

## Mixed small cell carcinomas of the uterine cervix: prognostic impact of focal neuroendocrine differentiation but not of Ki-67 labeling index

Lars-Christian Horn, MD, PhD<sup>a,\*</sup>, Bettina Hentschel, MA<sup>b</sup>, Karl Bilek, MD, PhD<sup>c</sup>,  
Christine E. Richter, MD<sup>a,c</sup>, Jens Einenkel, MD<sup>c</sup>, Cornelia Leo, MD<sup>c</sup>

<sup>a</sup>*Institute of Pathology, Division of Gynecologic Pathology, University of Leipzig, Leipzig D-04103, Germany*

<sup>b</sup>*Institute of Medical Informatics, Statistics and Epidemiology, University of Leipzig, Leipzig D-04103, Germany*

<sup>c</sup>*Department of Obstetrics and Gynecology, University of Leipzig, Leipzig D-04103, Germany*

### Abstract

Small cell neuroendocrine carcinomas sometimes represent a non-small cell component. Because of infection with the high-risk human papillomavirus of small cell carcinomas (SmCCs), several host cell regulatory proteins are altered, thus causing altered proliferative activity. Knowledge regarding the prognostic impact of focal neuroendocrine differentiation in mixed SmCCs and the value of proliferative activity in these tumors is very limited. Small cell carcinomas were selected for immunohistochemical staining with neuroendocrine markers and Ki-67. In cases with mixed tumors, the percentage of the SmCC component was calculated and correlated with survival. Of 677 tumors, 9 (1.3%) were classified as SmCCs after Grimelius staining (8/9 positive tumors) and immunohistochemical reaction against neuron-specific enolase, chromogranin A, synaptophysin (7/9 positive tumors), and CD56 (8/9 positive tumors); all specimens were positive for at least 2 of these. CD99 staining was completely negative. Two thirds of the SmCCs showed non-small cell differentiation. Four patients died of the tumor after a median time of 36.7 months (range, 15–56 months). Even an SmCC component of 17% was associated with a fatal course. Small cell carcinoma represented a significantly lower proliferation (Ki-67 labeling index) than did the non-small cell component in the same tumor (12.8% vs 70.8%;  $P < .001$ ). Even a small SmCC component in mixed carcinomas of the uterine cervix was associated with adverse outcome. Proliferative activity, determined by Ki-67 labeling index, is of no prognostic value.

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### Keywords:

Small cell carcinoma; Cervix uteri; Ki-67; MIB-1; Prognosis

### 1. Introduction

Small cell neuroendocrine carcinomas comprise a rare but aggressive subset of uterine cervical neoplasms with a high rate of recurrence and poor overall survival [1–3]. Some of the small cell carcinomas (SmCCs) of the uterine cervix are associated with a non-small cell component [1,3,4].

In concordance with squamous cells and adenocarcinomas of the uterine cervix, cervical SmCCs are associated with high-risk human papillomavirus in approximately two thirds

of cases [1,3,5]. It is well accepted that the oncoproteins encoded by E6 and E7 of high-risk human papillomavirus have the ability to bind host cell regulatory proteins, thus causing consecutive functional alterations, including increased proliferative activity. Contrary to the value of proliferative activity in cervical intraepithelial neoplasia lesions, determined by Ki-67 immunostaining, this parameter has no prognostic impact on invasive squamous cells and adenocarcinomas of the uterine cervix [6,7].

In contrast to squamous cells and adenocarcinomas of the uterine cervix [6,8], there is limited experience regarding the prognostic value of proliferative activity in SmCCs. In addition, there is no information about the impact of focal small cell neuroendocrine differentiation in mixed cervical carcinomas.

\* Corresponding author. Tel.: +49 341 97 150 46; fax: +49 341 97 150 09.

E-mail address: [hornl@medizin.uni-leipzig.de](mailto:hornl@medizin.uni-leipzig.de) (L.-C. Horn).

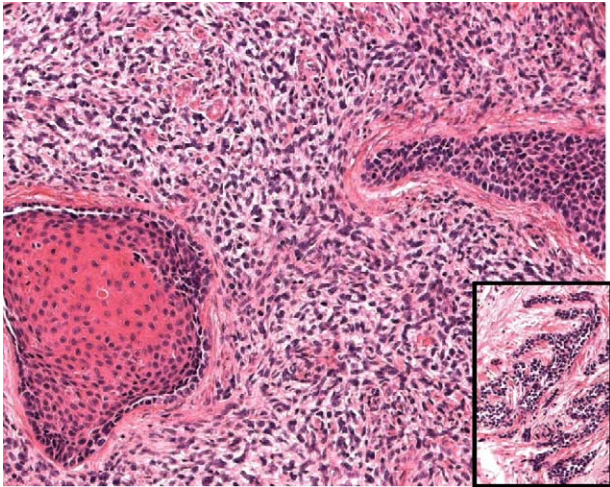


Fig. 1. Mixed small cell neuroendocrine and squamous cell carcinoma of the uterine cervix with diffuse infiltration pattern ( $\times 215$ ; inset  $\times 315$ ).

Therefore, we have determined the proliferative activity in SmCCs and the size of the neuroendocrine differentiation in mixed tumors.

## 2. Material and methods

### 2.1. Tumor specimens

Six hundred seventy-seven surgically treated carcinomas of the uterine cervix, histopathologically staged from pT1b1 to pT2b, were available from the Wertheim Archive of the Division of Gynecologic Pathology, Department of Pathology, of the University of Leipzig (Leipzig, Germany) [9]. All tumors were handled using a standardized protocol [10]. Tumors with approximately 2.5 cm in the largest dimension were processed completely for histological examination;

from larger tumors, one block per centimeter of their largest dimension was obtained.

Eleven cases showed complete or focal small cell differentiation on hematoxylin-eosin (H&E) staining. These cases were selected for clinical and immunohistochemical analysis.

The size of the different tumor cell components was calculated in percentage values by comparing the largest extension of each component with the largest dimension of the tumor. Follow-up information were obtained from clinical files.

### 2.2. Immunohistochemical analysis

After the reevaluation of all 677 cases, tumors with small cell appearances on H&E-stained slides were selected for Grimelius staining and immunohistochemical analysis.

To determine neuroendocrine differentiation, we included synaptophysin (DakoCytomation, Glostrup, Denmark; catalogue number M0776; 1:50), chromogranin (BioGenex, San Ramon, CA; catalogue number MU126-UC; 1:150), S100 (BioGenex; catalogue number MU058-UC; 1:250), neuron-specific enolase (DakoCytomation; catalogue number M0873; 1:300), CD99 (DakoCytomation; catalogue number M3601; 1:50), and CD56 (Ventana Medical, Tucson, AZ; catalogue number CD56-186, prediluted) for immunohistochemical staining. Sections known to stain positively were included in each batch and negative controls were also performed by replacing the primary antibody with mouse or goat ascites fluid (Sigma-Aldrich Biochemicals, St Louis, Mo).

Proliferative activity was determined using an antibody against the Ki-67 antigen (MIB-1, DakoCytomation; catalogue number M7249; 1:50). Two hundred cells were counted by creating the percentage between negatively and positively stained nuclei.

Table 1  
Clinicopathological characteristics and Ki-67 LI of SmCCs of the uterine cervix

No.	Age (y)	Tumor type	Overall size (cm)	Percentage of histological component (%)	Depth of invasion (%)	Stage	pLNM	Ki-67 LI (%)	Adjuvant therapy	Follow-up
1	57	SmCC	3.5 × 3	100	57	pT1b1	PN0 (0/27)	15	RX	DOD, 4.2 y
2	57	SmCC	3.5 × 2	100	77	PT1b1	pN0 (0/35)	22	RX	NED, 4.2 y
3	58	SmCC	4 × 2.5	17.5	88	pT2a	pN0 (0/28)	25	RX	DOD, 4.8 y
		SQCC		82.5				80		
4	25	SmCC	2.5 × 2	40	30	pT1b1	pN0 (0/37)	8	None	NED, 12.6 y
		SQCC		60				85		
5	49	SmCC	5 × 3.5	36	50	pT1b2	pN1 (8/46)	10	RX	DOD, 1.3 y
		SQCC		64				44		
6	61	SmCC	2.5 × 2.5	100	100	pT2b	pN0 (0/23)	2	RX	NED, 8.5 y
7	38	SmCC	4 × 3.5	55	86	pT1b1	pN0 (0/36)	12	RX	NED, 9.7 y
		SQCC		25				80		
		AC		20				70		
8	24	SmCC	2.5 × 2	36	57	pT1b1	pN0 (0/39)	9	None	DOD, 2.2 y
		AC		64				66		
9	57	SmCC	0.9 × 0.6	90	47	pT1b1	pNX	15	RX	?
		CIS		10				66		

pLNM indicates pelvic lymph node metastases; RX, radiation therapy; DOD, dead of disease; NED, no evidence of disease; SQCC, squamous cell carcinoma; AC, adenocarcinoma; CIS, carcinoma in situ.

### 3. Results

Of the 677 cases in which the H&E-stained slides were reevaluated histologically, 78.9% showed squamous cell, 5.6% showed adenocarcinomatous, and 11 tumors showed small cell histology; 14.2% of the carcinomas represented tumors of other histological types (eg, adenoid basal-cell carcinoma, unclassified carcinomas).

Two cases that were initially grouped into SmCCs were excluded from the study because of negative results with Grimelius staining or neuroendocrine markers. Thus, 9 of 677 carcinomas of the uterine cervix (1.3%) represented small cell differentiation (Fig. 1).

Of the 9 tumors, 8 showed positive Grimelius staining, 7 represented positive staining results with antibodies against neuron-specific enolase, chromogranin A, or synaptophysin, and 8 indicated positive staining for CD56. Staining results for CD99 were completely negative. Of the 9 tumors, 6 showed additional non-small cell components (see Table 1). The percentage of SmCC components in the present cases ranged from 17.5% to 55% in mixed carcinomas (Table 1).

Interestingly, the SmCC component represented a lower proliferative activity than did the non-small cell component in the same tumor, determined by Ki-67 labeling index (LI). Small cell carcinomas showed a Ki-67 LI of 13.1% on average (range, 2%-22%), which was significantly lower than that of the non-small cell component (70.8%; range 44%-85%;  $P < .001$ ; Table 1).

One patient was lost during follow-up. Of the remaining 8 patients, 4 died of the disease after a mean time of 36.7 months (range, 15-56 months) after surgery (Table 1). On autopsy, distant metastases were found at the lungs, liver, brain, bones, and mesenteric and para-aortic lymph nodes.

### 4. Discussion

Albores-Saavedra et al [11] first reported the occurrence of SmCCs of the uterine cervix. The frequency of these tumors ranges between 0.5% and 5% [12,13]. This is in accordance with our result of 1.3% (9/677 cases).

Small cell carcinomas are highly aggressive tumors with an early recurrence, irrespective of initial treatment [14], and a very low 5-year survival rate of 14% to 29% [1,3,13]. In our study, 4 patients died of the disease after a median time of 36.5 months (range, 15-56 months), representing a 5-year survival rate of 56%. In concordance to the literature [14], the aggressive course of the disease is characterized by the development of widespread hematogenous metastases.

The presence of non-SmCC differentiation has been reported in 25% of cervical SmCCs (range, 8.7%-42.3%; [1,3,4,12,15]). Two thirds of our tumors demonstrated mixed histology (Fig. 1). To the best of our knowledge, there are no data in the literature regarding the correlation between the size of neuroendocrine differentiation and the prognostic outcome of patients. The percentage of the

SmCC component of the examined tumor tissue in our study ranged from 17.5% to 55%. Conversely, the presence of 17.5% of the small cell neuroendocrine carcinomas was associated with poor prognostic outcome in our study (case 3 in Table 1). Thus, it is mandatory for pathologists and clinicians to recognize focal small cell neuroendocrine differentiation in cervical carcinomas for the selection of adjuvant treatment [16].

Surprisingly, the SmCC component of our cases represented a significantly lower proliferative activity than did the non-small cell component (Ki-67 LI, 13.1% vs 70.8%; see Table 1). Therefore, it can be assumed that high proliferative activity is not a prerequisite for aggressive tumor growth in SmCCs of the uterine cervix. The finding of low proliferative activity in cervical SmCCs is contrary to observations in small cell neuroendocrine carcinomas of the lung as well as of the gastrointestinal tract [17,18]. The causes for this diversity in proliferation are unclear at this time and further studies are required. Some reports on non-SmCC of the cervix have shown that proliferative activity has no prognostic impact on disease-free and overall survival [6,8]. However, Graflund et al [19] have reported a lower proliferative activity in tumor cells metastasized to pelvic lymph nodes than in cells of the primary tumor. This and the results of our study suggest that the lower proliferative activity of tumor cell compartments, which show a biologic aggressive potential (ability to metastasize to pelvic lymph nodes in non-SmCC and neuroendocrine differentiation with worse prognostic outcome), is one characteristic of highly aggressive tumor cell populations.

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