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ORIGINAL ARTICLE

Introducing a novel highly prognostic grading scheme based on tumour budding and cell nest size for squamous cell carcinoma of the uterine cervix

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Abstract

A novel histopathological grading system based on tumour budding and cell nest size has recently been shown to outperform conventional (WHO-based) grading algorithms in several tumour entities such as lung, oral, and oesophageal squamous cell carcinoma (SCC) in terms of prognostic patient stratification. Here, we tested the prognostic value of this innovative grading approach in two completely independent cohorts of SCC of the uterine cervix. To improve morphology-based grading, we investigated tumour budding activity and cell nest size as well as several other histomorphological factors (e.g., keratinization, nuclear size, mitotic activity) in a test cohort ($n = 125$) and an independent validation cohort ($n = 122$) of cervical SCC. All parameters were correlated with clinicopathological factors and patient outcome. Small cell nest size and high tumour budding activity were strongly associated with a dismal patient prognosis ($p < 0.001$ for overall survival [OS], disease-specific survival, and disease-free survival; test cohort) in both cohorts of cervical SCC. A novel grading algorithm combining these two parameters proved to be a highly effective, stage-independent prognosticator in both cohorts (OS: $p < 0.001$, test cohort; $p = 0.001$, validation cohort). In the test cohort, multivariate statistical analysis of the novel grade revealed that the hazard ratio (HR) for OS was 2.3 for G2 and 5.1 for G3 tumours compared to G1 neoplasms ($p = 0.010$). In the validation cohort, HR for OS was 3.0 for G2 and 7.2 for G3 tumours ($p = 0.012$).

In conclusion, our novel grading algorithm incorporating cell nest size and tumour budding allows strongly prognostic histopathological grading of cervical SCC superior to WHO-based grading. Therefore, our data can be regarded as a cross-organ validation of previous results demonstrated for oesophageal, lung, and oral SCC. We suggest this grading algorithm as an additional morphology-based parameter for the routine diagnostic assessment of this tumour entity.

Keywords: cervical carcinoma; cell nest size; budding; grading; prognosis; survival

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No conflicts of interest were declared.

Introduction

Squamous cell carcinoma of the uterine cervix (cervical SCC) is one of the most common cancer types among females [1–3]. It is almost exclusively caused by persistent infection with a distinct subgroup of carcinogenic human papillomaviruses (HPV) [4,5].

Cervical SCC shows a highly variable disease course, ranging from subclinical neoplasms to biologically aggressive carcinomas associated with metastatic spread and short patient survival, underlining the need for valid prognosticators of cervical SCC beyond tumour stage [6,7]. Current histopathological grading of cervical SCC is performed by analogy to the guidelines of the WHO Classification of Tumors of Female Reproductive Organs (WHO) and is primarily based on a tumour's degree of keratinization, mitotic activity, and nuclear pleomorphism [8]. However, WHO-based grading of cervical SCC does not allow accurate prognostic patient stratification and therefore remains a matter of debate [8,9]. Competing grading cervical SCC approaches [10,11], which partially showed prognostic value in clinically heterogenous cohorts, unfortunately mixed histomorphological factors like keratinization with grading-independent parameters (e.g., vascular invasion) and can therefore not be regarded as pure histopathological grading systems.

Tumour budding, a histomorphological parameter that describes the quantity of diffusely infiltrative growth of a given carcinoma, has been identified by us and others as a strong prognostic factor in a variety of SCC entities [12–20]. Furthermore, novel grading approaches of major clinical significance, which paired tumour budding with cell nest size, a second parameter that qualitatively measures a cancer's capability of cellular dissociation, have been established for oral, pulmonary, and oesophageal SCC [12–14]. While high tumour budding activity was recently reported to be associated with a highly increased risk of cervical SCC relapse in early-stage cervical cancer [21], the prognostic value of cell nest size remains unclear. In addition, the novel grading approaches combining tumour budding and cell nest size have not yet been investigated regarding their suitability for the purpose of cervical SCC grading.

To clarify these open questions, we evaluated tumour budding and cell nest size, as well as other histomorphological features (keratinization, nuclear size, mitotic activity, stromal content), in two comparatively large and completely independent cervical SCC cohorts (test cohort: 125 patients; validation cohort: 122 patients) from two German university hospitals. All histomorphological factors were correlated with staging and clinical outcome parameters (overall, disease-free [DFS], and disease-specific survival [DSS]) and assessed regarding their suitability for prognostic patient stratification.

Patients and methods

Test cohort and validation cohort

We investigated two distinct cohorts of primary resected SCCs of the uterine cervix. Cohort 1 (test cohort) comprised 125 resection specimens of cervical SCC, which were resected between 1991 and 2016 at Klinikum Rechts der Isar of the Technical University of Munich, Germany. The mean age of patients in cohort 1 was 47.8 years (range: 24–87 years). About two third of the carcinomas were confined to the uterus ($pT1b_{1/2}$: 83/125; 67%), one third (pT2/3/4: 42/125; 33%) showed more advanced tumour stages. Thirty-five cases harbored lymph node metastases (28%). At the time of resection, distant metastases were present in three patients (2%). Mean follow-up time for living patients at the end of the follow-up period was 89.3 months. Thirty-seven patients died during the observation period. Thirty of the 37 deaths were tumour specific. Thirty-three patients relapsed during follow up. The detailed distribution of clinicopathological factors in the test cohort is provided in Table 1 and supplementary material, Table S1.

Cohort 2 (validation cohort) included 122 cervical SCC resection specimens, which were resected between 2000 and 2016 at the University Hospital Erlangen, Germany. Mean patient age was 44.5 years (range: 24–84 years). Eighty-six/122 cervical SCCs were pT1 tumours (70.5%; pT2 29.5%), nodal metastases were present in 26/122 (21%) cases. Mean follow-up time was 106.8 months. During the observation period 17 patients died. All deaths were tumour specific. Twenty-two patients relapsed during follow up.

In both cohorts, tumours from patients who received neoadjuvant treatment as well as pT1a carcinomas were excluded. Approval for this study was obtained from the Ethics Committee of the Technical University of Munich (331/17 S).

Histological evaluation

Full block haematoxylin and eosin (H&E) stained cervical SCC slides of every case from each cohort were evaluated by at least one of a total of three

Table 1. Association of tumour budding, cell nest size, grade and clinicopathological factors with OS, DSS, and DFS (univariate) in the test cohort

	Overall	Events (OS)	Mean overall survival	*P* value	Events (DSS)	Mean DSS	*P* value	Events (DFS)	Mean DFS	*P* value
Age										
≤ Median	72	12	130.65	*0.113*	11	132.29	*0.003*	12	129.38	*0.002*
> Median	53	25	94.74		19	101.23		21	95.96	
pT										
1	83	12	131.25	*<0.001*	10	134.23	*<0.001*	12	130.66	*<0.001*
2	32	16	100.2		11	105.1		12	100.52	
3/4	10	9	46.34		9	46.34		9	39.47	
pN										
0	90	22	123.47	*0.008*	18	125.44	*0.043*	19	*122.96*	*0.017*
1	35	15	97,00		12	103.93		14	*95.49*	
pM										
0	122	34	118.29	*<0.001*	27	121.81	*<0.001*	30	117.76	*<0.001*
1	3	3	36.47		3	36.47		3	28.7	
UICC Stage										
1	67	8	134.61	*<0.001*	7	136.49	*<0.001*	8	134.15	*<0.001*
2	14	6	118.26		3	121.75		3	121.39	
3	40	19	95.01		16	100.57		18	92.13	
4	4	4	28.63		4	28.63		4	22.33	
Histological subtype										
Keratinizing	52	16	112.02	*0.013*	13	117.86	*0.048*	15	113.17	*0.029*
No keratinization	58	14	126.06		11	126.93		11	125,00	
Basaloid	15	7	72.86		6	77.45		7	52.67	
Nuclear size										
Small	16	4	124.89	*0.598*	3	125.67	*0.448*	4	118.29	*0.568*
Medium	54	15	119.84		11	123.22		12	119.93	
Large	55	18	109.46		16	113.51		17	110.08	
WHO Grade										
G1	3	0	–	*0.113*	0	–	*0.072*	0	–	*0.026*
G2	43	9	125.37		6	131.65		6	131.77	
G3	79	28	109.14		24	111.29		27	105.08	
Budding (10 HPF)										
0	47	3	144.06	*<0.001*	1	147.28	*<0.001*	1	147.28	*<0.001*
≤ 15	49	18	108.76		14	112.11		17	103.39	
>15	29	16	83.74		15	87.7		15	81.96	
Cell nest size										
Single cells	48	23	90.14	*<0.001*	21	95.32	*<0.001*	22	90.05	*<0.001*
2–4 cells	24	9	111.91		7	112.07		9	100.96	
5–15 cells	16	2	135.37		2	135.37		2	134.99	
>15 cells	37	3	144.02		0	–		0	–	
Grade (new)										
1 (score 2–3)	51	4	143.35	*<0.001*	1	147.5	*<0.001*	1	147.5	*<0.001*
2 (score 4–5)	22	6	124.23		4	124.42		6	112.34	
3 (score 6–7)	52	27	86.11		25	90.46		26	84.48	
Stromal content										
Scarce	23	2	142.53	*0.077*	1	144.64	*0.109*	1	144.67	*0.072*
Moderate	40	14	108.14		12	110.85		12	109.24	
Marked	54	19	109.08		15	114.45		18	107.24	
Extensive	8	2	112.09		2	119.39		2	110.31	
Mitotic count / 10 HPF										
<10	11	5	93.7	*0.134*	5	93.7	*0.307*	5	89.31	*0.269*
10–20	22	3	139.4		3	139.4		3	139,00	
21–30	32	12	108.93		8	116.32		9	110.89	
31–40	22	6	117.32		5	118.43		5	118.1	
>40	37	11	111.77		9	116.54		11	115.08	
L										
0	66	7	143.48	*<0.001*	3	145.83	*<0.001*	4	143.66	*<0.001*
1	59	30	83.62		27	88.66		29	82.32	
Pn										
0	96	19	130.48	*<0.00*	12	134.91	*<0.001*	12	132.09	*<0.001*
1	29	18	65,00		18	65,00		19	52.36	

Ultimate numbers refer to the number of events of a specific survival parameter or specific subgroup.

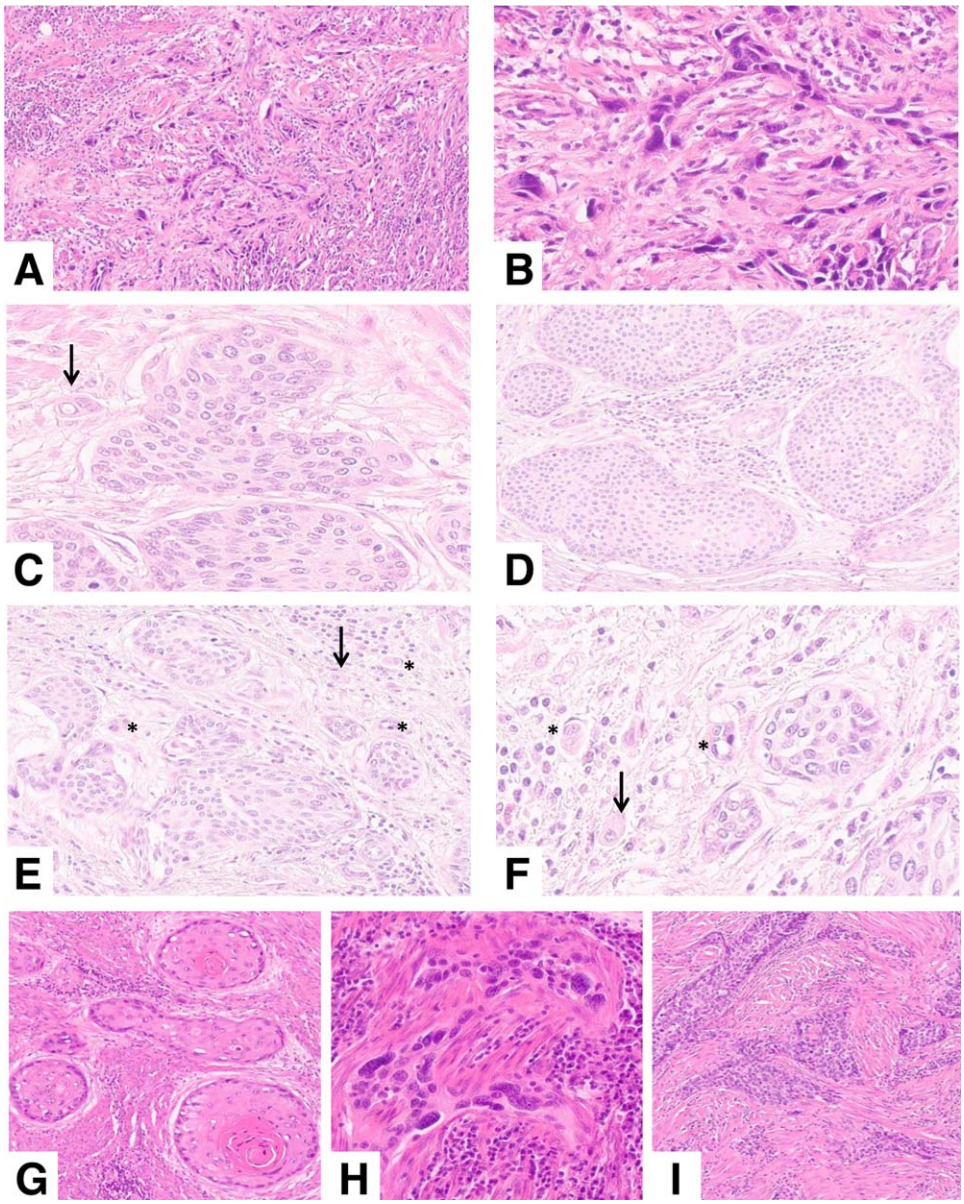

Figure 1. Histomorphological characteristics of SCC of the uterine cervix. (A)–(C) Tumour budding: overview (A) and details (B) of a cervical SCC with high tumour budding activity, indicated by the branching of numerous small tumour clusters of <5 cells into the surrounding tissue; (C) Details of low tumour budding activity with a single tumour cluster <5 cells (arrow) budding into the tumour stroma. (D)–(F) Cell nest size: intermediate magnification (D) of large and medium sized cell nests. Details (E, F) of small cell nest size (stars) and single cell invasion (arrows). (G)–(I) Histological subtype, nuclear size, and stromal content: representative images of a keratinizing cervical SCC (G), large tumour cell nuclei (H) and high stromal content (I). [Correction added on 28 Mar 2018, after first online publication: Error in figure 1 labelling, F, G and H to G, H and I]

pathologists (MJ, MB test cohort; JS, MJ, MB validation cohort), who were blinded to clinicopathological data and follow-up. Furthermore, 20 randomly selected cases from the validation cohort were evaluated by both MJ and MB consecutively in order to investigate interobserver reproducibility. All cervical SCCs were graded (G1–G3) in accordance with the WHO classification of female reproductive organs and divided into keratinizing as well as nonkeratinizing neoplasms (including basaloid carcinomas) [8].

Dissociation of small tumour complexes consisting of <5 neoplastic cells that 'bud' into the peritumoural stroma was defined as tumour budding. In analogy to previous studies [12–14], tumour budding activity was evaluated throughout the whole tumour area and scored within the tumour area showing the

highest budding activity. Tumour budding activity was classified as low and high if 1–14 or ≥15 budding foci were detected in 10 high power fields (HPFs), respectively (Figure 1).

Cell nest size was evaluated to assess the minimum size but not the quantity of invasive tumour cells clusters. Following the algorithm from previous studies [12–14], cell nests comprising >15 and 5–15 tumour cells were classified as large and intermediate, respectively. Cell nests consisting of 2–4 tumour cells were scored as small, while the term single cell invasion was reserved for tumours harboring singular, discohesive tumour cells without nested architecture. For every carcinoma, the smallest identifiable cell nest size was reported (Figure 1). For example, in a cervical SCC predominantly composed of large cell nests with only a single small cell nest, the respective cell nest size was classified as small.

Four categories were created to determine the quantity of tumour stroma: very low (≤10% of the whole tumour area), low (>10–25%), moderate (>25–50%), and high (>50%). Nuclear diameter was assessed by comparing the nuclear size of tumour cells with the nuclear diameter of tumour associated lymphocytes (small nuclei = ≤4 lymphocytes, intermediate nuclei = 4 lymphocytes, large nuclei >4 lymphocytes), reporting the largest identifiable nuclear size within a given carcinoma. Mitotic activity was evaluated in 10 HPFs within the tumour area showing the highest mitotic rate (Figure 1).

Composition of the novel grading system

As previously reported for pulmonary, oesophageal, and oral SCC [12–14], we established a novel grading system based on budding activity and the smallest identifiable cell nest size, attributing a score to both budding activity (1–3 points) and cell nest size (1–4 points). Tumours without budding activity had a score of 1, tumours with low budding activity (<15 buds per 10 HPF) received a score of 2 and tumours with high budding frequency (≥15 buds per 10 HPF) had a score of 3. Likewise, large cell nests (>15 cells) received 1 point, while intermediate (5–15 cells) and small (2–4 cells) nests were scored with 2 and 3 points, respectively. Single cells were scored with 4 points. As done previously, the sum of both scores resulted in a final grading score ranging from 2 to 7. In the novel cervical SCC grading score, which is identical to the novel grading proposed for oesophageal SCC [13] and will be referred to as 'Grade new' in the following sections of the manuscript, G1 (well-differentiated) tumours had a score ranging from 2 to 3, G2 (moderately differentiated) tumours had a score ranging from 4 to 5 and G3 (poorly differentiated) tumours had a score ranging from 6 to 7. The composition of the novel grading system is again summarized in Table 2.

Statistics

Statistical analysis was performed using the Statistic Package for Social Sciences 23.0 statistical software (SPSS, Chicago, IL, USA). Correlations of 'raw' morphological parameters with each other were calculated with Spearman's rank order correlation. Associations of grouped morphological characteristics with clinicopathological parameters were calculated with χ^2 (chi square) test as well as χ^2 test for trends and Fisher's exact test. Survival probabilities were plotted with the Kaplan–Meier method, a log-rank test was used to probe for the significance of differences in survival probabilities. Multivariate survival analysis was performed with the Cox proportional hazard model. P values ≤0.05 were considered significant. Analysis of interobserver variance was performed using the Cohens–Kappa algorithm. As only planned hypothesis testing was performed, no corrections for multiple testing were necessary in this study [22].

Results

Distribution of histomorphological features

In the test cohort, there was a comparable percentage of keratinizing (52/125, 41.6%) and nonkeratinizing (58/125, 46.4%) tumours, with 12% (15/125) of tumours classified as basaloid. Budding activity (per 10 HPF) was almost equally distributed with 47 out of 125 (37.6%) cases without any activity, 49 cases

Table 2. Algorithm for determining tumour grade from tumour budding activity and cell nest size scores in cervical SCC

Grading proposal for SCC of the uterine cervix	
Tumour budding activity/10 HPF	
No budding	1
< 15 budding foci	2
≥ 15 budding foci	3
Smallest cell nest size within the tumour core	
> 15 cells	1
5–15 cells	2
2–4 cells	3
Single cell invasion	4
Tumour grading	**Total score**
Well differentiated (G1)	2–3
Moderately differentiated (G2)	4–5
Poorly differentiated (G3)	6–7

with low budding activity (39.2%), and a slightly lower number of cases (29/125, 23.2%) with high tumour budding scores. The evaluation of the minimal cell nest size revealed single cell invasion in 48/125 cases (38.4%), 24 cases (19.2%) showed small cell nests (composed of 2–4 cells), 16 cases (12.8%) harbored intermediate nests (5–15 cells), and 37 (29.6%) out of 125 cases showed large cell nests (>15 cells). The nuclei were small in 16/125 (12.8%) of cases, while intermediate (54/125, 43.2%) and large (55/125, 44%) nuclei were observed in comparable frequency. A scarce stromal compartment was evident in 23/125 (18.4%) of cases, while 40 (32%) and 54 (43.2%) of cases had moderate and marked stromal components, respectively. An extensive stromal component was rarely observed (8/125, 6.4%) (supplementary material, Table S1). Some parameters had a slightly different distribution in the validation cohort ($n = 122$) with a higher number of keratinizing cases (63.1%) and cases with large nuclei (55.7%). In addition, tumours of this cohort tended towards lower budding activity and larger nest sizes (supplementary material, Table S2).

Correlation of morphological characteristics with each other

In the test cohort, high budding activity and small nest size were strongly correlated. Both factors were positively associated with nuclear size and stromal content, however, to a considerably lesser degree. No association of any morphological factors with mitotic activity was seen (supplementary material, Table S3). The association of budding and nest size was also found in the validation cohort but the weaker associations of both factors with the other morphological factors could not be recapitulated (supplementary material, Table S4).

Correlation of morphological and clinicopathological characteristics

In the test cohort, tumour budding activity as well as cell nest size were strongly and significantly associated with tumour stage (pT, $p < 0.001$, respectively) and Union for International Cancer Control/Tumour Node Metastasis (UICC/TNM) [7] stage ($p < 0.001$, respectively) but not with the occurrence of nodal (pN) and distant metastases (pM). Furthermore, smaller cell nest sizes and higher budding activity were associated with lymphatic invasion (L, $p < 0.001$ for both comparisons) and perineural spread (Pn, $p < 0.001$ and $p = 0.002$). Larger nuclear size and higher stromal content were associated with lymphatic and perineural spread but not with any other clinicopathological data. The correlations of all morphological and clinicopathological characteristics in the test cohort are given in supplementary material, Table S1.

In the validation cohort, we were able to confirm the association of tumour budding and cell nest size with pT and UICC/TNM stage as well as with perineural and lymphatic invasion. Furthermore, an additional association of small nest size and higher budding activity with nodal positivity was evident ($p = 0.003$ and $p = 0.001$, respectively). The correlations of all morphological and clinicopathological characteristics in the test cohort are given in supplementary material, Table S2.

Correlation of morphological features with survival

As depicted in Table 1, tumour budding activity was significantly associated with shortened overall survival (OS), DFS, and DSS in the test cohort ($p < 0.001$, respectively). For example, while tumours without budding showed a mean OS of 144.1 months,

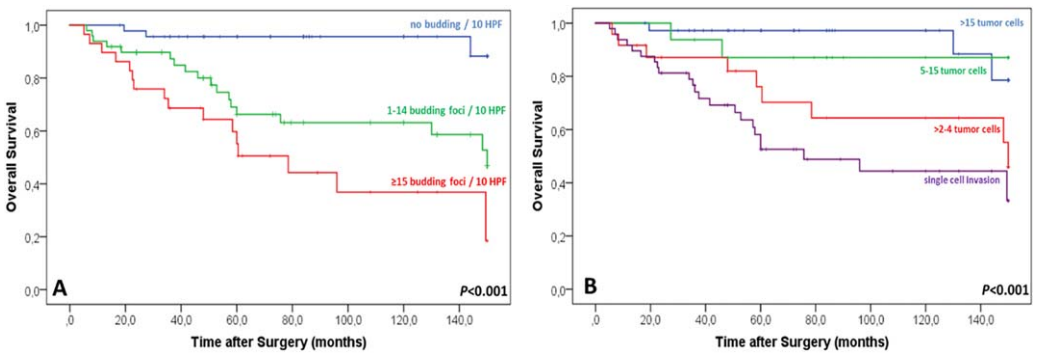

Figure 2. Impact of tumour budding activity (per 10 HPFs) (A) and cell nest size (B) on overall survival in the test cohort. *P* values were calculated using a log-rank test.

OS gradually decreased to 108.8 months for tumours with intermediate and to 83.7 months for tumours with high budding activity (Table 1 and Figure 2A). Likewise, with decreasing cell nest size, OS, DSS, and DFS also strongly decreased ($p < 0.001$, respectively). Patients whose tumours were composed solely of large cell nests had an OS of 144 months, while OS in patients with intermediate and small cell nests decreased to 135.4 and 111.9 months, respectively. Single cell invasion was associated with the worst OS of 90.1 months ($p < 0.001$) (Table 1 and Figure 2B). A weaker survival association was evident for the different histological subtypes of cervical SCC (e.g., for DFS: $p = 0.029$). Basaloid cervical SCCs had the shortest survival time (DFS: 52.7 months), while keratinizing tumours ranged in between (DFS: 113.2 months) and nonkeratinizing tumours had the best prognosis (DFS: 125 months). Other pure histomorphological factors such as stromal content, nuclear size, or mitotic activity showed no associated with survival in the test cohort. In the test cohort, conventional WHO-based grading was not prognostic for OS and DSS, but showed a weaker prognostic association with DFS. Table 1 depicts the associations of all histomorphological parameters and of all staging parameters (e.g., pT, pN, etc.) with patient survival.

In the validation cohort, we were able to validate the prognostic impact of both high budding activity and small cell nest size as these factors were each strongly associated with reduced patient survival (e.g., for OS: $p = 0.001$ for both parameters). Again, the other pure histomorphological factors such as mitotic count, nuclear size as well as stromal content showed no association with patient survival. Conventional WHO-based grading showed no prognostic impact. However, since follow-up duration was shorter in the validation cohort, fewer events were observed. Therefore, a significant impact of pT, pN, L, and UICC/TNM stage on OS and DFS was not evident, although the raw survival data showed some trends towards the expected directions.

Prognostic impact of the novel grading approach

In the test cohort, the novel grade ('Grade new') based on tumour budding and cell nest size had a significant impact on overall, disease-specific, and DFS (all comparisons $p < 0.001$) (Table 1, Figure 3A–C). Overall survival dropped from 143.4 months for G1 tumours to 124.2 months for G2 neoplasms and 86.1 months for G3 carcinomas. A significant impact of the new grade on OS and DFS was confirmed in the validation cohort ($p = 0.001$ and $p = 0.013$, respectively) (supplementary material, Figure S1A and S1B). In this cohort, OS for G1 tumours was even longer with 195.7 months, while patients with G2 tumours had an OS of 176.2 months, which was reduced to 116.1 months for G3 neoplasms. Furthermore, lower tumour differentiation (G2/G3)

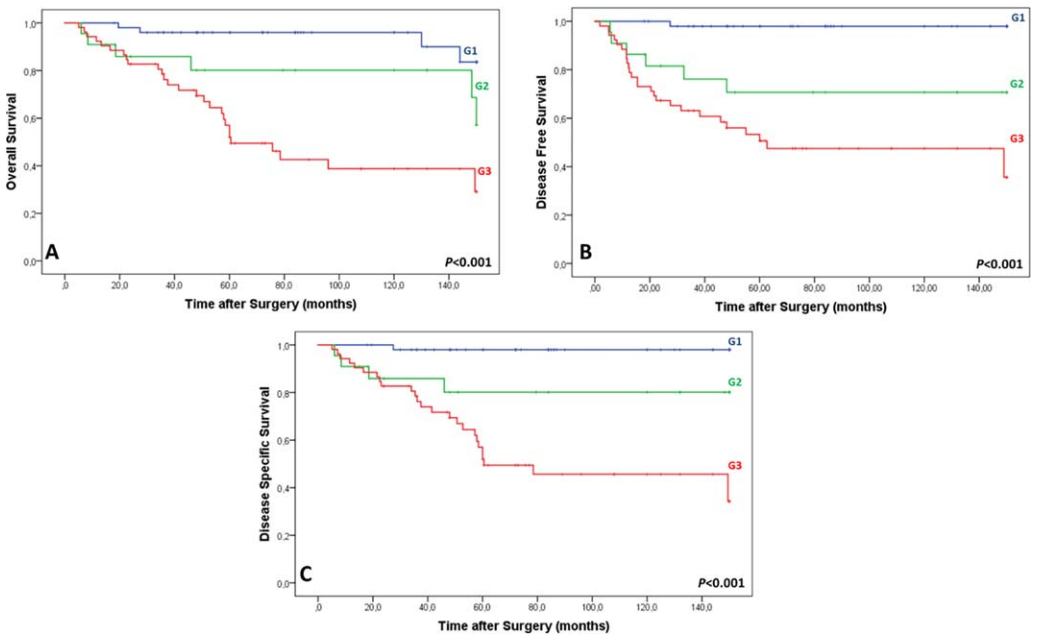

Figure 3. Association of the novel cervical SCC tumour grade with overall (A), disease-specific (B) and disease-free (C) survival in the test cohort. *P* values were calculated with a log-rank test.

Table 3. Multivariate analysis for overall survival, including the novel grading algorithm, age and UICC/TNM stage in the test cohort

		HR (OS)	Lower CI (95%)	Upper CI (95%)	*P* value
Age	*per year*	1.047	1.021	1.073	*<0.001*
UICC/TNM stage	*1*	1.00			*0.001*
	2	2.45	0.81	7.40	
	3	3.89	1.66	9.16	
	4	12.42	3.51	43.92	
Grade (new)	*G1*	1.00			*0.010*
	G2	2.29	0.61	8.43	
	G3	5.14	1.68	15.68	

HR, hazard ratio; CI, confidence interval.

according to the novel grade was significantly associated with higher pT- and UICC/TNM stage, lymphovascular invasion, and perineural spread (supplementary material, Table S1) in the test cohort. The association with pT-stage, lymphovascular invasion, and perineural spread was confirmed in the validation cohort. In addition, lower tumour differentiation according to the new grade (G2/G3) was significantly linked to nodal metastases (supplementary material, Table S2) in the validation cohort.

Multivariate survival analyses in the test cohort revealed a very strong significant stage and age-independent prognostic impact of the novel grading system for OS (Table 3), DSS (not shown), and DFS (supplementary material, Table S5). With G1 as a reference, OS hazard ratio (HR) for G2 tumours was 2.3 and increased to 5.1 for G3 tumours ($p = 0.010$, Table 3). When this analysis was repeated in the validation cohort, again a stage and age-independent significant impact on survival was observed for both OS (supplementary material, Table S6) and DFS (supplementary material, Table S7). For overall survival in the validation cohort, the HR for G2 was 3.0 and for G3 7.2 ($p = 0.012$) when G1 tumours were taken as a reference.

An exploratory analysis (20 cases, validation cohort) of the interobserver reproducibility revealed a high reproducibility of the novel grading system for cervical SCC between two independent pathologists (Kappa–Cohens value: 0.857).

Discussion

In this study, we investigated tumour budding and cell nest size, histopathological parameters which formed the basis for novel, highly prognostic grading approaches for pulmonary [14], oral [12], and oesophageal SCCs [13], regarding their potential to form the basis of a prognostically relevant histopathological grading system applicable to SCCs of the uterine cervix. We did this by examining primary resected cervical SCCs from two completely independent cohorts obtained from two German university hospitals. In analogy to pulmonary [14], oral [12], and oesophageal SCCs [13], tumour budding activity and cell nest size turned out to be the most significant prognostic histomorphological factors in both cohorts, paving the way for a combined, three-tiered, highly prognostic grading system based on both parameters.

Conventional histopathological grading of cervical SCC according to the WHO classification of tumours of the female reproductive organs [8] is conducted by considering a tumour's degree of keratinization, nuclear size, and mitotic activity. However, cervical SCC grading based on these parameters has been considered to be of questionable prognostic value, not only by the WHO itself [8], but also by the few studies that investigated several cervical SCC grading approaches [9]. The most important study by Zaino *et al* investigated the prognostic value of classical histomorphological parameters (e.g., mitotic count, keratinization, nuclear pleomorphism) as well as several grading systems that primarily incorporate these parameters and found their prognostic relevance to be highly limited [9]. These previous reports, that the prognostic value of the histomorphological factors keratinization, nuclear size, and mitotic count is, at best, marginal, are in accordance with the findings from our study, in which these parameters, as well as the WHO-based grading, failed to show significant prognostic discrimination in both of our cohorts. Competing cervical SCC grading schemes, like the efforts from Stendahl *et al* [10] or Crissman *et al* [11], at least partially based their approaches on additional factors such as invasion depth or vascular invasion, which represent grading-independent parameters that are usually reported separately in pathological classifications, as they do not provide information

regarding the histomorphological architecture of a given cancer. Therefore, these approaches are not suitable for a pure histopathological routine grading algorithm, as malignant tumours have to be graded only under consideration of architectural or cytomorphological criteria [23,24]. Including grading-independent factors (such as vascular invasion) in a histopathological grading algorithm would result in (hidden) double reporting of such factors in the TNM classification accompanying most pathological reports.

While the effect of cell nest size on survival has not yet been investigated in cervical SCC, tumour budding on its own has recently been linked with a dismal prognosis in a comparatively large series of early stage cervical cancers [21], a finding that is in line with the conclusions of various other studies, that investigated this parameter in SCCs of other anatomic sites [12–15,18–20,25,26].

Although a certain relation between tumour budding and cell nest size does certainly exist (as nicely exemplified by their high correlation), each of the two parameters on its own contains a slightly different set of information regarding a carcinoma's ability for cellular dissociation. While cell nest size describes the maximal degree of cellular discohesion from a qualitative point of view, tumour budding is a quantitative parameter that measures the numeric amount of dissociative growth within a given cancer. Therefore, we are convinced that the combination of both factors is more likely to accurately portray the malignant potential of a SCC than one these parameters alone. Furthermore, our initial data regarding the interobserver reproducibility of the novel grading system for cervical SCC show a high level of interobserver agreement underlined by a Cohens–Kappa value of 0.857, consistent with previous interobserver analyses of the novel grade performed for oral SCC [12].

Our data show the almost perfect transferability of the tumour budding/cell nest size-based grading approach to cervical SCC and can be regarded as yet another cross-organ validation of this grading system. This underlines the assumption that tumour budding and cell nest size have the potential to constitute the pillars of a highly prognostic grading system that is most likely applicable to all SCCs. Worthy of note, high budding activity and small cell nest size on their own as well as the novel grade were additionally associated with lymphatic invasion (test and validation cohort) and nodal metastases (validation cohort), potentially rendering these parameters, if validated by further studies, to be helpful for the prediction of lymphatic invasion and/or nodal metastases.

Our retrospective study is limited by the fact that our analyses were confined to resection specimens, so that the transferability of this novel grading to biopsy specimens, which is the subject of a current cross-organ study at our institution, remains yet unclear.

Taken together, our study on the clinical impact of several histomorphological parameters in two independent cervical SCC cohorts introduces a novel, highly prognostic histopathological grading scheme for SCCs of the uterine cervix, which combines the strongest histopathological factors and is easy to implement. Furthermore, as comparable tumour budding/cell nest size-based grading approaches have been proposed for SCCs of a variety of other anatomic sites, this study yet again underlines the potential of these parameters to serve as the basis for a common grading system that might be generally applicable to all SCCs in a pan-cancer fashion.

Author contributions statement

MJ and WW mainly wrote the manuscript with help from JS, KS, KB, AN, AH, MWB, and MCK. MJ, JS, and MB performed the histological analyses. WW and MJ performed statistical analyses with assistance from AMS, ArW, KS, FB, and AW. AS, AH, MK, MCK, MJ, GM, JS, and MCK obtained clinicopathological data.

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SUPPLEMENTARY MATERIAL ONLINE

Figure S1. Association of the novel cervical SCC tumour grade with overall (A) and DFS (B) in the validation cohort. *P* values were calculated with a log-rank test

Table S1. Association of morphological and clinicopathological factors in the test cohort. *P* values were calculated with χ^2 test, χ^2 test for trends and Fisher's exact test, respectively

Table S2. Association of morphological and clinicopathological factors in the validation cohort. *P* values were calculated with χ^2 test, χ^2 test for trends and Fisher's exact test, respectively

Table S3. Rank-order correlation of morphological factors in the test cohort

Table S4. Rank-order correlation of morphological factors in the validation cohort

Table S5. Multivariate DFS analysis including UICC/TNM stage, age, and the novel tumour grading algorithm in the test cohort

Table S6. Multivariate overall survival analysis including UICC/TNM stage, age, and the novel tumour grading algorithm in the validation cohort

Table S7. Multivariate DFS analysis including UICC/TNM stage, age, and the novel tumour grading algorithm in the validation cohort