Pathologically Tumor-free Resection Margin Distance as a Surrogate Parameter in Primary Vulvar Squamous Cell Cancer: Analysis of a Large Two-Center Patient Cohort

Breite des pathologisch tumorfreien Resektionsrandes als Surrogatmarker beim primären Plattenepithelkarzinom der Vulva: Analyse einer großen zweizentrischen Patientinnenkohorte

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Keywords
vulvar cancer, squamous cell cancer, resection margins

Schlüsselwörter
Vulvakrebs, Plattenepithelkarzinom, Resektionsrand

received 17.6.2024
accepted after revision 27.12.2024

Bibliography
Geburtsh Frauenheilk
DOI 10.1055/a-2508-8628
ISSN 0016-5751

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ABSTRACT

Background
This study evaluated the role of tumor-free margin distances and other prognostic factors, including age, lymph-node metastases, lymphatic vessel invasion, and local recurrence, on the survival of patients with vulvar squamous cell carcinoma (VSCC) undergoing primary surgical treatment.

Methods
A retrospective analysis reviewed the records of 232 VSCC patients who had undergone primary radical local excision with R0 resection between 2009 and 2021 at ANregiomed Hospital Ansbach and Erlangen University Hospital (Germany). Patients, aged 18 and older with no distant metastases, were grouped by resection margin distances (1 to ≤ 3 mm, 3 to ≤ 8 mm, > 8 mm) for survival analysis using the Kaplan–Meier and log-rank tests. A Cox proportional hazards regression model incorporating multiple covariates, selected using the best-subset selection method and guided by the Akaike information criterion (AIC), was used. In the next step, we conducted a separate analysis of the patients who experienced a local recurrence.

Results
The median age of the 232 patients analyzed was 69 years, with a median follow-up period of 10.5 years; 82 patients died. Survival varied significantly relative to resection margin distance ($p = 0.0022$), with the highest rates in the 1 to

≤ 3 mm group and the lowest in the > 8 mm group. Multivariate analysis revealed that age, lymphatic vessel invasion, and resection margin distance significantly influenced survival, with higher values associated with increased mortality. Out of 232 patients analyzed, 43 developed a local recurrence. In the group with resection margins of 1 to ≤ 3 mm, 37% of patients experienced a local recurrence. Among those with margins of 3 to ≤ 8 mm, 44% had a recurrence, while only 19% of patients with margins > 8 mm showed a local recurrence.

Conclusion

The study underscored the significance of tumor-free margin distance as a surrogate marker for survival in VSCC patients. In addition to lymphatic vessel invasion as the most critical prognostic factor, tumor-free resection margin distance and age emerged as significant predictors of overall survival. The findings advocate for tailored, function-preserving surgical approaches to improve patient outcomes.

ZUSAMMENFASSUNG

Hintergrund

Diese Studie untersucht die Bedeutung der Breite des tumorfreien Resektionsrandes und anderer prognostischer Faktoren, darunter Alter, Lymphknotenmetastasen, Lymphgefäßinvasion und Lokalrezidiv, für das Überleben von Patientinnen mit einem Plattenepithelkarzinom der Vulva (VSCC) nach der chirurgischen Primärtherapie.

Methoden

In einer retrospektiven Analyse wurden die Patientenakten ausgewertet von 232 Patientinnen mit VSCC, die zwischen 2009 und 2021 eine primäre radikale lokale Exzision mit R0-Resektion im ANregiomed Klinikum Ansbach oder Uniklinikum Erlangen erhielten. Die Patientinnen waren 18 Jahre alt oder älter und hatten keine Fernmetastasen. Die Patientinnen wurden für die Überlebensanalyse mit Kaplan-Meier-Test und Log-Rank-Test in Gruppen unterteilt gemäß der Breite des Resektionsrandes (1 bis ≤ 3 mm, 3 bis ≤ 8 mm, > 8 mm). Verwendet wurde ein Cox-Regressionsmodell mit proportionalen Hazards und mehreren Kontrollvariablen, die mithilfe der besten Teilmengenbestimmungsmethode geleitet von dem Akaike-Informationskriterium (AIC) ausgewählt wurden. Der nächste Schritt bestand aus einer separaten Analyse von Patientinnen mit Lokalrezidiv.

Ergebnisse

Das mediane Alter der analysierten 232 Patientinnen war 69 Jahre, mit einem medianen Nachbeobachtungszeit von 10,5 Jahren; 82 Patientinnen verstarben. Es gab signifikante Unterschiede im Überleben relativ zur Breite des Resektionsrandes ($p = 0{,}0022$), wobei die höchsten Raten in der 1–≤ 3-mm-Gruppe und die niedrigsten Raten in der > 8-mm-Gruppe zu verzeichnen waren. Eine multivariable Analyse zeigte, dass Alter, Lymphgefäßinvasion und Breite des Resektionsrandes signifikante Auswirkungen auf das Überleben hatten, wobei höhere Werte mit einer höheren Mortalität assoziiert waren. Von den 232 analysierten Patientinnen entwickelten 43 ein Lokalrezidiv. In der Gruppe mit Resektionsrändern von 1 bis ≤ 3 mm entwickelte sich bei 37% der Patientinnen ein Lokalrezidiv. In der Gruppe der Patientinnen mit Resektionsrändern von 3 bis ≤ 8 mm entwickelten 44% ein Rezidiv, während nur 19% der Patientinnen mit Resektionsrändern von > 8 mm ein Lokalrezidiv aufwiesen.

Schlussfolgerung

Die Studie unterstreicht die Bedeutung der Breite des tumorfreien Resektionsrandes als Surrogatmarker für das Überleben von Patientinnen mit VSCC. Neben dem wichtigsten prognostischen Faktor einer Lymphgefäßinvasion stellten sich die Breite des tumorfreien Resektionsrandes und das Alter als signifikante Prädikatoren des Gesamtüberlebens dar. Diese Ergebnisse unterstreichen die Bedeutung von individuellen funktionserhaltenden chirurgischen Ansätze bei der Verbesserung der Überlebensaussichten von Patientinnen mit VSCC.

Introduction

Vulvar cancers are relatively rare, ranking as the nineteenth most common cause of cancer among women in Europe, with around 16 500 new cases in 2020 [1]. In Germany, 3293 new cases were reported in 2019. These cancers primarily affect older women [2], but the incidence of vulvar cancer in women under the age of 60 years has been increasing in recent decades [3]. The term "vulvar cancer" can encompass various types of cancer that originate from different cells in the genital region. The labia majora are most often affected, and less frequently the labia minora and clitoral region [4]. The vast majority of the lesions involve squamous cell carcinoma.

Epidemiological risk factors associated with vulvar cancer include age, the prevalence of human papillomavirus (HPV) infection, smoking, human immunodeficiency virus (HIV) infection, vulvar intraepithelial neoplasia, and lichen sclerosus [4, 5].

The diagnosis of vulvar cancer typically involves a physical examination, vulvoscopy, and histological analysis of any suspicious areas [6].

For treatment planning and selection of appropriate therapy, the tumors are classified in accordance with internationally recognized criteria that assess the lesion's anatomic spread and assign it to different stages [7]. Vulvar cancer is staged using both the International Federation of Gynecology and Obstetrics (FIGO) system and the TNM classification system [8, 9]. The latest revision of the FIGO staging system was published in 2021 by the FIGO Committee on Gynecologic Oncology. This updated staging was informed by an analysis of data from the National Cancer Database for the period 2010–2017 [8]. The revised staging for vulvar carcinoma now comprises two substages in stage I, lacks a substage in stage II, incorporates three substages in stage III, and features two substages in stage IV. Notably, the update introduces a fresh definition of depth of invasion, aligns with the definition of lymph-node metastases used in cervical cancer, and allows the inclusion of findings from cross-sectional imaging in the staging of vulvar cancer [8]. The eighth edition of the TNM staging system for vulvar cancer was published in 2017 by the American Joint Committee on Cancer (AJCC) and the International Union Against Cancer (UICC) and applies to primary carcinomas [9].

The primary treatment for local disease without metastasis is surgery [10]. Radical local excision is recommended, with the aim of achieving histologically tumor-free margins. Over the years, the primary goal has been to secure tumor-free margin distances of at least 10 mm, allowing even narrower margins if the lesion is close to critical structures. However, recent extensive studies have failed to establish a clear link between the extent of tumor-free margins and the rates of local recurrence or overall survival improvement. Consequently, the evidence supporting the 10-mm margin distance requirement is now quite weak. Debate on the optimal tumor-free margin distance to reduce the risk of local recurrences and improve patients' overall survival is still ongoing [11, 12, 13, 14, 15, 16, 17, 18, 19, 20]. It appears that a minimum margin larger than 2–3 mm on histopathology might be sufficient, allowing better functionality with acceptable oncological outcomes.

However, determining the optimal size for the tumor-free margin distance is a continuing topic of discussion. The various guidelines for treating vulvar cancer used in Germany, the United States, and Europe illustrate the lack of a consensus on the matter. In Germany, the expert consensus guideline published by the German Society for Gynecology and Obstetrics (*Deutsche Gesellschaft für Gynäkologie und Geburtshilfe,* DGGG) and the German Cancer Society (*Deutsche Krebsgesellschaft,* DKG) suggests a minimum tumor-free margin distance of 3 mm on histological examination [21]. Unfortunately, an S3 guideline for vulvar carcinoma is not available in Germany. As a result, the S3 guideline for cervical carcinoma is often referred to by analogy [22, 23]. In contrast, the National Comprehensive Cancer Network (NCCN) guidelines in the United States recommend tumor-free margin distances of 1–2 cm for early-stage vulvar squamous cell carcinoma [24]. The European Society of Gynaecological Oncology (ESGO) advises that a narrow margin distance should be considered in order to preserve critical structures such as the clitoris, urethra, or anus [25].

The diagnosis and treatment of vulvar cancer has substantial physical and psychological effects on women. Several studies indicate that there is a risk of persistent sexual dysfunction after radiotherapy or vulvar surgery, potentially linked to the extent of vulvar tissue removal [26, 27, 28]. Reports of incontinence issues have also appeared after radical vulvectomy in cases in which a portion of the urethra is removed or the vulvectomy excision comes close to the urethra (within 1 cm) [29].

The goal of this two-center retrospective study was to assess the influence of the tumor-free margin distance on patient survival and local recurrence rates, while identifying additional prognostic factors for overall survival.

Methods

The retrospective two-center cohort analysis involved a review of clinical records and histopathological reports for patients who had undergone surgical treatment for primary vulvar squamous cell carcinoma between 2009 and 2021 at ANregiomed Hospital in Ansbach, Germany, and Erlangen University Hospital/Comprehensive Cancer Center Erlangen–European Metropolitan Region Nuremberg (CCC ER-EMN), Germany. The study was approved by the Ethics Committee of Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU) (reference number: 23–169-Br).

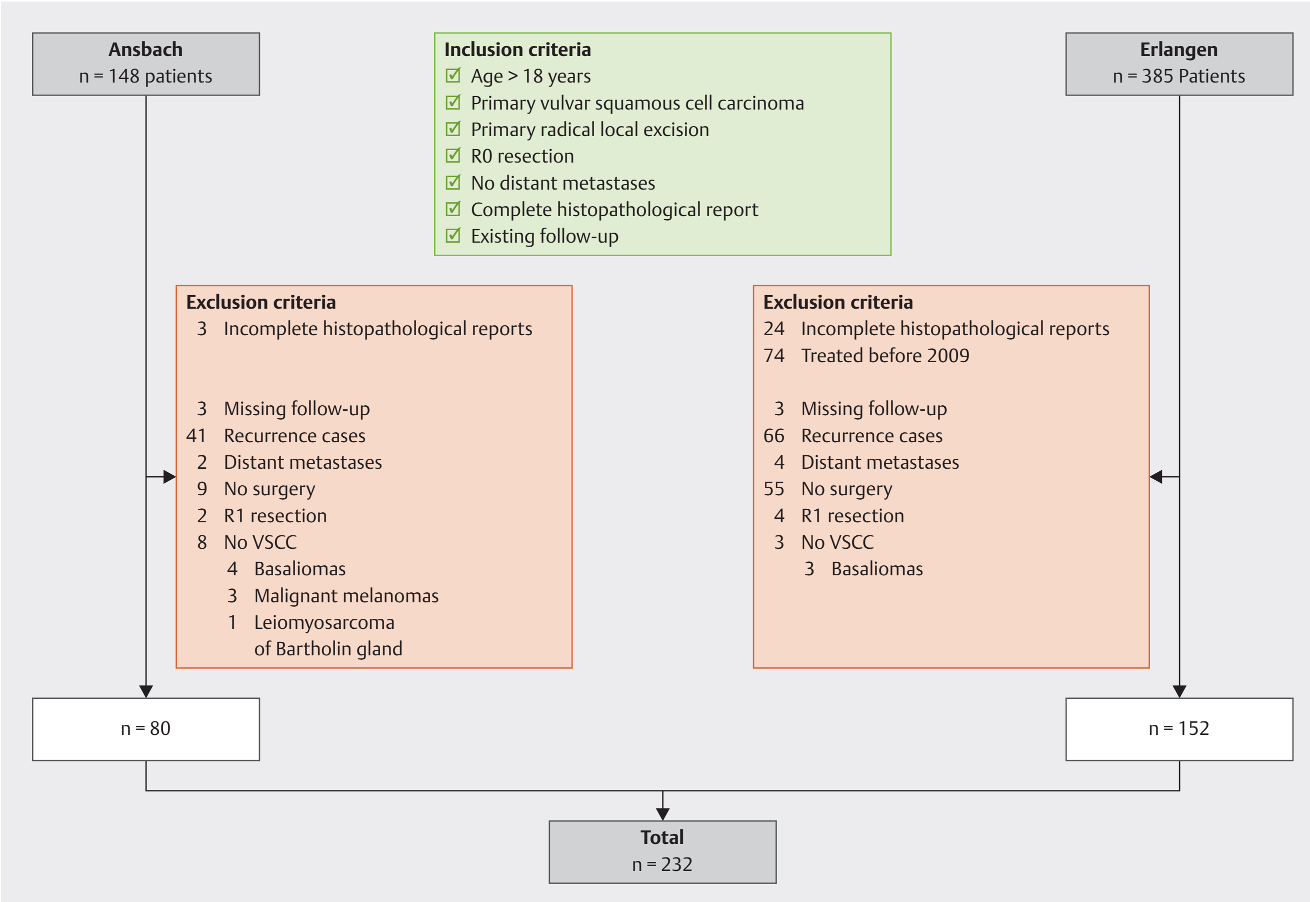
The inclusion criteria for the study consisted of age over 18, primary radical local excision with an R0 resection, no distant metastases, and availability of a complete histopathological report and follow-up data (▶ **Fig. 1**). In the next step, we conducted a separate analysis of patients with local recurrence.

Oncology data collection

The database systematically recorded tumor characteristics and various aspects of surgical treatment, including pathological staging (pTNM), tumor dimensions, histological type, depth of invasion, tumor margin distance status, presence or absence of lymphovascular space invasion, and perineural invasion, as well as the presence or absence of premalignant disease. The type of groin surgery performed and the date of last contact with the patient or death were also documented. The comprehensive data were extracted from both hospitals' internal registries and the registry of the Oncological Center at ANregiomed Hospital and Erlangen University Hospital/CCC ER-EMN. The data were compiled in an irreversibly anonymized format and exclusively analyzed using computers in the respective hospitals.

Classification of pathological margin distance

For analytical purposes, patients were further subclassified into three groups on the basis of the pathological resection margin distance: 1 to ≤ 3 mm, 3 to ≤ 8 mm, and > 8 mm. These three groups were selected on the basis of recommendations regarding the minimum histological margin distance found in the relevant specialist literature. Although achieving tumor-free margins of at least 8 mm is a long-standing goal, the evidence for it is sparse. Data suggest that histological margins of 5 mm or even 3 mm represent the minimum necessary to ensure oncological safety and long-term survival [16, 25]. In the present study, all gross specimens were processed in accordance with local protocols. Resection margin distances were determined on the basis of the smallest values indicated in the histopathological reports. In the event of uncertainties, the pathologist conducted a reassessment of the resection margin distance. The margin refers to the "ultimate" margin after the completion of surgical treatment, encompassing the thickness of any additional resections performed in the same surgical session or during a subsequent operation.



▶ **Fig. 1** Flowchart illustrating the patient selection process for the study. The diagram outlines the steps taken to identify eligible patients from the initial cohort. VSCC: vulvar squamous cell carcinoma.

Treatment approach

Treatment was carried out in accordance with the guidelines, given that both centers are certified gynecological facilities. Surgical treatment for vulvar squamous cell carcinoma consisted either of partial or complete (radical) vulvectomy or of wide resection of the tumor. Lymph-node staging involved a sentinel lymph-node procedure for unifocal tumors < 4 cm in size with a depth of invasion > 1 mm, without clinical evidence of lymph-node metastases. For tumors near the median line (< 1 cm), the sentinel lymph-node procedure was carried out bilaterally. In cases of tumors > 4 cm with clinically presumed or pathologically confirmed lymph-node metastases, or a sentinel lymph-node metastasis before the validation of the sentinel lymph-node procedure in vulvar cancer, a systematic inguinofemoral lymphadenectomy was conducted. After the surgical procedure, all of the patients' cases were discussed in the multidisciplinary tumor board to explore further therapy options. None of the patients included in the analysis received neoadjuvant radio(chemo)therapy or systemic therapy. Adjuvant therapy was administered on the decision of the local multidisciplinary tumor board.

Statistical analysis

The analyses were conducted using the Prism program, version 9.5.0 (GraphPad Software, Boston, Massachusetts, USA). In a univariate investigation of factors influencing survival time, a survival rate was calculated and depicted graphically for each level of the input variable, using the Kaplan–Meier estimator [30]. The trajectories of survival probabilities for different groups were compared using the log-rank test [31]. A significance level of 5% was applied.

A proportional hazards regression model (Cox model) was used to model survival time [32]. In this context, the hazard of individuals (hazard rate) was modeled on the basis of multiple covariates. The selection of relevant factors was carried out using a "best-subset selection" approach [33] with the Akaike information criterion (AIC) [31]. This involved considering all possible subsets of influencing factors and selecting the subset that provided the best model fit, according to the criterion used. The analysis was performed using JMP Pro, version 17.0 (JMP Statistical Discovery LLC, Cary, North Carolina, USA).

▶**Table 1** Distribution of patients by resection margin distance (RMD) categories at ANregiomed Hospital in Ansbach and Erlangen University Hospital. Patients are grouped into three RMD categories: 1 to ≤ 3 mm, 3 to ≤ 8 mm, and > 8 mm. For each hospital, the table shows the number of patients (n) and the corresponding percentage (%) relative to the hospital's total cohort. The "Total" row combines data from both hospitals, presenting the total number and percentage of patients in each RMD category across the entire study population (n = 232).

RMD (mm)	Hospital				Total	
	Ansbach		Erlangen			
	n	%	n	%	n	%
Total	80	100	152	100	232	100
1 to ≤ 3 mm	14	16	63	41	77	33
3 to ≤ 8 mm	29	45	64	44	93	44
> 8 mm	37	39	25	15	62	23

Results

Descriptive analysis

Of the 533 patients treated in the two hospitals during the study period, 232 met the inclusion criteria. Among the patients analyzed, 80 were affiliated with the ANregiomed Hospital in Ansbach, while 152 were associated with Erlangen University Hospital. Their overall median age was 69, with patients from ANregiomed Hospital having a notably higher median age of 74.5 years in comparison with those from Erlangen University Hospital, where the median age was 67.

Survival analysis

To calculate the survival time in years, the period between the date of surgery and the date of death (or the end of the study on December 31, 2022) was divided by 365 days. In total, 82 patients (35.34 %) died within a period of up to 10.5 years after surgery. Notably, a significantly higher percentage of patients at ANregiomed Hospital died (n = 39, 48.8 %), in comparison with Erlangen University Hospital (n = 43, 28.3 %). The mortality rate remained relatively stable during the first 2.5 years after surgery. Between 2.5 and 4.5 years postoperatively, there was only a modest number of recorded deaths, followed by a subsequent increase in fatalities from 4.5 years to approximately 7 years after surgery.

Resection margins

The resection margins in the operations ranged from 1 mm to 24 mm, with a mean of 5.6 mm. In the majority of cases, the resection margin was less than 8 mm. However, there were cases in which patients had considerably higher values, reaching up to 24 mm. The distribution also showed variation between the two hospitals; at ANregiomed Hospital in Ansbach, the mean resection margin was significantly larger at 7.87 mm in comparison with Erlangen University Hospital (4.4 mm).

▶ **Table 1** shows the distribution of patients across the different resection margin categories. At Erlangen University Hospital, the resection margin was less than 8 mm in over 80 % of the operations. In contrast, at ANregiomed Hospital, the resection margins in nearly half of the operations were more than 8 mm.

Tumor classification

The distribution of tumor classifications before treatment shows that a significant majority of the patients belonged to a single class for each evaluation criterion. For instance, 174 patients (69 %) had unaffected lymph nodes, and the tumor extension was classified as T1 b in 173 patients (68.7 %). All other tumor extensions were relatively rare, except for T1a, which was observed 48 times (20.69 %).

Univariate analysis

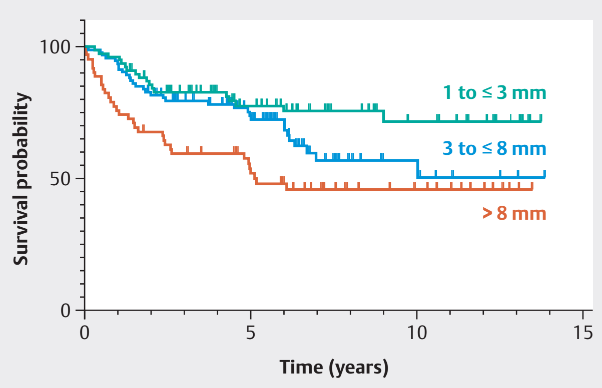
In the univariate analysis, the influence of both the resection margin distance and the tumor assessment criteria (T stage and N stage) on the overall survival time following surgery was assessed.

Survival time in relation to the resection margin

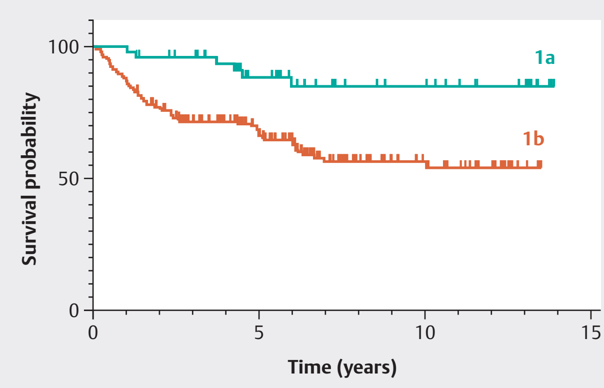
The estimated survival probabilities showed notable variations across the different classes (▶ **Fig. 2**). The group with a resection margin distance of 1 to ≤ 3 mm was associated with the highest survival rate. During the initial 4 years, the group with a resection margin distance of 3 to ≤ 8 mm closely mirrored the outcomes in the group with smaller resection distances. Nevertheless, a significant rise in fatalities occurred between the fourth and seventh year post-treatment. The group with a resection margin distance of > 8 mm consistently showed a lower survival rate throughout the entire period in comparison with the other cohorts. The log-rank test yielded a p value of 0.0022.

Survival time in relation to tumor size (T stage)

Noticeable variations in estimated survival probabilities were also apparent across different groups. The smallest tumor size (T1a) was associated with the highest survival rate, while the survival probability for the largest (T1 b) consistently lagged behind the reference at all time points. In this category, 61.3 % of the patients survived overall. Although there were only 11 patients, the survival probability associated with a large tumor size (T2, T3) was significantly lower. The log-rank test indicated a p value of 0.0001,



▶ **Fig. 2** Estimated survival probabilities based on the Kaplan–Meier method across resection margin distance categories. The figure depicts the estimated survival probabilities over time, calculated using the Kaplan–Meier method, and stratified by resection margin distance categories. The x-axis represents time in years since surgical resection, while the y-axis shows survival probability, ranging from 0 % to 100 %. Each curve corresponds to a specific resection margin category: 1 to ≤ 3 mm, 3 to ≤ 8 mm, > 8 mm.



▶ **Fig. 3** Estimated survival probabilities according to the Kaplan–Meier method for tumor stages T1a and T1 b. The figure illustrates the estimated survival probabilities over time for patients with tumor stages T1a and T1 b, calculated using the Kaplan–Meier method. The x-axis represents time in years since surgical intervention, while the y-axis shows survival probability, ranging from 0 % to 100 %. The survival curves correspond to tumor stages T1a and T1 b.

indicating a statistically significant difference in survival among the groups.

Due to the very low number of patients with large tumors (T2, T3), the main focus for the assessment of significant differences lay between groups T1a and T1 b. These specific groups are examined individually in ▶ **Fig. 3**. Once again, a distinct difference between the two groups was evident. The log-rank test yielded a p value of 0.0009 in the analysis of these two groups, indicating that the differences in survival were statistically significant in this case as well.

The correlation between larger tumor sizes and increased resection margin distances was also examined. The case numbers for T2 and T3 are limited, but the resection margin distances in group T1 b were significantly larger than those in group T1a.

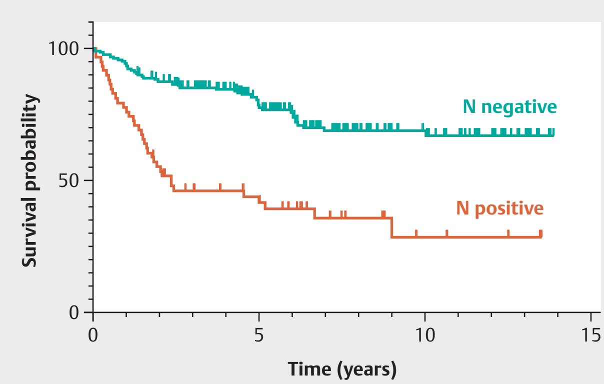
Survival time in relation to lymph-node involvement (N stage)

Examining lymph-node involvement presented a challenge, due to a substantial imbalance in the group sizes. Among the patients, 173 (68.7 %) did not have any lymph-node issues (N0), while the remaining individuals were distributed across six distinct classes. Survival periods were lower among patients with lymph-node involvement in comparison with those without. In view of the low case numbers in the groups, the subsequent analysis focused on comparing survival probabilities between patients without lymph-node involvement and all patients with lymph-node involvement (▶ **Fig. 4**). The log-rank test confirmed a statistically significant difference ($p < 0.0001$).

Multivariate analysis

In the multivariate analysis, the overall survival time was examined in relation to several influencing factors, including the resection margin distance (RMD), tumor extension (T), lymph-node involvement (N), depth of invasion, lymphatic vessel invasion (L), age, and hospital treating the patient. On the basis of the observations from the univariate analysis, lymph-node involvement (N) was treated as a binary variable ($N > 0$). In this context, a value of 0 indicated no lymph-node involvement, while a value of 1 represented the presence of lymph-node involvement.

The resection margin distance was not further divided into three classes; instead, the resection margin distance (RMD) in millimeters (mm) was treated as a continuous variable. In addition,



▶ **Fig. 4** Estimated survival probabilities according to the Kaplan–Meier method for patients with or without lymph-node involvement (N). The figure shows estimated survival probabilities over time for patients with or without lymph-node involvement (N), calculated using the Kaplan–Meier method. The x-axis represents time in years since surgical intervention, and the y-axis shows survival probability (0 % to 100 %). Survival curves are stratified by lymph-node involvement: one for patients with lymph-node involvement (N positive) and one for patients without (N negative).

▶**Table 2** Assessment of the significance of factors influencing survival time using the Wald test for both the selected best-subset model (left) and the full Cox model (right), which considers all potential factors for all patients. The table presents Wald test statistics and corresponding p values for each variable in both models. Assessed variables include hospital (Ansbach/Erlangen), age, tumor stage (pT1a vs. pT3, pT1 b vs. pT3, pT2 vs. pT3), lymphatic vessel invasion, depth of invasion, resection margin distance, and lymph-node involvement. The p values in the "Best-subset selection" column reflect the selected model, which includes only the most significant factors as determined by the Akaike Information Criterion (AIC). The p values in the "Cox model" column are derived from the full model, which incorporates all factors.

Variable	Best-subset selection	Wald test	p	Cox model	Wald test	p
Hospital (Ansbach/Erlangen)	0	0	1.000	0.0493	0.0324	0.857
Age (years)	0.0731	40.73	< 0.001	0.0689	33.6542	< 0.001
pT1a versus pT3	0	0	1.000	− 1.6459	2.1907	0.139
pT1 b versus pT3	0	0	1.000	− 1.2586	1.4702	0.225
pT2 versus pT3	0	0	1.000	− 0.8435	0.4998	0.48
Lymphatic vessel invasion	0.736	7.951	0.005	0.5436	3.0530	n/a *
Depth of invasion (cm)	0	0	1.000	0.0731	0.1546	0.694
Resection margin (mm)	0.1124	17.83	< 0.001	0.1077	13.0944	< 0.001
Lymph-node involvement (N-positive)	0	0	1.000	0.2315	0.4877	0.485

* Lymphatic vessel invasion was not applicable in the full Cox model, due to a strong correlation with positive lymph-node involvement.

three variables were taken into consideration: lymphatic vessel invasion (L), depth of invasion, and patient's age. The correlation between these variables and survival probability has not previously been investigated.

Modeling survival probability for all patients

Survival probability was initially modeled taking all patients into consideration. However, due to missing data for 19 patients for the variables of lymphatic vessel invasion and invasion depth, only 213 of the 232 patients were included in this analysis. The most influential factors, identified with a best-subset selection approach [33] using the AIC criterion [31], included age, lymphatic vessel invasion, and resection margin distance in millimeters.

▶ **Table 2** shows the parameter estimates from the Cox model and assesses influences using the Wald test [31]. The full model, including all of the factors analyzed (on the right), and the best-subset selection model (on the left) were compared. In the full model, only two factors — age and resection margin distance— were deemed significant at the $\alpha = 5\%$ level. In the selected model, lymphatic vessel invasion emerged as significant, with a p value well below 5 %.

The effects of individual variables were interpreted using exponentiated parameter estimates (hazard ratio) for the three most pivotal factors. All three variables showed a hazard ratio exceeding 1, indicating an elevated risk of mortality and reduced probability of survival. The 95 % confidence intervals show a range of values providing the level of confidence for these estimates. Alongside lymphatic vessel invasion, higher age and larger resection margin distances similarly showed adverse effects on the likelihood of survival.

Modeling survival probability for patients in Erlangen University Hospital

Survival times only among patients at Erlangen University Hospital were examined here. In view of the substantial variations between the hospitals, particularly with regard to resection margin distances and age variables, which markedly affect the modeling of survival probabilities, further investigation was needed to determine whether similar effects were observed when focusing solely on a single hospital. In addition, eight patients had missing data for the variables of lymphatic invasion or depth of invasion. The analysis was consequently based on the data for only 144 of the 152 patients at this point.

▶ **Table 3** again shows the parameter estimates in the Cox model, encompassing both the model selected using the procedure described and the comprehensive model incorporating all covariates. Importantly, this analysis focused solely on patients from Erlangen University Hospital. The findings indicate a substantial similarity to the broader analysis including all patients. Age, resection margin in millimeters, and lymphatic invasion retained a significant influence in the final model. In addition, despite a p value just above the significance threshold of 5 %, the depth of invasion was incorporated in the model in this instance.

All of the variables examined showed a hazard ratio exceeding 1. This implies that a higher value for each respective covariate led to a lower probability of survival. The 95 % confidence intervals indicate a range of values providing a level of confidence for these estimates.

▶**Table 3** Assessment of the significance of factors influencing survival time using the Wald test for both the selected best-subset model (left) and the full Cox model (right), which considers all potential factors for patients treated at Erlangen University Hospital. The table presents Wald test statistics and corresponding p values for each variable in both models. Assessed variables include age, tumor stage (pT1a vs. pT2, pT1 b vs. pT2), lymphatic vessel invasion, depth of invasion, resection margin distance, and lymph-node involvement. The p values in the "Best-subset selection" column reflect the selected model, which includes only the most significant factors as determined by the Akaike Information Criterion (AIC). The p values in the "Cox model" column are derived from the full model, which incorporates all factors.

Variable	Best-subset selection	Wald test	p	Cox model	Wald test	p
Age (years)	0.0847	28.03	< 0.001	0.0892	27.45	< 0.001
pT1a versus pT2	0	0	1.000	0.218	0.054	0.816
pT1 b versus pT2	0	0	1.000	0.214	0.078	0.781
Lymphatic vessel invasion	0.764	4.603	0.032	0.983	4.944	0.026
Depth of invasion (cm)	0.468	3.462	0.063	0.566	3.963	0.047
Resection margin (mm)	0.1216	5.698	0.017	0.119	4.901	0.027
Lymph-node involvement (N-positive)	0	0	1.000	− 0.399	0.801	0.371

▶**Table 4** Distribution of patients with local recurrence by resection margin distance (RMD) at ANregiomed Hospital in Ansbach and Erlangen University Hospital. The table categorizes patients with local recurrence (n = 43) into three RMD groups: 1 to ≤ 3 mm, 3 to ≤ 8 mm, and > 8 mm, reporting the absolute number (n) and percentage (%) of recurrences for each hospital. The "Total" row aggregates data from both hospitals.

RMD (mm)	Hospital				Total	
	Ansbach		Erlangen			
	n	%	n	%	n	%
Total	18	100	25	100	43	100
1 to ≤ 3 mm	5	28	11	44	16	37
3 to ≤ 8 mm	8	44	11	44	19	44
> 8 mm	5	28	3	12	8	19

Local recurrence

Out of 232 patients analyzed, 43 experienced a local recurrence. Of these, 18 were from ANregiomed Hospital in Ansbach and 25 from Erlangen University Hospital. ▶ **Table 4** shows the distribution of patients with local recurrence based on resection margin distance. In the group with tumor-free margins of 1 to ≤ 3 mm, 37 % experienced a local recurrence, with higher rates at Erlangen (44 %) compared to Ansbach (28 %). Among those with margins of 3 to ≤ 8 mm, 44 % had a recurrence, with similar rates at both hospitals. In the group with margins > 8 mm, only 19 % developed a recurrence, with lower rates at Erlangen (12 %) compared to Ansbach (28 %).

Discussion

This study examined a large two-center cohort of patients (n = 232) with primary vulvar squamous cell carcinoma (VSCC), in order to assess the impact of pathological tumor-free resection margin distances on the survival outcomes, while identifying additional prognostic factors influencing overall survival. The data show a consistently lower survival rate throughout the study period among patients with the largest resection margin distances (> 8 mm). Conversely, patients with resection margin distances of 1 to ≤ 3 mm had the highest survival rates. The data reveal intriguing nuances that warrant further discussion.

One interesting observation is related to the interplay among the various influencing factors. In the univariate analysis, a correlation is seen between tumor size and resection margin distances. Larger tumors were associated with wider resection margin distances, probably with an adverse effect on survival. The mean age of patients with larger tumors (T) was also higher. These associations suggest that the influences observed may have been partly driven by other correlated variables, potentially explaining why tumor size (T) was excluded from the final model. Alternatively, older patients might have often had more advanced disease at the same time of surgery, potentially necessitating larger resection margins. Several factors could have contributed to this. Firstly, advanced age was frequently associated with delayed diagnosis, which could lead to tumors being identified at a more advanced stage. Secondly, older patients might have experienced different tumor behaviors or more aggressive forms of cancer, influencing the surgical approach to ensure complete tumor removal and minimize the risk of local recurrence. Additionally, the presence of two distinct molecular subtypes of vulvar cancer – HPV-positive and HPV-negative – could also have played a significant role. The HPV-related subtype, which is often located in the anterior vulva near the clitoris and urethra, generally requires lower resection margins [8].

In the multivariate analysis, age, lymphatic vessel invasion, and resection margin distance (measured in millimeters) emerged as the most influential factors affecting overall survival.

To date, only a limited number of studies have investigated the impact of the tumor-free resection margin distance on survival in patients with VSCC. Woelber et al. analyzed a much smaller cohort of 102 VSCC patients and suggested that the resection margin did not have a significant influence on disease-free survival, after categorizing the patients into three groups (< 3 mm, ≥ 3–8 mm, and ≥ 8 mm) [34]. However, the median follow-up period in the cohort analyzed was only 31 months [34]. Raimond et al., who also separated the patients into three groups (< 3 mm, ≥ 3–8 mm, and ≥ 8 mm) analyzed 112 VSCC patients and did not observe any significant effect of the tumor-free resection margin distance on disease-free survival or overall survival [19]. However, as in the study by Woelber et al., the median follow-up in the cohort analyzed was only 25 months [19]. Micheletti et al. studied 114 patients with FIGO stages IB/II VSCC and postulated that a histological margin of 5 mm is the minimum distance required to ensure oncological safety and long-term survival in patients with lymph node-negative FIGO stage IB/II VSCC. The cohort was divided into groups according to the size of the tumor-free histological margin (< 5 mm and ≥ 5 mm, and later < 8 mm and ≥ 8 mm) to compare survival outcomes. The median follow-up period was 80 months [16]. Taran et al. assigned 128 patients with node-negative VSCC, who were treated with surgery alone and did not receive any adjuvant treatment, to three groups (1–3 mm, > 3 to 8 mm, and > 8 mm) and did not observe a significant effect of the pathologically tumor-free resection margin on disease-free or overall survival [35]. The median follow-up period for disease-free survival was 6.39 years, and for overall survival 6.29 years [35].

Against the background of the continuing discussion regarding the optimal tumor-free margin distance, the present study reassessed the significance of the pathological resection margin size on overall survival in a much larger cohort with a longer follow-up period. The most influential factors in the two-center cohort were identified. In contrast to other studies that similarly categorized patients into three groups (1 to ≤ 3 mm, 3 to ≤ 8 mm, and > 8 mm), the present analysis of the estimated survival probabilities found significant variations between the different resection margin classes ($p = 0.0022$) [19, 34, 35]. The cohort with a resection margin distance of 1 to ≤ 3 mm was associated with the longest overall survival, while the cohort with the widest resection margin (> 8 mm) consistently had a lower survival rate throughout the entire period.

In the multivariate analysis, a pronounced correlation of $r = 0.53$ emerged between lymphatic vessel invasion (L) and lymph-node involvement (N). This close association posed a challenge for pinpointing which of the two variables truly accounted for the variations in survival probabilities. Despite this complexity, the study addressed the issue by prioritizing lymphatic vessel invasion (L) as a more informative variable, contributing to an enhanced model quality.

The significant disparities in age and resection margin distance between the ANregiomed Hospital in Ansbach and Erlangen University Hospital prompted further discussion. In the ANregiomed Hospital, the median age of the patients (74.5 years) and the mean resection margin size (7.87 mm) were notably higher. These differences may have contributed to the higher proportion of deceased patients. These variations highlighted the importance of considering hospital-specific characteristics when interpreting the results. It was crucial to determine whether the outcomes reflected inherent differences in the patient population or hospital-specific practices.

To provide a comprehensive understanding of the variations observed, the analysis was bifurcated: firstly for all patients, and then specifically for those from Erlangen University Hospital. The aim was to discern whether potential influences were exclusively tied to the distinctions between the two hospitals. Conducting separate analyses was intended to ascertain whether any trends or outcomes that were observed were driven primarily by hospital-specific factors rather than universal factors affecting all patients.

The survival probability modeling in all VSCC patients identified three significant influencing factors: age, lymphatic vessel invasion (L), and resection margin distance. While age and lymphatic vessel invasion remained constant, the resection margin distance could be influenced by the operating surgeon and was integral to active therapy planning. These findings are consistent with observations in the study by Micheletti et al., which concluded that the highest long-term survival was observed in lymph node–negative patients [16].

The modeling of survival probability was then specifically conducted for the patients in Erlangen University Hospital. The results not only reinforced the consistency of the overall dataset, but also confirmed the identification of age, lymphatic vessel invasion, and resection margin distance (in millimeters) as primary determinants of overall survival. These insights showed remarkable consistency in the influences across different patient cohorts, emphasizing the relevance of these factors for the prognosis.

Our analysis of local recurrence data supported our earlier findings. Specifically, smaller tumors with resection margins of 1 to ≤ 3 mm were associated with a lower risk of local recurrence. Conversely, larger tumors treated with resection margins greater than 8 mm also showed a reduced risk of recurrence. However, the group with resection margins of 3 to ≤ 8 mm presented a more complex scenario. The optimal margin distance for minimizing recurrence risk in this range was less clear-cut. The variability in recurrence rates within this group suggested that a one-size-fits-all approach might not be effective. Instead, individualized treatment strategies that considered both tumor size and margin distance seemed necessary to optimize patient outcomes.

The study has several limitations. It was a retrospective analysis of patients who underwent primary surgical treatment for VSCC at ANregiomed Hospital in Ansbach and Erlangen University Hospital over a 13-year period. During this period, the surgical management of VSCC evolved, mainly through the dissemination of the sentinel-node procedure. Although VSCC is a disease mostly affecting older patients, information on the cancer-specific mortality rate was not available. The strengths of the study include the large, comprehensive cohort of patients with VSCC and the long follow-up period.

Conclusion

The study underlines the importance of tumor-free margin distance as a prognostic factor for survival in patients with VSCC. Patients with tumor-free margins exceeding 8 mm had notably higher mortality rates. This was likely due to the observed correlation between resection margin distances, tumor sizes, and age, where larger resection margins often signified more advanced disease and older age, both of which are linked to a poorer prognosis. Smaller resection margins did not indicate worse or reduced survival outcomes. Therefore, for smaller tumors and younger patients, resection with smaller margins was deemed appropriate, provided that healthy margins were ensured.

Tumor-free margin distance and age, along with lymphatic vessel invasion as the primary prognostic factor, played pivotal roles in predicting overall survival. Awareness of these key determining factors allowed a more informed and nuanced approach to treatment strategies, especially in order to customize surgical interventions to individual patients' characteristics. It may be useful to consider a more tailored surgical approach, with the aim of minimizing morbidity and preserving both function and sensitive areas on the vulva.

Financial Support

This study received no specific financial support from funding agencies in the public, commercial, or non-profit sectors.

Acknowledgement

The contribution of Eva Balog to the present work was made in fulfillment of the requirements of the Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU) for obtaining the degree of Doctor of Medicine (Dr. med.).

Conflict of Interest

The authors declare that they have no conflict of interest.

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