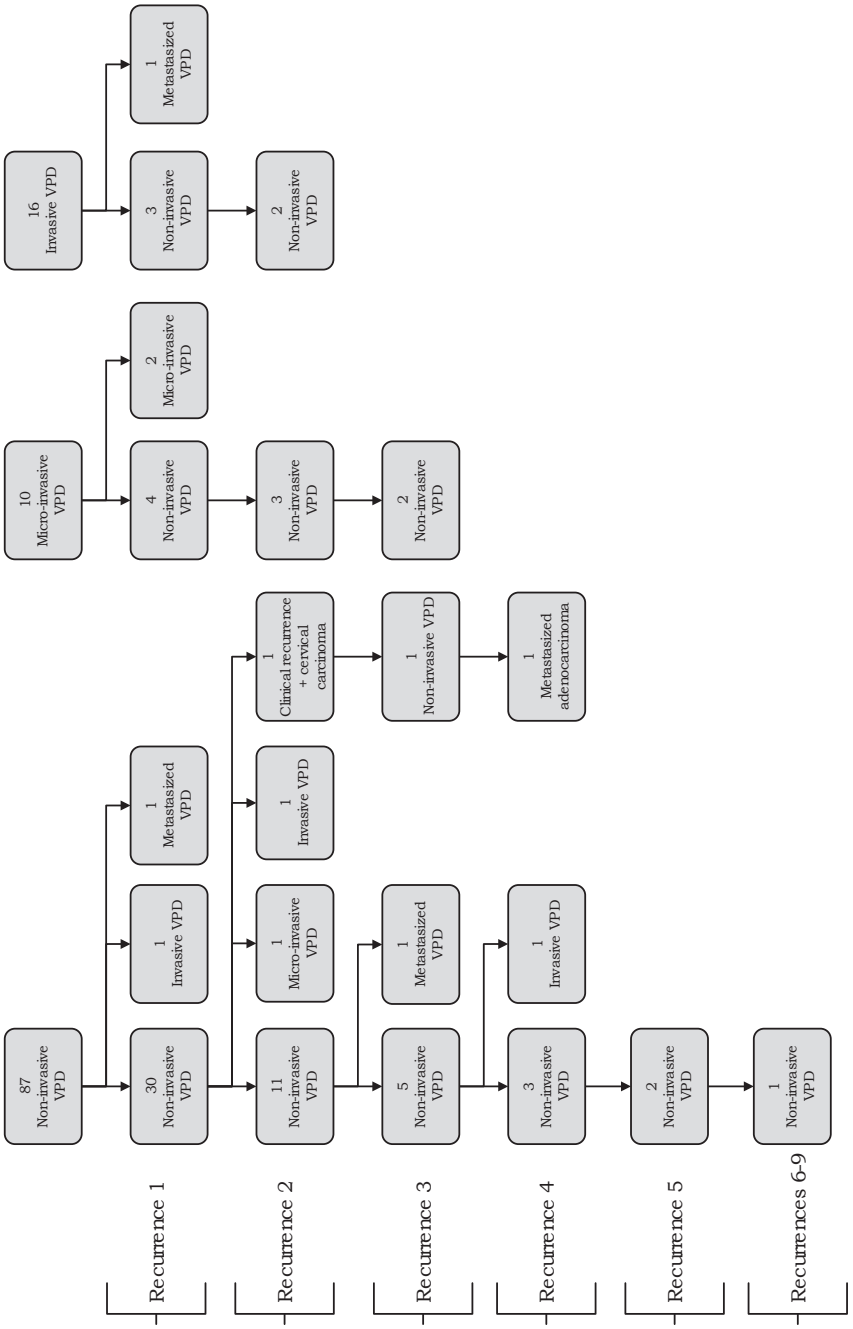

FIGURE 1 Recurrences in VPD.
VPD: vulvar Paget disease.

vical adenocarcinoma, which was the cause of death: invasion of the VPD was not reported.

In all 87 women with an initial diagnosis of non-invasive VPD, seven (8.0%) eventually progressed, see figure 1. Progression into invasion occurred after a median of 62 months after initial diagnosis, ranging from 31 to 165 months.

Of the 10 women with micro-invasive VPD, six women (60%) had one or more recurrences. Two had micro-invasive recurrences, and four had non-invasive recurrences. See figure 1.

Five women (31.3%) with invasive disease had one or more recurrences. In two cases the VPD was metastasised. Three women (18.7%) had non-invasive recurrences, see figure 1 for an overview of all recurrences in women with invasive VPD.

SURVIVAL

The estimated median DFS was 69.3 months for non-invasive, 39.3 months for microinvasive disease, and 26.5 months for invasive disease (*p* 0,301). There was no difference in the DFS between women with margins clear or positive for Paget cells after initial surgical treatment (*p* 0,491).

The median follow-up after the VPD diagnosis of all women was 38 months (range 0-451). Table 3 presents an overview of disease status at time of last follow-up. Five-year DSS was significantly better in women with initially

	Non-invasive VPD (n=87)	**Microinvasive VPD (n=10)**	**Invasive VPD (n=16)**
Alive:			
With disease	35 (40.2%)	3 (30.0%)	4 (25.0%)
No evidence of disease	33 (37.9%)	5 (50.0%)	2 (12.5%)
Disease status unknown	3 (3.4%)	1 (10.0%)	-
Dead:			
Of vulvar carcinoma	1 (1.1%)	-	8 (50.0%)
Other cause	7 (8.0%)	1 (10.0%)	2 (12.5%)
Cause unknown	8 (9.2%)	-	-

TABLE 3 Disease status at last follow-up.

VPD: Vulvar Paget disease

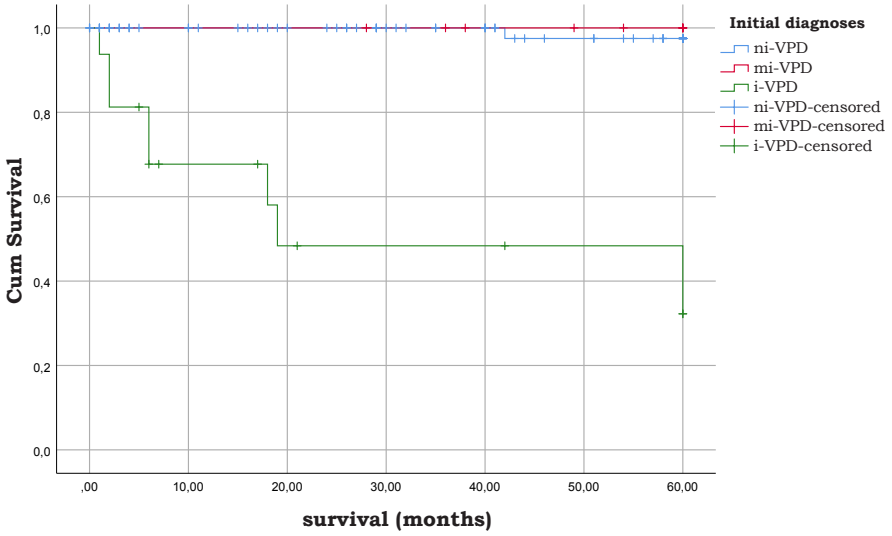

FIGURE 2 Disease specific survival in non-invasive versus micro-invasive and invasive VPD

Ni-VPD: non-invasive VPD, mi-VPD: microinvasive VPD, i-VPD: invasive VPD.
Women at risk: ni-VPD 87, mi-VPD 10, i-VPD 16.

non-invasive and microinvasive VPD compared to invasive VPD: 98.8%, 100.0% versus 50.0% (p<0.0005, figure 2). One patient with non-invasive VPD died from metastasised VPD at time of first recurrence 30.6 month after initial diagnosis. The five-year overall survival of non-invasive VPD women did not differ between women that were surgically treated, underwent topical treatment or without treatment (p=0.713).

DISCUSSION

This is the largest cohort study of women with vulvar Paget disease so far. We analysed clinical data of 113 VPD women who have been treated in all gynaecologic oncology university centres in the Netherlands.

We found that women had symptoms for several months to years before the diagnosis was finalised. Due to its rarity VPD is not often recognised at first evaluation by clinicians. Most medical specialist performed a vulvar

biopsy at the woman's first visit, confirming the diagnosis. The importance of early histological assessment is stressed, especially in cases of vulvar lesions not responding to therapy. In the majority of cases the pathologist had no difficulties with recognising the Paget cells in VPD.

VPD may be associated with invasion; we found 23% of cases at initial diagnosis were invasive, and in 2.7% disease was already metastasised. Progression of non-invasive VPD into invasive disease occurred in seven cases (8%) after a median time of five years. This long interval might suggest a de novo invasive lesion, instead of progression of the non-invasive VPD.

The main issue in surgical excision of VPD is obtaining free surgical margins, as the histological presence of disease does not overlap with the visibly affected skin due to intradermal localisation.[12] In our series, surgical margins were clear in only 9.4% of the women. A different surgical technique, such as Moh's Microsurgery seems to improve these figures.[13] With Moh's Microsurgery the lesion is excised through the epidermis and dermis, and 100% of the peripheral margins are examined immediately. Excision is repeated, enlarging the circumference each time, until the margins are clear. The DFS seems longer, and recurrence rate lower in women treated with Moh's Microsurgery.[13-15] Our study did not find that the margin status influences the DFS. Moreover, enlarging the excision will raise morbidity of treatment.

Topical 5% imiquimod cream is suggested as a new treatment option. It has shown to be effective for high grade vulvar squamous cell intraepithelial lesions.[16] Several case series and two small prospective studies have reported on imiquimod for VPD.[17, 18] We found that 20% of the women initially treated with imiquimod had a complete response, and 40% a partial response, although data of histological response were not available in most cases. Treatment schedules vary a lot with respect to frequency and overall duration. There is currently one study ongoing investigating the efficacy of topical 5% imiquimod cream in VPD in a standardised treatment schedule (Clinicaltrials.gov NCT02385188). Results can be expected at the end of 2018.

Care for women with VPD should take place in specialised centres. Women should frequently consult their physician whenever new lesions and/or symptoms arise. In case of non-invasive VPD, a watchful waiting or symp-

tomatic treatment with regular check-ups may be advised as recurrence rates are high and the presence of residual non-invasive disease does not influence the survival. Women with invasive VPD should be treated according the guidelines for vulvar squamous cell carcinoma. However, sentinel lymph node procedures are not recommended in these women. Recurrences after invasive VPD are not always invasive as well, a phenomenon which is also seen in squamous vulvar (pre)malignancies. Our study identified one case of non-invasive VPD with an invasive recurrence which led to the death of the patient. This patient was initially treated with topical imiquimod cream in a referring hospital: 31 months after initial diagnosis she was diagnosed with a vulvar adenocarcinoma and referred to a tertiary centre. Groin and bone metastases of a vulvar adenocarcinoma were found and the patient received chemotherapy. Disease progressed and she died 42 months after initial diagnosis. The pathology reports of the first biopsy samples taken in the referring hospital were not available for reviw. The available reports have suggested a possible relation with the breast carcinoma the patient was diagnosed with 9 years prior.

The main limitation of this study is that we only analysed clinical files and it was not possible to review pathology. The diagnosis of invasive VPD lacks a clear description: it can be hypothesised that the natural phenomenon of Paget cells in the skin adnexa are mistaken for invasive disease. Whereas an inevitable vulvar adenocarcinoma may be a second diagnosis in cases where the overlying, i.e. intact, epithelium contains Paget cells. This may be regarded as non-invasive VPD with an underlying vulvar adenocarcinoma. The prognosis of the patient may then depend on the adenocarcinoma, rather than the VPD. We therefore advise, combined with the rarity of the disease, that all cases should be reviewed by a specialist gynaecological and/or dermatological pathologist.

In conclusion, this study presents an overview of 113 VPD patients from the Netherlands. In case of a lesion suspicious for VPD, histology should confirm the diagnosis. Invasion occurs in about 14%, generally diagnosed with the first biopsy, which has a worse prognosis compared to non-invasive or micro-invasive disease. A symptomatic or watchful waiting policy is acceptable in women with non-invasive VPD. The risk of recurrence is high, but the risk of progression is limited and generally occurs after several years. Care for women with VPD should take place in centres with specialised clinicians and pathologists, experienced with this rare disease.

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CHAPTER 5

STOP ROUTINE SCREENING FOR ASSOCIATED MALIGNANCIES IN NON-INVASIVE CUTANEOUS VULVAR PAGET DISEASE?

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ABSTRACT

Background: Vulvar Paget disease (VPD) is extremely rare and thought to be associated with other malignancies.

Objective: The aim of this study was to evaluate the risk of developing breast, intestinal, and urological malignancies in patients with VPD compared with the general population, and in particular focus on the risk of malignancy in patients with cutaneous non-invasive VPD.

Methods: Data on the oncologic history of patients with any type of VPD between 2000 and 2015 were obtained from PALGA, a nationwide archive containing all pathology reports in the Netherlands. Follow-up data and a control group from the general population were obtained from the Netherlands Cancer Registry. After correction for age and calendar year at time of diagnosis, standardised incidence ratios (SIR) for the first three years after VPD diagnosis were estimated with 95% confidence intervals (95%CI).

Results: We identified 199 patients with a first diagnosis of VPD (164 non-invasive, 35 (micro-) invasive) between 2000 and 2015. The SIR of developing an associated malignancy in the first 3 years after diagnosis was 4.67 (95%CI 2.66-7.64). This was mainly due to the high incidence of intestinal malignancies among patients with secondary VPD. Subgroup analysis for cutaneous non-invasive VPD did not reveal a significantly increased risk for associated malignancies, i.e. SIR 2.08 (95%CI 0.76-4.62).

Conclusions: Of patients with VPD, 76.9% is diagnosed with cutaneous non-invasive VPD, and this group has no increased risk for developing malignancies of the breast, intestinal or urological tract. Our study suggests that routine screening for these malignancies in patients diagnosed with cutaneous non-invasive VPD may not be necessary.

INTRODUCTION

Vulvar Paget disease (VPD) is a rare skin disorder, most commonly seen in postmenopausal Caucasian women.[1] VPD causes itching or burning erythematosquamous plaques and is diagnosed when typical Paget cells are seen in the epidermis. VPD can be classified according to origin and invasion: VPD can be primary of cutaneous origin (type 1) or secondary to an intestinal (type 2) or urological (type 3) malignancy.[2] Most cases of primary disease, i.e. cutaneous, are non-invasive (type 1a). Cutaneous VPD can invade through the basal membrane (type 1b), or be seen in conjunction with a vulvar adenocarcinoma (type 1c). The difference between primary or secondary VPD cannot be made on histopathological assessment alone.[3] The aetiology and origin of Paget cells remains unknown.[1]

VPD has been considered to be associated with malignancies of the breast, intestinal and urological tract.[4,5] Some consider VPD secondary to intestinal or urological malignancies a Pagetoid spread rather than a separate entity. Therefore, by some primary non-invasive VPD may be considered the only 'true' VPD, and secondary VPD a 'Pagetoid phenomenon'.[6-9]

In the late 19th century, skin lesions like the nipple ulceration associated with breast cancer, became known as extramammary Paget disease (EMPD).[10-12] The histological characteristics of EMPD resembles mammary Paget disease, raising the suspicion of a comparable pathogenesis.[13] The term VPD is used for EMPD specifically localised at the female genital skin. In 1975, Friedrich et al. analysed 11 published articles, including 78 patients with VPD of whom 14 (18%) were diagnosed with breast cancer around VPD diagnosis.[4] The authors concluded that screening for breast cancer should be standard care in VPD patients.

In 1985, Chanda summarised 197 cases of EMPD in a literature review.[5] Of these patients, 128 had VPD. Simultaneous occurrence of a malignancy and VPD was reported in 12% of cases, and overall 29% of the patients were reported to have had a malignancy of either the breast, gynaecological, intestinal, or urological tract. The true association between these so-called "associated" malignancies and EMPD was questioned, as most malignancies were not diagnosed at the same time as EMPD, nor showed the two diseases a parallel course. However, Chanda concluded that clinicians should consider a search for malignancies of the gastrointestinal tract in cases of peri-

anal EMPD, or for genitourinary malignancies in genital EMPD. Ever since, the assumed association between VPD and malignancies has been generally accepted. Nowadays, international guidelines advise that women with VPD should be screened for associated malignancies.[14,15] However, the extent and timing of screening, and the preferred diagnostics, are not exemplified.

Our recent analysis of publications describing 10 or more patients with VPD, suggests that breast, intestinal and urological malignancies in VPD patients are rare: 3.2% had a history of breast cancer, 2.2% of intestinal cancer, and 3.9% of urological cancer.[1]

The aim of this study is to estimate the risk of developing breast, intestinal or urological malignancies in all patients with (non-)invasive VPD in comparison with the general population: moreover to evaluate whether all VPD patients should be routinely screened for malignancies.

PATIENTS AND METHODS

PATIENT SELECTION

The PALGA database, a nationwide network and registry of histo- and cytopathology in the Netherlands with national coverage since 1991, was searched for all cases of vulvar Paget disease and vulvar adenocarcinomas. All cases with a first diagnosis of VPD, non-invasive and invasive, between January 1st 2000 and December 31st 2014 were included. We excluded cases of adnexal gland tumours, mammary-like gland tumours or vulvar adenocarcinomas without evidence of VPD. Patients with vulvar localisation of an intestinal or urological malignancy were also excluded. We reported the type of VPD as reported in the pathology report: primary VPD (of cutaneous origin) which can be distinguished with the following immunohistochemical (IHC) profile: CK7+, CK20-. Secondary VPD of intestinal origin can be distinguished with the IHC profile CK7-, CK20+, CDx2+, and secondary VPD of urological origin with the IHC profile CK7+/-, CK20+, and Uroplakin-III+. Pathology reports that concluded with “vulvar Paget disease” but in which IHC were not available, were reported as VPD not otherwise specified (NOS), and assumed to be primary VPD, since this is the most common type.[1]

Follow up data were retrieved via the Netherlands Cancer Registry (NCR), which has national coverage since 1989 and registers all malignancies in the Netherlands. If follow-up data were not available from the NCR, we used the date of the last pathology report as the last date of follow-up.

ASSOCIATED MALIGNANCIES

VPD patients diagnosed with associated malignancies were identified from the pathology reports, as were the age at diagnosis of the associated malignancy and the date of diagnosis.

All invasive breast, intestinal or urological tumours were defined as potentially VPD associated. Besides invasive tumours, ductal carcinoma in situ (DCIS) of the breast was also included, because DCIS is considered to be a malignancy. Intestinal malignancies included tumours of the colon, rectum, rectosigmoid junction and anus. Urological malignancies included tumours of the kidney, ureter, bladder and urethra.

All women with histological confirmation of one or more aforementioned tumours between 2000 and 2015 were selected from the NCR. The total number of women living in the Netherlands by age category and calendar year was obtained from Statistics Netherlands.

STATISTICAL ANALYSIS

The incidence of associated malignancies in women with VPD was compared with the incidence of these malignancies in women from the general population. The expected incidence was extracted from data from the NCR and population data from Statistics Netherlands. Risks were stratified by age and calendar year diagnosis of malignancy. The standardised incidence ratio (SIR) was estimated as the ratio between the observed incidence of an associated malignancy in women with VPD divided by the expected incidence in women from the general population. SIRs were estimated until 36 months after VPD diagnosis, we assumed that malignancies diagnosed within 36 months after VPD diagnosis might have already been present, possibly as a premalignancy, at the time of VPD diagnosis. Furthermore, this time frame ensured a reasonable sample size of the

study cohort. We performed subgroup analyses for cases reported as primary non-invasive VPD only. Statistical models were based on a Poisson distribution using person time at risk as an offset.

Person time at risk was calculated as time from the date of first VPD diagnosis to the date of first histological confirmation of the associated malignancy of interest, date of death or date of last follow-up, whichever came first. Follow-up data were available up to January 1, 2015. Analyses were performed using STATA/SE 13.0 (StataCorp, College Station, TX). SIRs and their 95% confidence intervals (95%CI) were based on the Mid-P exact test,[16] and calculated using the OpenEpi Standardised Mortality Ratio Calculator (OpenEpi version 3.01)[17]. Figures were designed using Microsoft Office Excel 2007 and Visio 2007.

RESULTS

POPULATION

In total, 199 women with a first diagnosis of VPD in the Netherlands (~17 million inhabitants) between 2000 and 2015 were identified from the pathology reports. Non-invasive VPD was diagnosed in 164 (82.4%) patients, micro-invasive VPD in 12 (6.0%), and invasive VPD in 23 (11.6%). An overview of the types of VPD per diagnosis is presented in table 1. We considered NOS as primary cutaneous VPD in further analyses. The median age at diagnosis was 74 years (range 40-97), and did not differ between patients with non-invasive and (micro-) invasive disease (p=0.838). The median follow-up of all patients was 36 months (range 0-182) after VPD diagnosis.

	Non-invasive VPD	**(Micro-)invasive VPD**	**Total**
NOS	133 (66.8%)	25 (12.5%)	158 (79.4%)
Type 1, cutaneous	20 (10.1%)	8 (4.0%)	28 (14.1%)
Type 2, intestinal	8 (4.0%)	2 (1.0%)	10 (5.0%)
Type 3, urological	3 (1.5%)	0	3 (1.5%)
Total	**164 (82.4%)**	**35 (17.6%)**	**199 (100%)**

TABLE 1 Overview of type of VPD per diagnosis.

Overview of the different types of VPD per diagnosis. NOS: not otherwise specified. VPD: vulvar Paget disease.

ASSOCIATED MALIGNANCIES

Of all patients, 27 (13.6%) were diagnosed with breast cancer, 17 (8.5%) with an intestinal and 9 (4.5%) with a urological malignancy before, simultaneously with, or after the diagnosis with VPD. See supplemental table 1 for an overview. Three patients (1.5%) had a history of two associated malignancies.

Eighteen of the 27 patients (66.7%) with breast cancer were diagnosed before they were diagnosed with VPD: the median time difference was 102 months (range 3-213). Three of these 18 patients were diagnosed with breast cancer within 3 to 6 months prior to the VPD diagnosis, which was primary non-invasive in all three. Eight of the 27 patients (29.6%) were diagnosed with breast cancer after their VPD diagnosis, the median time difference between both diagnoses was 46 (range 1-116) months. Two of these eight patients were diagnosed with breast cancer within 2 months after the VPD diagnosis, which was primary non-invasive in both. It is uncertain whether these malignancies were detected with the VPD screening protocol. Time of breast cancer diagnosis in relation to VPD diagnosis was unknown for one patient.

In six (35.3%) of the 17 patients with intestinal malignancies and VPD, the intestinal malignancy was diagnosed before they were diagnosed with VPD, with a median time difference of 201 months (range 96-272). Two of the 17 patients (11.8%) were diagnosed with the intestinal malignancy and VPD simultaneously: both being type 2 VPD. Nine (52.9%) patients were diagnosed with an intestinal malignancy at a median time difference of 16 months (range 1-90) after VPD diagnosis.

Four of the 9 patients (44.4%) had a urological malignancy before the VPD diagnosis, with a median time difference of 152 months (range 103-249). Urological malignancies were diagnosed after VPD in the other five patients (55.6%), after a median time difference of 50 months (range 11-147).

Figure 1A shows the time difference between diagnoses of VPD and the associated malignancies for all patients, whereas figure 1B shows the time difference between VPD diagnosis and the associated malignancies for the subgroup of primary non-invasive VPD. The time difference between VPD diagnosis and for example urological malignancies was up to 250 months prior, and 150 months after VPD diagnosis.

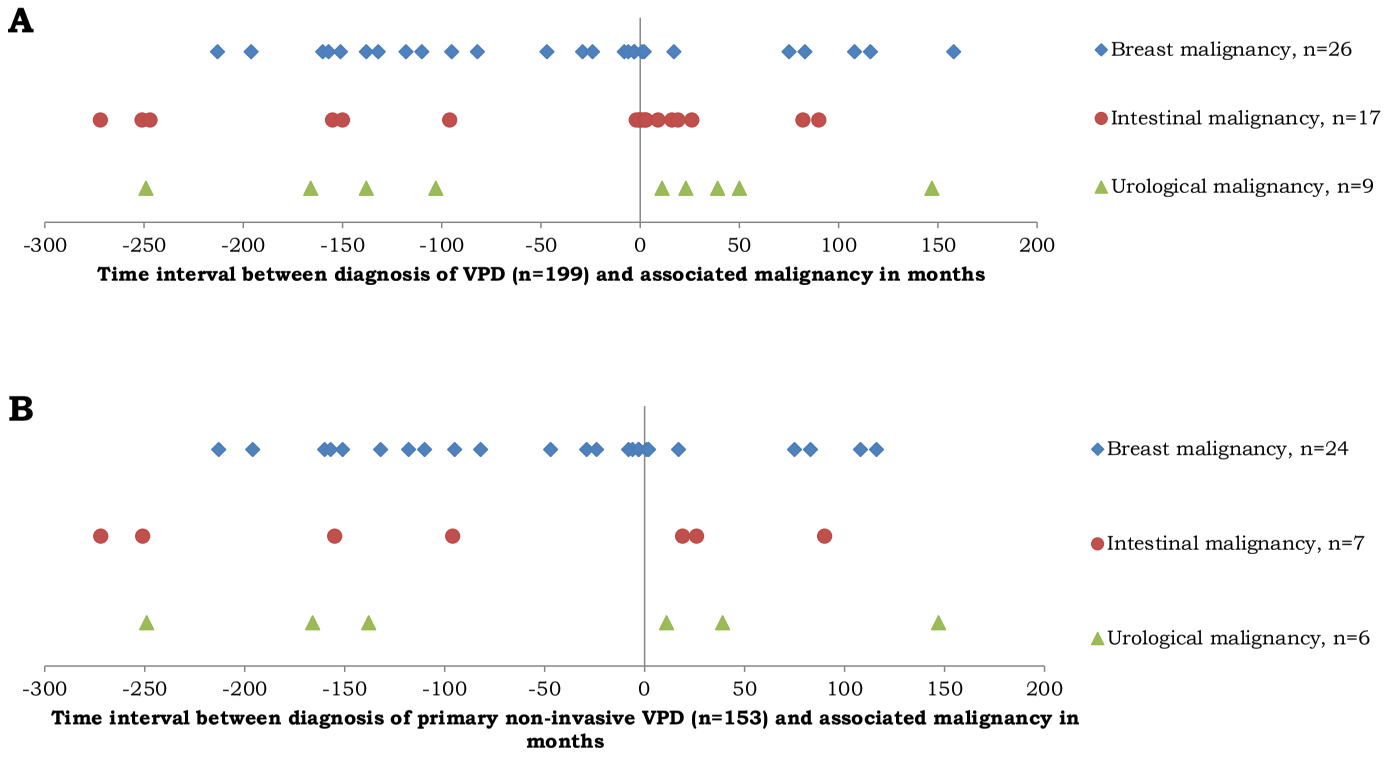

FIGURE 1 **(A)** Time difference between diagnosis of VPD (all types) and associated malignancy **(B)** Time difference between diagnosis of non-invasive primary VPD and associated malignancy.

VPD: vulvar Paget disease.

RISK OF DEVELOPING AN ASSOCIATED MALIGNANCY AFTER ANY TYPE OF VPD DIAGNOSIS

The cumulative risk of developing any of the associated malignancies within 36 months after diagnosis with any type of VPD was increased with a SIR of 4.77 (95%CI 2.66-7.64). This increased risk was mainly based on the significantly statistically increased risks of developing an intestinal malignancy, SIR 8.18 (95%CI 3.99-15.01), and for a urological malignancy: SIR 6.67 (95%CI 1.19-22.03). The SIR for developing a breast malignancy was not statistically significant 2.00 (95%CI 0.51-5.44), see table 2.

	Year after VPD	Obs	Exp	SIR	95% CI
	1	9	1.2		
	2	4	1.0		
	3	1	0.8		
Cumulative risk		**14**	**3.0**	**4.67***	**2.66-7.64**
	1	2	0.6		
	2	1	0.5		
	3	0	0.4		
Breast cancer		**3**	**1.5**	**2.00**	**0.51-5.44**
	1	6	0.4		
	2	2	0.4		
	3	1	0.3		
Intestinal malignancy		**9**	**1.1**	**8.18***	**3.99-15.01**
	1	1	0.1		
	2	1	0.1		
	3	0	0.1		
Urological malignancy		**2**	**0.3**	**6.67***	**1.12-22.03**

TABLE 2 Risk of developing an associated malignancy, all types of VPD.

Risk of developing an associated malignancy within 3 years after diagnosis with VPD, irrespective of type of VPD. VPD: vulvar Paget disease. Obs: observed cases per year and in total. Exp: expected cases per year and in total. SIR: standardised incidence ratio. 95%CI: 95% confidence interval. Statistically significant SIRs are marked with an asterisk (*).

RISK OF DEVELOPING AN ASSOCIATED MALIGNANCY AFTER PRIMARY NON-INVASIVE VPD DIAGNOSIS

See table 3 for the analysis restricted to patients with primary non-invasive VPD. The SIR for developing any of the associated malignancies was 2.08 (95%CI 0.76-4.62). The SIR for breast cancer was 2.50 (95%CI 0.64-6.80), the SIR for intestinal malignancies was 1.11 (95%CI 0.06-5.48), and the SIR for urological malignancies was 3.33 (95%CI 0.17-16.44). None of these results were statistically significant.

	Year after VPD	Obs	Exp	SIR	95% CI
	1	3	1.0		
	2	2	0.8		
	3	0	0.6		
Cumulative risk		**5**	**2.4**	**2.08**	**0.76-4.62**
	1	2	0.5		
	2	1	0.4		
	3	0	0.3		
Breast cancer		**3**	**1.2**	**2.50**	**0.64-6.80**
	1	0	0.4		
	2	1	0.3		
	3	0	0.2		
Intestinal malignancy		**1**	**0.9**	**1.11**	**0.06-5.48**
	1	1	0.1		
	2	0	0.1		
	3	0	0.1		
Urological malignancy		**1**	**0.3**	**3.33**	**0.17-16.44**

TABLE 3 Risk of developing an associated malignancy, non-invasive primary VPD.

Risk of developing an associated malignancy within 3 years after diagnosis with non-invasive primary VPD. VPD: vulvar Paget disease. Obs: observed cases per year and in total. Exp: expected cases per year and in total. SIR: standardised incidence ratio. 95%CI: 95% confidence interval. Statistically significant SIRs are marked with an asterisk (*).

DISCUSSION

This is the first population-based study investigating the incidence of possibly associated malignancies in patients with VPD. The risk of developing an associated malignancy within 3 years after VPD diagnosis of any subtype is significantly increased compared to the general Dutch female population. However, subgroup analysis of primary non-invasive VPD patients showed no significant increased risk to develop one of the associated malignancies. Therefore, there is no evidence for a routine screening program for these malignancies when diagnosed with non-invasive primary VPD.

All patients that were diagnosed with an intestinal or urological malignancy around the time of VPD diagnosis were diagnosed with secondary VPD (type 2 or 3). In patients with primary, i.e. cutaneous, non-invasive VPD the time interval between VPD diagnosis and the diagnosis of the associated malignancy varied greatly. We were therefore unable to identify a clear parallel course between primary non-invasive VPD and the so-called

associated malignancies. This raises the question of a consistent or comparable aetiology.

The main limitation of our study is the large group of cases labelled NOS. Our data is collected from two nation-wide databases. This means it was not viable to review the histopathological samples and perform additional IHC to define the origin. We relied on the assessment of multiple pathologists throughout the country, which reflects daily practice. We assumed patients with NOS VPD to have non-invasive cutaneous VPD. The distribution of the different types of VPD in our study cohort, resembles the distribution reported in literature.[1] It is possible this assumption caused us to overestimate the incidence of associated malignancies in this subgroup, since we might have included patients with VPD secondary to another malignancy.

Several studies report that perianal localisation of EMPD is a risk factor for intestinal or anal malignancies, and recent literature indicates that the location of the skin lesion influences the disease-specific survival.[18,19] Nonetheless, it is uncertain how the skin lesion and the intestinal malignancy are related to each other in case of secondary VPD. A recent epidemiological study by Karam et al. reported a higher risk of intestinal malignancies in EMPD.[20] They reported a SIR for any malignancy of 1.47 (95%CI 1.17-1.84), but did not find a statistically significantly increased risk for breast cancer (SIR 1.41, 95%CI 0.85-2.20), anorectal and colorectal malignancies (SIR 1.64, 95%CI 0.90-2.76), and bladder (SIR 2.39, 95%CI 0.65-6.13) or kidney/pelvic malignancies (SIR 0.93, 95%CI 0.02-5.21). However, this study included male and female patients with invasive disease. If the intestinal malignancy grows continuously to the epidermis, the question may be raised whether the EMPD lesion should be considered an expansion of the tumour rather than a separate entity. This holds especially in cases with invasive disease, as it is impossible to determine which lesion came first.

As suggested above, differentiation between the subtypes of VPD (primary cutaneous, secondary to an intestinal, or secondary to a urological malignancy) may be of great importance for the risk of developing other malignancies. However, currently there are no clear guidelines for this differentiation. It is generally accepted that the immunohistochemical profile of Paget cells can help to characterise different subtypes. As stated above,

the cutaneous phenotype is determined by a CK7+ and CK20- profile, the intestinal phenotype is determined by CK7-, CK20+, CDx2+, and the urological phenotype is determined by CK7+/-, CK20+, and Uroplakin-III+. These are the main markers used to distinguish primary from secondary VPD for almost two decades.[3,21-23] There are no studies assessing the sensitivity and sensibility of IHC in differentiating the different subtypes of in VPD, and the reliability of several other IHC stains can be discussed. For example GATA3, which is both reported to be sensitive as well as a potential pitfall in recognising VPD secondary to urothelial malignancies.[24,25]

Acknowledging this limitation, we assessed the risk of not screening for malignancy in those with a cutaneous IHC profile. Based on our data, refraining from additional screening for malignancies in case immunohistochemistry shows a cutaneous profile would have missed 5 patients over 15 years in the Netherlands, a country of ~17 million inhabitants. However, there are national screening programs for both breast and intestinal malignancies in which most patients would be screened anyhow. In case immunohistochemistry shows an intestinal or urological origin for the VPD lesion, a directed surveillance for the specific malignancies can be performed. To assist clinicians in the work-up of newly diagnosed VPD patients, we suggest a work-up according to the flowchart in figure 2. Even though our results indicate there may be no association with cutaneous VPD and underlying malignancy, screening may still be warranted depending on the clinical context.

Our study population contains 15 years of national data, and is the first to focus on primary non-invasive VPD. We were not able to match all cases reported in the PALGA database to patients registered in the NCR to obtain follow-up data. We therefore used the date of the last pathology report as the last date of follow-up. This may shorten follow-up times and therefore reduce the sample size: at 36 months after VPD diagnosis the cohort consists of 101 patients. With a small cohort the number of expected events, i.e. the development of one of the associated malignancies, based on the incidence in the general age and calendar year corrected population would be smaller than 1 patient per year. This causes the SIR to be disproportionately high for those years an associated malignancy was diagnosed.

For inclusion in the study we required that associated malignancies had to be histologically confirmed. It is possible we missed cases of associat-

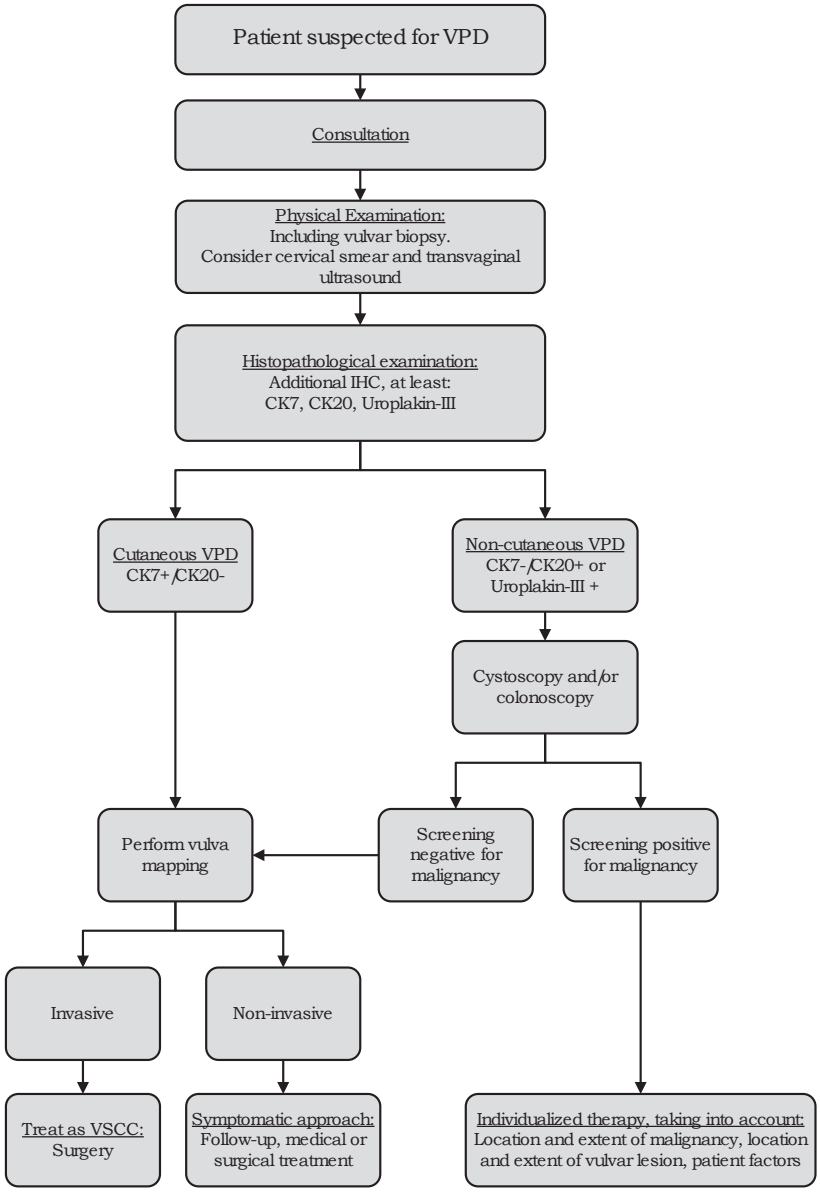

FIGURE 2 Flowchart for the workup of newly diagnosed VPD patients.

VPD: vulvar Paget disease. IHC: Immunohistochemistry. Vulvar mapping: ideally consisting of several punch biopsies taken from all suspicious lesions, histological slides are reviewed under the microscope and additional IHC is performed on all of the slides. VSCC: Vulvar squamous cell carcinoma.

ed malignancies that were diagnosed via clinical examination or imaging, without histology. However, this is corrected by including only histologically confirmed malignancies in our background file of the general population.

The main goal of this study was to estimate the risk of developing associated malignancies in patients with VPD and to evaluate the need for screening for these malignancies. Looking at all patients with any type of VPD, we find that the risk of developing an associated malignancy is increased. VPD can be a sign of an internal malignancy, and may be a valuable cutaneous sign prompting earlier diagnosis. A diagnoses of secondary VPD, with an IHC phenotype favouring non-cutaneous origin, should prompt a search for internal malignancy above and beyond physical exam.

The data in this study suggests promisingly that there is no statistically significant increase in cancer diagnosed in the first three years after primary non-invasive VPD compared with the general population. This may assist clinicians in reassuring and allaying concerns of patients with non-invasive VPD. Reassuringly, most of these patients are often at an age where they are offered screening for bowel, breast and cervical cancer. So, our main conclusion is that there is no evidence for routine screening in patients diagnosed with primary non-invasive VPD.

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SUPPLEMENT TABLE 1 Overview of patients with associated malignancy

	Diagnosis	Type	Malignancy	Before/After VPD diagnosis	Time between diagnoses (months)
1	ni-VPD	NOS	Breast	Before	157
			Urological	Before	166
2	ni-VPD	Type 2	Breast	Before	158
			Intestinal	After	2
3	ni-VPD	Type 2	Intestinal	After	82
			Urological	After	23
4	i-VPD	NOS	Breast	Unknown	Unknown
5	ni-VPD	NOS	Breast	Before	213
6	ni-VPD	NOS	Breast	Before	196
7	ni-VPD	NOS	Breast	Before	160
8	ni-VPD	NOS	Breast	Before	151
9	ni-VPD	Type 3	Breast	Before	138
10	ni-VPD	NOS	Breast	Before	132
11	ni-VPD	Type 1	Breast	Before	118
12	ni-VPD	NOS	Breast	Before	110
13	ni-VPD	NOS	Breast	Before	95
14	ni-VPD	Type 1	Breast	Before	82
15	ni-VPD	Type 1	Breast	Before	47
16	ni-VPD	NOS	Breast	Before	29
17	mi- VPD	NOS	Breast	Before	24
18	ni-VPD	Type 1	Breast	Before	8
19	ni-VPD	NOS	Breast	Before	6
20	ni-VPD	NOS	Breast	Before	3
21	ni-VPD	Type 1	Breast	Before	3
22	ni-VPD	NOS	Breast	After	1
23	ni-VPD	NOS	Breast	After	2
24	ni-VPD	NOS	Breast	After	17
25	ni-VPD	Type 1	Breast	After	75
26	mi- VPD	NOS	Breast	After	83
27	ni-VPD	NOS	Breast	After	108
28	ni-VPD	Type 1	Breast	After	116
29	i-VPD	Type 1	Intestinal	Before	272
30	ni-VPD	NOS	Intestinal	Before	251
31	ni-VPD	Type 2	Intestinal	Before	247
32	ni-VPD	NOS	Intestinal	Before	155
33	i-VPD	Type 2	Intestinal	Before	150
34	ni-VPD	NOS	Intestinal	Before	96
35	i-VPD	Type 2	Intestinal	Same time	-
36	ni-VPD	Type 2	Intestinal	Same time	-
37	ni-VPD	Type 2	Intestinal	After	2
38	ni-VPD	Type 2	Intestinal	After	3
39	ni-VPD	Type 2	Intestinal	After	9
40	ni-VPD	Type 2	Intestinal	After	16
41	ni-VPD	NOS	Intestinal	After	19

SUPPLEMENT TABLE 1 Continued

	Diagnosis	Type	Malignancy	Before/After VPD diagnosis	Time between diagnoses (months)
42	i-VPD	NOS	Intestinal	After	26
43	ni-VPD	NOS	Intestinal	After	90
44	ni-VPD	NOS	Urological	Before	249
45	ni-VPD	NOS	Urological	Before	138
46	ni-VPD	Type 3	Urological	Before	103
47	ni-VPD	NOS	Urological	After	11
48	ni-VPD	NOS	Urological	After	39
49	ni-VPD	Type 3	Urological	After	50
50	i-VPD	NOS	Urological	After	147

ni-VPD: non-invasive vulvar Paget disease, mi-VPD: micro-invasive vulvar Paget disease, i-VPD: invasive vulvar Paget disease, NOS: not otherwise specified.

CHAPTER 6

CERVICAL METASTASES ORIGINATING FROM A PRIMARY RECTAL ADENOCARCINOMA DUE TO A PAGETOID SPREAD

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ABSTRACT

Vulvar Paget disease is a rare skin disorder, considered an in situ adenocarcinoma. It is characterised by intraepithelial Paget cells, of which the origin is unclear. About 75% of cases have a cutaneous origin; the other 25% originate from an intestinal or urological malignancy. We report the first case of retrograde pagetoid spread from a rectal adenocarcinoma to the vulva and cervix. A 66-year-old woman presented with postmenopausal bleeding and a history of Crohn's disease. Gynaecological workup revealed vulvar and endocervical lesions consisting of Paget cells and adenocarcinoma, respectively. A rectal adenocarcinoma with in situ adenocarcinoma was diagnosed. The surgical specimen demonstrated Paget cells in the squamous epithelium of the anus and vulva. Immunohistochemistry demonstrated an intestinal phenotype of these cells. Genetic testing revealed the same TP53 mutation in tumour cells of the rectal adenocarcinoma and vulvar and endocervical lesions, demonstrating that the Paget cells originated from the same intestinal tumour.

INTRODUCTION

Vulvar Paget disease (VPD) is a rare skin disorder that causes erythematous scaling plaques.[1] It is characterised by the presence of Paget cells in the epidermis. The origin of these Paget cells remains largely unknown. It is hypothesised that these Paget cells originate from the skin appendages, from the mammary-like glands of the vulva, or from precursor Toker cells.[1] The classification of Wilkinson and Brown distinguishes 3 types of VPD: primary cutaneous disease, and secondary spread of an intestinal or an urological malignancy.[2] A few case reports reported a spread of cutaneous VPD via the vagina to the cervix.[3-7] We present the first case of spread of Paget cells via the vulva to the cervix secondary to a rectal tumour.

MATERIALS AND METHODS

PATIENT

A 66-year-old woman was referred to the department of Obstetrics and Gynaecology in our university medical centre for analysis of postmenopausal vaginal bleeding. In 1982, she was diagnosed with Crohn's disease and underwent multiple abdominal surgical procedures. She had a history of rheumatoid factor-negative polyarthritis and uses disease-modifying antirheumatic drugs. Her medical history also included hypertension, chronic obstructive pulmonary disease, a meningioma, and recurrent urinary tract infections. She was referred to the urologist for analysis of hematuria and the recurrent urinary tract infections. Analyses excluded the urethra or bladder as the origin of the hematuria. Therefore, she was referred to investigate whether the bleeding originated from the gynaecological tract.

For several months, the patient noticed irregular bleeding of unknown origin. Result of the last cervical smear, 5 years ago, was normal. Transvaginal ultrasonography showed a retroverted uterus with normal ovaries and little fluid in the pouch of Douglas. Palpation of the cervix was normal. Some suspicious tissue was visible in the external ostium of the cervix and biopsied for histological assessment. A probable adenocarcinoma of 1.3 cm was seen, suspected for an endometrial adenocarcinoma. The cer-

vical smear showed malignant cells suspicious for endometrial adenocarcinoma, and was negative for high-risk human papilloma virus. Shortly after that, a vague erythematous vulvar lesion was biopsied and showed Paget cells in the vulvar epithelium with an intestinal phenotype: cytokeratin 7 negative and cytokeratin 20 positive. The patient underwent a hysterectomy with salpingo-oophorectomy for the suggested endometrial adenocarcinoma. Histological analysis revealed that an adenocarcinoma of the endometrium was not present. Leydig cell hyperplasia was present in the hilus of the ovaries. The cervical epithelium showed several atypical tubular structures, mainly consisting of Paget cells.

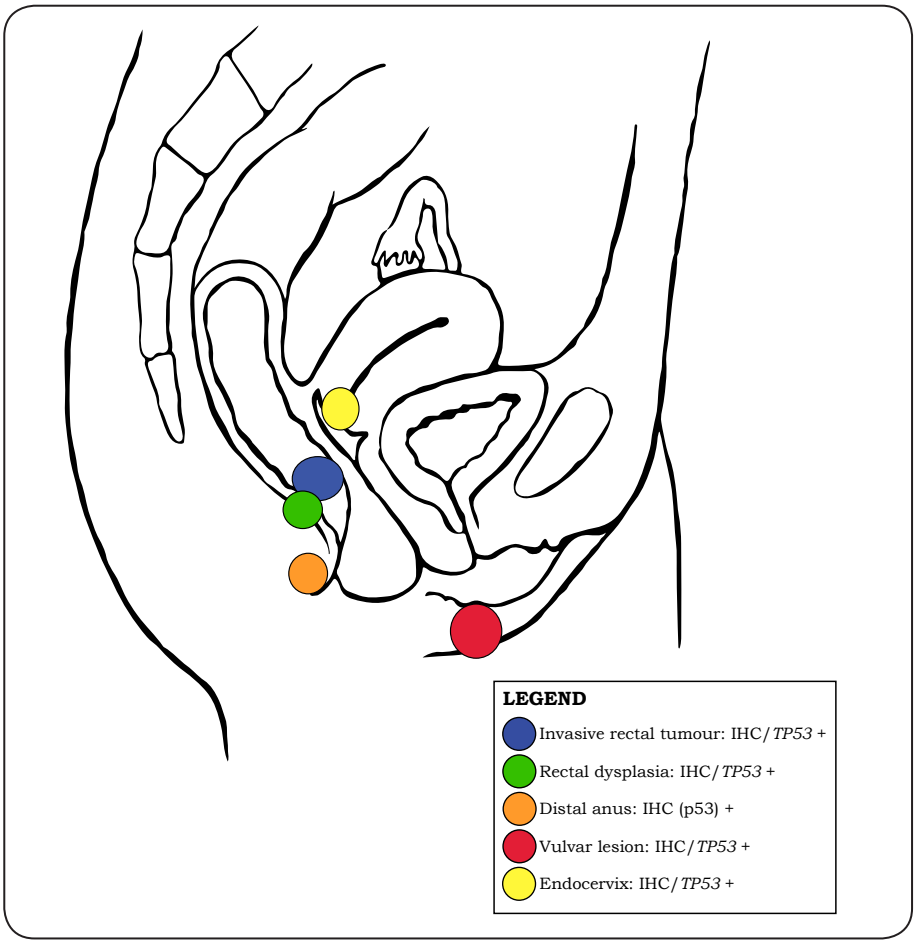

FIGURE 1 Overview of anatomical location of lesions

Additional colonoscopy was performed, and 2 polypoid lesions were biopsied at the pectinate line and revealed a rectal adenocarcinoma with a diameter of 1.5 cm, surrounded by in situ adenocarcinoma. Figure 1 presents the anatomical location of the several lesions. A positron emission tomographic scan ruled out lymph node metastases in the pelvis and groins, and an abdominoperineal resection was performed a few weeks later. Except for spontaneous discharge of an internal hematoma, the patient recovered well.

SAMPLES

The endocervical tissue, complete vulvar lesion and multiple sections of the intestinal tissue were embedded in paraffin. Subsequent sections were stained with haematoxylin and eosin (H&E) and Alcian Blue. Paget cells were seen in the endocervical epithelium, in the epidermis of the vulva and adjacent to the rectal tumour. Representative slides of the adenocarcinoma of the pectinate line, surrounding in situ adenocarcinoma, and vulvar and endocervical lesions were selected and examined by 2 expert pathologists (JB, PB).

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Immunohistochemistry (IHC) was performed using the routine steps of deparaffinisation, dehydration and antigen retrieval which was achieved after boiling with sodium citrate. Primary and secondary antibodies were used in the LabVision Autostainer 360 (ThermoFisher, Waltham, MA, USA), slides were counterstained with haematoxylin. CK7 (Clone OV-TL 12/30 EDTA 1:400; VWR ImmunoLogic, Duiven, the Netherlands), CK20 (Clone E19-1 citrate 1:100; VWR ImmunoLogic, Duiven, the Netherlands), CK8.18 (Clone CAM5.2 EDTA 1:10; BD Biosciences, San Jose, CA), and CDx2 (Clone CDx2-88 EDTA 1:50; VWR Klinipath, Duiven, the Netherlands) were used as primary antibodies. A p53 nuclear stain was performed as well (Clone DO-7 EDTA 1:400; ThermoFisher Scientific, Waltham, MA). Positive and negative controls were run for all samples.

GENETIC ANALYSIS

Selected representative slides of the rectal tumour, surrounding in situ adenocarcinoma, and vulvar and cervical lesions were verified by both expert pathologists (JB, PB) for mutation analysis. The neoplastic cells were marked in each slide, and the percentage of atypical cells was assessed. The fallopian tube was selected as normal tissue, to rule out a germ line mutation. Single molecule Molecular Inversion Probe base sequence analysis of the *TP53* and *CDKN2A* genes was performed in a NextSeq 500 (Illumina, San Diego, CA, USA). The selected gene panel analyzes >95% of the coding regions of *TP53* (NM_000546.4) and *CDKN2A* (P14:NM_058195.1 and P16: NM_000077.3).

RESULTS

IMMUNOHISTOCHEMISTRY

The endocervical and vulvar lesions, distal anus, rectal in situ adenocarcinoma, and adenocarcinoma had the same IHC profile: CK7 negative, and CK20, CK8.18 and CDx2 positive. These aforementioned lesions were now interpreted to be metastases of the rectal adenocarcinoma with Paget cells with an intestinal phenotype. p53 nuclear stain was strongly positive in the tumour cells in all slides as well. See figure 2 for an overview of the performed stains.

GENETIC ANALYSIS

TP53 mutation analysis revealed the same mutation (c.524G>A [p.{Arg175His }]) in all tissue samples: adenocarcinoma of the pectinate line, surrounding in situ adenocarcinoma, vulvar lesion, and endocervical epithelium. This mutation was not found in the healthy tissue of the fallopian tube, suggesting that all lesions originated from the same tumour.

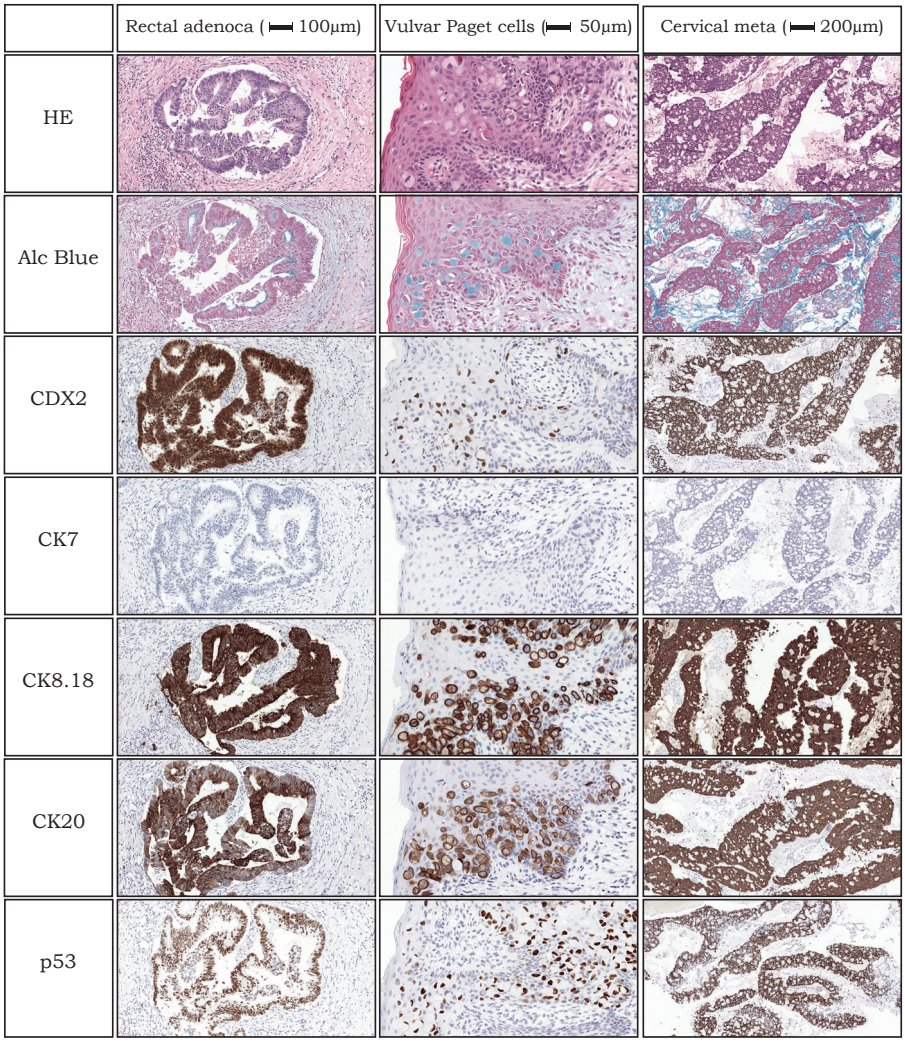

FIGURE 2 Overview of performed stains.

Overview of the different stains in the TP53+ rectal adenocarcinoma, vulvar Paget cells and the cervical metastasis. HE: Haematoxylin-Eosin, Alc Blue: Alcian Blue, CK: cytokeratin.

DISCUSSION

We have presented the first case of spread of Paget cells to the vulva and cervix, originating from a rectal tumour. Extension of VPD to the cervix has been reported in a few cases. Lloyd et al.[7] reported a patient with a vulvar

adenocarcinoma, the adjacent and overlying epidermis contained epithelial membrane antigen-positive cells, diagnosed as Paget cells. These cells were also present in the vaginal mucosa, but not in a continuous pattern. Four weeks after the vulvectomy, a hysterectomy was performed because of a symptomatic prolapse, and Paget cells were also seen in the cervical tissue. Another case report described a patient with VPD of a cutaneous origin confirmed by IHC.[3] A routine cervical smear revealed atypical cells and a cone biopsy was performed. In the histological tissue sample Paget cells were seen. Further sampling of the vaginal tissue was performed, also revealing Paget cells. Three case reports described patients with a cervical lesion or dysplasia.[4, 6, 8] Two of the patients had a history of non-invasive primary VPD. Histological samples of the cervical lesions in all 3 patients contained Paget cells. During postoperative follow-up, the vaginal tissues of 2 patients were sampled, which revealed Paget cells as well.

One study retrospectively investigated the incidence of abnormal cervical smears in VPD patients.[5] Three of the 19 VPD patients had an abnormal cervical smear result. In one case the cervical smear result was abnormal 24 months after diagnosis with VPD and vaginal spread of the Paget cells was confirmed with a biopsy. Four years later, the patient was diagnosed and treated for a mucinous anal adenocarcinoma and an urothelial carcinoma of the bladder. The second case had an abnormal cervical smear result 84 months after the diagnosis of VPD, the assessment of the endocervix revealed an invasive cervical adenocarcinoma with Paget cells, and follow-up smears remained positive for Paget disease. Histological samples revealed Paget cells in the vaginal tissue and invasive Paget disease in the urethra with metastases to the inguinal lymph nodes. A third patient had an atypical PAP smear result and the subsequent biopsies of the cervix and vagina contained Paget cells. The patients were lost to follow-up after diagnosis. These cases may resemble our presented case. However, because of the limited description of the cases and the lack of IHC, we are not able to make a comparison between the cases. However, it is emphasised that Paget cells have the ability to migrate to other locations than their primary origin.

Most of the above mentioned reports of spread of Paget cells also reported IHC profiles consistent with a primary, or cutaneous, origin. The retrospective study of Gu et al. reported 1 patient with intestinal and urological malignancies, associated with VPD, and one patient with invasion.[5] Re-

sults of IHC in these specific patients would have been valuable to identify the origin of the Paget cells and the possible relation to the other malignancies.

A recent Japanese study included 6 patients with perianal or vulvar Paget disease and suspected spread of Paget cells to the anal canal, to investigate the optimal surgical margin.[9] Five patients underwent anal mapping: 2 patients with perianal Paget disease (1 male and 1 female) had evidence of Paget cells at or close to the dentate line. The 3 other patients had VPD; the biopsies showed Paget cells of the anal verge, but not in the anal canal itself. Additional IHC to identify the origin of the Paget cells had not been performed in any of these cases.

Abnormal nuclear p53 is generally accepted as a marker for a mutation in the corresponding tumour suppressor gene at 17p13. Several studies have evaluated p53 IHC in vulvar Paget disease, with variable results.[10-15] In some studies p53 positivity was correlated to invasion; because of small sample sizes in the individual cases and variable definitions of invasion, it is uncertain whether this conclusion can be made. To our knowledge, *TP53* mutation analysis in VPD has not yet been reported.

In our case, the cervical, vulvar, and intestinal lesions all originate from the same intestinal tumour or in situ adenocarcinoma based on the *TP53* mutation analysis. Further research into the genetics of Paget cells may help identifying the origin of these particular cells. Our case strongly suggests that VPD with a cutaneous IHC profile has a different origin than secondary VPD. Additional research and evidence of this hypothesis may lead to further understanding of this rare disease and highlight possible clinical differences between primary and secondary disease.

In conclusion, this is the first report of spread of secondary, intestinal extramammary Paget disease to the vulva and cervix. All affected locations were genetically proven to originate from the same rectal tumour. This raises the question of whether secondary Paget's disease should be considered as a Pagetoid phenomenon, rather than a type of Paget's disease.

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CHAPTER 7

THE IMMUNE INFILTRATE IN VULVAR PAGET DISEASE

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ABSTRACT

Background: Non-invasive vulvar Paget disease (VPD) is a rare skin disorder mainly affecting elderly women. Recently, the immune modulator imiquimod was reported as an effective treatment option. Knowledge about the immune microenvironment of VPD is lacking.

Methods: This study investigates the basic characteristics of the immune infiltrate in VPD (n=10); moreover the influence of imiquimod was studied (n=6). Immunohistochemistry for CD4, CD8, CD14, CD20, CD56 and FoxP3 was performed. The infiltrates of VPD were compared to vulvar high-grade squamous cell intraepithelial lesions (HSIL) (n=43), a HPV induced vulvar premalignancy with known response to imiquimod cream, and healthy controls (n=30). Immune cell counts in samples taken before and after treatment were compared.

Results: The microenvironment in VPD differs from the healthy vulvar skin and vulvar HSIL. VPD is characterised by a decrease in immune cells in the epithelium and an abundant number of immune cells in the stroma, consisting predominantly of T cells. The intraepithelial CD8+/Foxp3+ ratio and number of CD56+ increased after imiquimod therapy, whereas the numbers of CD14+ cells decreased which may point to a treatment-induced type 1 immune response.

Conclusions: The epithelium in VPD contains less immune cells, but a dense stromal immune infiltrate. Changes in immune cell counts after immune modulation in relation to clinical responses should be further investigated.

BACKGROUND

Vulvar Paget disease (VPD) is an uncommon non-invasive skin disorder mainly seen in elderly women. VPD causes symptoms like pain, pruritus and a skin lesion, which may be scaling or ulcerated.[1] Patient's as well as doctor's delay play a significant role in recognising VPD because it presents like a dermal mycosis or eczema. The diagnosis is confirmed when so-called Paget cells are seen in histological skin samples. Paget cells are large, pleomorphic, pale cells with vacuolated cytoplasm in the epidermis. Secondary changes of the epidermis, such as acanthosis, papillomatosis, elongated dermal rete ridges and parakeratotic hyperkeratosis are frequently seen. An inflammatory infiltrate in the papillary dermis and stroma of VPD has been noted.[2] The mechanisms underlying of VPD and its treatment are a subject of discussion.

The aetiology of non-invasive VPD remains unclear; a commonly used hypothesis is that it is a premalignant abnormality of the skin adnexa, eccrine and/or apocrine glands.[2] The risk of malignant progression in patients with non-invasive VPD is about 8% (unpublished submitted data van der Linden et al.). Paget cells generally stain positive for carcinoembryonic antigen (CEA), cytokine 7 (CK7), epithelial membrane antigen (EMA) or mucin 1 (MUC1), MUC5AC and androgen receptors (AR). These Paget cells show variable expression for CK20, gross cystic duct fluid protein 15 (GCDFP-15) and the proliferation marker Ki-67.[3, 4] The mechanisms underlying malignant transformation in non-invasive VPD are unclear.

Currently, the treatment of choice is surgical excision of the skin lesion, which often causes severe morbidity and psychosexual problems.[1] Irrespective of the margin status, the recurrence rate is high (~35%), often resulting multiple surgical procedures.[1] Moreover, comorbidity and age may hamper optimal surgery. Clearly, there is need for less invasive treatment options. Topical 5% imiquimod cream is an immune modulator and has been reported to be effective in patients with anogenital warts, vulvar high-grade squamous cell lesions (HSIL) or actinic keratosis.[5, 6]

Recent literature also reports clinical efficacy of imiquimod for non-invasive VPD.[7-9] A literature review reported complete clinical and histological remission in 73% of the case reports and case series.[8] Two prospective

observational studies reported complete clinical and histological remission in 75% and 90% of the patients.[7,9] The mechanism of response of topical 5% imiquimod cream in VPD is unclear and also the basic composition of the immune infiltrate in VPD.[10] At present only two immune parameters in VPD are described; a higher number of FoxP3+ Tregs at the dermal-epidermal junction was associated with recurrent disease,[11] and CD163+ macrophages were detected more frequently in invasive VPD.[12]

The aim of this study was to investigate the microenvironment of VPD by examining the baseline composition of the immune infiltrate of the epithelium and stroma in VPD, and to compare these findings to the immune infiltrate seen in healthy vulvar tissue samples and vulvar HSIL. In addition, we compared the immune cell counts before and after treatment with 5% imiquimod. The results of this study may help to understand the rationale of effectiveness of topical 5% imiquimod cream treatment for VPD.

METHODS

PATIENT MATERIAL

We selected consecutive patients between 2000 and 2015 who underwent treatment with topical 5% imiquimod cream for non-invasive VPD at the Leiden University Medical Center (LUMC) and the Radboud university medical centre (Radboudumc), Nijmegen, the Netherlands.

We included diagnostic tissue samples prior to treatment, and, if available, samples taken after treatment with imiquimod. As assessed by the institutional review board of the Radboudumc and the LUMC, the study was not subject to the Dutch 'Medical Research Involving Human Subjects Act', i.e. it was exempt from approval. Clinical data were retrieved. Response to treatment is reported as the clinician reported the response in the medical files: complete response (CR) when there was no visible residual lesion, partial response (PR) when the lesion decreased in size, no response (NR) for cases in which the lesion size was stable or increased.

PATHOLOGY REVIEW

We obtained archival formalin fixed paraffin embedded (FFPE) blocks: 17 diagnostic biopsy samples taken before treatment of a total number of 10 patients. From 6 patients we obtained different samples taken after treatment: 4 biopsy samples and 3 surgical excision specimens. All haematoxylin and eosin (HE) slides were reviewed by a gynaecological pathologist (TB) blinded for treatment. Vulvar (in situ) melanoma was excluded by additional immunohistochemical staining (S100, MART, or HMB-45). CK7, p63, or CK8.18 staining was performed to confirm a cutaneous origin. In one case (patient 1), there was no evidence of disease in the sample after treatment.

IMMUNOHISTOCHEMISTRY

FFPE tissue blocks were retrieved from the pathology archives of the LUMC, and the Radboudumc, Nijmegen, the Netherlands. Ten blank 4µm slides were cut and blank slides were deparaffinised in xylene and rehydrated with ethanols. We selected CD4 as a marker for T-helper cells, CD8 for cytotoxic T-cells, CD14 for monocytes as an indicator for macrophages, CD20 for B-cells, CD56 for NK-cells, and FoxP3 for regulatory T-cells. We performed single stain IHC for CD4 (anti-CD4, mouse monoclonal, clone 4B12; DAKO 1:100), CD8 (anti-CD8+, mouse-IgG2b, clone 4B11; Leica 1:400), CD14 (anti-human CD14, mouse, clone M5E2; BD Biosciences 1:100), CD20 (CD20cy, mouse, clone M0755; DAKO 1:1600), CD56 (anti-CD56, mouse, clone M7304; DAKO 1:15) and FoxP3 (Anti-FOXP3, mouse, clone 236A/E7; ABCAM 1:400).

For all stains endogen peroxidise activity was blocked with 0.3% H_2O_2/MeOH solution for 20 minutes. Antigen retrieval was achieved in boiling 10/1 mM Tris/EDTA buffer for 10 minutes and washed with phosphate-buffered saline (PBS) twice for 5 minutes for CD4, CD8, CD14 and CD56. For CD20 and FoxP3 antigen retrieval was achieved in boiling citrate (pH 6.0) for 12 minutes, cooling for 2 hours and washed with demiwater twice for 5 minutes. Tissue sections were incubated with the primary antibody diluted in PBS containing 1% bovine serum albumin (BSA) overnight. The tissue sections were incubated with BrightVision-Poly/HRP for 30 minutes. The slides were washed 3 times with PBS

for 5 minutes between each step. The antigen-antibody reaction was visualised with 0.05M Tris-HCL buffer (pH 7.6) with 0.05% of 3,3'-diaminobenzidine (DAB) and H_2O_2 30% for 10 minutes in CD8, and with DAB+ for the other stains. All slides were counterstained with haematoxylin. Cells positive for the antigens stained brown. A tonsil was used as a positive control, and a section without primary antibody served as a negative control.

CELL COUNTS

Images were captured using the Pannoramic 250 digital slide scanner (3DHISTECH Ltd.). We selected five areas representing the density of the immune infiltrate in each sample. Images of these five areas were captured in each stained slide using Pannoramic viewer at 40x. Half of the images contained the epithelial layer and half of the image contained the subdermal stroma. Stromal and epithelial cells were manually counted using the cell counter plug-in in ImageJ 1.48v software (National Institutes of Health, USA) by two individual researchers (MvdL and SvdS). The mean cell count for epidermal and stromal cells per image was calculated. Cell counts are converted and presented as number of cells per mm^2. Each case was assigned a random subject number to ensure both researchers were unaware of the clinical characteristics of each case when analysing the immune infiltrate.

The T-cell and monocyte infiltrate in VPD patients were compared to the infiltrate seen in samples of vulvar HSIL patients and healthy controls as published previously.[13, 14] We used the median cell counts for CD4, CD8, CD14, and FoxP3 for healthy controls (n=30) and patients with vulvar HSIL (n=43).

STATISTICAL ANALYSIS

All data were analysed using Statistical Package for the Social Sciences software package 20 (IBM SPSS 20). Data are presented in a descriptive manner. GraphPad Prism 5.03 (Graphpad Software Inc.) was used to illustrate the data.

The average measures Intraclass Correlation Coefficient (ICC) was calculated using the Cronbach's alpha method for inter-rater reliability. In case of poor consistency, defined as Cronbach's alpha <0.7, cases in which the cell counts differed more than 25% were identified. In cases with less than 10 counted cells, the difference was accepted. In cases the counted cells were more than 10, the cases were reassessed to ensure the entire views were counted, and difficulties in differentiating stained cells and blur was discussed to resolve disagreement between the two researchers to decide on a representative cell count. A log-transformation was performed as the inter-observer residues were not normally distributed. The ICC was excellent for all stains except for the epithelial counts in the CD14 stain. This was due to one outlier in a case of an extremely dense infiltrate which covered the basal membrane. After sensitivity analysis in which the outlier was excluded, the ICC was acceptable. The ICC and sensitivity analysis are reported in supplement table 1.

The Shapiro Wilk test was used to determine a normal distribution. All variables were non-parametric. The Mann Whitney U test was used to compare independent groups: healthy controls versus vulvar HSIL patients versus vulvar Paget disease patients. The Wilcoxon Signed Rank test was used to compare the continuous variables between groups: first sample taken before versus first sample taken after treatment. Two sided *p* values <0.05 were considered statistically significant.

RESULTS

PATIENT CHARACTERISTICS

We selected 10 patients with non-invasive VPD who were treated with topical 5% imiquimod cream: 6 from the Radboudumc and 4 from the LUMC. The median age of the patients at time of diagnosis was 72 (63-90) and the median follow-up time was 39 months (range 4-166 months). Of the 10 patients, four (40%) had a complete response, 4 (40%) a partial response and 2 (20%) no response to treatment. See table 1 for an overview of the patient characteristics. An overview of the used patient materials is given in supplement table 2.

Characteristic	N=10
Age at diagnosis (years)	
Median	72 (63-90)
Imiquimod treatment (weeks)	
Median	13 (10-24)
Response to imiquimod treatment, n (%)	
CR	4 (40%)
PR	4 (40%)
NR	2 (20%)
Follow-up time (months)	
Median	39 (4-166)
Follow-up status	
Alive	9 (90%)
Dead, cause unknown	1 (10%)

TABLE 1 Patient characteristics.

CR: complete response, defined as no visible residual lesion. PR: partial response, defined as a decrease in lesion size. NR: no response, defined as stable disease or increase in lesion size.

Characteristic		Samples taken before treatment *n* (%) (*N*=17)	Samples taken after treatment *n* (%) (*N*=7)
Infiltrate	Strong	5 (29.4%)	4 (57.1%)
	Moderate	6 (35.3%)	1 (14.3%)
	Minimal	5 (29.4%)	2 (28.6%)
	None	1 (5.9%)	0
Cell type	Mixed	1 (5.9%)	2 (28.6%)
	Mixed, favouring granulocytes	1 (5.9%)	
	Mixed, favouring eosinophiles	0	1 (14.3%)
	Granulocytes	1 (5.9%)	0
	Lymphocytes	8 (47.0%)	1 (14.3%)
	Lymphocytes and granulocytes	1 (5.9%)	0
	Lymphocytes and eosinophiles	0	1 (14.3%)
	Neutrophiles	1 (5.9%)	0
	Unclear	4 (23.5%)	2 (28.6%)

TABLE 2 Semi-qualitative analysis of immune infiltrate VPD.

Characteristics of the immune infiltrate and cell types after semi-qualitative analysis of vulvar Paget disease samples taken before and after treatment with imiquimod cream.

SEMI-QUALITATIVE ANALYSIS OF THE IMMUNE INFILTRATE

To establish that immune cells are present in VPD, we characterised the immune infiltrate in the epithelium and stroma on H&E slides (supplemental figure 1 and table 2). The composition of the immune infiltrate of the diagnostic VPD biopsies showed a mixed morphology, consisting mostly of mononuclear cells (52.9%), admixed with granulocytes in a subset of cases (17.7%). The majority of the pretreatment samples showed a moderate to prominent immune infiltrate (11 of 17 samples, data not shown). On H&E no obvious difference in immune infiltrate composition was noted in the 7 specimens taken after treatment. In a subset of the specimens taken after treatment, eosinophilic granulocytes were present (28.6%).

IMMUNE MICROENVIRONMENT IN VPD

To characterise the type of immune cells present in VPD, we performed immunohistochemical stainings. We found a great variety between the five images of each slide, and between the multiple samples taken per

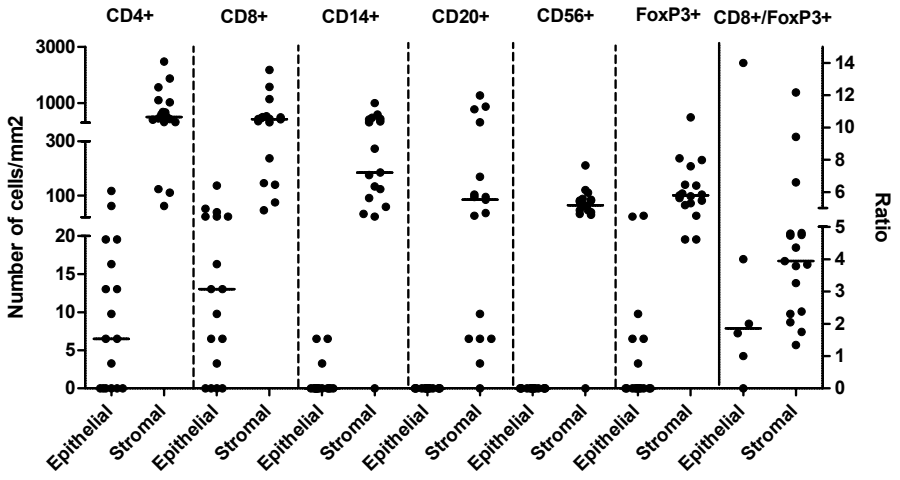

FIGURE 1 Distribution of immune cell types in non invasive VPD.

Number of median cell counts per sample for CD4 (T helper cells), CD8 (cytotoxic T-cells), CD14 (monocytes), CD20 (B cells), CD56 (NK cells), FoxP3 (regulatory T-cells) per mm2, and CD8+/FoxP3+ ratios in non-invasive vulvar Paget.

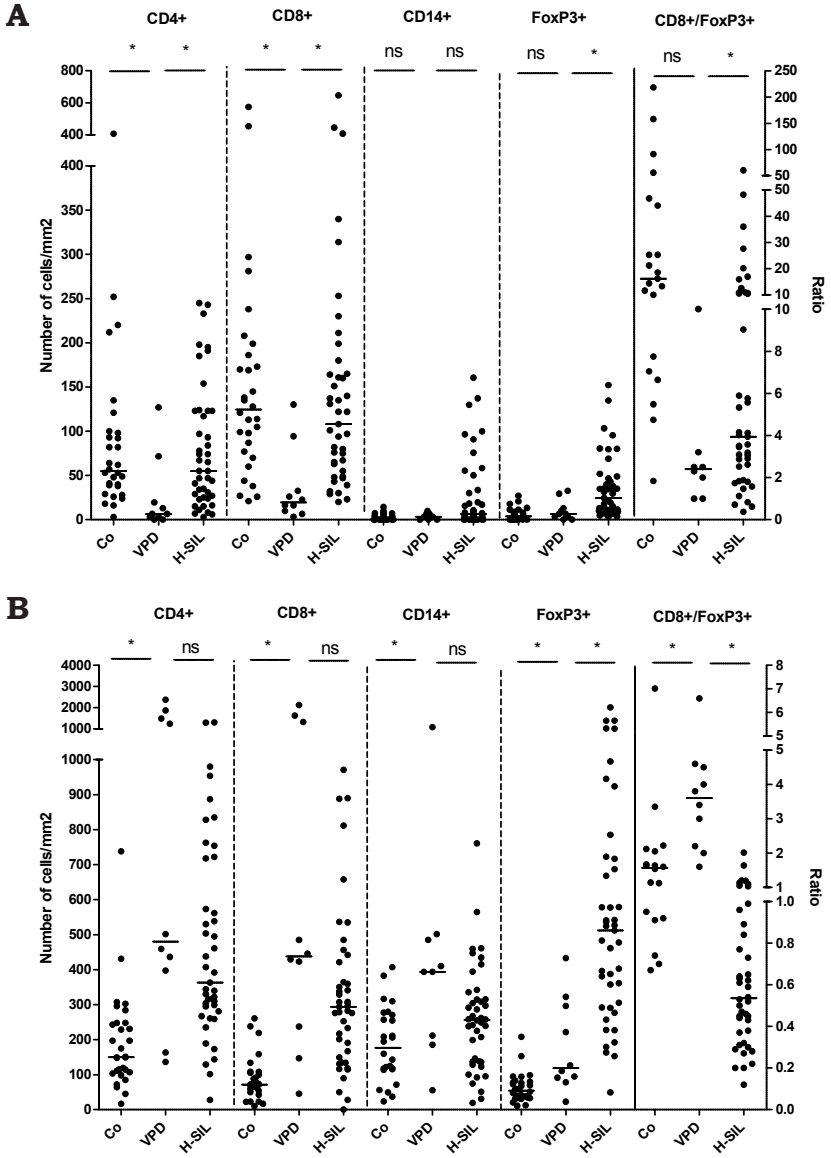

FIGURE 2 Distribution of immune cell counts in healthy controls, vulvar HSIL patients, and VPD patients.

*Number of CD4 (T helper cells), CD8 (cytotoxic T-cells), CD14 (monocytes) or FoxP3 (regulatory T-cells) per mm² and CD8+/FoxP3+ ratios. * is a significant (<0.05), or non significant (ns) p-value, analysed with the non-parametric Mann-Whitney U test used to determine differences between healthy controls versus vulvar Paget disease patients, and vulvar HSIL patients versus vulvar Paget disease patients. A: epithelial, B: stromal cell counts.*

patient. The vast majority of immune infiltrate consisted of T cells (94% of the epithelial compartment, and 67% of the stromal compartment, data not shown). The microenvironment of VPD is mainly infiltrated with CD4+ and CD8+ T-cells, followed by CD14+ cells, FoxP3+, CD56+ and CD20+ cells. See figure 1 for the distribution of the different cell types, and supplemental figure 2 for all stains in a representative case with a moderate immune infiltrate.

COMPARISON OF VPD TO HEALTHY CONTROLS AND VULVAR HSIL

The immune infiltrate of VPD was compared to healthy vulvar tissue and vulvar HSIL, a premalignant vulvar disease with a known response to treatment with 5% imiquimod cream. The median epithelial and stromal cell counts for 30 healthy controls, 43 vulvar HSIL and for 10 diagnostic VPD samples (prior to treatment) are presented in figure 2.

In the epithelial compartment of pretreatment VPD samples, the numbers of CD4+ and CD8+ T-cells are significantly decreased compared to healthy controls, and also was the CD8+/Foxp3 ratio. The numbers of intraepithelial CD14+ and FoxP3+ cells did not differ from healthy controls. In contrast, stromal numbers of CD4+, CD8+, Foxp3+ and CD14+ cells and the CD8+/Foxp3 ratio were significantly higher than healthy controls.

Both intraepithelial and stromal Foxp3+ cells were much more pronounced in vulvar HSIL compared to VPD, whereas the number of intraepithelial CD4+, CD8+, and Foxp3+ T-cells were decreased in VPD (figure 2). The CD8+/FoxP3 ratio was significantly lower in the epithelium of VPD compared to HSIL, and significantly higher in the stroma of VPD compared to HSIL.

In summary, we found that VPD lesions are highly infiltrated by immune cells. In the epidermis of VPD patients, numbers of CD4+ and CD8+ T-cells and the CD8+/Foxp3 ratio were lower compared to healthy controls. In contrast, VPD is immunologically characterised by high numbers of CD4+, CD8+, Foxp3+ and CD14+ cells in the stroma.

IMMUNE CELL COUNTS IN VPD BEFORE AND AFTER TOPICAL 5% IMIQUIMOD THERAPY

Immune cell counts before and after imiquimod therapy for the patient group and in individual patients are reported in table 3 and figure 3. Overall, there were no significant differences between samples taken before and after treatment. However, the numbers of CD8+ and FoxP3+ T cells in the epithelial compartment were increased after imiquimod treatment as was the ratio of CD8/FoxP3, reflecting a relative increase in CD8+ T cells. A decrease of CD14+ and an increase of CD56+ cells in the epithelium was seen in the post treatment samples. Due to the small sample size and differences in time point of post treatment samples it was not possible to draw any definite conclusions about changes in immune cell counts and relation to clinical responses in VPD patients who were treated with imiquimod.

Cell type	**Before treatment (n=10)** **Median (range)**	**After treatment (n=6)** **Median (range)**	***p*-value***
CD4+ (E)	16.3 (0.0-127.0)	13.0 (3.3-78.2)	0.600
CD4+ (S)	869.7 (397.4-2384.2)	804.5 (74.9-1742.6)	0.600
CD8+ (E)	19.5 (3.3-13.2)	76.5 (0.0-211.6)	0.173
CD8+ (S)	437.8 (45.6-2128.7)	945.5 (19.5-1708.8)	0.600
CD14+ (E)	3.3 (0.0-6.5)	0.0 (0.0-182.3)	0.496
CD14+ (S)	485.0 (55.3-1087.1)	136.7 (13.0-1204.3)	0.686
CD20+ (E)	0.0 (0.0-6.5)	1.6 (0.0-19.5)	0.414
CD20+ (S)	195.3 (6.5-953.7)	200.2 (0.0-2226.4)	0.753
CD56+ (E)	0.0 (0.0-3.3)	9.8 (0.0-19.5)	0.066
CD56+ (S)	97.6 (13.0-247.4)	84.6 (26.0-188.8)	0.917
FoxP3+ (E)	4.9 (0.0-32.6)	14.6 (0.0-61.8)	0.500
FoxP3+ (S)	118.8 (22.8-322.2)	200.2 (26.0-813.7)	0.463
CD8/FoxP3 ratio (E)	1.0 (0.0-2.5)	3.7 (2.0-6.6)	0.138
CD8/FoxP3 ratio (S)	4.2 (0.0-9.3)	3.4 (0.8-5.5)	0.674

TABLE 3 Comparison of immune cells before and after treatment.

Median cell counts per mm^2 in the first samples taken before or after treatment per patient.
** Significant p-values <0.05 by analysis with the non-parametric Wilcoxon Signed Rank test used to determine differences between samples taken before versus after treatment with imiquimod cream. (E) = epithelium, (S) = stroma.*

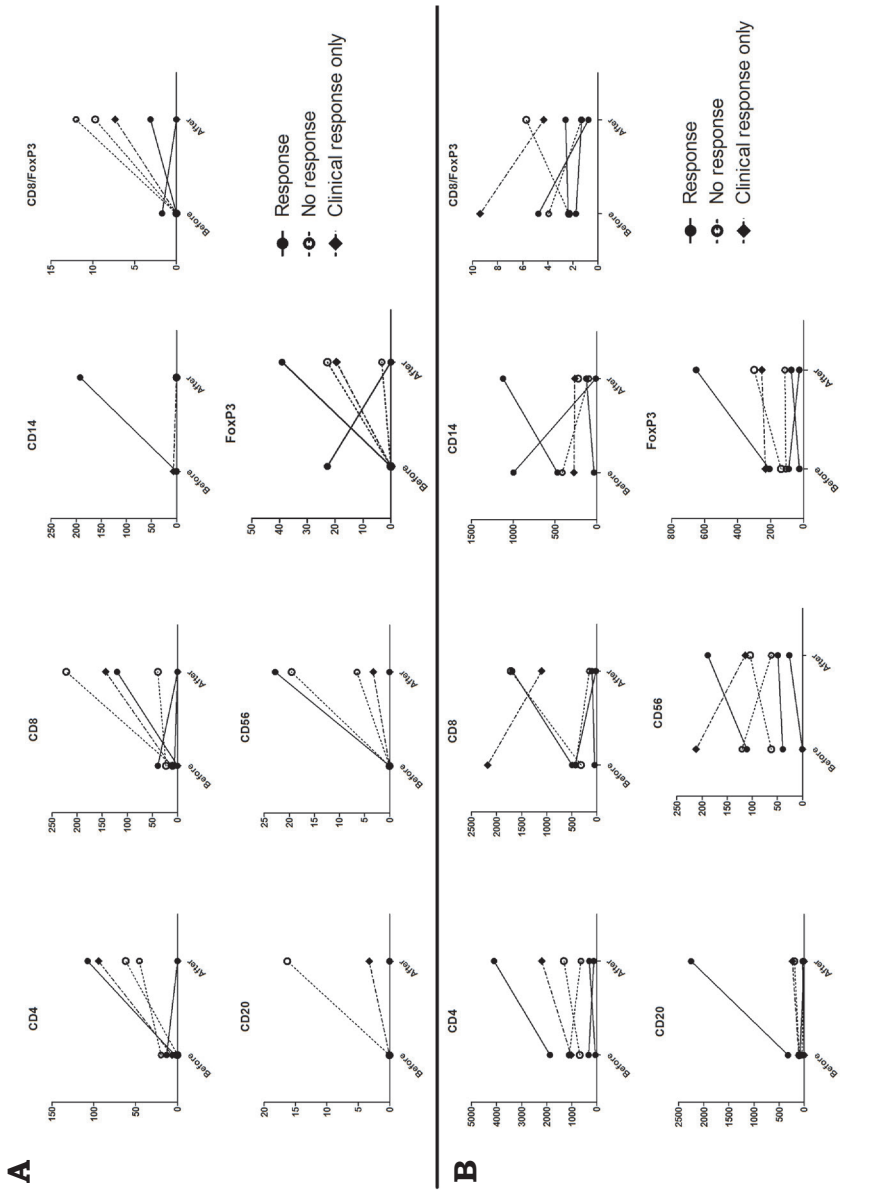

FIGURE 3 Immune cell types in non invasive VPD before and after imiquimod treatment in individual patients.

Number of median cell counts per sample for CD4 (T helper cells), CD8 (cytotoxic T-cells), CD14 (monocytes), CD20 (B cells), CD56 (NK cells), FoxP3 (regulatory T-cells) per mm^2, and CD8+/FoxP3+ ratios in non-invasive vulvar Paget disease before and after treatment with 5% imiquimod cream. A: epithelial compartment, B: stromal compartment.

DISCUSSION

This study is the first to describe the immune microenvironment in non-invasive vulvar Paget disease compared to the immune infiltrate to healthy controls and vulvar HSIL. We found that the epithelium of VPD has few immune cells, whereas the stromal compartment is highly infiltrated by CD4+, CD8+, Foxp3+ T cells, and CD14+ cells.

In the normal healthy vulvar skin, the immune infiltrate consists mainly of T-cells.[13] Pathological review of non-invasive VPD samples revealed that VPD is histologically characterised by a prominent stromal infiltrate consisting of a mixed population of immune cells with a prominence of T-cells. Monocytes, NK-cells and B-cells are present, but are much less abundant. The epithelial compartment of VPD seems immunocompromised as the numbers of CD4+ and CD8+ cells as well as the CD8+/Foxp3 ratio were significantly decreased compared to healthy controls, whereas the stromal compartment contains significantly more T-cells and monocytes. We hypothesise that the immune cells are unable to penetrate the intraepithelial compartment to clear the Paget cells. There are three possible explanations for this phenomenon. First, an acute immune response may occur at the onset of VPD. It is known that there is severe delay in diagnosing VPD, it is possible the acute immune response faded into a chronic infiltrate by the time the first histological biopsy is taken. Second, the Paget cells may excrete cytokines that suppress a local immune response and impede the immune cells to penetrate the basal membrane. This phenomenon where local immunity is suppressed was recently described in serous tubal intraepithelial carcinoma as a precursor for serous ovarian cancer.[15] Third, it is possible that Paget cells are not recognised by the immune system as malignant or aberrant cells. This may be due to limited or no neo-antigen production by Paget cells, which to our knowledge has never been investigated. Whether the immunocompromised epithelium or the strong stromal immune infiltration may point to the underlying pathogenesis of VPD, should be examined in future studies.

We observed a high variability in the immune microenvironment between patients, as well as between samples from one patient. A possible explanation is that the composition of the immune infiltrate is influenced by the experienced symptoms, or by topical treatments like dermatosteroids. Patients who experience severe symptoms like itching may scratch more

often, causing a non-specific chronic inflammation as often seen in lichen simplex chronicus.[16] On the other hand, the main symptom in psoriasis is pruritus, whereas psoriasis is associated with a minimal infiltrate. Therefore it is unlikely the observed heterogeneity is solely based on an itch-scratch-cycle.

In solid tumours, the tumour microenvironment has a key role in oncogenesis and tumour progression.[17] A strong Th1 cytotoxic microenvironment, consisting of CD8+ cytotoxic T cells, CD4+ T helper cells, M1 macrophages, NK-cells and dendritic cells, is associated with a more favorable prognosis and therapy responsiveness. In vulvar HSIL, high stromal CD8+/Treg ratio's and low amounts of epithelial macrophages are associated with better clinical outcomes.[14] A high CD8+/Treg ratio in the stroma or increased numbers of stromal CD4+ or CD8+ T-cells expressing the co-inhibitory markers TIM3 or NKG2A are associated with a prolonged recurrence free survival time, or an absence of recurrences.[13] In this study, we found a high CD8+/Foxp3 ratio in the stroma of VPD as well as high numbers of CD4+ and CD8+ cells, but the clinical significance should be examined in larger studies.

Compared to healthy controls and vulvar HSIL, high numbers of stromal CD14+ monocytes are present in VPD. The significance of these CD14+ cells in VPD is not clear. In general, CD14+ monocytes are marked as macrophages but can be precursors of (immature) inflammatory DCs as well; where CD14+ DCs are described to reside in the tissues and lymph nodes as interstitial DCs.[18-20] Subtyping can be done by CD163 for M1 or M2 macrophages and by CD11c or CD68 cells for DCs or DC-like-macrophages.[21] In melanoma and cervical carcinoma, an adequate immune response is reflected by if stromal M1 macrophages predominate the M2 macrophages.[21, 22] A recent study showed that M2 type CD163+ macrophages were associated with malignant progression in VPD.[12] Dendritic cells, and Langerhans cells in the epithelium, are important in the tumour micro-environment and are for example involved in the regression of melanomas.[23] Recently it is reported in melanoma research that DC's expressing galectin 9 (Gal9) reflect an IFNy related Th1 cell immune response, and are an independent predictor of favourable survival.[21] In dysplastic naevi a higher density of CD1a+ Langerhans cells was described as compared to both invasive melanoma and melanoma *in situ* illustrating the importance of presence of antigen presenting cells. Interestingly, it is known

that CD14+ cells can differentiate into CD1a+ Langerhans cells under inflammatory circumstances.[24] Indeed, in vulvar HSIL, HPV clearance after treatment with imiquimod was associated with a decreased number of intraepithelial CD14+ cells and an increased number of CD1a+ Langerhans cells.[6] Another study in patients with vulvar HSIL it was shown that the risk of recurrence was highly associated with a dense CD14+ macrophage intraepithelial infiltrate.[14] Further characterization of the CD14+ cells is needed to clarify the significance in VPD.

Imiquimod is an immune response modifier that triggers toll-like receptor 7 (TLR-7) which leads to T-cell activation and an anti-tumour response.[25, 26] In a melanoma-bearing humanised mouse model imiquimod was found to directly mobilise plasmacytoid dendritic cells and to trigger cytotoxic functions: the expression of type 1 IFN response genes was up regulated.[27] Topical immunotherapy with imiquimod cream was proven to be effective for vulvar HSIL.[5] Clinical responses after imiquimod treatment were associated with increased numbers of intraepithelial CD1a and CD8+ cells and a decrease in Treg cells in the stroma.[6,28] Although our sample size was too small to draw any definite conclusions from the biopsies taken before and after imiquimod therapy, we found that the numbers of CD8+ cells and the ratio of CD8/FoxP3 in the epithelial compartment were increased after imiquimod treatment whereas the numbers of CD14 decreased, suggesting a shift towards a type 1 IFNy related immune response which is the hallmark of an effective immune response. Recently, we completed a prospective study on treatment of VPD with topical 5% imiquimod cream (NCT02385188).[29] An analysis of pre- and post treatment immune infiltrate in treated patients will reveal if changes in immune cell counts correlate with clinical outcomes in VPD. Based on the results of this study we will further focus on the phenotyping of T-cells, DC's and macrophages in VPD.

In conclusion, the microenvironment of VPD differs from the healthy skin and vulvar HSIL. An immunocompromised epidermis and a stromal department rich of immune cells characterise VPD. Future research will focus on further specifying the T-cell population and myeloid cells in VPD, and correlation of changes in the immune infiltrate after imiquimod treatment to clinical outcomes.

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SUPPLEMENT TABLE 1 Intraclass correlation coefficient.

Cell type	Epithelial cell count	Stromal cell count
CD4+	0.905 (95%CI 0.780-0.959)	0.990 (95%CI 0.977-0.996)
CD8+	0.968 (95%CI 0.926-0.986)	0.990 (95%CI 0.976-0.996)
CD14+	0.655 (95%CI 0.186-0.854)	0.967 (95%CI 0.921-0.986)
CD14+ SA	0.748 (95%CI 0.405-0.893)	0.856 (95%CI 0.660-0.939)
CD20+	0.973 (95%CI 0.938-0.988)	0.995 (95%CI 0.989-0.998)
CD56+	0.970 (95%CI 0.931-0.987)	0.979 (95%CI 0.951-0.991)
FoxP3	0.804 (95%CI 0.547-0.915)	0.984 (95%CI 0.963-0.993)

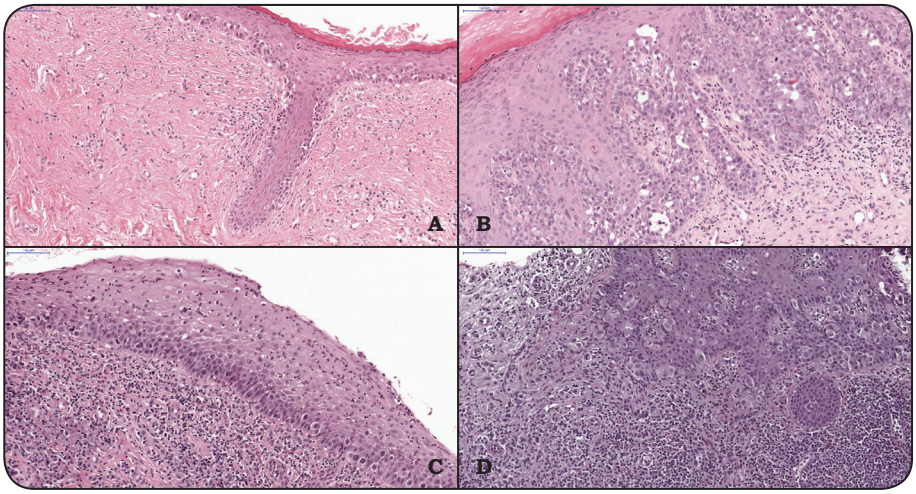
Intraclass correlation coefficient for the epithelial and stromal cell counts per staining protocol. SA: sensitivity analysis of ICC after exclusion of outlier.

SUPPLEMENT TABLE 1 Summary of available samples per patient.

Patient	Type of sample	Moment of sample	Treatment (weeks)	Weeks between sample and end of treatment	Clinical response	Histological response
1	Biopsy	Before treatment				
	Biopsy	After treatment	11	0	PR	CR
2	Biopsy	Before treatment				
3	Biopsy	Before treatment				
	Excision	After treatment	13	1	NR	NR
4	Biopsy	Before treatment				
5	Biopsy	Before treatment				
	Excision	After treatment	12	12	NR	NR
6	Biopsy	Before treatment				
	Biopsy	Before treatment				
	Excision	After treatment	13	125	PR	NR
7	Biopsy	Before treatment				
	Biopsy	After treatment	13	30	CR	NR
	Biopsy	After treatment	10	36	CR	NR
8	Biopsy	Before treatment				
	Biopsy	Before treatment				
	Biopsy	Before treatment				
	Biopsy	Before treatment				
	Biopsy	After treatment	11	6	CR	NR
9	Biopsy	Before treatment				
	Biopsy	Before treatment				
10	Biopsy	Before treatment				
	Biopsy	Before treatment				
	Biopsy	Before treatment				

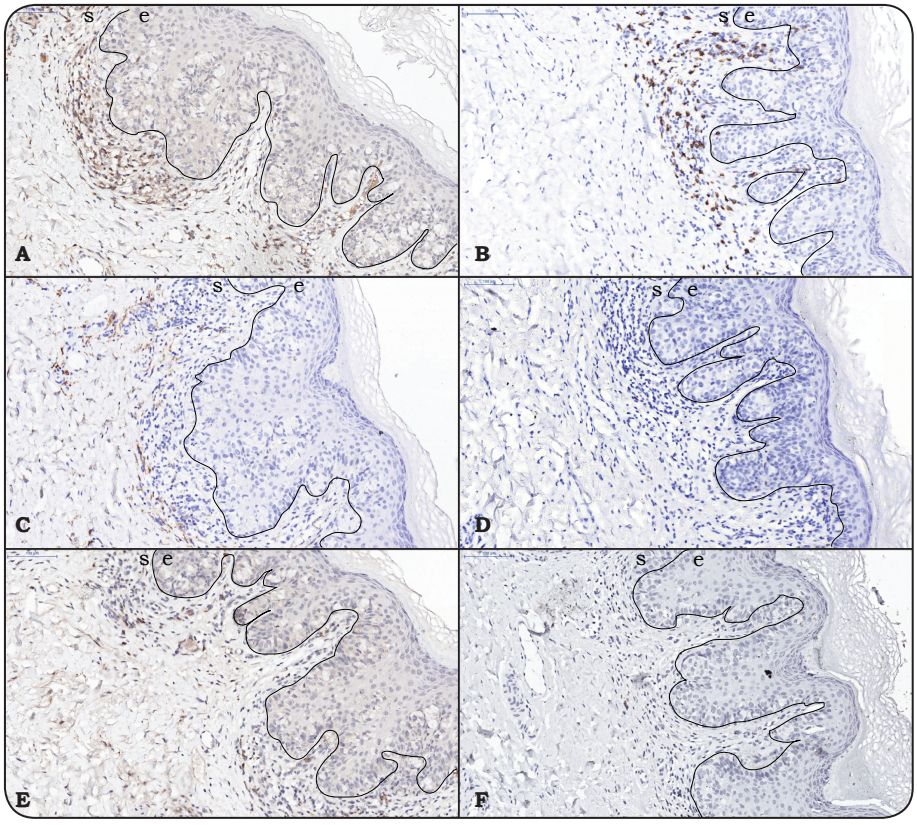
Overview of the available samples per patient, type of sample and moment the sample was taken, the number of weeks the patients received treatment with imiquimod cream, number of weeks between the end of treatment and moment the sample after treatment was taken, and the response to treatment. For clinical response; CR: complete response: no visible residual lesion. PR: decrease in lesion size. NR: no response, stable disease or increase in lesion size. For histological response; CR: no Paget cells present. NR: Paget cells present.

SUPPLEMENTAL FIGURE 1 Immune infiltrate density in non-invasive vulvar Paget disease.



Examples of the immune infiltrate in samples of 4 different patients with non-invasive vulvar Paget disease before treatment, H&E 20x. A: no infiltrate, B: minimal infiltrate, C: moderate infiltrate, D: strong infiltrate

SUPPLEMENTAL FIGURE 2 Immunohistochemistry in a moderately dense immune infiltrate.



Examples of the immunohistochemistry in the sample of 1 patient (subject 2) with a moderately dense immune infiltrate in non-invasive vulvar Paget disease before treatment with imiquimod cream. A: CD4, 20x. B: CD8, 20x. C: CD14, 20x. D: CD20, 20x. E: CD56, 20x. F: FoxP3, 20x. e: epithelium. s: stroma.

CHAPTER 8

THE PAGET TRIAL:

A MULTICENTER, OBSERVATIONAL COHORT INTERVENTION STUDY FOR THE CLINICAL EFFICACY, SAFETY, AND IMMUNOLOGICAL RESPONSE OF TOPICAL 5% IMIQUIMOD CREAM FOR VULVAR PAGET DISEASE

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ABSTRACT

Background: Vulvar Paget disease is a rare skin disorder, which is most common in postmenopausal Caucasian women. They usually present with an erythematous plaque that may show fine or typical "cake icing" scaling or ulceration that may cause itching, pain, irritation, or a burning sensation. Although most cases are non-invasive, vulvar Paget disease may be invasive or associated with an underlying vulvar or distant adenocarcinoma. The histological evidence of so-called "Paget cells" with abundant pale cytoplasm in the epithelium confirms the diagnosis. The origin of these Paget cells is still unclear. Treatment of choice is wide local excision with negative margins. Obtaining clear surgical margins is challenging and may lead to extensive and mutilating surgery. Even then, recurrence rates are high, ranging from 15% to 70%, which emphasises the need for new treatment options. A number of case reports, retrospective case series, and one observational study have shown promising results using the topical immune response modifier imiquimod.

Objective: This study aims to investigate the efficacy, safety, and immunological response in patients with non-invasive vulvar Paget disease using a standardised treatment schedule with 5% imiquimod cream.

Methods: Topical 5% imiquimod cream might be an effective and safe treatment alternative for vulvar Paget disease. The Paget Trial is a multicenter observational cohort study including eight tertiary referral hospitals in the Netherlands. It is ethically approved by the Medical-Ethical Committee of Arnhem-Nijmegen and registered in the Central Committee on Research Involving Human Subjects (CCMO) Register by as NL51648.091.14. Twenty patients with (recurrent) non-invasive vulvar Paget disease will be treated with topical 5% imiquimod cream three times a week for 16 weeks. The primary efficacy outcome is the reduction in lesion size at 12 weeks after end of treatment. Secondary outcomes are safety, immunological response, and quality of life. Safety will be assessed by evaluation of adverse events and tolerability of treatment. To evaluate the immunological response, various immunological markers will be tested on biopsy specimens taken before, during, and after treatment. Quality of life will be assessed with three questionnaires taken before, during, and after treatment.

Results: First results are expected in the summer of 2018.

INTRODUCTION

Cutaneous Paget disease was first described in a series of patients with nipple ulceration and an underlying breast carcinoma. This became known as mammary Paget disease (MPD).[1] When the same condition was reported on the scrotum and vulva, these were named extramammary Paget disease (EMPD).[2, 3]

The presence of so-called Paget cells in the basal layers of the epithelium is pathognomonic for this rare disease. The origin of these large cells with abundant clear, pale cytoplasm, which often contain mucin, remains unclear. The most common hypothesis is that Paget cells originate from adnexal structures, such as apocrine glands or multipotent stem cells in the basal layer of the epidermis.[4, 5] Other theories suggest the anogenital area contains mammary- like glands, or that Toker cells, also seen in the nipple in mammary Paget disease, are precursor cells for EMPD.[6-8]

The incidence rate of EMPD is 0.11 per 100,000 person-years, based on an epidemiological study with data of the Netherlands Cancer Registry.[9] Vulvar Paget disease (VPD) causes pain, itching or a burning sensation, and a skin lesion which can be described as a scaling, erythematous plaque which sometimes shows ulceration. VPD typically presents in postmenopausal Caucasian women.[8]

Vulvar Paget disease can be divided in primary VPD, which is cutaneous and secondary VPD, which is non-cutaneous. Table 1 illustrates the different types of VPD.[10].

Primary EMPD (cutaneous)	Type 1a	Associated with non-invasive, intraepithelial disease
	Type 1b	Associated with invasive disease
	Type 1c	Associated with an underlying adenocarcinoma
Secondary EMPD (non-cutaneous)	Type 2	EMPD originates from intestinal adenocarcinoma
	Type 3	EMPD originates from urothelial carcinoma

TABLE 1 Different types of vulvar Paget disease

VPD is associated with different malignancies, mainly an underlying vulvar, intestinal or urological malignancy, and breast cancer. About 20% of patients are

reported to have an associated malignancy in their history. Therefore screening for underlying carcinoma is advised, although there is no evidence for screening, and no consensus on the extent of the additional diagnostic procedures.[11,12]

Historically, the treatment of choice for VPD is wide local excision with clear margins, which is not always easy to realise on the vulva. Because Paget cells are found widely spread throughout the anogenital area, it is almost impossible to obtain clear surgical margins.[13,14] The recurrence rates of VPD are high: 15 to 70%, independent of margin status. The risk of recurrence is highest in the first year after treatment.[15] To improve obtaining clear surgical margins, Moh's Microsurgery (MMS) has been evaluated for treatment of VPD. In MMS, the lesion is excised and the entire margin is examined immediately.[16] In case the margin is not clear the excision is repeated, enlarging the circumference until the margins are clear. This technique may lead to lower recurrence rates.[17] However, large vulvar excisions may require plastic reconstruction.

Extensive vulvar surgery can cause permanent mutilation and functional impairment.[18-22] To address this problem, alternative treatment options such as photodynamic therapy, radiotherapy, chemotherapy, laser treatment and recently topical 5% imiquimod cream have been used in patients with VPD, with varying degrees of success.[23-30]

Topical 5% imiquimod cream is an immune response modifier. It binds to toll-like receptor 7, inducing an innate and cell-mediated immune response.[31] It has antiviral and antitumour properties and is registered for the treatment of condylomata acuminata, actinic keratosis and superficial basal cell carcinomas. Also Imiquimod has shown to be effective for human papilloma virus (HPV) induced usual vulvar intraepithelial neoplasia (uVIN).[32, 33] The mechanism of action of imiquimod and local immunity in VPD are not known.

More recently, a number of case reports, case series and one observational trial that reported on the use of topical 5% imiquimod cream for VPD, showed that imiquimod may be an effective treatment option.[34, 35] A systematic review also concluded it is an effective alternative for VPD.[36] However, most studies described limited numbers of patients, various treatment schedules and short follow-up periods. Therefore, it is impossible to pool data from previous study to make final conclusions about the

efficacy. The authors of the systematic review also mentioned the risk of publication bias: only positive results may be published retrospectively.[36]

OBJECTIVE

The objective of this study is to assess the clinical efficacy, safety, and local immunity of topical 5% imiquimod cream in patients with non-invasive vulvar Paget disease.

METHODS/DESIGN

STUDY DESIGN

This study is a multi-centre, prospective, open-label observational cohort study in patients with histologically proven, non-invasive VPD. Patients will be treated with topical 5% imiquimod cream three times a week for 16 weeks, with follow-up of one year after the end of treatment.

STUDY SETTING

As VPD is rare, with an estimated incidence of 4 to 7 cases per year in the Netherlands, the trial will be carried out in 7 tertiary referral hospitals with a vulvar clinic in the Netherlands. Vulvar clinics are outpatient multidisciplinary clinics with participation of both gynaecologists and dermatologists who are specialised in disorders of the vulva.

Participating centres are:
- Antoni van Leeuwenhoek, Netherlands Cancer Institute, Amsterdam;
- Catharina Ziekenhuis, Eindhoven;
- Erasmus Medical Center, Rotterdam;
- Leiden University Medical Center;
- Radboudumc, Nijmegen;
- University Medical Center Groningen;
- University Medical Center Utrecht.

PARTICIPANTS

All patients with histologically proven non-invasive cutaneous VPD visiting or referred to a participating clinic will be asked to participate in this study. We estimate to include one patient per centre per year because of the rarity of the disease.

Inclusion criteria are: non-invasive VPD (primary or recurrence after earlier surgery or imiquimod treatment more than 6 months ago), age 18 and older, willing and able to comply with the protocol and provide informed consent in accordance with institutional and regulatory guidelines. Most patients are expected to be elderly, postmenopausal women, who may suffer from co-morbidities. All patients are instructed on how to apply the imiquimod cream by their clinician, according to the leaflet provided by the manufacturer, and using a mirror. In cases the patient is physically unable to apply the cream, a health care provider (for example a nurse of the nursing home, or via domiciliary care) will receive written instructions. If the patient consents, a printed photograph in which the affected skin is marked will be provided. Main exclusion criteria are: current invasive VPD, underlying adenocarcinoma and treatment of the vulva with topical 5% imiquimod cream during the last 6 months.

SAMPLE SIZE

Based on the estimated incidence of VPD in the Netherlands, viability is set at 20 inclusions. Our sample size considerations are based on the response rate. The primary outcome variable is the response at 12 weeks after end of treatment with topical 5% imiquimod cream. The only observational study on this topic, at time of conception of this trial, reported a response in 9 out of 10 women.[35] Assuming a complete response rate of 80%, a cohort size of 20 patients is sufficient to estimate the complete response rate with a standard error of 9%, using the normal approximation for the binomial distribution. As we presume that the dropout rate will not exceed 20%, a maximum of 25 patients will be included. When 20 patients have been treated with topical 5% imiquimod cream for at least 8 weeks, we will stop recruitment.

STUDY INTERVENTION

All patients will be treated with topical 5% imiquimod cream 3 times a week for 16 weeks. This treatment schedule is based on the treatment schedule for condylomata acuminata and on a previous randomised controlled trial of imiquimod 5% for usual vulvar intraepithelial neoplasia.[31, 33] The healthy skin around the visible lesion can be protected with an indifferent basic ointment. Patients are allowed to use topical 3% lidocaine in vaseline ointment if they experience pain at the application site. There must be a one hour interval between the application of different topical agents. Patients are also allowed to use paracetamol. In case of severe pain, when paracetamol and 3% lidocaine ointment are insufficient, it is permitted after consultation with the clinician to stop the treatment with topical 5% imiquimod cream for one week at a time. Patients are allowed to stop/delay treatment for a total of 3 weeks within the assigned treatment period.

In case of a suspected secondary bacterial infection, fucidin cream or ointment 20 mg/g will be prescribed. The patient will apply the fucidin cream or ointment 3 times a day, according to the prescription. No other local products than imiquimod cream, lidocaine, indifferent moisturisers or fucidin are allowed to be applied at the lesion site. On individual basis, other topical products will be considered as a protocol violation.

STUDY SCHEDULE

Patients will visit the clinic 7 times during the study; the final visit will be one year after the end of treatment (Table 2). One consultation will take place by telephone. During these consultations, pain will be measured by means of the visual analog scale (VAS) score for pain. Pain, burning and itching will be asked on a four-point likert scale. During the visits, the clinical response will be evaluated by vulvar examination and bi-dimensional measurement of the visible lesions. The histological effect will be assessed by pathological assessment of the presence of Paget cells in the biopsy sample(s) taken 12 weeks after the end of treatment. All biopsy samples, taken before, during and after treatment, will be taken around the same location. The site of the first biopsy is most likely the most evident lesion, causing a clinically visible lesion. The site of this biopsy will

	0 w	Baseline	4 w	10 w	16 w	28 w	40 w	52 w	68 w
Mapping and mammography	X								
Written informed consent	X								
Require histological samples (incl. mapping) and mammography results from referring hospital	X								
Imiquimod 5% cream		*Start*			*Stop*				
Consultation by phone				X					
Consultation at outpatient clinic of involved research clinic, containing:		X	X		X	X	X	X	X
• Vulvar examination, measurement and photo documentation		X	X			X			X
• VAS score		X	X	X	X	X	X	X	X
• Tolerability questionnaire			X	X	X				
• Biopsy	X		X			X			
• EQ5D		X	X			X			
• DLQI		X	X			X			
• FSDS		X	X			X			
• Review patient diary			X	X	X				

TABLE 2 Study schedule presenting an overview of all study activities.

be recorded in the Case Report File, to ensure other biopsies will be taken at the same area. Quality of life will be assessed before, during and after treatment, using 3 questionnaires on general health (EQ5D), dermatological quality of life (DLQI) and (if applicable) sexual functioning (FSDS).

Safety will be evaluated by documentation of all adverse effects, recorded by the clinician, and by the patient in the patient diary.

The immunological effect will be assessed by comparing the results of an additional immunohistochemistry stains performed on all 3 samples taken around the same location at baseline, 4 weeks after start of treatment and 12 weeks after end of treatment. All biopsies will be taken at about the same location, to ensure the local microenvironment is as similar as possible in all samples. There are limited data on the tumour microenvironment in VPD; currently we perform a pilot study to investigate the parameters in the immune infiltrate in VPD. We are investigating which immune cells are present in VPD, and will use this knowledge to further explore which immune cells respond to the topical imiquimod cream, and which role they play in the origin and treatment of VPD.

STUDY ENDPOINTS

The main study outcome is the clinical response. This will be assessed by determination of the reduction in lesion size 12 weeks after the end of treatment. This will ensure any local skin effects caused by treatment will be healed at time of examination. All measurements during the study will be conducted by the same trained and experienced local clinician. Photographs for documentation will be taken with a ruler alongside the lesions. The comparison between the lesion size at the start of treatment and 12 weeks after the end of treatment can lead to the following outcome:

- Complete response (CR): defined as disappearance of the lesion and histological confirmation of disappearance;
- Partial response (PR): defined as decrease by ≥50% of total lesion size;
- No response: defined as <50% decrease of total lesion size;
- Progressive disease: defined as ≥25% increase of total lesion size or progression into invasive disease and/or adenocarcinoma.

Secondary outcomes are the safety, quality of life and the assessment of local immunological response. These outcomes will be assessed according to the following criteria:

- Safety: all adverse events that occur during the study will be collected by the clinician at every consultation (at the clinic or via telephone) and by the patient using a standardised patient diary.
- Quality of life: results of the 3 questionnaires (EQ5D, DLQI and, if applicable, FSDS) taken before, during and after treatment will be compared.
- Local immunological response will be assessed by a set of markers, to be determined, in tissue samples obtained by vulvar biopsy before, during and after treatment.

STATISTICAL ANALYSIS

An intention-to-treat (ITT) and per protocol (PP) analysis will be performed. The population included in the ITT analysis is defined as all patients that have started treatment with topical 5% imiquimod cream. PP analysis will include patients that have completed treatment with topical 5% imiquimod cream according to protocol: during 8 weeks. Two-tailed *P* values <0.05 will be considered statistically significant. Our primary study parameter

is the clinical response to topical 5% imiquimod cream. Twelve weeks after the end of treatment the clinician will examine the vulva of the patient, and assign the patient in one of the response categories as defined above. Estimates of the percentage responders per response category will be presented, with corresponding 95% confidence intervals. The relation between treatment duration and dose versus response will be explored. Safety will be analysed in a descriptive manner, presenting all adverse events (local and systemic) in all subjects treated with topical 5% imiquimod cream. Also the use of painkillers, lidocaine ointment and discontinuation of treatment will be reported.

Quality of life will be assessed by three questionnaires. The EQ5D results will be converted to the crosswalk index values, using the Crosswalk Index Value Calculator.[37] The DLQI results will be categorised according to the instruction manual, ranging from 'no effect at all at patients life' to 'extremely large effect on patients life'.[38] The result of the FSDS is the sum of the answers. Descriptive statistics will be used to present the changes outcomes during treatment versus before treatment, and after treatment versus before treatment. A sub-analysis of responders and non-responders will be conducted.

The immunological results will be counted and compared between the different biopsy samples. These data will be reported in a descriptive manner.

ETHICS

This study will be conducted according to the principles of the Declaration of Helsinki (2008) and the Medical Research Involving Human Subjects Act (Dutch: WMO). The protocol has been medical-ethically approved by the Medical-Ethical Committee of Arnhem-Nijmegen to be conducted in all 8 centres (NL51648.091.14). Before enrolment to the study, written informed consent will be obtained from all patients.

RESULTS

The study opened for enrolment in January 2015. Currently, 17 patients are participating in this trial. The first results are expected in the summer of 2018.

DISCUSSION

Currently, this study is the first prospective study examining the clinical efficacy of topical 5% imiquimod cream in patient with non-invasive VPD using a standardised treatment schedule during 16 weeks. In addition, this study will also be the first to investigate the safety, quality of life and immunological response of 5% imiquimod cream therapy in patients with VPD.

Until now, about 25 retrospective case series have been published on this topic. These studies show high success rates. The effectiveness of topical 5% imiquimod cream for VPD in these cases might be overrated due to publication bias in these retrospective cases. Most of the retrospective series have used different treatment schedules. The prospective trial of Marchitelli et al. used a different treatment schedule per patient.[35] In most case studies, treatment was continued until the patient obtained a complete response. The pilot study by Cowan et al. investigated the clinical response after 12 weeks of treatment in eight patients with non-invasive VPD.[34] Patients applied the cream three times a week. Six patients had a clinical and histological complete response; the other two had a partial response with histological persistence. In our study, all 20 consecutive patients will be treated according to the same treatment schedule: 3 times a week for 16 weeks. Currently, there are no guidelines for topical 5% imiquimod treatment for VPD. We based the treatment schedule on the treatment schedule for condylomata acuminata, as this is a registered indication and therefore we consider this treatment schedule to be safe for the genital skin.[31] Furthermore, VPD may be considered a vulvar premalignancy, and the same treatment schedule is used in a previous randomised controlled trial of imiquimod 5% for usual vulvar intraepithelial neoplasia.[33]

There are very limited data concerning the influence of VPD on everyday life of the patient. It is reported that vulvar surgery may contribute to decreased quality of life and sexual functioning compared to healthy patients. As VPD has high recurrence rates, we assume (repetitive) surgical treatment may have significant psychosexual effects on the patients. Topical treatment with 5% imiquimod cream will not induce scarring, nor will it alter the anatomy of the vulva. Because there is a lack of data on this specific topic, we will investigate the quality of life with 3 different questionnaires before, during and after treatment.

The mechanism of action and immunological effects of 5% imiquimod cream in vulvar Paget disease are uncertain. It is likely that imiquimods' immune modulating effect induces a local immune response resulting in clearance of the Paget cells. Investigating the immunological response in biopsy specimen taken before, during and after treatment will provide insight in the local effects of imiquimod in the skin and also in the underlying mechanisms of action. Unfortunately, there is no current literature on this topic. Therefore we are conducting a pilot study, investigating the microenvironment of VPD, to assess which markers may be valuable in understanding the immunological response in VPD.

In conclusion, VPD remains an elusive disease. Surgery has been the treatment of choice over a century. Due to high recurrence rates and the vulnerable patient population affected by the disease, there is a need for other, less invasive treatment options. Topical 5% imiquimod cream may be an attractive alternative. Our trial will investigate the clinical efficacy of topical 5% imiquimod cream in 20 patients with a standardised treatment schedule. This study will also evaluate the safety, quality of life and immunological response while using 5% imiquimod cream.

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CHAPTER 9

GENERAL DISCUSSION

This thesis focuses on glandular lesions of the vulva, with a special interest in vulvar Paget disease (VPD). Due to its' rarity there is limited knowledge on VPD. Worldwide there are few available guidelines on VPD: two guidelines from the Royal College of Obstetricians and Gynaecologists include VPD and several local guidelines, generally based on expert opinion and general practice. The aim of this thesis was to improve care for patients with VPD and improving the knowledge on this disease by studying some clinical challenges.

In the Netherlands (population ~ 17 million), each year 10 to 15 women are diagnosed with any type of VPD. Due to the rarity of VPD, many clinicians are not familiar with this disease and may not recognise it. Fortunately the histopathological signs of VPD are very typical, so once a biopsy has been taken and the pathologist is familiar with vulvar pathology, the diagnosis VPD can be made rather quickly.

However, the risks and consequences of VPD are unclear. This means most patients get limited information on the diagnosis VPD, and what to expect.

For a long time, clinicians have been convinced that VPD is a life threatening malignant disease which may either metastasise to, or should be considered as a metastasis of malignancies of the breast, intestinal or urological tract. Mostly, extensive locoregional surgery is advised to women who present with VPD. These statements are the main topics in this thesis, and are all rebutted to some extent.

This leads us to the final conclusions which are important for all clinicians who may encounter VPD patients:

- Non-invasive VPD is a chronic skin disorder with a limited malignant potential;
- The risk of having a concurrent malignancy is very small;
- Treatment can be less invasive than extensive (perpetual) locoregional surgery.

PATHOGENESIS

VPD is considered to be an adenocarcinoma in situ of the vulvar skin. Little is known about the exact pathogenesis of VPD. The diagnosis is confirmed when typical Paget cells are seen in the vulvar skin but the origin

of these cells remains elusive. We are now almost 150 years after VPD was first described. And still, no-one has ever proven the origin of this disease.

In the previous century, the skin adnexa were reported as the possible origin of Paget cells.[1,2] A relatively recent study from 2006 investigated twelve cases of VPD or anal extramammary Paget disease (EMPD, *i.e.* Paget disease located elsewhere than the breast).[3] All cases showed Paget cells in the interfollicular epidermis, hair follicles, sebaceous and apocrine glands. In all cases, the Paget cells expressed CK19, a marker for follicular stem cells.

A Russian study from 2016 investigated 178 specimens of 146 patients with primary anogenital EMPD.[4] In more than 90% of the cases the skin adnexa were affected with disease, ranging from 3% of the apocrine secretory coils to 82% of the hair follicles.

The above mentioned studies suggest that the skin appendages of the vulva are a relatively common location for Paget cells. In mammary Paget disease (MPD) Paget cells are also suggested to have a glandular origin.[5] In MPD, the origin of the Paget cells is also unknown: the main hypothesis is that Paget cells arise from ductal carcinoma cells, either invasive or not invasive, which migrated to the epidermis of the nipple. This is called the epidermotropic theory.[6] A second theory is the transformation theory, in which keratinocytes transform in a malignant manner into Paget cells, suggesting VPD has a malignant potential. A third theory is the Toker cells theory[7]. Toker cells, which are polygonal, rather large, pale epithelial cells that may look like Paget cells. These cells are especially present in the nipple region, and are seen as precursor cells that may transform into Paget cells. The origin of Toker cells is unclear; these cells were first described in the nipple and later on also suggested as precursor cells for VPD.[8,9] The difference between Toker cells and Paget cells is defined as 'the lack of cytological stigmata of malignancy', such as cytologically atypia, which can be seen in Paget cells.[7] Besides, Toker cells contain melatonin.

Paget cells were first described in the nipple of cancerous breasts. There has always been a close link between MPD and EMPD, though, limited research compared the two diseases. The main findings are the great variety in the presence of Paget cells and their molecular profiles in both MPD as well as EMPD.[2,10]

However, the natural behaviour of Paget cells in both the breast and vulva seems to overlap. From a histological point of view, it is almost impossible to distinguish the vulvar skin from the skin of the nipple: the nipple and the vulva contain the same tissue, which led to the suggestion of breast-like tissue in the vulva. Mammary-like glands (MLG) are first described by Hartung in 1872 in a case of a patient who lactated from a vulvar lesion.[11] In 1994 van der Putte was the first to suggest EMPD originated from the MLG.[12]

In another study, the previously mentioned Russian study group investigated 181 cases of EMPD and found that all 4 cases with EMPD and an invasive carcinoma showed alterations in the MLG.[13] MLG showed ductal carcinoma in situ, columnar cell change and columnar cell hyperplasia. In 6 cases the authors saw colonization of the MLG by Paget cells. The authors concluded that rare cases of primary EMPD may originate in the MLG, where the neoplastic Paget cells migrate into the epidermis and later may breach through the basal membrane.

A different research group recently investigated whether MLG adenocarcinomas are immunohistochemically comparable to breast cancer.[14] There are five different molecular types of breast cancer, see table 1. The author investigated whether four subtypes of breast cancer (luminal A, luminal B, HER2 and basal-like) were present in the MLG adenocarcinomas. This study reported it is impossible to distinguish a MLG adenocarcinoma from VPD, solely based on immunohistochemistry (IHC). Most cases of non-invasive VPD were luminal A (70%), whereas only 29% of invasive VPD and 14% of MLG adenocarcinoma were of this subtype. HER2 overexpression was more common in invasive VPD than in the non-invasive VPD: 43% versus 30%, which may trigger more aggressive tumour behaviour. The authors suggest this may be the reason why non-invasive VPD has a low malignant potential. And in cases of HER2 overexpression in invasive VPD, patients may respond to trastuzumab therapy. Previous studies reported HER2 overexpression in non-invasive EMPD also,[15-19] and two case reports include patients with HER2 positive EMPD with good responses to trastuzumab in combination with paclitaxel chemotherapy.[20,21]

As reported in chapter 3 of this thesis, studies that report on HER2 overexpression in VPD show various results.[23] Only one study investigated the presence of Ki67 in VPD, and found it was highly expressed (defined as

Molecular type	Characteristics
Luminal A	• Hormone receptor positive, and • HER2 negative, and • Low levels of Ki-67 • Low-grade malignancy, relatively good prognosis
Luminal B	• Hormone receptor positive, and • HER2 positive, or • HER2 negative with high levels of Ki-67 • Slightly worse prognosis.
Basal-like (triple-negative)	• Hormone receptor negative, and • HER2 negative, and • More common in *BRCA1* gene mutation carriers.
HER2-enriched	• Hormone receptor negative, and • HER2 positive, • High-grade malignancy, worse prognosis • Targeted therapy possible.
Normal-like	• Like Luminal A, but with slightly worse prognosis.

TABLE 1 Molecular types of breast cancer[22]

more than 5%) in about 30% of cases, but did not appear to correlate to the prognosis of the disease.[24] VPD also seems to lack the hormone receptors.[25] This seems to strengthen the hypothesis that VPD is indeed most comparable to the breast cancer type Luminal A.

It is evident that Paget cells are related to glands. To truly understand the course of disease, it is necessary to understand where these cells originate and how they migrate and cause a skin lesion. One of the main issues in clarifying the pathogenesis is that, even though the vulva seems to be a small part of the female body, many theories are believed within different groups of clinicians, pathologists and researchers. For example, some consider MLG to be the answer, whereas others deny the existence of such glands. From a pathological point of view, it can be very interesting to differentiate all separately involved glands and discuss whether these are involved. This causes the literature on this topic to become more and more divided into believers of separate types of glands. Whereas from a more pragmatic clinical point of view, it doesn't matter which type of gland the cells come from as long as their malignant potential is clear, and how the symptoms they cause can be optimally cured.

Over the last several years, tumour immunology became an important topic within oncology. In many tumours the local microenvironment is investigated. For VPD little is known about the microenvironment. Chapter 7 describes the study we conducted to explore the immune infiltrate in non-invasive VPD. We found a mixed-cell immune infiltrate mainly consisting of T-cells, macrophages and natural killer (NK)-cells were also present. We compared our findings to the immune infiltrate found in healthy controls and high-grade squamous intraepithelial lesions (H-SIL) of the vulva. The immune infiltrate in VPD differs from healthy controls, suggesting the presence of Paget cells either influences, or is influenced by the immune infiltrate. Another recent study investigated the immune checkpoint inhibitors CTLA4 and PD-L1 in both EMPD and MPD.[26] PD-L1 was not expressed in any of the tissue samples. CTLA4 was expressed occasionally in both mammary as well as extramammary tissue samples. The influence and role of the tumour microenvironment in VPD is yet to be determined. Understanding the tumour microenvironment will help to improve treatment and may enlighten the pathogenesis of the disease.

CLINICAL PRACTICE

Any doctor can perform a vulvar biopsy in a patient with a vulvar lesion. In case the pathology report comes back with the conclusion: "Non-invasive primary/cutaneous Paget disease" the patient should be referred to an experienced gynaecologic oncologist, gynaecologist or dermatologist with a special interest for vulvar disease. The flowchart in figure 1 will aid clinicians in which diagnostics and treatment options can be considered.

However, the patient needs to be informed of the diagnosis, which will lead her to asking questions:

Q: What is vulvar Paget disease?
A: Vulvar Paget disease is a chronic skin disease that causes an irritated, itching, scaling redness of the vulva. We don't know how it originates. We found typical cells, called Paget cells in the skin of the vulva.

Q: My family and I googled Paget and found something with bone disease, is that the same?

A: No, both diseases are first described by an English doctor, James Paget, in the late 19th century, but there is no relation between VPD and the bone disease.

Q: My family and I googled Paget and found breast cancer, do I have cancer?

A: No, in the late 19th century a doctor called James Paget had 15 patients with a red, scaling nipple who all turned out to have breast cancer. A couple of years later, a French doctor called Dubreuilh, had a patient with a vulvar lesion comparable to the nipple lesion Paget described. That's where the name comes from. However, one in seven women will develop breast cancer in their live –unrelated to vulvar disease. Have you recently been checked? Otherwise I will examine you and order a mammogram to be sure.

Q: Can VPD be related to other cancers? Intestinal cancer runs in my family?

A: We used to think VPD is related to intestinal or urological cancer. We found that there are 3 types of VPD: VPD as a presentation of intestinal cancer, VPD as a presentation of urological cancer and strict cutaneous VPD. The pathologist, who microscopically examined the biopsy sample, has run additional tests on the tissue. We found that in your case, the lesion originates from the skin only. So we do not have to check for intestinal or urological disease. Unless you have other symptoms of these diseases? In that case we should evaluate to be sure. There is no information on VPD being hereditary.

Q: Am I going to die from this disease?

A: The risk that you will die from non-invasive VPD is extremely low. Invasive VPD, which is cancer, does influence survival severely. We know that +/- 8% of the patients with non-invasive VPD may develop invasion over several years; 92% does not. Therefore it is important we monitor the lesion very closely.

Q: Why did I get this disease?

A: We don't know why certain patients get VPD, we also have not been able to identify risk factors for developing VPD. We know the disease is most commonly seen in elderly, postmenopausal patients. However, younger patients may also be affected. In general, VPD is extremely rare; each year about 10-15 new patients are diagnosed in the Netherlands (~17 million inhabitants).

Q: Are my children or sisters at risk?
A: This has not been a topic of research because the disease is extremely rare, but we have never found a report of different family members with VPD.

Q: Why are you referring me to another hospital?
A: Because each year, only 10-15 patients in the Netherlands are diagnosed with VPD. For such rare disease, we think it is important you see doctors who are experienced with treating and monitoring it. For sure, referring you to an oncology centre has no relation with a poor prognosis but is related to a very low incidence and the premalignant character of the disease.

Q: How is it treated?
A: That depends on the severity of your symptoms, the location and size of the lesion, your overall health and preference. There are different treatment options: a wait-and-see policy (not all lesions should be treated), it may be treated with a somewhat aggressive cream called topical 5% imiquimod cream, or surgery.

FUTURE RESEARCH

Research so far has proven that Paget cells are generally located in the epidermis, close by the basal membrane and in the glands and hair follicles of the vulvar skin. The main challenge in VPD remains the clarification of the pathogenesis. Once the true origin of Paget cells is discovered, the natural course of disease can be evaluated. The main theories so far have focused on which part of the skin Paget cells arise from: skin appendages, Mammary-like glands, Toker cells. However, the effect Paget cells have on their surrounding cells is unknown. Maybe Paget cells originate in subtracting hair follicles in the degenerating vulvar skin, and spread through the epidermis, being part of a natural development in the skin, with other factors causing the irritative symptoms?

Pathologists faced with diagnosing VPD have no specific guidelines for reporting their findings regarding this disease. The pathologist's conclusions have an enormous impact on the patient's diagnosis and treatment. We advise the pathological report should at least describe whether the disease is invasive, and a immunohistochemistry panel to define the origin of disease.

Still, a lot of debate is going on about the natural behaviour of Paget cells. They are thought to originate in the basal membrane of the skin or skin appendages, and migrate to the surface of the skin. But what happens in cases with invasive disease: how do they migrate the other way, and suddenly be able to invade through the basal membrane to the dermis and cause cancer? And what is the difference between 'invasive VPD' and 'VPD with an underlying adenocarcinoma'? If the adenocarcinoma originates in the deeper layers or glands of the skin, and extends to the epidermis, are surrounding epidermal Paget cells part of that malignancy? Or does the patient have two diagnoses: a vulvar adenocarcinoma and non-invasive VPD?

Further research should be focussed on bringing the pathological and clinical implications together. Ideally combining clinical data and tissue samples from different countries, from both MPD and EMPD patients together. As long as case series are the highest level of evidence, we are unable to solve the mysteries of Paget cells. An important step in resolving the origin of Paget cells may be their genetic makeup. The techniques to evaluate the genetics of different tumours and diseases are improving, which will reveal the origin of Paget cells and their correlations to the surrounding healthy tissue and cancerous tissue.

Also the tumour microenvironment can provide knowledge about the origin and natural behaviour of Paget cells. This will be further investigated in the Paget Trial, where the microenvironment in biopsy samples taken before, during and after treatment with 5% imiquimod cream for non-invasive VPD.[27]

When the origin of Paget cells is discovered, the true nature of the disease can be further explored and care for the few patients who suffer from this rare disease can be really improved. Moreover, the effectiveness of topical 5% imiquimod cream may be better understood and treatment schedules can be improved.

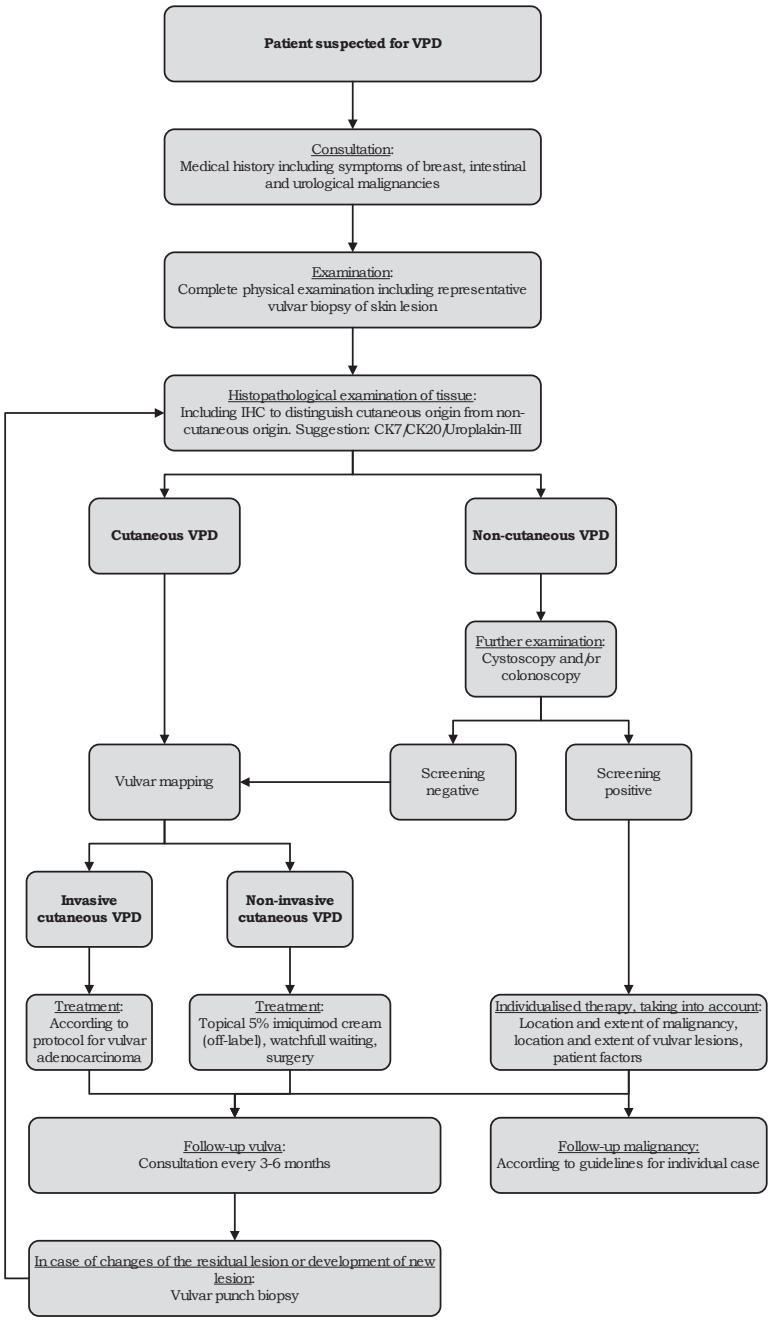

FIGURE 1 Flow-chart for the work-up for patients with VPD

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CHAPTER 10

SUMMARY/SAMENVATTING

SUMMARY

Vulvar Paget disease (VPD) is a rare skin lesion most commonly seen in postmenopausal Caucasian women. It causes pain, itching or a burning sensation and presents as a scaling erythematous plaque. The symptoms resemble the nipple ulceration called mammary Paget disease, which is associated with breast cancer. Both types of Paget disease are confirmed when typical Paget cells are seen in a histological biopsy sample. **Chapter 1** describes the history of the disease and explains the anatomy of the vulva, followed by the pathogenesis and treatment of vulvar malignancies.

The incidence and survival of different glandular vulvar malignancies is described in **chapter 2**. Data of PALGA, a nation-wide database registering all histo- and cytopathology in the Netherlands, was used to identify all cases of glandular vulvar malignancies between 2000 and 2015. Additional data were retrieved via the Netherlands Cancer Registry. A total of 197 patients with a glandular vulvar malignancy were identified. Of these patients 55% had a primary malignancy while 45% had secondary malignancies: expansion of another tumour in 17% and metastases or recurrences of another malignancy in 28%. There is a great variety of different diagnoses of primary glandular vulvar malignancies: 11 different types were identified. VPD was most common, 30% of primary glandular vulvar malignancies was (micro-)invasive VPD. We found an overall incidence rate of glandular vulvar malignancies of 0.9 – 2.5 per 1,000,000 women per year. Five-year survival for patients with a primary glandular vulvar malignancy was 68.5%, which is comparable to patients with a vulvar squamous cell carcinoma. Most of the secondary vulvar malignancies originated from (ano-) rectal malignancies. The great variety in diagnoses combined with the low incidence should lead to routine pathologic revision of these lesions and treatment in specialised gynaecologic oncology centres.

Chapter 3 presents an overview of the clinical aspects, histopathology, molecular genetics, and treatment options for VPD. Historically, surgical excision has been the treatment of choice. Recent studies show that imiquimod cream may be an effective and safe alternative.

In **chapter 4** the current treatment schedules, recurrence rates and survival of patients with VPD were studied in a retrospective cohort study.

Data was collected in eight tertiary clinics throughout the Netherlands about 113 patients diagnosed with VPD between 1991 and 2016. The median age at time of diagnosis was 73 years. Non-invasive VPD was the most common diagnosis (77%). Most women were surgically treated (65%) while about 25% of the women with non-invasive disease used topical treatment. Almost 40% of women had one or more recurrences. Of women with non-invasive VPD 8% developed invasion during follow-up. There were no disease specific deaths reported in women with non-invasive VPD. The 5 year DSS was over 98% in non-invasive and microinvasive VPD, and significantly worse in invasive VPD: 50%. This study concludes that non-invasive VPD does not seem to affect survival of patients, and should be considered as a chronic vulvar skin disorder with a limited invasive potential. In case of invasive disease survival decreases significantly. Based on the high recurrence rate lifelong surveillance of all VPD women is advised.

VPD has always been associated with mammary Paget disease. The origin of both diseases is still unknown. However, many clinicians consider patients with VPD at risk for different other malignancies, such as breast cancer, intestinal or urological malignancies. The aim of **chapter 5** was to evaluate the risk of developing breast, intestinal, and urological malignancies in patients with VPD compared with the general population, and in particular with the focus on the risk of malignancy in patients with cutaneous non-invasive VPD. Data on the oncologic history of patients with any type of VPD between 2000 and 2015 were obtained from PALGA; follow-up data and a control group from the general population were obtained from the Netherlands Cancer Registry. After correction for age and calendar year at time of diagnosis, standardised incidence ratios (SIR) for the first three years after VPD diagnosis were estimated with 95% confidence intervals (95%CI). A total of 199 patients with a first diagnosis of VPD (164 non-invasive, 35 (micro-)invasive) between 2000 and 2015 were identified. The SIR of developing an associated malignancy in the first 3 years after diagnosis was 4.67 (95%CI 2.66-7.64). This was mainly due to the high incidence of intestinal malignancies among patients with secondary VPD. Subgroup analysis for cutaneous non-invasive VPD did not reveal a significantly increased risk for associated malignancies i.e., SIR 2.08 (95%CI 0.76-4.62). In conclusion, of patients with VPD 76.9% is diagnosed with cutaneous non-invasive VPD, and this group has no increased risk for developing malignancies of the breast, intestinal or uro-

logical tract. This study suggests that routine screening for these malignancies in patients diagnosed with cutaneous non-invasive VPD may not be necessary.

The association of VPD with intestinal malignancies is further explored in **chapter 6**. This chapter reports the first case of retrograde pagetoid spread from a rectal adenocarcinoma to the vulva and cervix. A 66-year-old woman presented with postmenopausal bleeding and a history of Crohn disease. Gynaecological workup revealed vulvar and endocervical lesions consisting of Paget cells and adenocarcinoma, respectively. A rectal adenocarcinoma with in situ adenocarcinoma was diagnosed. The surgical specimen demonstrated Paget cells in the squamous epithelium of the anus and vulva. Immunohistochemistry demonstrated an intestinal phenotype of these cells. Genetic testing revealed the same TP53 mutation in tumour cells of the rectal adenocarcinoma and vulvar and endocervical lesions, demonstrating that the Paget cells originated from the same intestinal tumour.

The origin of Paget cells remains elusive. New effective treatment therapies such as immunotherapy with topical Imiquimod cream suggests the microenvironment in VPD may play an important role in the origin. **Chapter 7** describes the immune cell infiltrate in the microenvironment of vulvar Paget disease. The basic characteristics of the immune infiltrate in VPD were reported in samples of 10 patients; moreover the influence of imiquimod was studied in 6 patients. Immunohistochemistry for CD4, CD8, CD14, CD20, CD56 and FoxP3 was performed. The infiltrates of VPD were compared to vulvar high-grade squamous cell intraepithelial lesions (HSIL) (n=43), a HPV induced vulvar premalignancy with known response to imiquimod cream, and healthy controls (n=30). Immune cell counts in samples taken before and after treatment were compared. The microenvironment in VPD differs from the healthy vulvar skin and vulvar HSIL. VPD is characterised by less immune cells in the epithelium and an abundant number of immune cells in the stroma, consisting predominantly of T cells. The intraepithelial CD8+/Foxp3+ ratio and number of CD56+ increased after imiquimod therapy, whereas the numbers of CD14+ cells decreased which may point to a treatment-induced type 1 immune response. Changes in immune cell counts after immune modulation in relation to clinical responses should be further investigated.

Chapter 8 describes the study protocol of an ongoing study on topical 5% imiquimod cream for vulvar Paget disease, the Paget Trial (NCT02385188). This trial investigates the clinical efficacy, safety and immunological response. The challenge of obtaining clear surgical margins may lead to extensive and mutilating surgery in patients with VPD. Even then, recurrence rates are high, ranging from 15% to 70%, which emphasised the need for new treatment options. A number of case reports, retrospective case series, and one observational study have shown promising results using the topical immune response modifier imiquimod. This multicenter observational cohort study includes eight tertiary referral hospitals in the Netherlands. Twenty patients with (recurrent) non-invasive vulvar Paget disease are treated with topical 5% imiquimod cream three times a week for 16 weeks. The primary efficacy outcome is the reduction in lesion size at 12 weeks after end of treatment. Secondary outcomes are safety, immunological response, and quality of life. Safety will be assessed by evaluation of adverse events and tolerability of treatment. To evaluate the immunological response, various immunological markers will be tested on biopsy specimens taken before, during, and after treatment. Quality of life will be assessed with three questionnaires taken before, during, and after treatment.

At last, **chapter 9** hypothesises on the origin of Paget cells and the comparison with breast cancer and mammary Paget disease. It also includes a section to assist clinicians with counselling patients and a work-up for diagnostics and treatment. Finally, suggestions for future research are made.

SAMENVATTING

Vulvaire morbus Paget (VPD), ofwel de 'ziekte van Paget aan de schaamlippen', is een zeldzame huidaandoening die vooral bij postmenopausale vrouwen van het Kaukasische ras voorkomt. Het veroorzaakt pijn, jeuk en branderigheid, en op de vulva is een schilferend erytheem te zien. De symptomen lijken op die van de tepelafwijking 'ziekte van Paget', ofwel mammaire morbus Paget. Als deze afwijking wordt gezien is er vaak sprake van borstkanker. Beide types van de ziekte van Paget worden vastgesteld indien er typische Paget cellen zichtbaar zijn in een weefselbiopt. **Hoofdstuk 1** beschrijft de geschiedenis van de ziekte van Paget, de anatomie van de vulva, gevolgd door de pathogenese en behandeling van vulvaire maligniteiten.

De incidentie en overleving van verschillende typen maligniteiten van de klieren van de vulva wordt in **hoofdstuk 2** beschreven. Hiervoor is data gebruikt van PALGA: een nationale database waarin alle histo- en cytologische onderzoeken in Nederland worden geregistreerd. Alle casus van maligniteiten van vulvaklieren tussen 2000 en 2015 zijn geïdentificeerd. Aanvullende gegevens zijn opgezocht in de Nederlandse Kanker Registratie. Totaal zijn er 197 patiënten gediagnosticeerd met een maligniteit van de vulvaire klieren. Van deze patiënten had 55% een primaire vulva maligniteit, en 45% secundair aan een andere maligniteit: doorgroei van een andere tumor in 17%, en een metastase of recidief van een andere tumor in 28%. Er is een groot aantal verschillende typen primaire maligniteiten van de vulvaire klieren: er werden 11 verschillende types beschreven. VPD was het meest voorkomend: 30% van de primaire maligniteiten was (micro-)invasieve VPD. De incidentie van maligniteiten van de vulvaire klieren was 0,9 – 2,5 per 1.000.000 vrouwen per jaar. De vijfjaarsoverleving voor patiënten met een primaire maligniteit van de vulvaire klieren was 68,5%, vergelijkbaar als de overleving van patiënten met een plaveiselcelcarcinoom van de vulva. De meeste secundaire vulva maligniteiten hadden een intestinale origine: een (ano-)rectaal carcinoom. Gezien de grote verscheidenheid aan diagnoses, en de lage incidentie van deze aandoeningen zou revisie van de pathologie routinematig moeten plaats vinden, behandeling dienst plaats te vinden in gespecialiseerde gynaecologische oncologie centra.

Hoofdstuk 3 geeft een overzicht van de klinische aspecten, histopathologie, moleculaire genetica en behandeling van VPD. Van oudsher is chirurgie de

eerste keus behandeling. Recente literatuur laat echter goede resultaten zien van imiquimod crème, wat een effectief en veilig alternatief kan zijn.

In **hoofdstuk 4** wordt een retrospectieve studie naar de huidige behandelschema's, recidiefkansen en overleving van patiënten met VPD beschreven. In acht Nederlandse tertiaire centra werd data over 113 patiënten die tussen 1991 en 2016 met VPD zijn gediagnosticeerd verzameld. De mediane leeftijd ten tijde van diagnose was 73 jaar. Niet-invasieve Paget was de meest voorkomende diagnose (77%). De meeste patiënten werden chirurgisch behandeld (65%), 25% van de vrouwen die niet-invasieve Paget had kreeg een topicale behandeling. Bijna 40% van de patiënten had één of meer recidieven. Van de vrouwen met niet-invasieve VPD ontwikkelde 8% invasieve ziekte gedurende de follow-up periode. Er werden geen overlijdens veroorzaakt door niet-invasieve VPD gevonden. De ziekte specifieke vijfjaarsoverleving was groter dan 98% in niet-invasieve en microinvasieve VPD, maar significant slechter in de invasieve groep met een overleving van 50%. Dit onderzoek concludeert dat niet-invasieve VPD de overleving niet lijkt te beïnvloeden, en beschouwd kan worden als een chronische vulvaire huidaandoening met een beperkte maligne potentie. In het geval van invasieve ziekte is de overleving significant slechter. Op basis van de hoge recidiefkansen wordt geadviseerd alle patiënten met VPD levenslang te controleren.

VPD werd altijd geassocieerd met mammaire Paget. De origine van beide aandoeningen is nog altijd onbekend. Desondanks worden patiënten met VPD beschouwd als patiënten met een hoger risico op andere maligniteiten, zoals borstkanker, of maligniteiten van het maagdarm- of urogenitale kanaal. Het doel van **hoofdstuk 5** was om het risico op geassocieerde maligniteiten van de borst, intestinaal of urologisch, te onderzoeken bij patiënten met primaire, vanuit de huid ontstane, niet-invasieve VPD. Gegevens van de oncologische voorgeschiedenis van alle patiënten gediagnosticeerd tussen 2000 en 2015 met alle typen VPD werden opgezocht in PALGA; follow-up data en een controle groep uit de algemene populatie werden samengesteld uit data van de Nederlandse Kanker Registratie. Na correctie van leeftijd en kalenderjaar ten tijde van diagnose werden gestandaardiseerde incidentie ratio's (SIR) berekend met een 95% betrouwbaarheidsinter val (95% BI) voor de eerste drie jaar na de diagnose VPD. In totaal werden 199 patiënten met een eerste diagnose van VPD (164 niet-invasief, 35 (micro-)invasief) tussen 2000 en 2015 geïdentificeerd. De

SIR voor het ontwikkelen van een geassocieerde maligniteit in de eerste drie jaar na de diagnose VPD was 4,67 (95% BI 2,66-7,64). Dit werd voornamelijk veroorzaakt door de hoge incidentie van darmmaligniteiten bij patiënten met secundaire VPD. Subgroep analyse voor patiënten met cutane, niet-invasieve VPD liet geen significant verhoogd risico op geassocieerde maligniteiten zien: SIR 2,08 (95% BI 0,76-4,62). Concluderend heeft 76,9% van de patiënten met VPD de cutane, niet-invasieve variant. Deze groep heeft geen verhoogd risico op het ontwikkelen van maligniteiten van de borst, maagdarm- of urogenitale kanaal. Deze studie suggereert dat het standaard verrichten van aanvullend onderzoek naar geassocieerde maligniteiten ten tijde van de diagnose cutane, niet-invasieve VPD niet zinvol is.

De associatie van VPD met darmmaligniteiten wordt in **hoofdstuk 6** verder onderzocht. Dit hoofdstuk beschrijft de eerste casus van een retrograde pagetoïde spread van een rectaal adenocarcinoom naar de vulva en cervix. Een 66 jarige vrouw meldde zich met klachten van postmenopauzaal bloedverlies en een voorgeschiedenis van de ziekte van Crohn. Gynaecologisch onderzoek toonde vulvaire en endocervicale afwijkingen met respectievelijk Paget cellen en een adenocarcinoom. Een rectaal adenocarcinoom met adenocarcinoma in situ werd vastgesteld. Het chirurgisch verwijderde weefsel bevatte Paget cellen in het plaveiselepitheel van de anus en vulva. Immunohistochemie toonde een intestinaal fenotype aan. Genetisch onderzoek liet dezelfde TP53 mutatie zien in de tumor cellen van het rectaal adenocarcinoom, de vulvaire en de endocervicale weefsels. Dit bevestigt dat de darmtumor de oorsprong van de Paget cellen is.

De origine van Paget cellen blijft dubieus. Nieuwe behandelmethodes zoals immunotherapie met topicale Imiquimod crème suggereert dat de het micromilieu van de cellen een belangrijke rol kan spelen in het ontstaan. **Hoofdstuk 7** beschrijft het immuuncel infiltraat in het micromilieu van VPD. De basale kenmerken van het infiltraat als gezien in de weefselbiopten van 10 patiënten met VPD worden beschreven. Tevens wordt de invloed van imiquimod op het immuun infiltraat bestudeerd in de weefsels van 6 van deze patiënten. Immunohistochemie werd verricht voor CD4, CD8, CD14, CD20, CD56 en FoxP3. Het infiltraat bij patiënten met VPD werd vergeleken met het infiltraat gezien bij patiënten met vulvaire hooggradige squameuze intraepitheliale laesies (HSIL) (n=43), een HPV geïnduceerde vulvaire premaligniteit met een bekende respons op imiquimod behandeling, en gezonde controle weefsels (n=30). Immuuncel aantallen in weefsels

genomen voor en na behandeling werden vergeleken. Het micromilieu in VPD verschilt van de gezonde vulvaire huid en van vulvaire HSIL. VPD wordt gekarakteriseerd door een immuungecompromitteerd epitheel en immuunrijk stroma, voornamelijk bestaand uit T cellen. De intraepitheliale CD8+/FoxP3+ ratio en het aantal CD56+ cellen zijn verhoogd na behandeling met imiquimod, terwijl het aantal CD14+ cellen afgenomen was. Dit kan duiden op een door de behandeling geïnduceerde type 1 immuunrespons. Veranderingen in de aantallen immuuncellen na immunotherapie in relatie tot de klinische respons zullen verder onderzocht moeten worden.

Hoofdstuk 8 beschrijft het studie protocol van een lopen onderzoek naar topicale 5% imiquimod crème voor vulvaire morbus Paget, de Paget Trial (NCT02385188). Deze trial onderzoekt de klinische effectiviteit, de veiligheid en immunologische respons. Het is zeer moeilijk om bij de chirurgische behandeling van VPD vrije snijvlakken te verkrijgen, dit kan leiden tot herhaaldelijke en mutilerende ingrepen. Zelfs als het lukt, worden recidiefkansen beschreven variërend van 15% tot 70%. Dit benadrukt de noodzaak voor nieuwe behandelopties. Een aantal case reports, retrospectieve case series en een observationele studie hebben veelbelovende resultaten van het topicale immuunmodulerende middel imiquimod beschreven. Deze multicenter observationele cohort studie loopt in acht tertiaire centra in Nederland. Twintig patiënten met (een recidief) niet-invasieve VPD worden met topicale 5% imiquimod behandeld, 3 maal per week, gedurende 16 weken. De primaire uitkomstmaat is de effectiviteit, gemeten als afname van omvang van de laesie 12 weken na het eind van de behandeling. Secundaire uitkomstmaten zijn veiligheid, immunologische respons, en kwaliteit van leven. Veiligheid wordt onderzicht met het evalueren van bijwerkingen en verdraagbaarheid van de behandeling. De immunologische respons wordt onderzocht door verschillende immunologische markers te testen op weefselbiopten die zijn genomen voor, tijdens en na behandeling. Kwaliteit van leven wordt onderzocht met behulp van een drietal vragenlijsten, ingevuld voor, tijdens en na behandeling.

Ten slotte wordt in **hoofdstuk 9** verder in gegaan op de mogelijke origine van Paget cellen en de vergelijking met borstkanker en mammaire morbus Paget. Het omvat ook een ondersteuning voor artsen om patiënten met VPD te counselen, en een flowchart voor de diagnostiek en behandeling van de aandoening. Afsluitend worden er suggesties voor verder onderzoek gedaan.

CHAPTER 11

APPENDICES

ABBREVIATIONS

BIBLIOGRAPHY

CURRICULUM VITAE

DANKWOORD

ABBREVIATIONS

BCC	Basal cell carcinoma
CEA	Carcinoembryonic antigen
CI	Confidence Interval
CK	Cytokeratin
DCIS	Ductal carcinoma in situ
DFS	Disease free survival
DSS	Disease specific survival
EMPD	Extramammary Paget disease
FIGO	International Federation of Gynecology and Obstetrics
HE	Haematoxylin and Eosin staining
HPV	Human papilloma virus
HSIL	High grade squamous intraepithelial lesion
ICD-O	International Classification of Disease for Oncology
IFL	Inguinofemoral lymphadenectomy
IHC	Immunohistochemistry
IKNL	Netherlands Comprehensive Cancer Centre (in Dutch: Integraal Kankercentrum Nederland)
ISSVD	International Society for the Study of Vulvovaginal Disease
ITT	Intention to treat; statistical analysis
MLG	Mammary-like-glands
MMS	Moh's microsurgery
MPD	Mammary Paget disease
NCR	Netherland Cancer Registry (in Dutch: Nederlandse Kankerregistratie, NKR)
NOS	Not otherwise specified
PDT	Photodynamic therapy
PP	Per protocol; statistical analysis
SCC	Squamous cell carcinoma
SIR	Standardised Incidence Ratios
SLN	Sentinel lymph node
TNM	Tumour Node Metastasis
VIN	Vulvar intraepithelial neoplasia
VPD	Vulvar Paget disease
VSCC	Vulvar squamous cell carcinoma
WHO	World Health Organisation

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CURRICULUM VITAE

Michelle van der Linden werd op 31 mei 1988 geboren in Heemstede. Na anderhalf jaar in Hillegom, het dorp waar haar ouders zijn opgegroeid, te hebben gewoond verhuisde het gezin naar Wijchen. Haar middelbareschooltijd bracht zij door op het Lindenholt College te Nijmegen, alwaar zij in 2006 haar vwo diploma behaalde. Zij werd ingeloot voor de studie geneeskunde aan de Radboud Universiteit in Nijmegen. Het vak gynaecologie leek haar altijd een mooi specialisme, en het liep dan ook als een rode draad door haar studie: van verpleegstage in het eerste studiejaar, tot extracurriculaire activiteiten op het gebied van seksuele gezondheid binnen de internationale studievereniging IFMSA. In 2011 begon Michelle haar wetenschappelijke carrière met een onderzoeksstage bij de Cochrane Menstrual Disorder and Subfertility Group aan de Auckland University, Auckland, Nieuw-Zeeland (Prof. dr. C. Farquhar en Prof. dr. J.A.M. Kremer). Deze onderzoeksstage resulteerde in de publicatie van een Cochrane review en herinneringen aan een mooie reis. Naar aanleiding hiervan wilde zij zich na haar afstuderen verder bekwamen in de wetenschap. Na de afsluitende keuzecoschappen foetale geneeskunde aan de afdeling Verloskunde en Gynaecologie van het Leids Universitair Medisch Centrum, en gynaecologie aan de desbetreffende afdeling van het TweeSteden Ziekenhuis te Tilburg begon zij in oktober 2014 als wetenschappelijk onderzoeker op de afdeling Gynaecologische Oncologie aan het Radboudumc te Nijmegen onder begeleiding van Dr. Joanne de Hullu. Dit heeft uiteindelijk geleid tot het proefschrift wat u nu in handen heeft. Sinds april 2017 werkt Michelle als arts-assistent niet in opleiding op de afdeling Verloskunde en Gynaecologie van het Rijnstate Ziekenhuis te Arnhem. Zij zal aan het eind van dit jaar beginnen met de opleiding tot gynaecoloog in cluster Nijmegen (opleider Dr. A.C. Bolte). In haar vrije tijd is Michelle te vinden bij Arnhemse roeivereniging Jason, waar zij met haar ploeg traint, borrelt of haar bestuurlijke werkzaamheden als Roeicommissaris uitvoert. Sinds 2015 woont Michelle in Arnhem.

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Lieve Marline, dankzij jou heb ik: een bureau toegewezen gekregen in de tuin, altijd kunnen navragen hoe je precies alle CMO papierwerkjes in moest vullen, geleerd hoe je officieel een studie zou moeten monitoren, gemiddeld 25 mailtjes per dag aan onzin geschreven, een dubieuze vermelding als bedpartner in jouw proefschrift, een jaarlijkse chocolade-pepernotentraditie, de ghetto van Colombia bezocht, Sri Lanka's belangrijkste chefkok ontmoet, nooit –d/-t foutjes in mijn teksten, een superleuke collega, en nu ook nog eens een fantastische paranimf. De afgelopen jaren waren lang niet zo leuk zijn geweest zonder jou!

Mijn lieve oma's, waarvan ik er drie mocht hebben, die allen op hun eigen manier hebben meegeleefd met mijn onderzoek.

En natuurlijk mijn ouders, van wie ik hou en zonder wie ik niet zou bestaan. Lieve Els, ik was een jaar of 6 toen jij in mijn leven kwam en ik vond het meteen leuk. Zo leuk, dat ik 7 jaar later -ietwat onverwacht- mijzelf fulltime in jouw leven uitnodigde. Ik waardeer enorm hoe je daar mee bent omgegaan en hoe je sinds het begin voor mij klaar hebt gestaan en alles wat je mij hebt meegegeven.

En dan, Meines Paps. Ik heb het geprobeerd hoor, achter de worst bij de Hema. Maar het werd tóch iets anders. Ik ben je enorm dankbaar voor hoe jij je altijd (en nog steeds) hebt ingespannen om te zorgen dat ik kon doen wat nodig was om mijn doelen te bereiken. Ik heb niet alleen mijn lengte van jou, we kunnen allebei verhalen vertellen met een introductie die zo lang is dat niemand meer naar de clou luistert, en als ik een gevatte opmerking maak ben jij de eerste die 'm doorheeft. Ik ben trots om je dochter te mogen zijn.