Vulvar cancer : pathogenesis, molecular genetics and treatment

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CHAPTER 1

General introduction and outline of the thesis

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Vulvar cancer

Vulvar cancer (VC) is a rare gynaecological malignancy that accounts for 3-5% of all female genital tract malignancies (1-3) with an incidence rate of 1-3 per 100,000 women in developed countries. This incidence rises with age, with a peak incidence between 60 and 70 years of age (1, 4-6). In the Netherlands (17 million inhabitants) around 300 new patients are diagnosed with vulvar cancer each year (7). Over the last decades the overall incidence has risen (Figure 1), probably because of a higher life expectancy and due to an increase in human papilloma virus (HPV) infections (4, 5). The majority of VCs (90%) are vulvar squamous cell carcinomas (VSCC)(1, 6). Less frequent histological types are malignant melanoma, Bartholin gland carcinoma, invasive Paget's disease, and basal cell carcinoma. Sarcomas and verrucous carcinomas are extremely rare (1, 6, 8). `

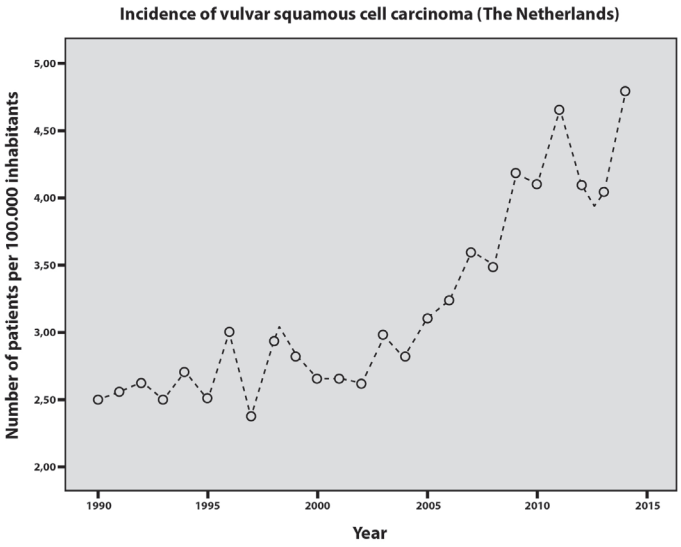

Figure 1: Incidence of vulvar cancer (The Netherlands) (7)

Dissemination of VC occurs through three different routes. The most common pattern of spread is spread by direct extension and lymphogenic to the inguinofemoral lymph nodes. Pelvic lymph node metastases are uncommon, with an incidence of 2-12%, and are seldom found in the absence of groin lymph node metastases (1, 6, 9). Haematogenous spread is very rare, especially in the absence of a groin lymph node metastasis (1, 6, 8-10).

FIGO stage

The International Federation of Gynecology and Obstetrics (FIGO) staging system has been adjusted in 2009 (Table 1) (1, 11, 12). Because prognosis is strongly dependent on the status of the lymph node(s) (13) the number and morphology (size and presence of extra-capsular growth) of involved lymph nodes are taken into account. The FIGO 2009 classification provides an adequate prognostic discrimination between the different stages (12, 14).

Table 1: FIGO 2009 staging system of vulvar cancer

Stage	
I	Tumours confined to the vulva or perineum, no nodal metastasis Ia: Tumour ≤ 2 cm with stromal invasion ≤ 1 mm Ib: Tumour > 2 cm or stromal invasion > 1mm
II	Tumour of any size with extension to adjacent perineal structures (lower urethra, lower vagina, anus), no nodal metastasis
III	Tumour of any size with or without extension to adjacent perineal structures (lower urethra, lower vagina, anus), with inguino-femoral nodal metastasis IIIa: 1 node metastasis (≥ 5 mm) or 1-2 node metastasis(es) (< 5 mm) IIIb: ≥ 2 node metastases (≥ 5 mm) or ≥ 3 node metastases (< 5 mm) IIIc: node metastases with extra-capsular spread
IV	Iva: Tumour invades any of the following: upper urethra and/or vaginal mucosa, bladder mucosa, rectal mucosa, or fixed to pelvic bone, or fixed or ulcerated inguinofemoral nodes IVb: Any distant metastasis including pelvic nodes

Treatment of vulvar cancer

Surgery is the cornerstone of treatment for VC (1, 8). Before 1980, surgery for all VC stages was extensive and consisted of radical vulvectomy with en-bloc lymfadenectomy of the groins and enlarged pelvic nodes (1, 8, 15). The rationale behind this radical surgical procedure was to remove all possible cancer infiltrated tissue by removing the vulvar lesions, the inguinofemoral lymph nodes and the lymphatics in between (8). This treatment strategy led to a high risk of morbidity with reported complication frequencies of up to 90% (16, 17). Most common complications are wound infections, wound breakdown, lymphocysts, lymphedema and psychosexual consequences (6, 8, 15). Furthermore, closure of large skin defects after radical vulvectomy was often insufficient, which could result in postoperative necrosis (6).

During the last decades, treatment for VC has evolved into a more conservative and individualized multidisciplinary approach, without compromising prognosis (1, 6, 8, 9, 15). Nowadays, the extent of disease determines the extend of surgery needed (Figure 2) (9). Micro-invasive VC (stage 1A), defined as a single lesion of ≤ 2 cm with a depth of invasion of ≤1 mm, can be treated with a wide local excision only. Treatment of the groins can be safely omitted, because there is almost no chance of groin metastases in

these patients (1, 6, 15). Surgery for early-stage VC infiltrating > 1 mm consists of wide local excision with uni- or bilateral inguinofemoral lymphadenectomy (IFL) via separate groin incisions or staging of the lymph nodes with the sentinel lymph node (SN) procedure (1, 8). The rationale to justify the use of separate incisions in groin treatment of VC is that the mechanism of lymphatic spread is by embolization rather than by permeation (18). The overall incidence of lymph node metastasis is about 30% (6, 9) and the risk for lymph node metastases rises as the stage of disease, size of the lesion and depth of invasion increases (1, 6, 9). Appropriate groin treatment in order to prevent a groin recurrence is the most important factor in reducing mortality from early stage VC due to the high mortality rate of a groin recurrence. A SN procedure is considered safe in patients with a unifocal vulvar tumour < 4 cm without enlarged or clinically suspicious lymph nodes upon palpation, ultrasonography or CT-scan, with groin recurrence rates of 2,3-3% (16, 19, 20). Unilateral IFL is safe for patients with a lateralized tumour (medial margin of the tumour > 1 cm from the midline) without suspicious groins at physical examination (8, 15). The chance of having positive contralateral lymph nodes for patients with unilateral tumours and negative ipsilateral lymph nodes is low (0.9 – 2.8%) (16, 21, 22). Bilateral IFL should be performed in case of midline tumours, lateral tumours of > 4 cm and in case of positive ipsilateral lymph nodes (1). Due to these treatment adjustments and especially due to the introduction of the SN procedure, morbidity has dramatically decreased. Still, postoperative morbidity remains a major concern, particularly after IFL. One or more complications after an IFL are reported in up to 66% of patients (10, 16, 17).

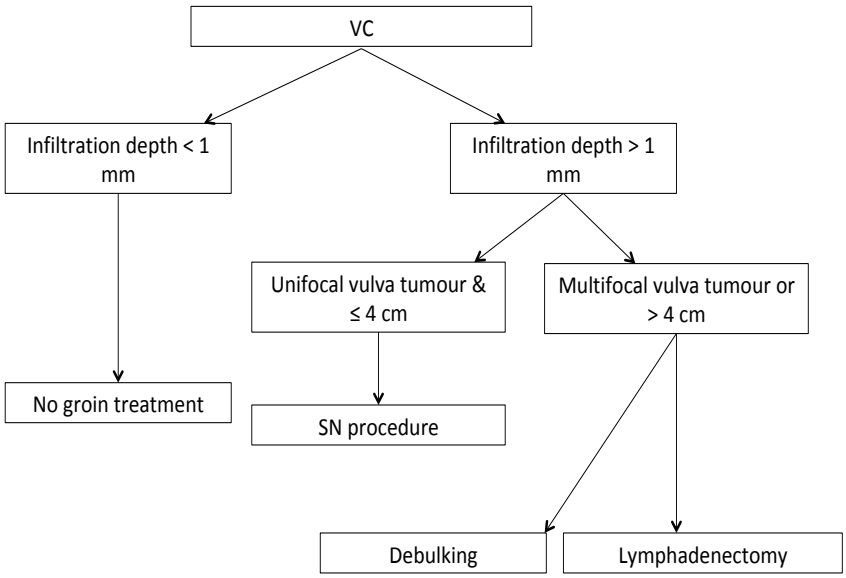

Figure 2: overview of treatment of vulvar cancer (VC). SN: sentinel node

Extensive groin surgery is necessary for patients with a suspicion of groin metastases or in case of a positive SN. Standard treatment at this moment is uni- or bilateral IFL. However, nodal debulking (i.e. removal of enlarged lymph nodes in the groins) might be a good alternative for IFL. A study by Hyde et al. in which nodal debulking was compared with IFL, both followed by radiotherapy, found no difference in groin recurrence rate. However, this study did not evaluate complication rate for both surgical procedures (23).

There are several important clinical issues in the treatment of VC and developing an appropriate, individualized treatment strategy is one of the major challenges. Treatment is often difficult and associated with high complication rates since VC patients are often fragile and elderly patients with high co-morbidity rates (4, 24). This emphasises the need to choose a treatment modality with the lowest morbidity and risk of complications. In the course of the years there have been important developments in less aggressive treatment strategies. Still, there remain major questions on the optimal treatment of VC. Especially the influence of tumour free margins after radical local excision and adequate treatment of the groins are crucial questions. Furthermore, recurrence rate after primary treatment remains high and prevention of these recurrences is a vital clinical challenge in order to further reduce morbidity and complication rates (25, 26).

Adjuvant therapy

Currently, local re-excision is advised in case of positive margins after primary local surgery. Adjuvant radiotherapy can be considered in patients when re-excision is impossible or when re-excision is contra-indicated. Re-excision should also be considered in patients with close tumour-free margins (< 8 mm), especially when there are other risk factors for local recurrence (8, 25, 27-31).

Adjuvant radiotherapy to the groins clearly improves prognosis in patients with involved groin lymph nodes (32, 33) and is indicated after nodal debulking of the groins, in case of two or more groin metastases after inguinofemoral lymphadenectomy or when groin metastases have extranodal growth (1, 8, 9, 15, 27-30).

Prognosis

Prognosis for VC patients is generally good, with an overall five-year survival of 70%. An early diagnosis of VC is important for improved prognosis (6, 9). Five-year survival is 80-90% for patients who present with early-stage VC, regardless of tumour diameter and expansion to the vagina and/or urethra (6, 15, 32, 34). This decreases to 25-67% if groin lymph nodes are affected, largely depending on the number of involved lymph nodes and their growth pattern (6, 9, 12, 32). Five-year survival is 75% for patients with one or two lymph node metastases and decreases to 24% for patients with five or six involved lymph nodes (8). Patients with extranodal growth of a lymph node metastasis

have a 5-year survival of 34% compared to 66% for patients with intranodal growth (12). VC related mortality usually results from failure to control the disease once it has progressed beyond the site of origin. In these patients diagnosis is often delayed by the patient or physician (6).

For patients with a local recurrence disease-specific survival decreases from 90% to 69% (35). Furthermore, disease-specific survival is worse for patients who develop a local recurrence within two years compared to patients that develop a local recurrence more than two years after primary treatment (53% versus 76%) (35, 36).

The majority of groin recurrences (~ 70%) develop within the first year after primary treatment, with a median time until recurrence of 7 months (35, 37, 38). Prognosis for patients with a groin recurrence is very poor. Most patients die of disease within two years after development of the groin recurrence (25, 34, 35, 38). On the contrary, a recently published study found an overall survival rate of 50% for 30 patients with a groin recurrence after 7 years. Especially patients who received multimodal treatment for their groin recurrence performed better (39).

Local recurrence

Recurrent disease is an important clinical challenge in the treatment of VC. Despite all developments in treatment strategies, recurrence rates of VC are still high: 12-37% of VC patients develop a recurrence (25, 26, 40) of which 50% are local (25, 26, 37, 40). There are several known risk factors for a local recurrence. The width of the tumour free margin is considered the most important predictive factor for local recurrences. It is known that tumour-positive margins are associated with recurrence and poor prognosis. The minimal safe tumour-free margin is one of the most relevant clinical questions in the primary surgical treatment of VC, especially given the treatment-related morbidity associated with radical surgery in the genital area. Most current guidelines advise a minimal tumour-free margin of 8 mm (27-30) which is based on a study by Heaps et al. The authors found that patients with a tumour-free margin of ≥ 8 mm did not develop a local recurrence (41). However, other studies on the tumour-free margin distance report contradictory results (18, 42-49). Another strong prognostic factor is tumour positive lymph node(s) (9, 25, 35, 40, 50). Intriguingly, tumour positive lymph nodes increase the chance of a groin recurrence as well as the chance of a local recurrence (50). This might be explained by a biological more aggressive tumour behaviour if lymph node metastases are present. Also the number of tumour positive lymph nodes (9), the size of nodal metastases and the presence of extranodal growth and the number of removed lymph nodes during IFL are known prognostic factors (6, 40). Other risk factors for recurrent disease are higher age (40, 50), greater tumour size (25, 50), depth of invasion of > 2 mm (40, 50) and lymph vascular space invasion (LVSI) (37, 40).

Pathogenesis of vulvar cancer

The pathogenesis of VC can typically be sub classified into HPV-independent and HPV-dependent VC (3, 9, 40, 51, 52). These two different types of VC have different epidemiological, clinical, pathological and molecular characteristics and it becomes more and more clear that both tumour types should be considered as two separate entities (3).

HPV-independent VC account for around 70% of all VC, usually occur in older patients and are associated with lichen sclerosus (LS) and mutations in *TP53*. The presumed precursor lesion in this type of VC is differentiated vulvar intraepithelial neoplasia (dVIN) (3, 51, 53). The exact mechanisms involved in the progression from LS and dVIN into VC are currently unclear.

HPV-dependent VC account for around 30% of all VC and have vulvar high grade squamous intraepithelial lesions (HSIL) as a precursor lesion (2, 3). The most prevalent HPV-types found in these VCs are HPV 16 in 60-78% of the cases, followed by HPV 18 in 5-16% of the cases (9, 54-60). Other encountered HPV types are HPV 31, 33 and 45 (3). This tumour type is more common in younger patients (35-65 years) and is associated with smoking, a higher number of sexual partners, and a compromised immune status (3, 9, 51).

Although HPV-independent and HPV-dependent VC are pathologically distinct, the clinical relevance of this distinction has not yet been established. In another tumour type with a similar dualistic classification, head and neck squamous cell carcinomas (HNSCC), the HPV presence has proven to improve prognosis. In addition, HPV-dependent HNSCC show a better response to adjuvant therapy (61-63). In VC, the prognostic significance of HPV on survival has been debated and is not yet fully understood (54). There is some suggestion that HPV-dependent VC, similar to HPV-dependent HNSCC, have a more favourable prognosis compared to HPV-independent VC (55-58, 60). However, other studies could not confirm this prognostic advantage (3, 54, 59, 64).

Vulvar pre-malignancies

About 50-80% of VC patients present with an epithelial disorder adjacent to the VC (3, 65, 66). Most VCs originate in these intraepithelial lesions, which precede the development of invasive disease by a variable period of time (3). The most recent classification system of the International Society for the Study of Vulvovaginal Disease (ISSVD) distinguishes between the HPV-independent precursor lesion dVIN (Figure 3a) and the HPV-dependent lesion HSIL (formerly known as usual VIN) (Figure 3b). The characteristics of these vulvar pre-malignancies are described in table 2.

Table 2: vulvar precursor lesions

	dVIN	HSIL
Synonym	VIN, differentiated type	Usual type VIN or VIN 2/3
HPV status	Negative	Positive (HPV 16-18)
Proportion	2-10%	± 90%
Characteristics	Older women	Younger women
	LS related	Smoking related
	TP53 mutations	Promiscuity
	Often adjacent to VC	Compromised immunity
		Often multifocal
Progression rate	±80% if untreated	9-16% if untreated

VIN: vulvar intraepithelial neoplasia
HSIL: high grade squamous intraepithelial lesion
HPV: human papilloma virus
LS: lichen sclerosus

Molecular features

More detailed information on the molecular background of VC and specifically information on genetic and epigenetic changes can provide valuable insight in the pathogenesis of VC. Previous studies on different types of cancer have shown that genetic and epigenetic alteration status can help in predicting prognosis and guide targeted therapy (67-71). Malignant transformation is determined by a sequence of genetic and epigenetic events often involving dysregulation of the cell cycle control. Cell cycle alterations are mainly caused by alterations in the p53 or pRb (p16/pRb/cyclin-D1) pathways. P53 overexpression is found in 40-81% and *Tp53* mutations in 20-30% of the VC patients and is unrelated to HPV-infection. The pRb pathway is mediated by inactivation of Rb through its phosphorylation. The P16 protein can act as an inhibitor by preventing this phosphorylation. Loss of cell cycle control via this pathway is thus caused by somatic mutations in Rb or by disrupted p16 function through somatic mutations or promoter hypermethylation. Promoter hypermethylation of p16 is common and this gene is currently considered the most frequently inactivated tumour suppressor gene in cancer (72, 73).

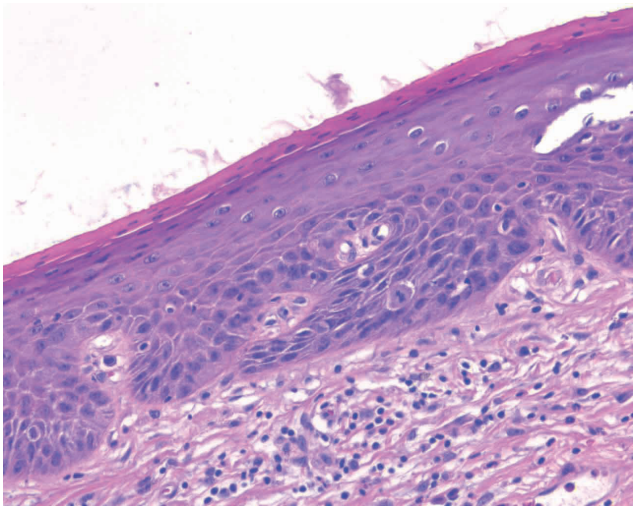

Figure 3a: Differentiated vulvar intraepithelial neoplasia (dVIN)

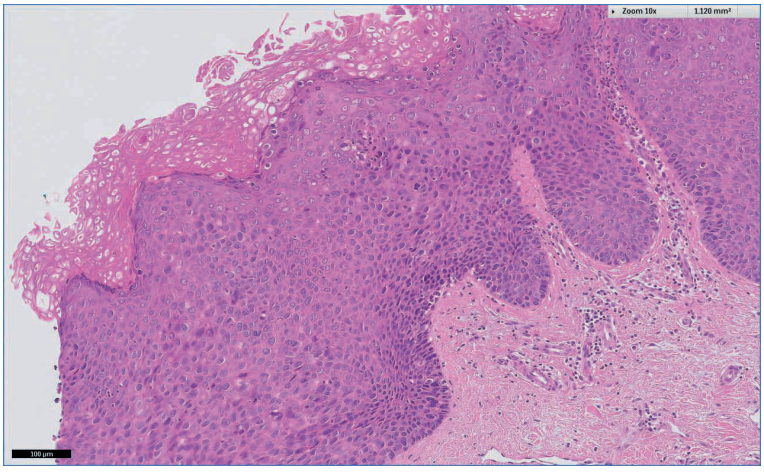

Figure 3b: Vulvar high grade squamous intra-epithelial lesion (HSIL)

At this moment, most is known about the molecular mechanisms involved in the development of vulvar HSIL and HPV-dependent VC (3, 74). This knowledge is partially acquired due to the great similarities with cervical cancer in which the role of HPV has been studied extensively (75). In HPV-dependent VC, the immune system fails to produce an effective response to high-risk HPV. This leads to virus persistence and integration and replication of the viral DNA in epidermal cells (75). The longer the infection persists, the longer the viral oncoproteins E6 and E7 can interfere with important cell cycle control mechanisms, which will lead to escape from programmed cell death and transformation (52, 75-77). E6 degrades the tumour suppressor p53, which leads to absence of cell cycle arrest. E7 inactivates the retinoblastoma tumour suppressor gene product, which results in hyperproliferation of tumour cells and overexpression of p16 and p14 (3, 76, 77). As a result, p16 has proven to be an excellent surrogate marker for high risk HPV infection.

HPV-independent VCs have been much less studied and the molecular mechanisms involved in its development have not yet been fully elucidated. Somatic mutations in *TP53*, leading to an aberrant function of the p53 protein, have been detected in a high percentage of HPV-independent VC and dVIN and seem to have an important function in the pathogenesis of VC (52, 76-79). Because aberrant p53 expression has also been described in precursor lesions of the vulva, this may be an initiating event in vulvar carcinogenesis (53). This is supported by a study by Rolfe et al. in which a *TP53* mutational analysis identified an identical genotype in the adjacent precursor lesion in 50% of the VC patients (n=27) (78). Studies on somatic mutations in VC other than in the *TP53* gene are limited. Holway et al. studied eight vulvar cancer patients and identified *PTEN* mutations in five of eight vulvar cancer patients, suggesting that *PTEN* is frequently altered in VCs (79). In a study on 108 VC samples published by Trietsch et al., somatic mutations were found in *CDKN2A* (13%), *HRAS* (9%), *PIK3CA* (7%) and *PP2R1A* (3%) (80).

Future research can further elucidate the molecular features involved in the pathogenesis of VC. The current developments in molecular diagnostics and especially (epi)genetic testing will provide a substantial contribution to our knowledge on this pathogenesis, in particular on the HPV-independent VC. At this moment it is unclear whether the different types of VC indeed represent a difference in clinical behaviour and thus whether this subdivision has clinical relevance. Differences in clinical behaviour might cause a change in treatment strategy of VC patients. Gaining knowledge of the pathogenesis will contribute to the development of a more individualized treatment strategy for VC patients.

Thesis aim and outline

The objectives of this thesis can be subdivided into clinical questions and questions regarding the pathogenesis of vulvar cancer. The overarching theme however is to use these data to improve and personalise the treatment of patients with vulvar cancer. The clinical section is covered in chapter 2-4. **Chapter 2** reports on the influence of the histological margin distance and local recurrence rate. In this study we combine the results of a meta-analysis of the currently available literature with a retrospective cohort study in the LUMC. **Chapter 3** describes the clinical outcome of vulvar cancer patients treated for groin lymph node metastasis, comparing extensive inguinofemoral lymphadenectomy with debulking of enlarged lymphnodes. **Chapter 4** presents a review on recurrent VC and literature concerning treatment of recurrent VC.

The second section of this thesis (chapter 5-7) is devoted to work that intends to improve our molecular understanding and diagnosis of vulvar (pre)cancers. It starts, in **chapter 5**, with a review on the (epi)genetic alterations in VC and its precursor lesions described in the current literature. In **chapter 6** we investigated whether stathmin immunostaining can be used in the differential diagnosis of vulvar precancerous lesions. In **chapter 7** a comprehensive genetic landscape of a large series of vulvar precursor lesions and VC is presented, including the clinical relevance.

The general discussion in **chapter 8** gives an overview of the findings presented in this thesis and a glance at future perspectives in the developments in treatment of VC and insight of the pathogenesis of VC.

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