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Margin status revisited in vulvar squamous cell carcinoma

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HIGHLIGHTS

- Local recurrences occur in 43% of the vulvar cancer patients within ten years after treatment.
- Pathologic tumor free margin distance had no effect on the local recurrence rate.
- Patients with dVIN (with or without LS) in the margin have higher local recurrence rates.

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ABSTRACT

Objective. To determine the incidence of local recurrence of vulvar squamous cell carcinoma in relation to tumor- and/or precursor lesion free pathologic margins.

Methods. Consecutive patients with primary vulvar squamous cell carcinoma surgically treated in two Dutch expert centers between 2000 and 2010 were included. All pathology slides were independently reviewed by two expert gynecopathologists, and local recurrence was defined as any recurrent disease located on the vulva. Time to first local recurrence was compared for different subgroups using univariable and multivariable Cox-regression analyses.

Results. In total 287 patients with a median follow-up of 80 months (range 0–204) were analyzed. The actuarial local recurrence rate ten years after treatment was 42.5%. Pathologic tumor free margin distance did not influence the risk on local recurrence (HR 1.03 (95% CI 0.99–1.06)), neither using a cutoff of eight, five, or three millimeters. Multivariable analyses showed a higher local recurrence rate in patients with dVIN and LS in the margin (HR 2.76 (95% CI 1.62–4.71)), in patients with dVIN in the margin (HR 2.14 (95% CI 1.11–4.12)), and a FIGO stage II or higher (HR 1.62 (95% CI 1.05–2.48)).

Conclusions. Local recurrences frequently occur in patients with primary vulvar carcinoma and are associated with dVIN (with or without LS) in the pathologic margin rather than any tumor free margin distance. Our results should lead to increased awareness among physicians of an ongoing risk for local recurrence and need for life-long follow-up. Intensified follow-up and treatment protocols for patients with dVIN in the margin should be evaluated in future research.

1. Background

In patients treated for early-stage vulvar squamous cell carcinoma, local recurrences are reported in up to 40% in the first 10 years after primary diagnosis [1]. A recent systematic review from our group estimated an annual local recurrence rate of 4% without plateauing despite adequate treatment [2]. In literature, data on prognostic factors related to local recurrences are mostly limited to classical clinico-pathologic factors. These data are heterogeneous and not sufficiently robust to propose individualized treatment and follow-up guidelines related to the risk on local recurrences [2].

Even though the current surgical approach has less morbidity than before, radical surgery of the vulva is still mutilating. Currently, the recommended surgical tumor-free margin distance varies between

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different guidelines, ranging between one to two centimeters [3–5]. The pathologic tumor margin distance with cut-off value of eight millimeters has frequently been challenged as a prognostic factor. However, studies investigating a lower cut-off value are scarce, retrospective and without proper central pathologic review. Therefore data available so far are insufficient to draw conclusions on which pathologic tumor free margin distance is safe without increasing the local recurrence rate [6,7].

Besides the tumor-free margin distance, vulvar precursor lesions in the skin adjacent to the tumor and/or in the margin could be of prognostic significance. Two different pathways with their own precursor lesions have been identified so far in the development of vulvar squamous cell carcinoma; the first and most common pathway is associated with lichen sclerosus (LS) and differentiated vulvar intraepithelial neoplasia (dVIN). The second pathway is caused by a persistent human papillomavirus (HPV) infection with high-grade squamous intraepithelial lesions (HSIL) as associated precursor [8]. Data from recently published studies indicate that the presence of LS in the resection specimen of vulvar carcinoma may strongly increase the risk of local recurrences [9,10].

Currently no stratification of vulvar carcinoma patients with respect to their risk for local recurrence is possible. However, identification of patients at such low risk that they can be discarded from follow-up, for example after two or five years, would have significant clinical benefit. Simultaneously for high-risk patients new strategies might be developed and evaluated to prevent local recurrences.

The main aim of this study was to determine the incidence of local recurrence of vulvar squamous cell carcinoma, in a clinically well-defined consecutive patient series from two expert centers. The secondary aims were to assess the relation of local recurrence to tumor- and/or precursor lesion free pathologic margins determined by extensive pathology review, and based on these results to identify different risk groups, allowing future individualized treatment and follow-up strategies.

2. Methods

This study is reported in accordance with the Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) [11].

2.1. Patients

Consecutive patients with vulvar squamous cell carcinoma treated at the Radboud university medical center and University Medical Center Groningen from January 2000 – December 2010 were eligible for analysis. Both centers are expert centers for the treatment of vulvar carcinoma. Eligibility criteria for this study were: primary diagnosis of vulvar squamous cell carcinoma and primarily surgically treated at one of the two participating centers. Patients who suffered from multifocal disease, or patients who received neoadjuvant chemotherapy and/or radiotherapy, definitive (chemo) radiation or palliative treatment were not included.

Clinical data of all patients treated for vulvar carcinoma in both centers were prospectively stored in a database and completed by retrospective review of the patient charts. We identified eligible patients for this study from this database. To ensure completeness, we searched the Dutch nationwide registry of histopathology and cytology (PALGA), which resulted in nine additional patients in the two centers.

2.2. Treatment and follow-up

The surgical treatment of the vulva consisted of either a radical vulvectomy or wide local excision of the tumor. For a wide local excision, the intention was to obtain surgical tumor-free margins of at least 10 mm. In patients with a macro-invasive tumor (depth of invasion > 1 mm), a sentinel node procedure and/or an inguinofemoral lymphadenectomy was performed. Adjuvant therapy with re-excision was recommended in patients with a tumor-positive margin. When re-excision was not possible, adjuvant radiotherapy to the vulva was recommended. In patients with a pathologic tumor free margin of <8 mm close follow-up was performed. When patients had an indication for radiotherapy to the groins, adjuvant radiotherapy on the vulva was considered in patients with pathologic tumor free margin distance of <8 mm. Adjuvant radiotherapy to the groin was indicated for patients with ≥2 metastatic lymph nodes, or in case of extra nodal growth. From 2000 until 2006, patients from both our centers participated in the GROningen INternational Study on Sentinel Nodes in Vulvar cancer (GROINSS-V) I study, while from 2006 onwards patients participated in the GROINSS-V II study. Long-term follow-up data of patients with early-stage disease who were included in the GROINSS-V I study have been published previously [1]. After treatment, patients were examined routinely every two to three months during the first two years after completion of primary treatment, every six months during the third and fourth year and yearly thereafter.

2.3. Histopathologic review

For this study, all formalin-fixed and paraffin-embedded and hematoxylin and eosin stained slides, were reviewed in a standardized way by two independent expert gynecopathologists (JB and HH), blinded for the results of treatment and follow-up data. Eight test cases were analyzed by both gynecopathologists independently, after which agreement scores for these eight cases were calculated and a consensus meeting was organized to reduce the interobserver variability. Before the consensus meeting percentage of agreement was 62–66% for the assessment of the presence of LVSI, differentiation grade and the presence of LS in the pathologic margin, 100% for the assessment of LS, HSIL and/or dVIN adjacent to the tumor and 100% for the assessment of HSIL and dVIN in the pathologic margin. The intraclass correlation coefficient for the smallest pathologic margin was 0.98 and for the depth of invasion 0.94. After the consensus meeting, interobserver variety was minimal for all variables analyzed. Histopathological review of the included patients was performed by one of the two expert gynecopathologists and included the following variables: tumor type, pathologic tumor free margin distance for the basal and lateral margin separately, presence of low-grade squamous intraepithelial lesions (LSIL), high-grade squamous intraepithelial lesions (HSIL), lichen sclerosus (LS) or differentiated vulvar intraepithelial neoplasia (dVIN) adjacent to the tumor and/or in the pathologic margin (according the ISSVD classification [8]), depth of invasion, tumor thickness, tumor diameter, presence of lymphangio-invasion, growth pattern and grade of differentiation. If re-excision was performed, both the slides of the primary excision and re-excision were reviewed.

2.4. Data handling

All data were entered in an anonymous database using Castor EDC in which patient identity was protected by study-specific unique patient numbers [12]. The codes of these specific numbers were only known to two dedicated data managers for each center separately. The use of these procedures combined with the fact that patients did not object against use of their clinical data or tumor material, meant that, according to Dutch law, no further patient or IRB approval was needed.

2.5. Endpoints

The primary endpoint was time to local recurrence of vulvar carcinoma. Local recurrence was defined as any newly diagnosed invasive squamous cell carcinoma located on the vulva, and time to local recurrence was defined as the period of time in months from the date of primary surgery to the date at which recurrence was identified by histopathology. The end of follow-up was defined as date of last

follow-up or date of death. Patients were reported lost to follow-up if no information on the last 24 months was available at time of data collection. Follow-up data were collected until January 1st, 2018. The median follow-up time was 80 months (range 0–204 months).

2.6. Definitions

The tumor specimens were formalin-fixed and paraffin-embedded (FFPE) in tissue blocks and all pathologic tumor free margin distances were measured on hematoxylin and eosin (H&E) stained slides from these blocks. Multiple sections at the tumor edges and margin were performed, and all reviewed in order to detect the smallest pathologic margin. The pathologic tumor free margin distance was defined as the distance in millimeters from the tumor edge to the end of the specimen, measured along the epithelium after formalin fixation using a ruler. All lateral tumor free margins were measured. Besides, the basal tumor free margin was measured. We determined the closest pathologic tumor free margin by taking into account both the lateral and basal margins. In case re-excision was performed, the closest margin after re-excision was assessed. The depth of invasion was measured from the epithelial-stromal junction of the most superficial adjacent dermal papillae to the deepest point of invasion as recommended by The International Society of Gynecological Pathologists (ISGYP) and The International Federation of Gynecology and Obstetrics (FIGO) [13]. Regarding the precursor lesions; we noted the presence of these lesions as 1) adjacent to the tumor; but not in the pathologic margin or as 2) precursor lesion in margin; located in the pathologic margin. For the variable presence of precursor lesion in the pathologic margin, the latter had to be present. For the variable presence of precursor lesion in the excised specimen either one of the two previously described variables; a precursor lesion adjacent to the tumor or in the margin or both were present. The following patient, tumor and treatment characteristics were collected: age, FIGO stage (2009), TNM stage, tumor localization, treatment given (primary and adjuvant), histopathologic outcomes and follow-up data.

2.7. Statistical analysis methods

Continuous variables were summarized using the median and range, discrete variables were described by frequencies. The local recurrence rate was determined using the Kaplan-Meier method. Censoring was applied to patients alive without local recurrence at last follow-up and patients who died. Time to first local recurrence was calculated from the date of primary surgery and compared for each prognostic factor performing univariate Cox-regression analysis; hazard ratios (HR) with 95% confidence intervals (CI) were presented. A p-value of <0.05 was considered to be statistically significant. Variables that had a p-value < 0.200 or were considered clinically relevant were incorporated in a multivariable Cox-regression analysis. The Akaike information criterion (AIC) and Bayesian information criterion (BIC) values were analyzed to compare the relative quality of the different Cox-regression models. A lower AIC of BIC indicates a better fit of the model compared to the other models. Together with the significance of the variables, the multivariable model with the best fit was chosen. Data analysis was performed using SPSS software (version 25.0, Armonk 2017) [14] and the statistical software R (version 3.5.0), with the survival package.

3. Results

During the study period, 435 patients were primarily surgically treated for vulvar carcinoma. For a variety of reasons, 148/435 patients were excluded, see flowchart in Supplementary Fig. 1. In total, data from 287 patients were analyzed. Median age was 73 years (range 26–100), and all TNM and FIGO stages were represented, except FIGO stage IVB. Fifty-two patients were lost to follow-up for at least two years at time of data collection; median follow-up of these patients was 64 months (range 0–196). Clinical and histopathologic characteristics of the study population are listed in Table 1, and did not differ between the two centers.

The actuarial local recurrence rates five and ten years after primary treatment were 28.3% and 42.5%, respectively, see Fig. 1. This rate did not differ between patients from both treatment centers individually: 5 and 10-year local recurrence rates were 26,0% and 30,5% and 42,6% and 43,5% respectively ($p = 0.679$). Median time to local recurrence was 32 months (range 0–202 months) and this did not differ significantly per precursor lesion subgroup ($p = 0.08$).

3.1. Pathologic margin in relation to local recurrences

The pathologic tumor free margin distance had no effect on the local recurrence rate (continuous HR 1.03 (95% CI 0.98–1.06)). No differences in local recurrence rate were observed, neither for the cut-off values ≥ 8 mm versus <8 mm, nor for different cut-off values (3–8 mm) ($p = 0.308$). Exclusion of patients with adjuvant radiotherapy on the vulva also did not indicate more local recurrences in relation to a smaller tumor-free margin distance. Because of small subgroups, patients were categorized using the cutoff point of eight, five and three millimeters, as shown in Table 2 and Supplementary Table 1.

The local recurrence rate ranged from 28.1% for patients with HSIL, 30.7% for patients with no precursor lesion, 44.2% for patients with LS, 44.8% for patients with dVIN, and 76.4% for patients with both LS and dVIN in the resection margin 10 years after treatment, respectively (see Fig. 2). Univariable analyses of all included patients using binary variables showed that dVIN and/or LS in the margin, was associated with more local recurrences compared to no dVIN and/or LS in the margin (dVIN and LS present; HR 2.58 (95% CI 1.55–4.32); dVIN present HR 2.39 (95% CI 1.54–372), LS present HR 1.56 (95% CI 1.02–2.39), see Table 2. There was no difference in local recurrence rate when HSIL was present in the margin compared to no HSIL in the margin.

Fig. 2 shows that patients with dVIN in the margin, with or without LS, have significant higher local recurrence rates compared to patients without any precursor lesion in the margin (HR 3.32 (95% CI 1.79–6.16) and HR 2.28 (95% CI 1.10–4.71) respectively). Furthermore, within the subgroup of patients with dVIN in the excised specimen, the local recurrence rate was higher in patients with dVIN located in the margin compared to patients without dVIN in the margin but adjacent to the tumor (10- year local recurrence rate 60.5% versus 41.6% respectively, $p = 0.002$), see Fig. 3.

3.2. Presence of precursor lesion in the excised specimen in relation to local recurrences

Univariable Cox-regression analyses were performed using a binary variable for the presence of a precursor lesion in the excised specimen. The presence of dVIN, LS or both dVIN and LS in the excised specimen was associated with a higher local recurrence rate (HR 1.80 (95% CI 1.08–2.99) and HR 1.61 (95% CI 1.03–2.52), 1.58 (95% CI 1.04–2.41) respectively) compared to patients without these precursor lesions present. The presence of HSIL was associated with a lower local recurrence rate (HR 0.32 (95% CI 0.14–0.75) compared to patients without HSIL (see Supplementary Table 2).

Univariable Cox-regression analyses dividing patients in five subgroups based on the presence of a precursor lesion in the excised specimen (no precursor lesion, dVIN and LS, dVIN, LS and HSIL) showed no difference in local recurrence rate between the subgroups of precursor lesions and the subgroup without precursor lesions (see Supplementary Fig 2).

3.3. Tumor characteristics in relation to local recurrences

The growth pattern, presence of lymph-vascular space invasion (LVSI), grade of differentiation, tumor diameter and depth of

Table 1
Clinical and histopathologic characteristics of the study population.

	Median (range)	Total 287 patients N (%)
Clinical characteristics		
Age at primary treatment (years)	73 (26–100)	
FIGO stage 2009/TNM stage		
- IA/T1aN0M0		9 (3)
- IB/T1bN0M0		124 (43)
- II/T2N0M0		5 (2)
- IIIA/T1,2N1a,bM0		70 (24)
- IIIB/T1,2N2a,bM0		13 (5)
- IIIC/T1,2N2cM0		58 (20)
- IVA/T1,2N3M0, T3NanyM0		5 (2)
- IVB/TanyNanyM1		0(0)
- Missing		3 (1)
Local surgery at primary diagnosis		
- Wide local excision		233 (81)
- Radical vulvectomy		46(16)
- Exenteratio posterior		5 (2)
- Skinning vulvectomy		3[a](1)
Groin treatment at primary diagnosis[b]		
- SN		168
- IFL		115
- Primary radiotherapy		1
- Debulking		13
- No treatment		15[c]
Adjuvant therapy		
- Radiotherapy to the vulva		49 (17)
- Re-excision		17 (6)
- Chemotherapy		2 (1)
- None		219 (76)
Status		
- Alive		152 (53)
- Died of vulvar carcinoma		57 (20)
- Died of intercurrent disease		68 (24)
- Died of unknown cause		10 (3)
Histopathologic characteristics		
Tumor diameter[d]	29.5 mm (1.5–130.0)	
Depth of invasion[e]	5.6 mm (0.5–25.0)	
Location		
- Central		211 (74)
- Lateral		72 (25)
- Unknown		4 (1)
Grade of differentiation		
- Grade 1		83 (29)
- Grade 2		127 (44)
- Grade 3		74 (26)
- Not assessed		3 (1)
Growth pattern		
- Spray		105 (36)
- Invasive		96 (34)
- Confluent		69 (24)
- Mixed		7 (2)
- Not assessed[f]		10 (3)
LVSI		
- No		221 (77)
- Yes		62 (22)
- Not assessed[f]		4 (1)
Margin after (re)excision[g]	9.0 mm (0–35.0)	
- Tumor positive		14 (5)
- <3 mm		36 (13)
- <5 mm		59 (21)
- <8 mm		130 (46)
- ≥8 mm		155 (54)
Presence of precursor lesion		
- Lichen sclerosus and dVIN		133 (46)
- dVIN		64 (22)
- Lichen sclerosus		34 (12)
- HSIL		30 (11)
- None		26 (9)
Presence of precursor lesion in margin		
- Lichen sclerosus and dVIN		39 (14)
- dVIN		26(9)
- Lichen sclerosus		104 (36)
- HSIL		15 (6)
- None		103 (36)

Abbreviations: SN: sentinel node, IFL: inguinofemoral lymphadenectomy LS: lichen sclerosus, LSIL: low-grade squamous intraepithelial lesions, HSIL: high-grade squamous intraepithelial lesions, dVIN: differentiated vulvar intraepithelial neoplasia, LVSI: lymph-vascular space invasion.

[a] Three patients underwent a skinning vulvectomy because of vulvar intraepithelial neoplasia, coincidentally these patients also had invasive squamous cell carcinoma, that was excised sufficiently.

[b] Patients undergoing two different groin surgeries are counted in both treatment groups.

[c] Nine patients did not receive groin treatment because of a microinvasive tumor, 3 because of comorbidity, 1 wish of the patient, 1 patient had advanced metastatic disease and in 1 case the reason was unknown.

[d] Not able to assess in 11 cases.

[e] Not able to assess in 4 cases.

[f] Not able to assess due to small tumors.

[g] Not able to assess in 2 cases.

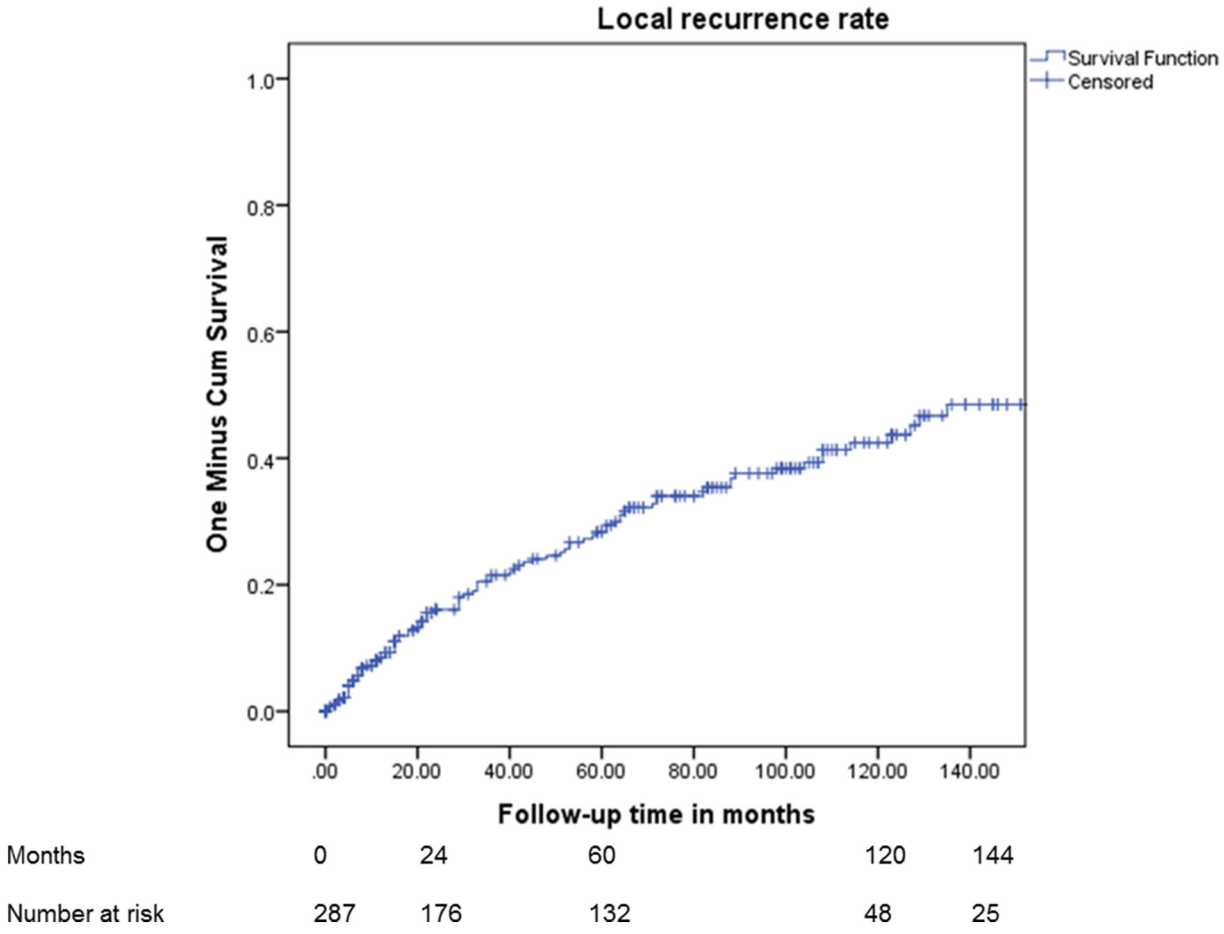


Fig. 1. Local recurrence rate.

invasion had no effect on the local recurrence rate, as displayed in Table 2.

3.4. Multivariable analyses

Our multivariable model shows that the presence of dVIN combined with LS or without LS in the margin is associated with a higher local recurrence rate (HR 2.76 (95% CI 1.62–4.71), $p < 0.001$, HR 2.14 (95% CI 1.11–4.12), $p = 0.023$). Furthermore, FIGO stage II or higher also resulted in a higher local recurrence rate (HR 1.62 (95% CI 1.05–2.48), $p = 0.028$), displayed in Supplementary Table 3.

4. Discussion

In this study, we show that local recurrences are not associated with (any) tumor free margin distance, but strongly with dVIN in the pathologic margin. Our research was carried out on large, clinically well-documented consecutive series of vulvar squamous cell carcinoma patients primarily treated with surgery with expert histopathologic revision.

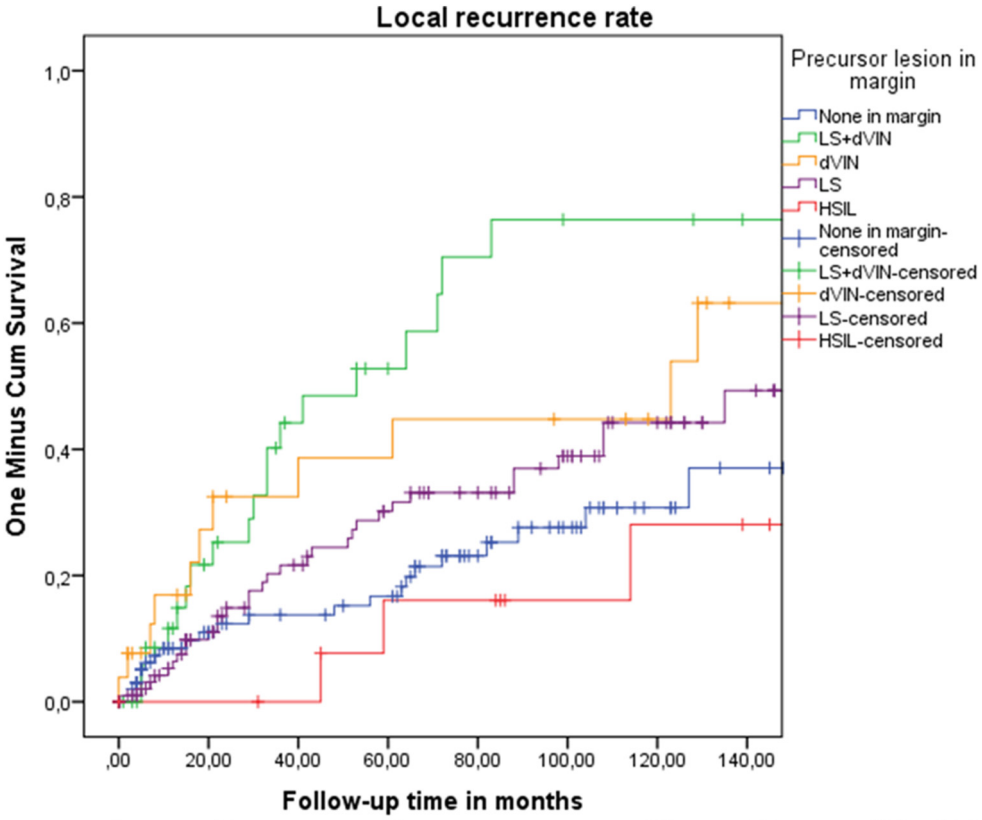
In our study a pathologic tumor free margin distance of <8, 5 or 3 mm is not associated with a higher local recurrence rate compared to a wider tumor free margin. Most guidelines recommend a surgical tumor free margin of ≥15 mm [3] or 10–20 mm [4]. The European Society of Gynecologic Oncology (ESGO) guideline recommends a surgical excision margin of at least 10 mm, while a narrower margin is considered acceptable when the tumor lies close to midline structures (clitoris, urethra, anus) and preservation of their function is desired [5]. For the ESGO vulvar cancer guideline the current (preliminary and unpublished) data and data from other more recent studies and reviews that questioned the influence of tumor free margin distance were taken into account [2,6,7,15]. Due to a limited amount of patients with a pathologic tumor free margin of <3 mm in our cohort, we could not determine the prognostic impact of this subgroup. Our data in larger number of patients clearly indicates that pathologic tumor fee margins of ≥3 mm do not relate with the local recurrence rate. In our cohort, in which a surgical margin distance of >10 mm was pursued, approximately 7% of the patients had a tumor positive margin and 8% a tumor free margin between 0 and 3 mm at primary excision. De Hullu et al. previously showed that in case of an intended surgical tumor free margin of 10 mm, 50% of the patients have a pathologic tumor free margin of ≤8 mm [16]. This might be due to smaller surgical margin if the tumor was close to important midline structures, shrinkage at fixation, but also because not all of the tumor is macroscopically visible. Therefore, we now recommend an intended surgical tumor free margin of 10 mm, but also not to excise unnecessary tissue close to important midline structures such as the clitoris (see flowchart Fig. 4). Future implementation of a smaller tumor free margin will expose less patients to the potential harmful and often mutilating therapies. In selected patients Mohs microsurgery technique, widely applied in patients with skin cancer (eg. in the face), where close but free margins are accepted, might also be useful in vulvar carcinoma cases where small surgical margins are needed because important structures (clitoris, anus) need to be preserved. However currently no data on Mohs and vulvar carcinoma exist.

No differences in local recurrences were found between patients that did or did not receive adjuvant radiotherapy on the vulva (Supplementary Table 1). Therefore, one should be reluctant with adjuvant radiotherapy on the vulva in case of small pathologic margins, since the morbidity of this therapy is high.

Table 2
Clinical and histologic characteristics related to local recurrence (univariable).

	Hazard ratio (95% confidence interval)	
	Whole cohort (n = 287)	Vulvar radiotherapy excluded (n = 236)
Patient- and treatment characteristics		
Type of local surgery		
- Wide local excision	1.0	1.0
- Skinning vulvectomy	1.90 (0.47–7.77), p = 0.369	1.83 (0.45–7.51), p = 0.398
- Radical vulvectomy	1.05(0.55–1.97), p = 0.887	1.08 (0.52–2.25), p = 0.841
- Exenteratio posterior	0.00 (0.00–4.05^{e+204}), p = 0.964	0.00 (0.00–4.8^{e+200}), p = 0.964
FIGO stage		
- IA, IB, II	1.0	1.0
- IIIA, IIIB, IIIC, IVA	1.45 (0.95–2.21), p = 0.084	1.51 (0.96–2.39), p = 0.075
Adjuvant radiotherapy on the vulva		
- No	1.0	–
- Yes	0.84 (0.46–1.54), p = 0.569	–
Location		
- Central tumor	1.0	1.0
- Lateral tumor	1.17 (0.73–1.85), p = 0.508	1.16 (0.72–1.89), p = 0.543
Pathologic margin		
Tumor free margin distance (continuous)	1.03 (0.99–1.06), p = 0.153	1.03 (0.98–1.08), p = 0.309
Tumor free margin distance		
- <8 mm	1.0	1.0
- ≥8 mm	1.25 (0.81–1.93), p = 0.308	1.29 (0.79–2.09), p = 0.307
Tumor free margin distance		
- <5 mm	1.0	1.0
- ≥5 mm	1.13 (0.63–2.05), p = 0.678	0.92 (0.44–1.91), p = 0.814
Tumor free margin distance		
- <3 mm	1.0	1.0
- ≥3 mm	0.93 (0.47–1.85), p = 0.831	0.62 (0.25–1.53), p = 0.298
Dvin and lichen sclerosus in margin		
- No	1.0	1.0
- Yes	2.58 (1.55–4.32), p < 0.001	2.57 (1.47–4.50), p = 0.001
dVIN in margin		
- No	1.0	1.0
- Yes	2.39(1.54–3.72), p < 0.001	2.55(1.58–4.11), p < 0.001
Lichen sclerosus in margin		
- No	1.0	1.0
- Yes	1.56 (1.02–2.39), p = 0.040	1.30 (0.83–2.04), p = 0.260
HSIL in margin		
- No	1.0	1.0
- Yes	0.54(0.20–1.48), p = 0.233	0.46(0.15–1.47), p = 0.189
Tumor characteristics		
Growth pattern		
- Invasive	1.0	1.0
- Spray	1.60 (0.94–2.70), p = 0.081	1.46 (0.82–2.59), p = 0.201
- Confluent	1.36 (0.77–2.41), p = 0.292	1.34 (0.74–2.44), p = 0.336
- Mixed	0.56 (0.08–4.11), p = 0.564	0.51 (0.07–3.77), p = 0.508
LVSI		
- No	1.0	1.0
- Yes	1.00 (0.58–1.72), p = 0.99	0.98 (0.54–1.77), p = 0.933
Grade of differentiation		
- Good	1.0	1.0
- Moderately	1.45 (0.88–2.40), p = 0.148	1.40 (0.82–2.37), p = 0.219
- Poor	1.41 (0.79–2.50), p = 0.242	1.45 (0.79–2.68), p = 0.234
Tumor diameter (continuous)	1.01 (0.99–1.02) p = 0.666	1.00 (0.99–1.02), p = 0.865
- <40 mm	1.0	1.0
- ≥40 mm	0.92 (0.55–1.54), p = 0.763	0.81 (0.44–1.51), p = 0.511
Depth of invasion (continuous)	1.02 (0.97–1.07) p = 0.381	1.03 (0.98–1.09) p = 0.281

Abbreviations: HSIL: high-grade squamous intraepithelial lesions, dVIN: differentiated vulvar intraepithelial neoplasia, LVSI: lymph-vascular space invasion.



Months		0	24	60	120	144	Univariable Cox-regression HR (95% CI)
Number at risk							
-	None in margin	103	64	56	14	9	1.0
-	LS and dVIN	39	20	9	3	1	3.32 (1.79-6.16), p<0.001
-	dVIN	26	12	10	6	1	2.28 (1.10-4.71), p=0.042
-	LS	104	66	47	19	9	1.45 (0.84-2.49), p=0.183
-	HSIL	15	15	10	6	5	0.83 (0.28-2.41), p=0.728

Fig. 2. Local recurrence rate by presence of precursor lesions in margin.

Our study shows a significant difference in local recurrence rates related to the presence of precursor lesions of the LS related pathway, which is in line with the study of Yap et al. [9] DVIN in the margin, whether or not in combination with LS, leads to higher local recurrence rates. Therefore, it is important for both the clinician and the pathologist to recognize dVIN. Efforts should be made to explore on how to improve clinical recognition of dVIN by clinician. Recognition of dVIN by the pathologist is of equal importance. Van den Einden et al. described histologic characteristics that are most important in the recognition of dVIN [17]. In addition, the authors concluded that it is of added value to revise specimens with an unclear diagnosis and/or clinical suspicion for dVIN by an expert gynaecopathologist. Immunohistochemistry could be helpful in some cases; p16 could be used to exclude HPV-related lesions and p53 might be useful in non-HPV related lesions. However, the exact diagnostic advantage of these and other immunohistochemistry stainings should be further researched to help identify dVIN.

Treatment of precursor lesions should be key in lowering the local recurrence rate. Our data show that patients with both dVIN and LS or dVIN alone in the pathologic margin have significantly higher local recurrence rates. The local recurrence rate 10 years after treatment is as high as 76% in patients with dVIN and LS in the margin compared to 31% for patients with no precursor lesion in the margin ($p < 0.001$, see Fig. 2). This might be explained by the concept of field cancerization; the vulva is a field with genetically altered cells with a high risk of developing a precursor lesion and/or carcinoma. Additionally, within the group of patients with dVIN in the resection specimen, patients with dVIN in the pathologic margin suffered significantly more from local recurrences compared to patients with dVIN adjacent to the tumor ($p = 0.002$, Fig. 3). Therefore, we recommend to excise lesions suspicious for dVIN during resection of the primary tumor, while re-excision should be considered if dVIN is present in the pathologic margin (see flowchart Fig. 4).

Treatment of underlying dermatoses must be one of the major focuses during follow-up. In patients with LS treatment with topical corticosteroids may reduce the risk for developing vulvar carcinoma [2,18,19]. Newer treatments such as lipo-injection, ablative laser treatment, and photodynamic therapy have been suggested, but no data are available on whether these therapies also reduce the malignant potential of LS or not. For dVIN, the malignant potential is higher and the time for progression to vulvar carcinoma shorter compared to LS [20–23]. Local (re)excision of dVIN is first choice of treatment. However, clinically it is often difficult to identify the exact location and borders of dVIN. Besides, excision can be mutilating especially when close to functional midline structures. In the future, alternative local treatment regimens such as targeted- or immunotherapy in these patients should be explored.

None of the studied histopathologic characteristics of the tumor were associated with a higher local recurrence rate. This finding is in line with the hypothesis that mostly all local recurrences are 'de novo' tumors arising in a premalignant field. In previously reported studies, authors artificially classified local recurrence in 'de novo' tumors and

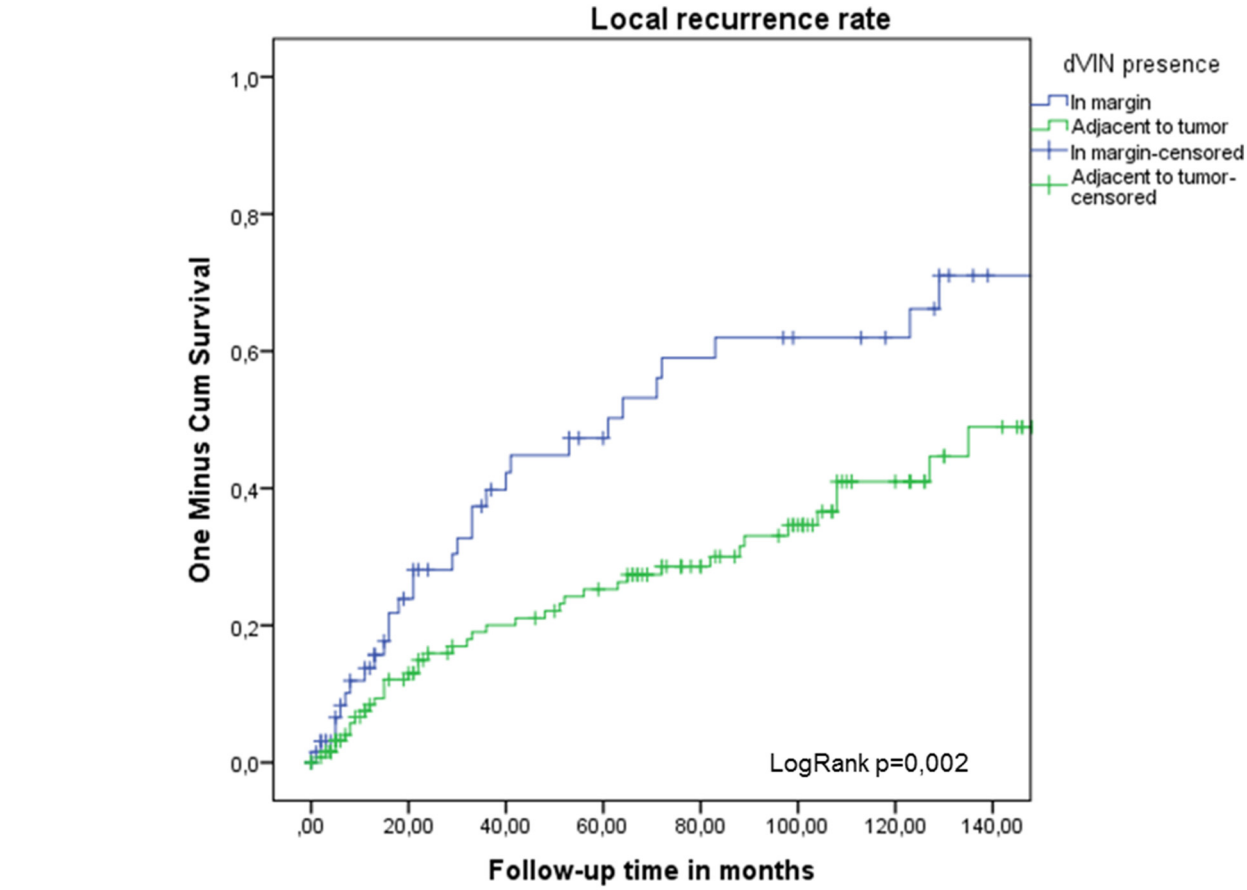


Fig. 3. Local recurrence rate in patients with dVIN; presence of dVIN adjacent to the tumor versus dVIN in the margin.

'true' local recurrence based on either the location (> or <2 cm from primary tumor, or ipsilateral or contralateral side of the vulva) or time to local recurrence (< or >2 years) [1,6,9,24,25]. In our opinion, both classifications are too arbitrary and currently have no clinical consequences and therefore not used in our study. Future research should be performed on clonal or genetic relationship analyses to distinguish a 'de novo' tumor and a 'true' local recurrence.

In our study cohort, we showed an ongoing risk for local recurrence. The first local recurrence was diagnosed as long as 202 months after primary treatment. No subgroup of patients could be identified with a negligible risk for local recurrence (Fig. 2 and Supplementary Fig. 2) and there were no differences in median time to local recurrence between the different precursor lesion groups ($p = 0.08$) (see Fig. 2 and Supplementary Fig. 2). Therefore, we recommend life-long follow-up for all patients treated for vulvar carcinoma with the aim to identify local recurrences as early as possible, but also to identify and treat precursor lesions to prevent a local recurrence.

Oonk et al. reported that 65% of the recurrences were detected at routinely scheduled follow-up meetings, indicating the need for patient education regarding the ongoing risk for local recurrence [26]. In addition, identification of high-risk patients may further improve patient empowerment, patient education and may also lead to individualized follow-up schedules. The (early) detection of a local recurrence might be improved by self-examination by the patient and/or her partner, besides the instruction to contact the treating physician at time of any symptoms [26,27]. Currently there is no literature on the efficacy of self-examination for early detection of a local recurrence in vulvar carcinoma.

Our study is the first examining the pathologic margin for both the presence of vulvar carcinoma and precursor lesions in a structured way with revision of all slides by two independent expert gynecopathologists. Furthermore, our study is performed in accordance with the Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK) rules. Due to the retrospective character of this study, we were not able to reconstruct the exact location of the local recurrence in all patients. Our study did not have a prospective nature; therefore, we had to deal with missing data. A high number of patients are included with dVIN in the pathological margin without adjuvant treatment, due the lack of a standardized treatment protocol for these patients at that time besides the fact that a part of the dVIN lesions were not detected at initial histopathologic examination. As a result of the small number of patients with a pathologic tumor free margin < 3 mm, we were not able to determine the prognostic impact of this subgroup with small pathologic tumor free margins.

In conclusion, our study shows a high local recurrence rate in patients surgically treated for vulvar carcinoma. No relation was found with pathologic tumor free margin distances. Based on our study we advise to lower the recommended cut-off for a save pathological tumor free margin distance to ≥3 mm. We found the local recurrence rate to

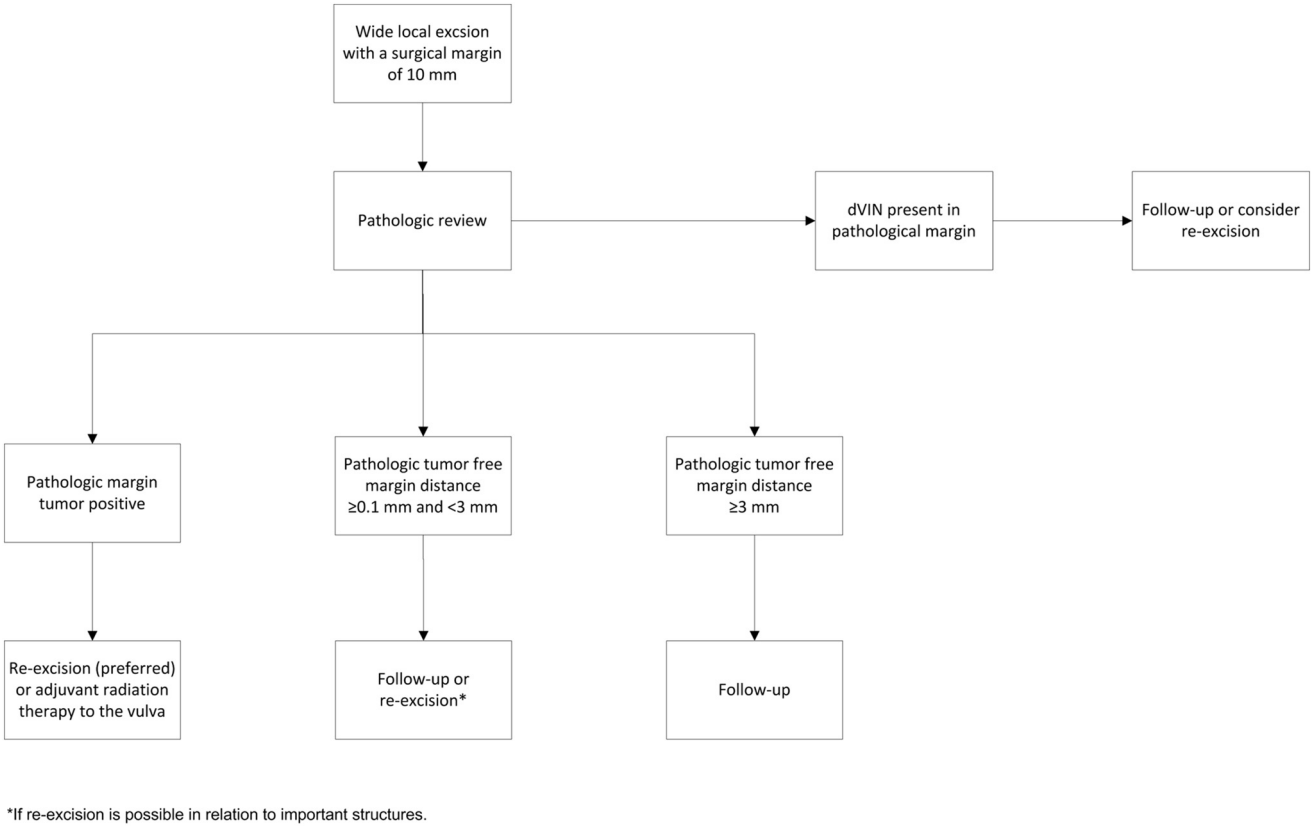


Fig. 4. Recommendations for adjuvant therapy after wide local excision of vulvar squamous cell carcinoma.

be especially related to the presence of dVIN (whether or not with LS) and we were unable to identify a subgroup with such a low risk that follow-up could be omitted. Our data reinforce that patients and their doctors need to be aware of the lifelong increased risk for local recurrence after surgical treatment for vulvar carcinoma, especially in patients with dVIN in the margin.

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Authors' contributions

NG, AP, GB, HH, JB, AZ, JH, MO contributed to conceptualization; NG, AP, GB, AZ, JH and MO contributed to methodology; NG, AP, HH and JB performed data acquisition; NG, AP and GB performed analysis; NG, AP, GB, HH, JB, AZ, JH, MO interpret the data for the work; NG, AP, GB, HH, JB, AZ, JH, MO wrote the manuscript; NG, AP, GB, HH, JB, AZ, JH, MO approved the final version to be published.

Declaration of Competing Interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygyno.2019.05.010.

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