Vulvar squamous cell carcinoma : genetics, morphology and clinical behaviour

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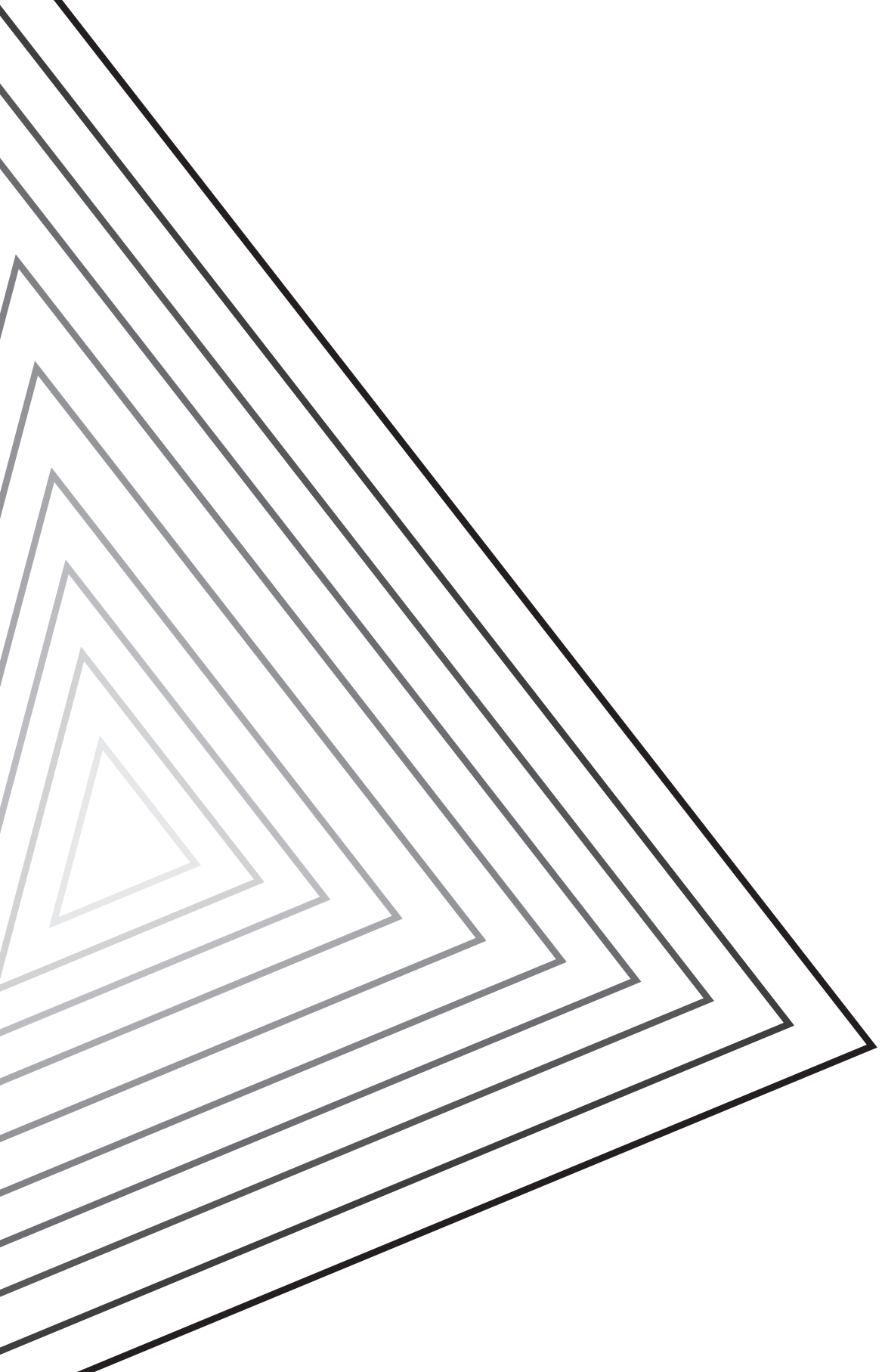

Chapter 5

Spindle cell morphology is related to poor prognosis in vulvar squamous cell carcinoma

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ABSTRACT

Background

Vulvar cancer is the fourth most common gynaecological malignancy, with an annual incidence of 2/100,000 women. Although most cases of early stage vulvar cancer have a good prognosis, recurrence and rapid tumour progression can occur. We investigated the prevalence of spindle cell morphology in vulvar cancer and its association with survival.

Methods

This retrospective cohort study included 108 patients with primary vulvar squamous cell carcinoma who were treated at the Leiden University Medical Center 2000–2009. Paraffin-embedded tissue was examined for the presence of spindle cell morphology. Survival and histology data were compared between cases with spindle and without spindle cell morphology.

Results

Twenty-two (20%) tumours showed spindle cells infiltrating the stromal tissue. All spindle cell tumours were human papillomavirus (HPV)-negative. Spindle cell morphology was strongly associated with poor prognosis and with a high risk of lymph node involvement at the time of diagnosis (relative risk 2.26 (95%CI 1.47-3.47)). Five-year disease-specific survival was lower in patients with vs. without spindle cell morphology (45.2% vs. 79.7%, respectively; P=0.00057).

Conclusion

Vulvar spindle cell morphology occurs frequently and seems to develop through the non-HPV pathway. It is associated with a worse prognosis than conventional vulvar squamous cell carcinoma.

Keywords

Vulva; carcinoma, squamous cell; carcinoma, spindle cell; prognosis

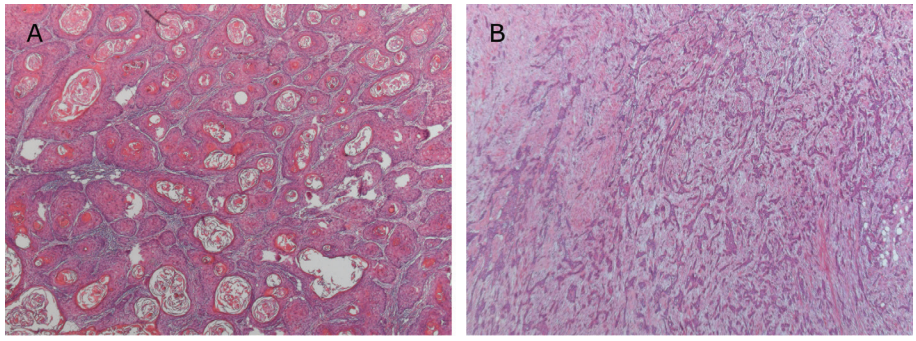
Introduction

Vulvar cancer is the fourth most common gynaecological malignancy in developed countries with an incidence of approximately 2 cases per 100,000 women each year (1-3). This cancer is mostly seen in post-menopausal women, and the average age at diagnosis is 70 years Nearly 90% of all vulvar carcinomas are vulvar squamous cell carcinoma (VSCC). (4). Squamous cell carcinomas of the vulva can be divided into two etiological types (5;6). The first type is seen mainly in younger patients and is associated with human papillomavirus (HPV) infection (7). The second type is seen mostly in elderly patients and seems to develop independent of HPV infection. This type of carcinoma is associated with lichen sclerosis and mutations of the *TP53* gene (7;8).

Vulvar carcinoma generally has a good prognosis when detected and treated at an early stage, but the most common treatment modalities, i.e. surgical removal and/or radiotherapy, can be mutilating and have high morbidity rates (9-11). A small proportion of patients suffer from early recurrence, rapid progression of tumour growth and death (12).Identifying risk factors for poor survival is important to further reduce the mortality and morbidity of vulvar cancer patients, but few studies have found new factors of clinical importance (13). To date, lymph node involvement is the only accurate prognostic factor for survival and recurrence (14).

A specific histological type of squamous cell carcinoma, termed spindle cell carcinoma, is seen occasionally. Spindle cell carcinoma is also referred to as pseudosarcoma or carcinosarcoma. Spindle cells are thin, slender, elongated cancer cells that infiltrate stromal tissue and that occur either as single cells or as cords of cells rather than as groups or islands (Figure 1). Spindle cell carcinoma is seen in many different types of cancer and is associated with a worse prognosis in cancers of the oral cavity, oesophagus and lung (15-19). Spindle-shaped epithelial cancer

Figure 1 *VSCC and spindle cell morphology*



*Representative images of vulvar squamous cell carcinoma (**A**) and vulvar squamous cell carcinoma with spindle cell morphology (20x magnification) (**B**).*

cells have lost their typical epithelial characteristics, and seem to gain the ability to infiltrate the underlying stroma and metastasize (20).

Spindle cell carcinomas consisting of spindle cells alone have been reported only incidentally in vulvar carcinoma, and it is thought to be an aggressive tumour type that occurs in 1% to 5% of all vulvar squamous cell carcinoma patients (15;21-27). Some VSCCs show a specific pattern of invasion in which there is infiltration of the stroma by single cells or cords of tumour cells adjacent to the 'conventional' squamous cell carcinoma cells. This pattern is also called 'spray pattern invasion'. This spray pattern invasion is seen more often than the rare vulvar spindle cell carcinoma and has been described before (28-30). A subpopulation of these vulvar carcinomas with a spray pattern of invasion clearly shows areas of infiltrating cells that resemble spindle cell carcinoma. We suggest that these tumours be termed, 'vulvar squamous cell carcinomas with spindle cell morphology', and describe the criteria used to characterize these tumours in the Methods section. The characteristics of tumours with this pattern of invasion and their association with survival have, to our knowledge, not been studied in a large cohort of patients. We hypothesise that tumours with spindle cell morphology, i.e. tumours consisting of both 'conventional' squamous cell carcinoma cells and spindle cells, may share some of the aetiology and clinical behaviour with the aggressive spindle cell carcinomas. We considered whether spindle cell morphology is a possible risk factor and studied its prevalence and possible relation with prognosis.

Materials and Methods

Patients

All patients who were primarily surgically treated for primary VSCC between 2000 and 2009 at the Leiden University Medical Center, a referral centre for gynaecological cancers in the Netherlands, were considered for inclusion in this study.

Clinical and follow-up data were retrieved from patient medical records and from the cancer registration database. Patients were excluded if they had received systemic immunosuppressive therapy, chemotherapy or radiotherapy in the pelvic area prior to surgery. Tumour staging was performed according to the FIGO system using histologically confirmed TNM data. We used the 1995 staging instead of the revised 2009 staging because of the retrospective design of the study (31;32). The patients were followed-up until December 2012. Patient samples were handled according to the medical ethical guidelines described in the Code of Conduct for Proper Secondary Use of Human Tissue of the Dutch Federation of Biomedical Scientific Societies.

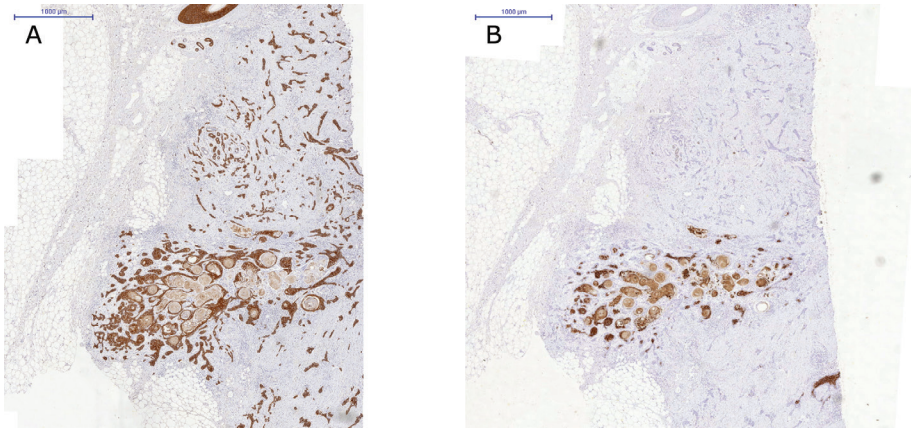
Microscopy and immunohistochemistry

All formalin-fixed, paraffin-embedded tissue blocks from the selected primary VSCC patients that were stored in the Leiden University Medical Center archives were examined microscopically for the presence of spindle-shaped tumour cells on haematoxylin and eosin-stained slides. Tumours from which only biopsies were taken were excluded from the study.

One series of consecutive 4-µm paraffin-embedded tissue sections was taken from all tumour-containing tissue blocks and stained as described previously (33) using primary antibodies for pankeratin AE1AE3 (mAB 3412, 1:2000, Millipore, Billerica, MA, United States of America), keratin 10 (DE-k10, 1:50, DAKO, Glostrup, Denmark) and keratin 14 (LL002, 1:2000, Abcam, Cambridge, United Kingdom). All slides were digitalized using a Mirax slide scanner and analysed using Panoramic Viewer (version 1.15.50, 3DHistec, Budapest, Hungary).

Since there is no established definition of spindle cell morphology, we categorized the samples as follows. Tumour cells were categorized as spindle cells if they had an elongated shape, infiltrated the underlying stromal tissue as single cells or as cords of cells instead of as islands of cells and were keratin 14-positive and keratin 10-negative as markers of dedifferentiation (Figure 2). When at least 10 spindle cells were seen per high power field using a 40 x 0.65 objective, independent of the proportion of spindle cells in relation to the solid component of the cancer, the tumour was categorized as having spindle cell morphology. Spindle-shaped cells in close proximity (<0.5 mm) to the solid component of the tumour were not marked as spindle cell carcinoma cells but were considered to be part of the spray pattern invasive border of the solid tumour.

Figure 2 *Keratin expression in VSCC with spindle cell morphology*



*A representative image of a sample of vulvar squamous cell carcinoma with spindle cell morphology stained for keratin 14 (**A**) and for keratin 10 (**B**). Note that the spindle-shaped cells are positive for keratin 14 but negative for keratin 10, while the solid component of the tumour is positive for both keratin 14 and 10 (Scale bar, 1000 µm).*

HPV analysis

DNA extracted from formalin-fixed, paraffin-embedded tumour tissue was used for HPV type analysis. To prevent and check for contamination, sections of a paraffin block without tissue, which were cut before each tumour sample, served as negative controls. All such controls were negative in the PCR analysis. The INNO-LiPA HPV Genotyping *Extra* Amp kit for in vitro diagnostic use (Innogenetics, Gent, Belgium), which is a highly sensitive hybridization assay, was used for HPV typing as described previously (34). This assay is able to detect oncogenic and common HPV types (34).

Statistical analysis

Statistical analyses were conducted using the IBM SPSS Statistics software package (version 20, IBM-SPSS Statistics, Armonk, NY, USA). The independent *t*-test was used to compare baseline variables, and Fisher's exact test was used to analyse categorical and normally distributed numerical data. The Shapiro-Wilk test was used to test for normality. The Mann-Whitney U test was used for data with a skewed distribution. Kaplan-Meier curves, the log rank test and Cox Proportional Hazard regression analysis were performed to analyse the differences in survival between the spindle and non-spindle groups. A P-value less than or equal to 0.05 was considered significant, corresponding to 95% confidence intervals. All tests were two-tailed. Results for normally distributed numerical data are presented as mean with standard deviation (SD), and results for skewed numerical data are presented as median with interquartile range (IQR).

Disease-free survival was defined as survival from the date of surgery until the first recurrence or death or until the end of study follow-up. The overall survival of the patients was measured from the date of surgery until death from any cause or until the end of study follow-up. Disease-specific survival was measured from the date of surgery until death from vulvar cancer or until the end of study follow-up. Recurrent disease in the vulvar area was characterized as 'local recurrence', whereas recurrences in the inguinal region were characterized as 'regional recurrence'. Recurrent disease on the contralateral side of the vulva was considered to be a second primary tumour.

Results

Between January 2000 and December 2009, 129 patients were treated surgically for primary VSCC at the Leiden University Medical Center. Nine patients were excluded because they had a history of chemotherapy, pelvic radiotherapy or immunosuppressive therapy for vulvar cancer or for another disease prior to the current diagnosis. Eleven patients were excluded because their tumours were biopsied but

no further surgical treatment was given. Tumour tissue from one patient was of poor quality and was not analysed further. Thus, a total of 108 patients with primary VSCC met all of our inclusion criteria. Table 1 lists the characteristics of these patients.

The median follow-up time was 39 months (IQR 16.3–70.5), and the mean age at diagnosis with vulvar carcinoma was 69.9 years (SD 14.1). Symptoms first

Table 1 *Characteristics of the vulvar squamous cell carcinoma patients in this study.*

Characteristic			Value	
Follow up†	– mo		39.0	(16.3-70.5)
Age at diagnosis‡	– year		69.9	(14.1)
Duration of symptoms†	– mo		5.0	(2.0-18.0)
FIGO stage	– n	(%)		
Stage 1			30	(27.8)
Stage 2			36	(33.3)
Stage 3			30	(27.8)
Stage 4			12	(11.1)
Treatment	– n	(%)		
Radical vulvectomy			69	(63.9)
Radical local excision			39	(36.1)
Adjuvant radiotherapy			43	(39.8)
Adjuvant chemotherapy			1	(0.9)
HPV positive	– n	(%)	18	(16.7)
Lymph node metastases	– n	(%)	41	(38.0)
Unilateral			29	(26.9)
Bilateral			12	(11.1)
Extracapsular growth			17	(15.7)
Tumor size‡	– mm		32.1	(22.2)
Infiltration depth†	– mm		6.0	(4.0-11.0)
Vasoinvasion	– n	(%)	15	(13.9)
Lymphangioinvasion			3	(2.8)
Perineural growth			4	(3.7)
Positive resection margins			21	(19.4)
Disease status	– n	(%)		
Complete remission			83	(76.9)
Local recurrence			22	(20.4)
Second primary tumour			10	(9.3)
Regional recurrence			9	(8.3)
Regional metastases			8	(7.4)
Distant metastases			24	(22.2)
Died			59	(54.6)
Disease-specific death			28	(25.9)
5-year overall survival	– %	(SD)	51.9	(5.0)
5-year disease specific survival	– %	(SD)	72.9	(4.5)
5-year disease free survival	– %	(SD)	30.7	(4.9)

**Significant difference (P<0.05), †Median (interquartile range), ‡Mean (standard deviation).*
Abbreviations: N = number; mo = months; mm = millimetre.

occurred at a median of 5.0 months (IQR 2.0–18.0) before diagnosis. Microscopic evaluation showed a mean tumour size of 32.1 mm (SD 22.2) and a median infiltration depth of 6.0 mm (IQR 4.0–11.0). Lymph node metastases were found in 41 of the 108 patients (38.0%). Thirty patients (27.8%) had a tumour that was FIGO stage 1, 36 patients (33.9%) stage 2, 30 (27.8%) stage 3, and 12 (11.1%) stage 4. Forty-three patients (39.8%) received adjuvant radiotherapy to the groin and/or vulva; 38 of these patients received adjuvant radiotherapy because they were stage 3 or higher, and 5 of them received it because a tumour-free resection margin of less than 8 mm was obtained. One patient received adjuvant radiotherapy and chemotherapy because of close tumour-free resection margins plus the presence of multiple inguinal lymph node metastases.

At the endpoint of the study period, 20% of the patients had developed local recurrent disease and 26% had died from the disease, giving a 5-year disease-free survival of 31% (SD 4.9) and a 5-year disease-specific survival of 73% (SD 4.5).

Comparison of the clinical outcomes for patients with spindle and non-spindle cell morphology

Of the 108 tumours that were analysed, 22 tumours (20%) were identified as having spindle cell morphology and 86 (80%) as being non-spindle cell carcinomas. Table 2 summarizes the outcome measurements for patients with carcinomas with spindle cell and non-spindle cell morphology. The mean age did not differ between the two groups. Vulvar spindle cell carcinoma patients were diagnosed at a higher FIGO stage than non-spindle patients, though the duration of symptoms before diagnosis did not differ.

Forty-one of the 108 patients had lymph node metastases at the time of diagnosis, 15 of which were also positive for spindle cells. This gives a relative risk of lymph node metastasis in spindle cell morphology patients of 2.26 (95% CI 1.47–3.47) compared with patients without spindle cell morphology. Eighteen of 108 patients tested positive for HPV [type 16 (n=10), 18 (n=2), or 33 (n=5); one patient tested positive for both HPV types 16 and 33]. None of these patients had carcinomas with spindle cell morphology.

Compared to spindle cell morphology patients, more non-spindle patients achieved complete remission (83.7% vs. 50.0%, P=0.003). At the endpoint of the study period, 22.7% of the spindle cell morphology patients and 19.8% of the non-spindle cell patients had developed local recurrent disease, and 9.1% and 8.1% of patients, respectively, developed regional recurrences (P=1.000). In terms of disease-specific death, 50% of the spindle cell patients and 19.8% of the non-spindle cell patients died from the disease (P=0.002).

Overall survival differed significantly between patients with and without vulvar spindle cell morphology (Figure 3A), with 5-year overall survival of 27.3% (SD 9.5)

Table 2 *Comparison of the clinical outcomes for patients with spindle and non-spindle cell morphology.*

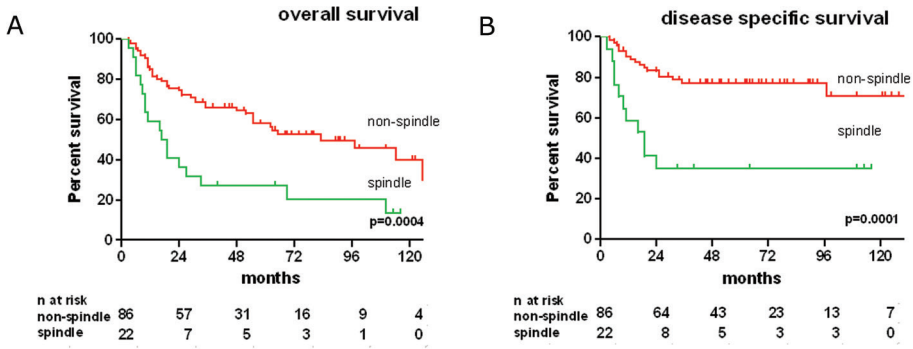
Outcome			Spindle n=22	(20.4%)	Non-spindle n=86	(79.6%)	P-value
Follow-up†	– mo		18.0	(8.8-46.0)	48.0	(123.0-77.5)	0.026*
Age at diagnosis‡	– yr		71.0	(13.2)	69.7	(14.4)	0.617
Duration of symptoms†	– mo		4.0	(2.8-12.5)	5.0	(2.0-22.0)	0.951
FIGO stage at first operation	– n	(%)					0.002*
Stage 1			3	(13.6)	27	(31.4)	
Stage 2			4	(18.2)	32	(37.2)	
Stage 3			8	(36.4)	22	(25.6)	
Stage 4			7	(31.8)	5	(5.8)	
Treatment	– n	(%)					
Radical vulvectomy			14	(63.6)	55	(64.0)	1.000
Radical local excision			8	(36.4)	31	(36.0)	
Adjuvant radiotherapy			14	(63.6)	29	(33.7)	0.020*
Adjuvant chemotherapy				(0.0)	1	(1.2)	
HPV positive	– n	(%)			18	(20.9)	0.011*
Lymph node metastases	– n	(%)	15	(68.2)	26	(30.2)	0.002*
Unilateral			8	(36.4)	21	(24.4)	0.001*
Bilateral			7	(31.8)	5	(5.8)	
Extracapsular growth			8	(36.4)	9	(10.5)	0.006*
Tumor size‡	– mm		38.7	(21.4)	30.5	(22.2)	0.610
Infiltration depth†	– mm		8.0	(4.5-12.0)	6.0	(3.0-11.0)	0.242
Vasoinvasion	– n	(%)	6	(27.3)	9	(8.3)	0.077
Lymfangioinvasion			1	(4.6)	2	(2.3)	0.499
Perineural growth			1	(4.6)	3	(3.5)	1.000
Positive resection margins			7	(31.8)	14	(16.3)	0.131
Disease status	– n	(%)					
Complete remission			11	(50.0)	72	(83.7)	0.003*
Local recurrence			5	(22.7)	17	(19.8)	0.771
Second primary tumour			2	(9.1)	8	(9.3)	1.000
Regional recurrence			2	(9.1)	7	(8.1)	1.000
Regional metastases			1	(4.5)	7	(8.1)	0.443
Distant metastases			7	(31.8)	17	(19.8)	
Died			18	(81.8)	41	(47.7)	0.007*
Disease specific death			11	(50.0)	17	(19.8)	0.002*
5-year overall survival	– %	(SD)	27.3	(9.5)	58.2	(5.6)	0.00041*
5-year disease specific survival	– %	(SD)	45.2	(11.4)	79.7	(4.6)	0.00057*
5-year disease free survival	– %	(SD)	25.0	(12..5)	44.3	(6.6)	0.149

**Significant difference (P<0.05), †Median (interquartile range), ‡Mean (standard deviation). Abbreviations: N = number; mo = months; mm = millimetre.*

vs. 58.2% (SD 5.6; P=0.00041), respectively. Disease-specific survival was significantly worse for spindle cell morphology patients than non-spindle cell morphology patients, with a 5-year disease-specific survival of 45.2% (SD 11.4) vs. 79.7% (SD 4.6; P=0.00057; Figure 3B). Five-year disease-free survival did not differ significantly between the two groups (25.0% vs. 44.3%, P=0.149).

Univariate Cox regression analysis for disease-specific death resulted in a hazard ratio of 3.50 (95% CI 1.63–7.52, P=0.001) for spindle cell morphology patients compared with non-spindle cell patients. After correction for the possible con-

Figure 3 *Survival curves*



*Overall survival (**A**) and disease-specific survival (**B**) in the 108 vulvar squamous cell carcinoma patients in this study. There were significant differences in both overall and disease-specific survival for patients that had carcinomas with vs. without spindle cell morphology (relative risk of dying 1.7 vs. 2.5; 95% CI 1.3–2.3 and 1.4–4.6; P=0.0043 and P=0.0041, respectively). Red is non-spindle, green is spindle.*

founders age and HPV infection, the hazard ratio became 2.71 (95% CI 1.26–5.81, P=0.011). When correcting for more tumour characteristics (tumour size, infiltration depth, positive resection margins), vulvar spindle cell morphology patients had a hazard ratio for disease-specific death of 2.51 (95% CI 1.12–5.64). Adding lymph node metastasis and regional or distant metastasis to the correction model gave a hazard ratio of 4.1 (95%CI 1.61–10.60) for patients with carcinomas with spindle cell morphology (Table 3).

Discussion

This study is, to our knowledge, the first to investigate vulvar spindle cell morphology in a large group of patients with VSCC. Spindle cell morphology occurred frequently in our series and was found in 20% of the VSCCs examined. Carcinoma with spindle cell morphology has not been reported as a separate entity in vulvar cancer prior to this report. A spray pattern of invasion, which also includes spindle cell morphology, has been reported in a few papers (28-30) and was found in 11 out of 26 VSCCs by Drew *et al* (29). In the latter study, which was relatively small, a spray pattern of invasion was significantly associated with poor survival after correction for FIGO stage alone.

Our study of a large cohort of VSCC patients shows that patients with spindle cell morphology have a worse prognosis than patients with conventional squamous cell carcinoma of the vulva. The disease-specific and overall survival was almost half that of non-spindle squamous cell carcinoma patients. After correcting for multiple possible confounders, vulvar spindle cell morphology remains an

Table 3 *Multivariate analysis of prognostic variables.*

		P-value	HR	95% CI for HR
Step 0	Spindle cell morphology	.001	3.503	1.632 - 7.517
		P-value	**HR**	**95% CI for HR**
Step 1	Spindle cell morphology	.011	2.706	1.262 - 5.805
	Age	.493	1.001	0.998 - 1.003
	HPV infection	.970	.000	N.A.
		P-value	**HR**	**95% CI for HR**
Step 2	Spindle cell morphology	.026	2.512	1.119 - 5.639
	Age	.850	1.000	0.997 - 1.002
	HPV infection	.971	.000	N.A.
	Tumour size	.205	1.016	0.991 - 1.042
	Infiltration depth	.906	1.004	0.940 - 1.072
	Positive resection margins	.335	1.734	0.566 - 5.306
		P-value	**HR**	**95% CI for HR**
Step 3	Spindle cell morphology	.003	4.135	1.613 - 10.601
	Age	.348	.999	0.996 - 1.001
	HPV infection	.964	.000	N.A.
	Tumour size	.050	1.028	1.000 - 1.057
	Infiltration depth	.562	.984	0.930 - 1.040
	Positive resection margins	.047	3.183	1.015 - 9.979
	Lymph node metastasis	.509	1.364	0.543 - 3.427
	Metastasis:			
	No metastasis	.000	Ref.	
	Locoregional metastasis	.001	14.807	3.204 - 68.425
	Distant metastasis	.000	39.734	11.341 - 139.214

Variables added in step 1: age, HPV. Variables added in step 2: tumour size, infiltration depth, positive resection margins. Variables added in step 3: lymph node metastases, infiltration depth, distant metastases.
Abbreviations: HR = hazard ratio

independent prognostic factor. None of the patients with spindle cell morphology showed HPV infection, strongly suggesting that these tumours arise through the non-HPV pathway (8;35).

The vulvar spindle cell morphology patients reported having symptoms for a similar length of time prior to diagnosis than non-spindle cell patients, but vulvar spindle cell carcinoma patients presented with higher FIGO stages, possibly because of the aggressive character of spindle cells and the rapid progression of this type of tumour.

Although this series is the largest reported in the literature, the number of patients in this study is still too small to correct for all potential confounders. However, even after correcting for multiple tumour characteristics that are likely to be part of the causal path (tumour size, infiltration depth, positive resection margins, lymph

node metastasis and distant metastasis), vulvar spindle cell morphology patients had a hazard ratio for disease-specific death of 4.14 (95% CI 1.61–10.60) relative to non-spindle cell patients, suggesting that the poor prognosis of these patients is associated with spindle cell morphology and is independent of other tumour characteristics. Caution has to be taken when interpreting multivariate analysis in small cohorts of patients. Given the distinct differences in survival, we suggest that this retrospective study be replicated at other large institutions to determine the added prognostic value of spindle cell morphology in a prognostic model.

Regarding the survival and overall incidence of spindle cells, selection bias could have been introduced by our inclusion of patients who were treated primarily by surgery. Patients that were treated by excision biopsy alone were not selected for this series, and such patients usually have smaller tumours and a better prognosis. On the other hand, patients with inoperable tumours, and likely a worse prognosis, were also not selected, so patient selection cannot fully explain the differences in survival. Notably, correcting for tumour size did not change the finding that spindle cell morphology patients have a significantly worse prognosis.

These results may have implications for the clinical management of VSCC. Spindle cells can be detected by a trained pathologist and, if necessary, be visualized using commonly available keratin stains. Given our finding that spindle cell morphology does not affect the risk of local recurrent disease, the current advice to perform a local radical tumour resection with 8-mm tumour-free margins after fixation seems appropriate (36). However, the increased risk of lymph node metastasis and poorer prognosis could influence the choice to perform adjuvant chemotherapy or radiotherapy in vulvar spindle cell morphology patients.

Conclusion

VSCC with spindle cell morphology appears to be an aggressive tumour type. In this series of patients, this cancer type had a worse prognosis than conventional VSCC as well as an increased risk of lymph node metastases at the time of diagnosis.

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