Vulvar cancer : pathogenesis, molecular genetics and treatment

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CHAPTER 2

Tumour-free margins in vulvar squamous cell carcinoma: does distance really matter?

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Abstract

Background: There is no consensus on the width of tumour-free margins after surgery for vulvar squamous cell carcinoma (VSCC). Most current guidelines recommend tumour-free margins of ≥8 mm. The aim of this study was to investigate whether a margin of <8 mm is associated with an increased risk of local recurrence in VSCC.

Methods: A meta-analysis of the available literature and a cohort study of 148 VSCC patients seen at a referral centre from 2000 to 2012 was performed. The primary end-point of the cohort study was a histologically confirmed ipsilateral local recurrence within 2 years after primary treatment in relation to the margin distance.

Results: Based on 10 studies, the meta-analysis showed that a tumour-free margin of <8 mm is associated with a higher risk of local recurrence compared to a tumour-free margin of ≥8 mm (pooled risk ratio, 1.99 [95% confidence interval {CI}: 1.13–3.51], p=0.02). In the cohort study, we found no clear difference in the risk of local recurrence in the <8 versus ≥8 mm group; however, 40% of the patients in the <8 mm group received additional treatment. Tumour-positive margin was the only independent risk factor for local recurrence in the multivariable analysis (hazard ratio, 0.21 [95% CI 0.08–0.55]).

Conclusions: This work provides important data to question the commonly used 8 mm margin as a prognosticator for local recurrence. More research is needed to address the question of whether additional treatment improves the prognosis in patients with a tumour-free margin of < 8 mm.

Introduction

The fundamental goal of curative oncological surgery is complete tumour resection (1, 2). Tumour-positive margins, usually expressed in millimetres of distance from the tumour to the nearest line of resection, are strongly associated with recurrence and poor prognosis (2-5). The minimal safe tumour-free margin is an important clinical issue in several tumour types where tissue-sparing surgery is desired (e.g., head and neck squamous cell carcinomas, breast cancer, soft tissue sarcomas, and penile cancer) (2, 6-10). The definition of a minimal safe tumour-free margin varies between 1–10 mm for different tumour types (2, 8, 9). Level one evidence is not available, and consensus or guidelines on the optimal tumour-free margin for many tumours are lacking (2, 11-14). Nonetheless, important clinical decisions are based on these tumour-free margins including the need for additional treatment (re-excision or (chemo)radiotherapy), which is associated with additional discomfort for patients, treatment-related morbidity, and increased health care costs (4, 7, 14-16).

Vulvar cancer is a rare malignancy, accounting for around 5% of all gynaecological cancers, with squamous cell carcinoma as the most common histologic subtype (17, 18). Surgery is the treatment of choice for most patients, but can lead to significant morbidity when the tumour is near the clitoris, urethra, or anus (3, 19). Patients with vulvar squamous cell carcinoma (VSCC) are at high risk for developing local recurrent disease. Approximately 25% of patients experience a local recurrence after primary treatment (20, 21). Although most local recurrences develop within 2 years, late "recurrences" often occur in VSCC as shown in a recent long-term follow-up study that found an overall local recurrence risk of 27.2% after 5 years, and 39.5% after 10 years (22). Local recurrences are considered the result of residual tumour cells after inadequate surgical margins and arise around the surgical scar. Late recurrences are unlikely to arise from residual tumour cells after inadequate resection, and are better defined as second primary tumours. Second primary tumours in VSCC arise from a persistent precancerous field, which encompasses altered cells with high premalignant potential (14, 23, 24). In VSCC, both human papillomavirus (HPV) (high-grade squamous intraepithelial lesion) and non-HPV (differentiated vulvar intraepithelial neoplasia) related precancerous lesions have been defined and are frequently identified surrounding VSCC (18, 25). This so-called "field effect" is considered to be responsible for the increased risk of the developing second primary tumours in patients with VSCC (24).

Given the treatment-related morbidity associated with radical surgery in the genital area, the minimal safe tumour-free margin is one of the most relevant clinical questions in the primary surgical treatment of VSCC. Additional treatment is generally advised when the tumour-free margin (i.e. the histological margin after fixation) is involved or close,

but a uniform definition for "close margin" is lacking (12, 13, 26). The Royal College of Obstetricians & Gynaecologist guidelines on the surgical treatment of VSCC (27) advises a minimal tumour-free margin of 10 mm, while the Dutch and the American National Cancer institute and National Comprehensive Cancer Network guidelines recommend a minimal tumour-free resection margin of 8 mm (12, 13, 26). To reach this, a surgical margin of 1-2 cm around the tumour is recommended. These guidelines are based upon relatively small studies (3-5, 19, 28-32). Additionally, it is not clear if additional treatment reduces the risk for local recurrence in VSCC (33).

The aim of this study was to investigate whether a tumour-free margin <8 mm is associated with local recurrence after primary surgery for VSCC. For this purpose, a systematic review and meta-analysis of the available literature was performed. Additionally, a large cohort study was conducted at a referral centre for patients with VSCC.

Methods

I. Systematic review and meta-analysis

Search eligibility and search strategy

A systematic review of the literature on the tumour-free margin status related to risk of recurrence in VSCC was performed. Relevant studies were identified from a literature search of PubMed, Embase, Web of Science, Cochrane database, and ScienceDirect. The search was conducted in October 2015. A combination of Medical Subject Headings and free text words were formulated after consulting a medical librarian. Our search included the terms vulvar neoplasm, vulva(r) carcinoma, surgical margin, histo(patho) logical margin, clinical margin, excision margin or margin (Appendix A). Studies on local recurrence risk in relation to the tumour-free margin in VSCC were eligible for inclusion. Exclusion criteria were languages other than English, Dutch, German, French, or Italian. Studies that compared local recurrence risk for patients with tumour-positive margins with tumour-free margins were also excluded because we focused on comparison of close versus wide margins. All articles were assessed based on the title, abstract, or full article. The electronic search was complemented with a manual search of references from relevant articles.

Data extraction and risk of bias assessment

For all studies, we extracted the following data: number of included patients, definition of local recurrence, number of local recurrences, and additional treatment (including reexcisions and radiotherapy). Two articles that reported on a tumour-free margin of 1 cm were also included. Three studies that only reported data on a smaller tumour-free margin (3 or 5 mm) were excluded because our focus was a tumour-free margin of 8

mm. When possible, patients with a tumour-positive margin were analysed as a separate patient group. The number of local recurrences was based on the definitions used in included articles. A risk of bias analysis was performed. All studies were evaluated for selection, performance, attrition, detection, and reporting bias according to the 'methods guide for comparative effectiveness reviews' (34).

II. Cohort study

Patient and tumour characteristics

A cohort study was performed of consecutive patients who were surgically treated for primary VSCC between 2000 and 2012 in the Leiden University Medical Centre. Histological slides were collected from the pathology archive, and patient characteristics were gathered from electronic patient charts after approval by the institutional review board.

All gross specimens were handled according to the local protocol, and minimal tumour-free margins were measured on haematoxylin and eosin stained slides from formalin-fixed, paraffin-embedded tissue blocks. To assure uniform assessment, minimal margin measurements were revised by an expert gynaecopathologist (TB) blinded to the patient's recurrence status. For this revision, slides were scanned with the Philips Ultra-Fast Scanner, and the Philips Digital Pathology Solutions software was used to measure the histological margins using a digital ruler. The tumour-free margin was defined as the closest distance from the invasive tumour to the lateral or basal resection margin.

Surgical treatment of the vulva consisted of a vulvectomy (removal of part or all of the tissues from the vulva; i.e. labia majora, labia minora, and the clitoris) or wide local excision (removal of the tumour with a macroscopic margin of at least 1 cm). Additional treatment was generally started within 6 weeks after the primary surgery and consisted of reexcision or radiotherapy. Additional treatment was recommended for patients with tumour-positive margins and was considered for patients with a tumour-free margin <8 mm who had other risk factors (advanced tumour stage, positive lymph nodes, or lymphovascular space invasion). All patients were discussed in a multidisciplinary meeting. When considered feasible, a reexcision was performed. Otherwise, the patient received additional radiotherapy comprising a total dose of 50.4 Gy in fractions of 1.8 Gy, with five fractions per week administered. Follow-up consisted of outpatient visits every 2–3 months during the first 2 years after treatment, every 4–6 months during the third and fourth years, and annually thereafter.

Definition of local recurrence

We defined a local recurrence as a histologically confirmed recurrence of VSCC within 2 years that was located on the ipsilateral side of the vulva as the primary tumour. A 2-year period after primary treatment was chosen because up to 80% of all local recurrences of VSCC occur within this time period after the initial treatment (5, 21, 22, 28, 29, 35). A new tumour developing more than 2 years after primary treatment and/or on the contralateral side of the vulva was considered a second primary tumour.

Statistical analysis

Statistical analysis for the meta-analysis was performed using Review Manager 5.3. A random effects analysis was carried out to estimate the pooled risk ratio for the association between the tumour-free margin (<8 mm versus ≥8 mm) and local recurrence risk.

For the cohort study, statistical analysis was performed with SPSS version 20.0. We divided the patients into groups with a tumour-positive margin and a tumour-free margin of <8 mm and ≥8 mm. The chi-square test was used to compare baseline characteristics between groups. A competing risk analysis (accounting for death as a competing risk) was performed to estimate local recurrence risk. In a post hoc analysis, local recurrence risk was also determined for other tumour-free margin cutoff values (2, 4, and 6 mm). Univariable and multivariable analyses were performed with the Cox proportional hazard model. Multivariable analysis included all variables with a p-value <0.1 in the univariable analysis because these variables were considered important factors for the probability of developing a recurrence.

Results

Meta-analysis

A total of 368 articles were identified through an electronic literature search. Seven articles were added through a complementary manual search for articles. Based on the title of the article, 292 articles were excluded. From the remaining 83 articles, the abstract was reviewed, after which another 43 articles were excluded. Ten cohort studies published between 1990 and 2015 investigating the association between tumour-free margin and local recurrence risk were included (Figure 1) (3-5, 19, 28-32, 36). The range of included patients was 79–205, and the mean follow-up time ranged from 31 to 110 months. The risk of bias analysis did not reveal any major bias in the included studies, although in most articles, the evaluated biases were not described.

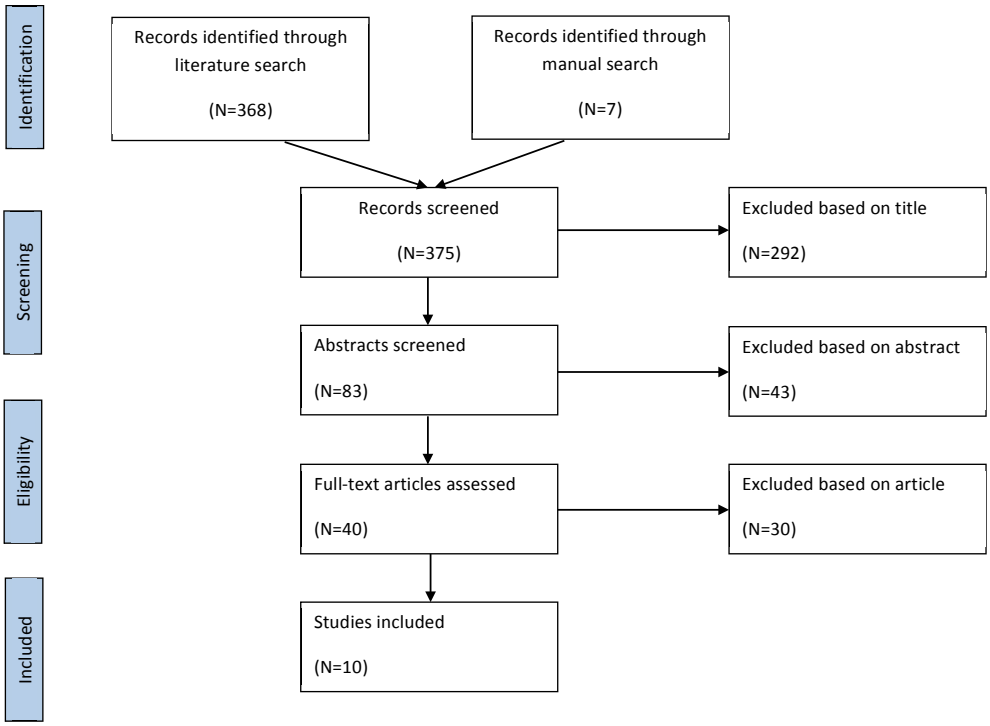

Figure 1: Flowchart illustrating inclusion and exclusion of articles for the meta-analysis

Eight studies compared local recurrence risk for patients with a tumour-free margin of <8 mm with a tumour-free margin of ≥8 mm. Two studies compared a tumour-free margin of <1 cm with ≥1 cm. Study descriptions are summarised in table 1 and the risk of bias analysis in supplementary table 1. Due to the retrospective character of the included studies, data extraction was often difficult. The included studies present heterogeneous data regarding tumour and treatment characteristics. None of the studies distinguished local recurrences from second primary tumours, and local recurrences were included independent of time interval or distance to the primary resection. Eight articles reported on additional treatment after the primary treatment (3-5, 28-31, 36). The influence of additional treatment on local recurrence risk was specified in one study (29). In nine studies, patients with a tumour-positive margin could be distinguished from the total group of patients with a tumour-free margin of <8 mm (3-5, 19, 28-30, 32, 36).

Table 1: Descriptive characteristics of the studies included in the meta-analysis

				Local recurrence risk				
Reference	**Design and inclusion period**	**No of partici-pants**	**Definition local recurrence**	**Tumour-positive margin**	**Tumour-free margin < 8 mm**	**Tumour-free margin ≥ 8 mm**	**Adjuvant treatment**	**Notes**
Baiocchi, 2015	Cohort study, (1980 – 2013)	205	Not specified	0	18/79	29/126	RT: 2 (2%) of 126 ≥ 8 mm and 8 (10%) of 79 < 8 mm	Influence of adjuvant therapy on local recurrence rate not further specified
Chan, 2007	Cohort study, (1984 – 2002)	90	Not specified	1/7	12/53	0/30	RT: 18 (20%) of 90 on the groins and/or perineum	Influence of adjuvant therapy on local recurrence rate not further specified
De Hullu, 2002	Cohort study, 15 years (1982 – 1997)	79	Histologically confirmed recurrence within 2 or 4 years after primary treatment	0/2	9/38	0/39	RT: 1 patient with a tumour positive margin	
Groenen, 2010	Cohort study, (2000 – 2005)	93	The period from the date of surgery till the clinically and histologically confirmed date of relapse	3/13	11/50	7/30	Re-excision: 13 (26%) of 50 < 8 mm and 7 (54%) of 13 tumour-positive RT: 11 (22%) of 50 < 8 mm and 2 (15%) of 13 tumour-positive	Influence of adjuvant therapy on local recurrence rate further specified. The number of local recurrence was comparable between the patients that did and did not receive adjuvant therapy
Heaps, 1990	Cohort study, (1957 – 1985)	135	Not specified	4/7	17/37	0/91	NS	Patients with a local recurrence more often had a stage 3 or 4 VSCC
Iacoponi, 2013	Cohort study, (2000 – 2010)	87	The appearance of tumour in a new location after a minimum disease-free period of 6 months	0/1	7/22	24/65	RT: 35 (40%) of 87	Influence of adjuvant therapy on local recurrence rate not further specified

Rouzier, 2002	Cohort study, (1978 – 1999)	215	Any tumour recurrence involving the skin and the subcutaneous tissues located around the vulvectomy scar or involving the skin bridge between the vulvectomy and groin dissection areas	NS	15/44	18/171	RT: 30 (68%) of 44 < 1 cm	Patients with a tumour-positive margin are included in the group of patients with a tumour-free margin < 1 cm 1 cm as cut-off value instead of 8 mm
Tantipalakorn, 2009	Cohort study, (1987 – 2005)	116	A recurrence within 2 cm of the primary tumour site, > 2 cm of the primary tumour site and skin bridge recurrence (in the dermis and the subcutaneous tissue between the groin and vulvar incisions)	0	8/24	17/92	NS	All patients had stage 1 or 2 VSCC Three types of recurrence described, but not specified for each patient group
Viswanathan, 2013	Cohort study, (1988 – 2009)	205	Recurrence free survival: the interval from diagnosis of primary disease to the date of first evidence of disease recurrence or progression or death from any cause.	9/20	44/116	9/69	RT: 11 (16%) of 69 ≥ 1 cm, 24 (21%) of 116 < 1 cm, 4 (20%) of 20 tumour-positive	1 cm as cut-off value instead of 8 mm Influence of adjuvant therapy on local recurrence rate not further specified
Woelber, 2011	Cohort study, (1998 – 2008)	102	Not specified	0	7/72	3/30	RT: 22 (31%) of 72 < 8 mm and 4 (13%) of 30 ≥ 8 mm	Influence of adjuvant therapy on local recurrence rate not further specified

NS: non-significant;
RT: radiotherapy;
VSCC: vulvar squamous cell carcinoma

Four studies (4, 19, 28, 31) found an increased risk of local recurrence for patients with a tumour-free margin <8 mm, with risk ratios ranging from 3.2 to 84.7. It should be emphasised, though, that in one of these studies (risk ratio, 3.2 [95% confidence interval {CI}: 1.8 – 5.9]), patients with a tumour-positive margin were included in the group of patients with a tumour-free margin of <8 mm (31). Six studies (3, 5, 29, 30, 32, 36) found no clearly increased risk of local recurrence when comparing <8 mm versus ≥ 8 mm.

Pooled random effects meta-analysis of these studies involving 1278 VSCC patients and 255 local recurrences showed a twofold increase in the risk of local recurrence for patients with a tumour-free margin <8 mm versus ≥ 8 mm (risk ratio 1.99 [95%CI 1.1 – 3.5], Figure 2). I^2 of the pooled analysis was 73%. After exclusion of the two studies that used 1 cm as cutoff instead of 8 mm, the risk ratio for local recurrence was 1.8 (95% CI 0.9–3.9) (Supplementary figure 1) (4, 31). After exclusion of the study that included patients with a positive margin in the <8 mm group, the pooled risk ratio was 1.88 (95% CI 0.99–3.5) (31).

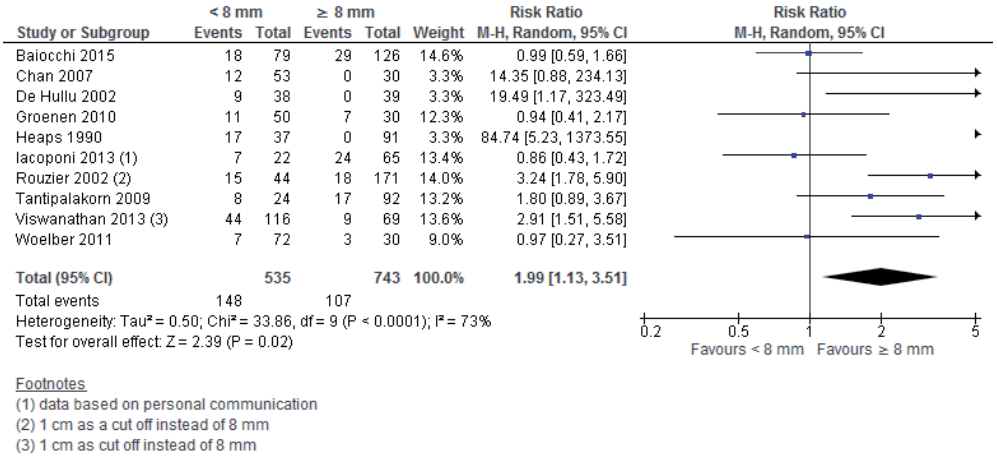

Figure 2: Meta-analysis. CI: confidence interval

Cohort study

Patient characteristics

Between January 2000 and December 2012, 192 patients underwent primary surgical treatment for VSCC at the Leiden University Medical Centre and 148 patients met the inclusion criteria for our study. The 44 patients that were excluded had a tumour with an infiltration depth of <1 mm or no residual tumour in the surgical specimen after excision biopsy at another hospital. Patient characteristics are described in table 2. Thirty patients (20%) had a tumour-positive margin, 92 (62%) had a tumour-free margin <8mm, and 26 (18%) had a tumour-free margin of ≥8 mm. The patient groups

Table 2: Patient characteristics (n=148)

Clinicopathological characteristics	**Tumour-positive margin (n=30)**	**Tumour-free margin < 8mm (n= 92)**	**Tumour-free margin ≥ 8mm (n= 26)**	**p-value**
Age (mean in years)	75	68	69	0.109
FIGO 2009				0.237
Stage I	12 (40.0%)	57 (62.0%)	18 (69.2%)	
Stage II	2 (6.7%)	2 (2.2%)	0 (0%)	
Stage III	15 (50.0%)	32 (34.8%)	8 (30.8%)	
Stage IV	1 (3.3%)	1 (1.1%)	0 (0%)	
Tumour size				< 0.001
Tumour size ≤ 40mm	13 (43.3%)	73 (79.3%)	23 (88.5%)	
Tumour size > 40mm	17 (56.7%)	19 (20.7%)	3 (11.5%)	
Depth of invasion				0.527
Depth of invasion ≤ 4mm	8 (26.7%)	37 (40.2%)	12 (46.2%)	
Depth of invasion > 4mm	22 (73.3%)	55 (59.8%)	14 (53.8%)	
LVSI				0.177
Yes	8 (26.7%)	16 (17.4%)	2 (7.7%)	
No	22 (73.3%)	76 (82.6%)	24 (92.3%)	
Primary treatment vulva				0.190
Radical local excision	12 (40.0%)	54 (58.7%)	13 (50.0%)	
Vulvectomy	18 (60.0%)	38 (41.3%)	13 (50.0%)	
Additional therapy				<0.001
Vulvar radiotherapy	20 (66.7%)[1]	22 (23.9%)	1 (3,8%)[3]	
Re-excision	7 (23.3%)[1]	15 (16.3%)	0 (0%)	
None	4 (13,3%)[2]	55 (59,8%)	25 (96,2%)	
Lymph node status				
Tumour-positive lymph nodes in the groin(s)	17 (56.7%)	33 (35.9%)	9 (34.6%)	0.108
Extracapsular spread	8 (26.7%)	13 (14.1%)	3 (11.5%)	0.210
Recurrence				
Local recurrence[4]	9 (30.0%)	9 (9.8%)	3 (11.5%)	0.010
Total recurrences[5]	12 (40.0%)	24 (26.1%)	6 (23.1%)	0.009
Median follow up time (months)	16	44	47	0.033

FIGO: Federation of Gynaecology and Obstetrics
LVSI: lymphovascular space invasion

[1] One patient with a tumour-positive histological margin received radiotherapy and reexcision as adjuvant treatment.

[2] Although indicated, four patients with a tumour-positive margin did not receive adjuvant therapy; one patient had metastasised disease and received palliative treatment only, one patient could not undergo radiotherapy because of severe comorbidity, one patient suffered from impaired wound healing and therefore an expectant management was and one patient died a few days postoperatively.

[3] This patient had an indication for postoperative radiotherapy on the inguinal region and simultaneously received radiotherapy on the vulva.

[4] Local recurrence: a histologically confirmed recurrence within 2 years after primary tumour on the ipsilateral side of the vulva.

[5] Total recurrences: all histologically confirmed recurrences on the vulva, irrespective of time and localisation.

were comparable for age, Federation of Gynaecology and Obstetrics (FIGO) stage, depth of invasion, primary treatment of the vulva, and lymph node status. Tumour size was larger in patients with a tumour-positive margin. Review of the tumour-free margins by an expert gynaecopathologist resulted in adjustment of the patient group in six cases (five patients initially had a tumour-free margin ≥8 mm according to the pathology report, but after revision had a tumour-free margin of <8 mm; another patient had a tumour-free margin <8 mm, but after revision it was ≥8 mm). Median follow-up time was 42 months (mean, 53.8 [range, 0–174] months).

Additional treatment was given to 26 of 30 patients (87%) with a tumour-positive margin (Figure 3). In the group with a tumour-free margins <8 mm, 37 of 92 patients (40%) received additional treatment (16% reexcision, 24% radiotherapy). Within this group, the mean tumour-free margin for patients who did and did not receive additional treatment was 3.1 mm (range, 0.31–7.85 mm) and 4.4 mm (range, 0.8–7.86), respectively.

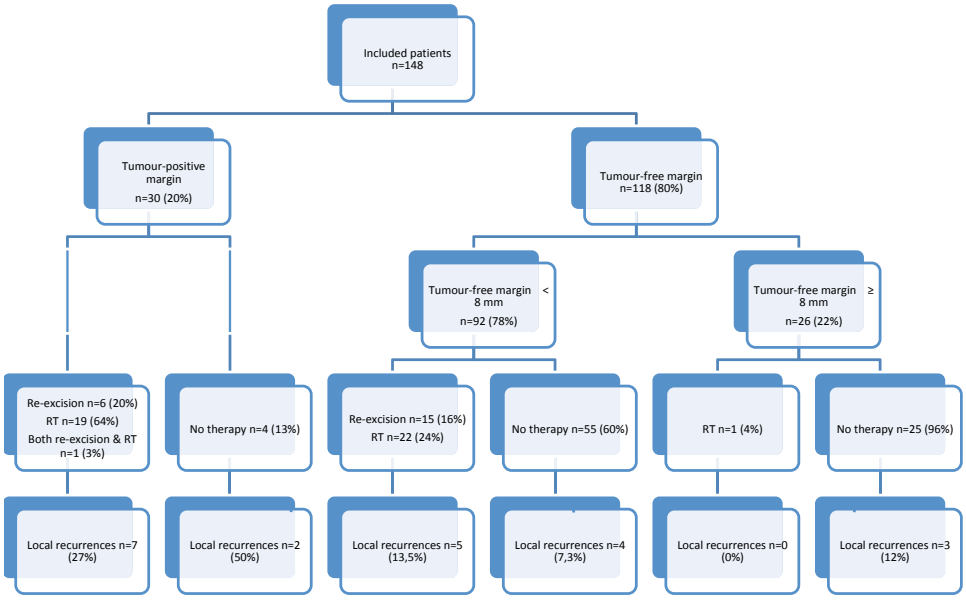

Figure 3: flowchart of margin distance, adjuvant treatment strategy and local recurrence rate

Risk of recurrence

Twenty-one of 148 patients (14%) developed local recurrences on the ipsilateral side of the vulva within 2 years after primary treatment. Another 21 patients developed a new tumour on the contralateral side of the vulva and/or more than 2 years after primary treatment, which were considered second primary tumours in this study (Table

2). In nine of these patients, the tumour developed on the ipsilateral side of the vulva. The competing risk analysis showed a cumulative incidence for local recurrence of 31% for patients with a tumour-positive margin, 10% for patients with a tumour-free margin of <8 mm, and 12% for patients with a tumour-free margin of ≥8 mm (p=0.01; Supplementary figure 2). There was no significant difference regarding local recurrence risk between the group of patients with a tumour-free margin of <8 mm versus ≥ 8 mm (hazard ratio [HR], 1.18 [95%CI: 0.32 – 4.35]).

Figure 3 displays the number of local recurrences in the different patient groups, taking additional treatment into account. Within the <8-mm group, there was no clear difference in local recurrence risk for patients who received additional treatment compared to patients who had no additional treatment (14% versus 7%, p=0.323). Of note, patients who received additional treatment more often had a higher FIGO stage, positive lymph nodes, and extracapsular growth of lymph node metastases, which are all known risk factors for local recurrence (data not shown) (20, 21). When analysing other tumour-free margins of 2, 4, and 6 mm, we found no differences in local recurrence risk (Table 3).

Table 3: local recurrence rate for other tumour-free margins

	2 mm	4 mm	6 mm
< tumour-free margin	2/19 (10.5%)	4/44 (9.1%)	7/74 (9.5%)
≥ tumour-free margin	10/99 (10.1%)	8/74 (10.8%)	5/44 (11.4%)
p-value	NS	NS	NS

NS: non-significant

Analysis of all "recurrences", irrespective of time and localisation on the vulva, showed no significant difference between the group of patients with a tumour-free margin of <8 mm and those with ≥8 mm (p=0.766) (data not shown).

Univariable and multivariable analysis

The results of the univariable and multivariable analyses for local recurrent disease of the vulva are shown in table 4. In univariable analysis, FIGO stage, positive lymph nodes, extracapsular growth, and the presence of a tumour-positive margin were associated with local recurrence. The only predictive factor for risk of local recurrence in the multivariable analysis was the presence of a tumour-positive margin versus a tumour-free margin of <8 mm (HR, 0.21 [95%CI 0.08–0.55]). A tumour-free margin of <8 mm did not clearly increase the risk of local recurrence compared to a tumour-free

margin of ≥8 mm in both univariable and multivariable analysis (HR, 1.18 [95% CI: 0.32–4.35] and HR, 1.09 [95% CI: 0.28–4.19], respectively). We performed a separate multivariable analysis on the influence of additional treatment on local recurrence risk in the group of patients with a tumour-free margin of <8 mm and corrected for FIGO stage, positive lymph nodes, extracapsular growth, and tumour-free margin distance. Patients who received additional treatment had a HR of 1.16 (95% CI: 0.23–5.84) for local recurrence compared to patients who did not (data not shown).

Table 4: Univariable and multivariable analysis for local recurrence

Predictors of local recurrence	Univariable analysis		Multivariable analysis	
	Hazard Ratio (CI)	p-value	Hazard Ratio (CI)	p-value
Age	1.02 (0.99 – 1.05)	0.281		
Tumour characteristics				
Tumour diameter ≤4 cm	1			
Tumour diameter >4 cm	1.43 (0.56 – 3.70)	0.456		
Tumour infiltration <4mm	1			
Tumour infiltration ≥4mm	1.48 (0.59 – 3.67)	0.396		
FIGO				
Stage 1&2	1		1	
Stage 3&4	2.73 (1.15 – 6.51)	**0.023**	1.67 (0.59 – 4.76)	0.339
Lymph node status				
Tumor negative	1		1	
Tumor positive	2.73 (1.15 – 6.51)	**0.023**	1.67 (0.59 – 4.76)	0.339
Extra capsular growth				
No	1		1	
Yes	3.20 (1.24 – 8.29)	**0.017**	2.53 (0.79 – 8.13)	0.120
Additional vulvar treatment				
No	1			
Yes	1.93 (0.81 – 4.59)	0.132	*	
HPV				
Negative	1			
Positive	0.24 (0.03 – 1.80)	0.240		
Margins				
< 8 mm versus positive margin	0.22 (0.09 – 0.55)	**0.001**	0.21 (0.08 – 0.55)	**0.001**
≥ 8 mm versus positive margin	0.25 (0.07 – 0.94)	**0.041**	0.29 (0.08 – 1.11)	0.070
< 8 mm versus ≥ 8 mm	1.18 (0.32 – 4.35)	0.808	1.09 (0.28 – 4.19)	0.903

CI: confidence interval
FIGO: Federation of Gynaecology and Obstetrics
HPV: human papillomavirus
*No multivariable analysis as in the group ≥8 mm only one patient was additionally treated

Discussion

Most guidelines recommend a tumour-free margin of ≥8 mm in the surgical treatment of VSCC (12, 13, 26), a recommendation that is mostly consensus based and supported by a lower level of evidence. To investigate whether tumour-free margins <8 mm are associated with an increased local recurrence risk in patients with primary VSCC, a meta-analysis was performed. This analysis showed a twofold increase in the local recurrence risk for patients with a tumour-free margin of <8 mm versus ≥8 mm. Nevertheless, there were substantial challenges regarding this meta-analysis. All included studies were retrospective, which made extraction of the necessary data difficult. Furthermore, it is imaginable that recurrences were missed due to the retrospective character of the studies, causing a reporting bias. Moreover, the included studies presented highly heterogeneous results. This might be partly explained by the different definitions of local recurrence used in the studies and missing data on additional treatment. Besides the meta-analysis, we performed a cohort analysis using a strict definition of local recurrence and considering the effect of additional treatment. In our cohort study, local recurrence risk within 2 years after primary surgery on the ipsilateral side of the vulva was 14%. We found no clear difference in local recurrence risk for patients with a tumour-free margin of <8 mm versus ≥ 8 mm. Importantly, a post hoc analysis of tumour-free margins of 2, 4, and 6 mm showed no difference in local recurrence risk. In a multivariable analysis, tumour-positive margins were the only independent risk factor for local recurrence.

In the meta-analysis, a total of 1278 VSCC patients from 10 studies were included. However, as mentioned above, statistical heterogeneity between the studies included was considerable (I^2=73%), and the results are therefore not easy to apply to individual patients. The local recurrence risk was 20%, which is consistent with the local recurrence risk found in other studies (20, 21). Definitions of local recurrence were different or not reported in the included studies (Table 1) (3, 5, 19). Other studies did not describe the distance to the primary tumour and/or the time span until a local recurrence (4, 28-32, 36). This can result in an overestimation of local recurrence risk because 'true local recurrences', as well as 'second primary tumours', are considered local recurrences. One study found that 14/52 (27%) 'local recurrences' were detected more than 2 years after primary treatment (28), and other studies showed that the maximum time to local recurrence could be as long as 166 months (4, 30, 36). It is unlikely that the size of the tumour-free margin has an influence on these 'late recurrences' or rather 'second primary tumours', which is also illustrated by the finding that remote site vulvar recurrences in general have a longer time to recurrence than primary site recurrences (31, 32). Indeed, in our cohort study we found that 21/42 (50%) newly developed tumours developed after more than 2 years or on the contralateral side of the vulva. Analysis of all "recurrences" in our cohort study, irrespective of time and localisation on the vulva, showed no significant

difference between patients with a tumour-free margin of <8 mm versus ≥8 mm (p=0.729). However, to definitely distinguish local recurrence from a second primary tumour, clonal or genetic relationship analysis should be performed (24).

Currently, there is very limited evidence on the effect of additional treatment (reexcision or adjuvant radiotherapy) with respect to the reduction of local recurrences after surgery in different tumour types. Importantly, randomised trials are lacking. A recent cohort study in 85 breast cancer patients with short tumour-free margins (≤ 2 mm) after breast-conserving surgery found a similar local recurrence risk for patients who underwent a reexcision (53%) versus those that did not (47%) (p=0.67) (37). To our knowledge, there are no studies on the impact of reexcision on local recurrence risk after primary treatment for VSCC. One cohort study including 34 VSCC patients with a tumour-free margin <8 mm investigated the influence of adjuvant radiotherapy and found a reduction in isolated local recurrence risk from 33% to 5% after adjuvant radiotherapy (33).

Missing data on additional treatment was a major limitation in the interpretation of the results of the meta-analysis, which hampered any conclusions on treatment effects. Only one study specified additional treatment in patients with a tumour-free margin <8 mm and found no difference in local recurrence risk (29). In our cohort study, 40% of patients in the <8 mm group received additional treatment. In these patients, the local recurrence risk was not different than that of patients who did not receive additional treatment. However, it should be considered that the patient group receiving additional treatment more often had a higher FIGO stage, positive lymph nodes, and extracapsular growth of lymph node metastases, which are all known prognostic factors that could influence the local recurrence risk (20, 21). Due to these limited data, it is not possible to make a final conclusion on the value of adjuvant treatment in patients with a tumour-free margin of < 8 mm.

In this meta-analysis and cohort study, we focused on 8 mm as a cutoff value for the tumour-free margin because this tumour-free margin is recommended in the Dutch and US guidelines (12, 13, 26). A post hoc analysis in our cohort study for tumour-free margins of 2, 4, and 6 mm showed no difference in local recurrence risk. There are few other studies that examined tumour-free margins other than 8 mm in the surgical treatment of VSCC (3 and 5 mm). In two studies, no difference in local recurrence risk was found for a tumour-free margin of 3 or 5 mm (5, 38). In contrast, Viswanathan et al. described a significantly reduced local recurrence risk for tumour-free margins ≥5 mm (HR 0.53 [95% CI 0.3–0.9]) (4). Two other studies defined a positive margin as <3 mm and found an increased local recurrence risk for patients with a 'tumour-positive' margin. However, these studies did not describe whether patients with tumour-positive margins were also included in the <3 mm patient group (39, 40).

In summary, currently, there is no firm evidence on the optimal length of the tumour-free margin in the treatment of VSCC. Due to the low incidence of vulvar cancer, there are no large prospective studies concerning this important clinical issue. This work provides important data to question the commonly used 8 mm margin as a prognosticator for local recurrence. More research is needed to address the question of whether additional treatment improves the prognosis in patients with a tumour-free margin smaller than 8 mm and what the best cutoff for the tumour-free margin would be.

Conflict of interest statement
None declared

Appendix A

Search string for meta-analysis:
("Vulvar Neoplasms"[Mesh] OR "vulva carcinoma"[all fields] OR "vulvar carcinoma"[all fields] OR "vulva carcinomas"[all fields] OR "vulvar carcinomas"[all fields] OR "Vulvar Neoplasm"[all fields] OR "Vulva Neoplasms"[all fields] OR "Vulvar Neoplasms"[all fields] OR "Cancer of Vulva"[all fields] OR "Vulva Cancers"[all fields] OR "Cancer of the Vulva"[all fields] OR "Vulva Cancer"[all fields] OR "Vulvar Cancer"[all fields] OR "Vulvar Cancers"[all fields] OR "vulval carcinoma"[all fields] OR "vulval carcinomas"[all fields] OR "Vulval Neoplasm"[all fields] OR "Vulval Neoplasms"[all fields] OR "Vulval Cancers"[all fields] OR "Vulval Cancer"[all fields] OR "vulva neoplasia"[all fields] OR "vulvar neoplasia"[all fields] OR "vulval neoplasia"[all fields]) AND ("surgical margin"[all fields] OR "histological margin"[all fields] OR "surgical margins"[all fields] OR "histological margins"[all fields] OR "surgical excision margin"[all fields] OR "surgical excision margins"[all fields] OR "clinical margin"[all fields] OR "clinical margins"[all fields] OR "margin assessment"[all fields] OR "histopathologic margin"[all fields] OR "excision margin"[all fields] OR "tumor margin"[all fields] OR "tumour margin"[all fields] OR "histopathologic margins"[all fields] OR "excision margins"[all fields] OR "tumor margins"[all fields] OR "tumour margins"[all fields] OR "margin"[all fields] OR "margins"[all fields])

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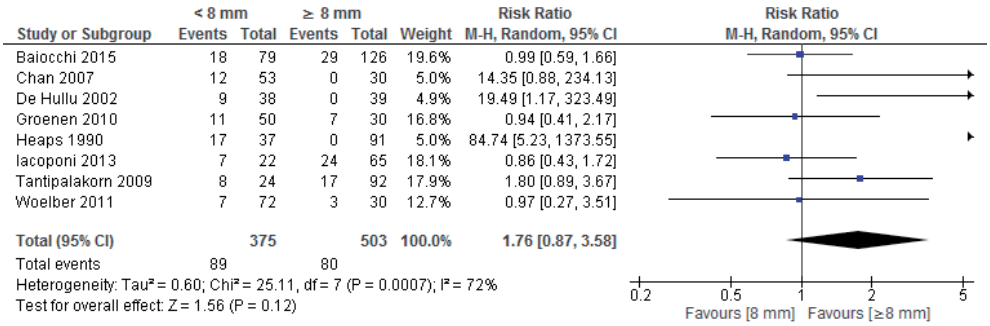
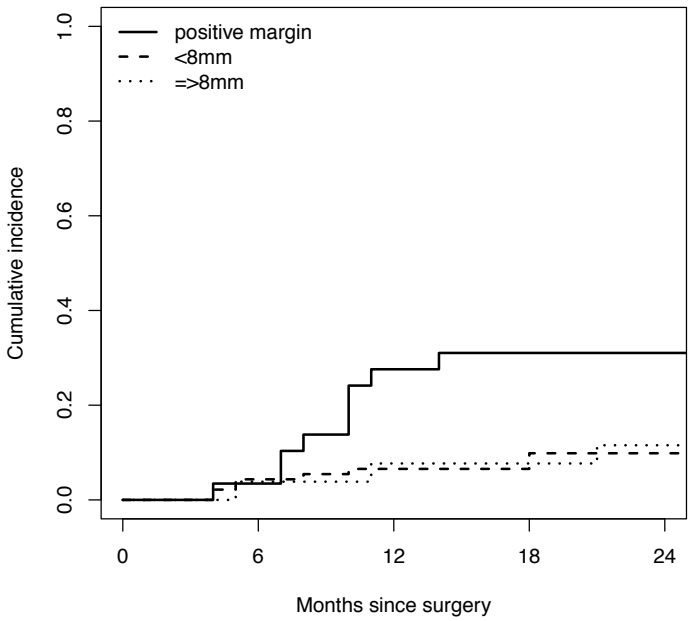
Supplementary data



Supplementary figure 1: Results meta-analysis after exclusion of two studies with 1 cm as a cutoff



Supplementary figure 2: Cumulative incidence for local recurrence

Supplementary table 1: Risk of bias analysis

Study	Selection bias (consecutive patients or a random sample)	Performance bias (Unequal co-interventions during follow-up?)	Attrition bias (loss to follow-up related to outcome?)	Detection bias (unequal length of follow-up?)	Reporting bias (no histological confirmation)
Baiocchi, 2015	Not described	Not described	Not described	Not described	Not described
Chan, 2007	No bias	Not described	Not described	Not described	Not described
De Hullu, 2002	No bias	No bias	No bias	Not described	No bias
Groenen, 2010	No bias	No bias	Not described	Not described	No bias
Heaps, 1990	No bias	Not described	Not described	Not described	Not described
Iacoponi, 2013	Not described	No bias	Not described	Not described	No bias
Rouzier, 2002	No bias	Not described	Not described	Not described	No bias
Tantipalakorn, 2009	Not described	No bias	Not described	Not described	Not described
Viswanathan, 2013	No bias	Not described	Not described	Not described	No bias
Woelber, 2011	No bias	Not described	Not described	Not described	Not described