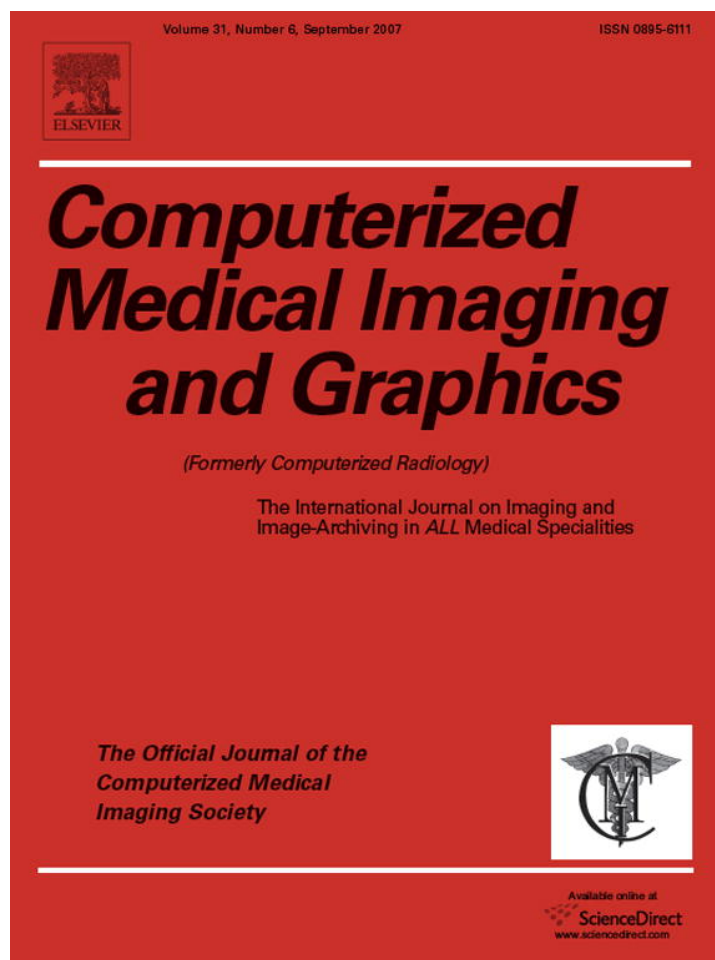


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Evaluation of the invasion front pattern of squamous cell cervical carcinoma by measuring classical and discrete compactness

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Abstract

The invasion front pattern of squamous cell carcinoma (SCC) is a conspicuous histological phenomenon, which is assessed without precise criteria. The current study was performed to introduce the classical (C_C) and discrete compactness (C_D) as new morphometric parameters for quantification of this pattern. A retrospective analysis of 76 surgically treated patients with cervical carcinoma was conducted and the pattern of invasion was qualitatively classified as *closed*, *finger-like* or *diffuse*, respectively, by two pathologists. After digitization of the histological slides with a field of view of $10.4\text{ mm} \times 8.3\text{ mm}$, tumor areas were labeled and C_C and C_D were computed based on the drawings (binary images). Additionally, intraindividual variation of compactness was evaluated for 12 selected tumors. The qualitative pattern assessment by the pathologists was moderately reproducible with an interobserver agreement of 72% and a κ coefficient of 0.44. The values of C_C and C_D referring to the invasion front patterns assigned by both pathologists were significantly different between the three classified groups ($p \leq 0.01$ and $p \leq 0.0001$), so that, both theoretically and in practice, compactness regards the same morphological feature. In due consideration of the analysis of the area under the ROC (receiver operating characteristic) curves and the variation coefficient of different tumor regions, C_D is more suitable for practical use than C_C . Tumors with a microscopic invasion into the parametria and with lymph-vascular space invasion were found to have a lower value of C_D , which indicates a more *diffuse* pattern of invasion ($p = 0.028$ and $p = 0.033$). We conclude that the discrete compactness C_D is a new and reproducible parameter for a computer assisted quantification of the invasion front pattern and, thus, defines a further phenotypic feature of SCC of the uterine cervix.

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Keywords: Squamous cell carcinoma; Invasion front pattern; Cervical cancer; Compactness; Morphometry

1. Introduction

Different patterns of tumor invasion are a well-known histological feature of squamous cell carcinomas resulting from the interaction between the tumor cells and their environment of the individual patient. However, the molecular mechanisms of tumor invasion involving cell–cell and cell–matrix adhesion, degradation of extracellular matrix and cell migration are incompletely understood at present [1].

Several investigators found relationships between the pattern of invasion and other morphological features as well as clinical outcome, but their results are inconsistent. Tumors with a diffuse tumor-stromal border were more likely to have lymph-vascular space invasion [2], a larger maximum depth of cervical invasion [3] and a higher probability of distant metastases within 5 years [4]. The prognosis for patients whose tumor exhibited a closed invasion front pattern has been reported to be better than those with diffuse invasion [4–6]. However, these findings have not been substantiated by others [2,3,7–10].

Similar studies have been performed with squamous cell carcinomas of various other genital and extragenital locations such as vulva [11], penis [12], head and neck or the oropharynx [13,14]. A multivariate analysis of 77 patients with carcinoma of the oropharynx revealed that the pattern of invasion

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was the only histologic factor that was predictive of survival [14].

There is a wide variation in histopathologic definitions, which is probably the major reason for both the lack of reproducibility and their limited predictive potential [15]. Another cause of inconsistencies could have resulted from the subjectivity of the pathologist's assessment; therefore, we developed a means of an objective quantitative characterization of the tumor invasion front. In principle, there are various possibilities for quantification, e.g. descriptions of tumor boundary lengths or areas (size, number or diameter of tumor cell clusters), or methods using differential-geometric properties such as local curvature or the fractal dimension. What appears most feasible and illustrative to us is a geometric shape description referred to as *compactness*. This is a dimensionless intrinsic image object property defined as quotient of the squared contour boundary length and the enclosed area. A circular area provides the minimum compactness of 4π . A specific way of measuring shape compactness of digital images makes use of the pixel contact perimeter introduced by Bribiesca and the result is called *discrete compactness* [16].

In a previously published analysis of our group, an image processing chain for three-dimensional (3D) reconstruction of selected parts of the tumor invasion front based on histological serial sections was introduced [17]. The 3D discrete compactness, which relies to internal voxel contact surfaces, was used to give a quantitative characterization of the 3D reconstructed invasion fronts of 13 specimens of cervical carcinoma. However, such analyses are very laborious and not applicable for routine use.

Therefore, the objective of the present study was to verify the feasibility of a computer assisted assessment of invasion front pattern by computation of the classical and discrete compactness for the two-dimensional (2D) case based on routine haematoxylin- and eosin-stained (H&E) sections and to evaluate their relationship to other histopathologic parameters.

2. Materials and methods

2.1. Patients

Between January 1997 and December 2002, 205 women with cervical cancer FIGO stages IB–IIB underwent a radical hysterectomy with pelvic and possibly paraaortic lymphadenectomy as the primary treatment at the Department of Obstetrics and Gynecology, Leipzig University.

The present study includes 76 patients with squamous cell carcinomas and a minimal tumor area of approximately $1.5\text{ cm} \times 1.5\text{ cm}$ at the slide. This limitation was appointed to eliminate possible size artifacts in the computation of compactness, so that smaller samples were not considered ($n=21$). Remaining patients had to be excluded from the analysis because of other histological types ($n=38$), the available tumor tissue was either not representative or was too sparse for the evaluation of the invasion front due to previous cervical conization ($n=45$), or the paraffin-embedded tissue blocks and the slides were no longer available from the archive ($n=25$).

Five patients with tumors $>5\text{ cm}$ in diameter were pretreated with three courses of neoadjuvant chemotherapy with 75 mg cisplatin/ m^2 every 3 weeks to reduce the tumor volume.

2.2. Histopathologic evaluation

H&E-stained sections were evaluated by one gynecologic pathologist (L.-C.H.). Tumors were classified according to the WHO and graded either as well, moderately, or poorly differentiated [18]. In cases treated with neoadjuvant chemotherapy no grading was stated (GX). The presence or absence of direct extension into the parametrium or vaginal cuff, vessel invasion, and the presence or absence of metastases to pelvic and possibly paraaortic lymph nodes were recorded. The peritumoral inflammatory response was graded semiquantitatively as none, weak, moderate and strong, according to the density of the lymphocytic stromal infiltration. For statistical analysis tumors were summarized in two combined categories due to small numbers in each group. Depth of stroma invasion was assessed according to the thickness of the deepest invasive tumor in relation to the thickness of the cervical wall of each patient. For analysis, the depth was condensed into three levels as follows: $\leq 1/3$, $>1/3$ but $\leq 2/3$, and $>2/3$.

Before evaluation of the invasion front pattern, all sections were screened microscopically by the pathologist and the section with the most prominent portion of the tumor was selected for this study. Pattern of invasion was classified as *closed*, *finger-like* or *diffuse* (Fig. 1) by the pathologist in the region of interest (ROI) determined by the microscope parameters (see below). A second independent pathologist assessed all sections again in a blinded fashion to evaluate the interobserver variation (A.S.).

2.3. Quantification of invasion front pattern—computation of compactness

The digitization of the selected sections was carried out manually using the AxioVision 3.1 software directly reading from a digital $2/3''$ one chip CCD-camera (AxioCam MRC[®]) mounted on top of a transmitted light microscope (Axioskop 2 plus[®]) equipped with a $1.25\times$ objective (Plan-NEOFLUAR) and a $0.63\times$ video adapter (all mentioned products made by Carl Zeiss, Jena, Germany). The raw digitization area is 1300×1030 pixels corresponding to a field of view of $10.45\text{ mm} \times 8.28\text{ mm} = 86.5\text{ mm}^2$ (=ROI) at a nominal squared pixel size of $8.04\text{ }\mu\text{m} \times 8.04\text{ }\mu\text{m}$ (Fig. 2).

The section was placed in such position that the invasion front runs across the image in the landscape format and the tumor structures take 50–70% of the whole image area. The image editing software Corel PHOTO-PAINT 11 was used to delineate the region of tumor (manual segmentation) and by this to define a binary tumor template image. Two observers neither provided with histopathologic nor other diagnostic information carried out this step. As main visual criterion within the H&E-stained sections, specifically saturated regions were carefully delineated where more haematoxylin was aggregated due to an enlarged cell nuclei density, whereas the latter occurs in the tumor tissue exhibiting enlarged cell nuclei because of strong

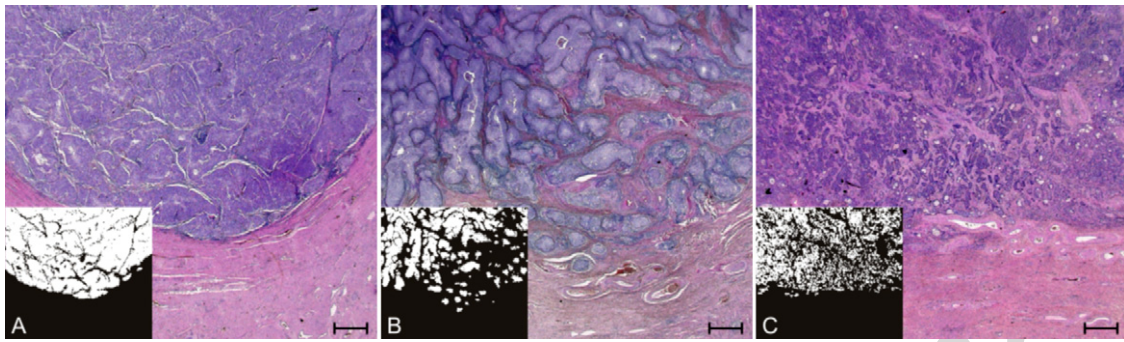


Fig. 1. Typical invasion front patterns with values of classical and discrete compactness (haematoxylin/eosin; scale bar = 500 μm). The inset shows the binary image for the computation: (A) *Closed* ($C_C = 139$; $C_D = 0.9978$): the interface between advancing tumor and stroma is well defined and smooth regular. (B) *Finger-like* ($C_C = 5\,902$; $C_D = 0.9661$): the invasion front is characterized by an irregular interface at the advancing edge of the tumor and a formation of islets of carcinoma cells. (C) *Diffuse* ($C_C = 19\,007$; $C_D = 0.9087$): Tumor cells invade the cervical stroma as nests and strands or as single cells and the interface is highly irregular.

aneuploidy in tumor cells. While basically this segmentation step could be automated, e.g. using a supervised trained color classifier, the reason for accomplishing it manually was to focus this work entirely on the appropriateness of the compactness as morphometric measure, whilst ensuring reference segmentation quality with a minimum of false negatives or false positives for the tumor tissue segmentation under the given spatial resolution of the digital images. Concerning possible incorrect tumor region segmentations, e.g. due to failures of some automatic segmentation procedure will be subject of forthcoming work.

Principally, there are two ways to implement the computation of shape compactness from digital images using on the one hand the external perimeter of pixels bordering the contour and the area of involved pixels (Fig. 3, green), or on the other hand the internal pixel contact perimeter (Fig. 3, red). Accordingly, digital **classical compactness** C_C is simply defined as $(L_E)^2/n$ in which L_E denotes the contour perimeter of a shape of n pixels. The contour perimeter was obtained using a Euclidean distance based approach [19]. This direct compactness implementation, however, does lack of sufficient

robustness, i.e. boundary length enlargements due to noise virtually could lead to the same compactness like real boundary shape changes (Fig. 3; note the changes due to the modification of the position of two pixels). **Discrete compactness** C_D , which is more robust, relies to internal pixel contact perimeter and is defined as $C_D = L_C - L_{C_{\min}} / L_{C_{\max}} - L_{C_{\min}}$ [16]. Herein, L_C denotes the number of sharing edges within a shape consisting of n pixels, whereas correspondingly $L_{C_{\max}} = 2(n - \sqrt{n})$ is the theoretical maximum of sharing edges achieved with a squared object also consisting of n isotropic pixels. Contrasting to Bri-biesca, we define $L_{C_{\min}} = 0$ in order to consistently allow for objects consisting of neighboring pixels even without sharing edges, so that $C_{D_{\max}} = 1$ for a square, and $C_{D_{\min}} = 0$ for a diagonal pixel chain. A circular area, however, build up from discrete pixels, obviously would be evaluated little less compact than a square.

2.4. Variation of compactness

Intraindividual variation of compactness was evaluated for 12 tumors in accordance with a selection within the order of

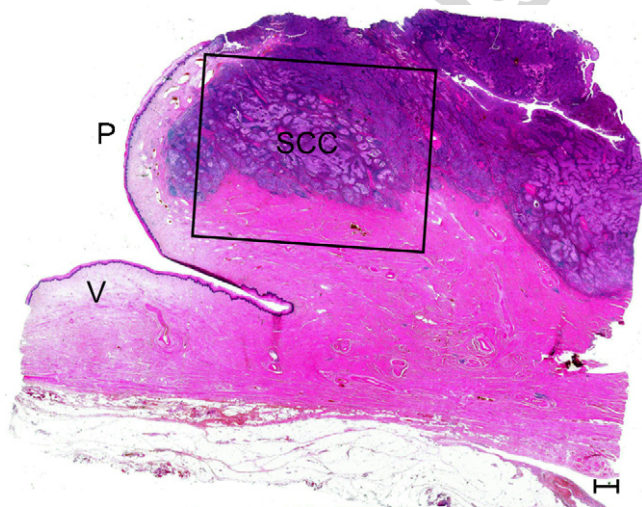


Fig. 2. Lengthways/radial section through the cervix (P: portio) and proximal vagina (V) with marking of the region of interest with a size of 86.5 mm² (definition see text; haematoxylin/eosin; scale bar = 1 mm). The squamous cell carcinoma (SCC) has an overall size of 3.5 cm \times 3.0 cm \times 1.4 cm (pT1b1).

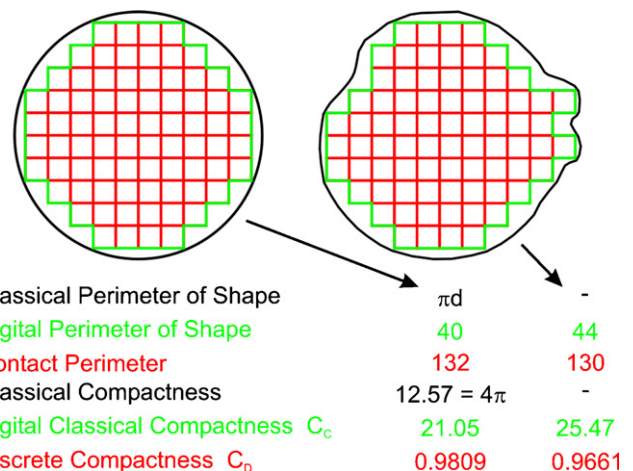


Fig. 3. Comparison of different measurements of compactness based on perimeter of shape using rectangular tessellation (e.g. pixel; $n = 76$). The small modification of two pixels changes the value of digital classical compactness C_C about 21.0% and the discrete compactness C_D about 1.5% [Ref. [16] modified].

rank of the discrete compactness (rank: 1, 4, 9, 18, 27, 36, 41, 50, 59, 68, 73 and 76). Three additional slides descended from different paraffin-embedded blocks of each selected tumor were examined and C_C as well as C_D were calculated. However, ROIs could not be adjusted exactly as described above as sometimes the tumor structures cover only 20–30% of the whole image area.

2.5. Statistical analysis

Kolmogorov–Smirnov test of fit (K–S-test) was applied to test for a normal distribution. The normality hypothesis was accepted if exact p -values (two-tailed) were higher than a α significance level of 0.4. The result is expressed as mean \pm standard deviation (S.D.). The variation coefficient (VC) is the quotient of S.D. and mean and was used to compare the values of dispersion.

Interobserver agreement was evaluated using the κ statistics. Differences in compactness among groups were analyzed by the nonparametric Mann–Whitney U -test or the t -test if random samples were normally distributed. Differences in proportions were evaluated by the χ^2 or the Fisher test, whichever was appropriate.

Receiver operating characteristic (ROC) curves and their measure, the area under the curve (AUC), were used for judging the discrimination ability of both types of compactness.

Exact p -values were two-tailed, and p -values less than 0.05 were considered significant. Statistical analysis was performed using SPSS (Statistical Package for the Social Sciences, Release 11.0, SPSS Inc., Chicago, USA).

3. Results

3.1. Patient characteristics and qualitative assessment of the invasion front

In Table 1 the distribution of patients according to pertinent clinical and histopathologic variables is shown. The mean age of the patients was 46.3 years (range 24–78 years).

The invasion front patterns were assessed as *closed* in 9 (11.8%), *finger-like* in 50 (65.8%) and *diffuse* in 17 (22.4%) patients by pathologist 1, and the assignment was 9, 51 and 16 by pathologist 2, respectively. The interobserver agreement between the pathologists was 72.4%, with κ coefficient of 0.444.

3.2. Quantification of the invasion front

The K–S-test confirms a Gaussian distribution of discrete compactness (C_D) of all tumors (mean: 0.9556; S.D.: 0.0254; min: 0.8927; max: 0.9978) but the distribution of classical compactness (C_C) is not normal with a right-sided skewness (mean: 7188; S.D.: 6255; min: 107; max: 26 038). The variation coefficient of C_D and C_C is 2.6% and 87.0%, respectively.

The C_D of the three groups of the invasion front pattern assigned by both pathologists is highly significantly different ($p \leq 0.0001$; Fig. 4, top). The subjective cut off between the *closed* (C) and the *finger-like* (B) pattern is sharp (especially clear for pathologist I), but the values of C_D of the groups with

Table 1

Patient characteristics ($n = 76$) and morphological findings in correlation to classical (C_C) and discrete compactness (C_D)

	Total		C_D		C_C	
	n	(%)	Median	p^a	Median	p^a
Age at surgery						
≤ 35	15	(19.7)	0.9490	NS	5966	NS
> 35	61	(80.3)	0.9603		5762	
pT						
1b1	33	(43.4)	0.9658 ^b	NS	4482 ^c	NS
1b2	12	(15.8)	0.9565		5414	
2a	8	(10.5)	0.9700		3720	
2b	23	(30.3)	0.9478 ^b		8736 ^c	
Parametrial involvement						
Absent	53	(69.7)	0.9637	0.028	4482	0.026
Present	23	(30.3)	0.9478		8736	
Relative depth of stroma invasion						
$\leq 1/3$	9	(11.8)	0.9734	NS	1134	NS
$> 1/3$ and $\leq 2/3$	11	(14.5)	0.9637		4482	
$> 2/3$	33	(43.4)	0.9596		5067	
pN						
N0	46	(60.5)	0.9576	NS	5185	NS
N1	30	(39.5)	0.9574		6122	
Histological grade						
G1	8	(10.5)	0.9714	NS	6320	NS
G2	45	(59.2)	0.9559		6278	
G3	18	(23.7)	0.9528		5339	
GX	5	(6.6)	–		–	
L						
L0	17	(22.4)	0.9661	0.033	5067	NS
L1	59	(77.6)	0.9559		5966	
V						
V0	64	(84.2)	0.9588	NS	5185	NS
V1	12	(15.8)	0.9534		6666	
Inflammatory response						
None/weak	28	(36.8)	0.9574	NS	6732	NS
Moderate/strong	48	(63.2)	0.9600		4860	
Recurrent tumour (within 60 months)						
None	59	(77.6)	0.9603	NS	5067	NS
Yes	17	(22.4)	0.9559		7724	

NS: not significant ($p \geq 0.05$).

^a Mann–Whitney U -test or t -test.

^b pT1b1/pT2b: $p = 0.020$.

^c pT1b1/pT2b: $p = 0.034$.

a *diffuse* (A) and a *finger-like* (B) pattern overlap in a wider range.

The distribution of C_C referring to the very same invasion front patterns is also significantly different ($p \leq 0.01$; Fig. 4, below). The overlap of values between the group with a *diffuse* (A) and a *finger-like* (B) pattern is more pronounced.

To compare the inherent capacity for discriminating the *diffuse* and the *finger-like* pattern of invasion, the area under the ROC curve was computed for both types of compactness (Fig. 5). For pathologist I and II the AUCs of C_D are 0.802 and 0.836, respectively, while in contrast, the estimated AUCs of C_C are 0.708 and 0.749. Accordingly, the difference of the discrimination ability is approximately 0.09 for both pathologists.

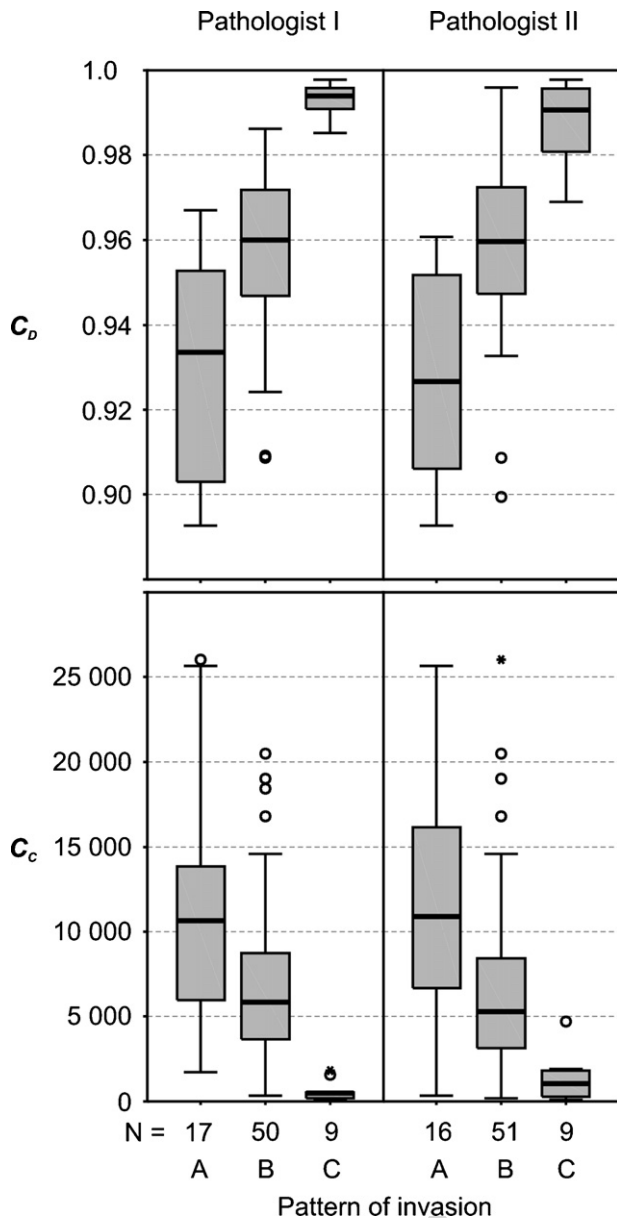


Fig. 4. Correlation between the assessment of pathologist I and II (pattern of invasion: A = diffuse; B = finger-like; C = closed) and discrete (C_D) respective classical compactness (C_C). The differences in C_D for the comparison of all groups are highly significant ($p \leq 0.0001$). For both pathologists, the values of C_C of group A differ significantly from group B (pathologist I: $p = 0.01$; pathologist II: $p = 0.002$) and the differences between groups B and C are highly significant ($p \leq 0.0001$).

The ROC analysis for discriminating the closed and the finger-like invasion front pattern reveals a difference of approximately 0.03 with a higher accuracy for C_D , too (data not shown).

3.3. Relationship of compactness and conventional histological features

The relationship of compactness and conventional clinicopathological parameters is presented in Table 1. There was no significant relation between the values of discrete or digital classical compactness and age, depth of cervical stroma invasion,

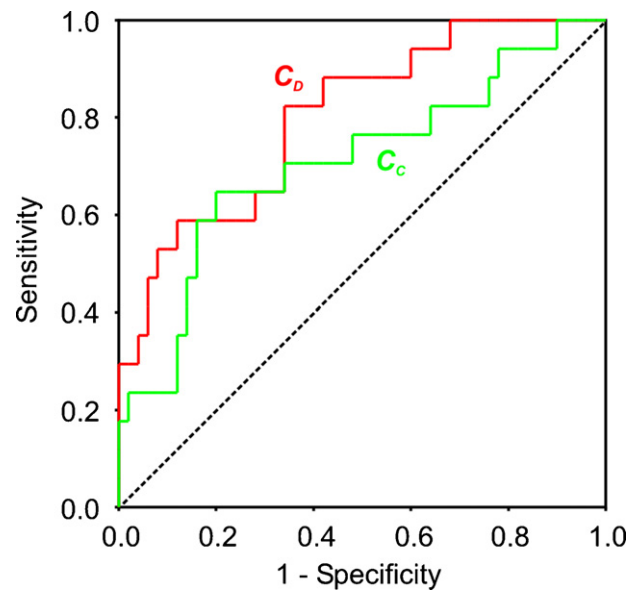


Fig. 5. The graph shows the ROC curves of C_D (red) and C_C (green), which have an area of 0.802 and 0.708, applied to discriminate the diffuse and the finger-like pattern of invasion. This “true” invasion front status was assessed by the gynecologic pathologist (I). The dashed line is called the chance diagonal and represents the practical lower bound for the ROC area of 0.5. (For interpretation of the references to colors in this figure legend, the reader is referred to the web version of the article.)

pelvic lymph node metastases, grading, blood vascular space invasion, host response and recurrence as well as number of metastatic lymph nodes. Primary tumors with a microscopic invasion into the parametria (pT2b) had a lower value of C_D and a higher value of C_C compared to those tumors without parametrial involvement ($p = 0.028$ and $p = 0.026$) and to tumors in stage pT1b1 ($p = 0.020$ and $p = 0.034$). Moreover, tumors with lymphovascular space invasion were found to have a more diffuse invasion front pattern in comparison to those with L0-status (C_D : $p = 0.033$).

However, these relations between clinicopathological parameters and the invasion front pattern were not evident when the tumor border was subjectively assessed by both pathologists (χ^2 test).

3.4. Intraindividual variation of compactness

The mean of variation coefficient of four different tumor regions evaluated for 12 tumors was 0.7% for C_D and 43.8% for C_C (Table 2). Particularly, high variation coefficients above 60% were found for tumors with a relative low mean of C_C and a relative high mean of C_D , which indicates a closed pattern of invasion (see cases with rank: 50, 73, 76).

4. Discussion

The pattern of the tumor invasion front was an object in many previous studies and is one component of various invasive front grading systems. Unfortunately, assessments by pathologists are influenced by an appreciable degree of subjectivity. The κ statistics is at present regarded as the best test of reproducibility

Table 2
Intraindividual variation of compactness of 12 tumors by measuring four slides from different paraffin-embedded blocks (VC_{mean}: mean of variation coefficient)

Rank dependent on C _D	C _D				C _C			
	Original	Intraindividual variation			Original	Intraindividual variation		
		Mean	S.D.	VC		Mean	S.D.	VC
1	0.8927	0.9122	0.0141	1.5	13,853	14,727	1 761	12.0
4	0.9028	0.9079	0.0096	1.0	4,224	5,198	1 540	29.6
9	0.9242	0.9232	0.0073	0.8	7,366	9,474	3 132	33.0
18	0.9389	0.9421	0.0066	0.7	14,581	14,482	1 594	11.0
27	0.9504	0.9523	0.0068	0.7	8,024	8,972	2 579	28.7
36	0.9559	0.9535	0.0055	0.6	10,640	10,129	2 771	27.4
41	0.9603	0.9600	0.0063	0.6	9,406	6,421	2 216	34.5
50	0.9664	0.9685	0.0102	1.0	3,816	2,079	1 351	65.0
59	0.9736	0.9762	0.0029	0.3	6,624	5,046	1 496	29.6
68	0.9862	0.9864	0.0020	0.2	1,051	1,496	495	33.1
73	0.9957	0.9938	0.0039	0.4	107	553	748	135.3
76	0.9978	0.9834	0.0111	1.1	139	4,656	4,043	86.8
			VC _{mean}	0.7			VC _{mean}	43.8

of inter- and intraobserver agreement and compares how the observed agreement differs from that expected by chance alone. However, there is a disagreement about what value of κ is considered acceptable, but it could be argued that a system designed to be used in prognosis and treatment planning should generate a κ value of at least 0.41, i.e. ‘reasonable’ or ‘moderate’ agreement [20,21]. A κ coefficient between 0.61 and 0.80 is considered to represent good agreement, values above 0.81 as very good agreement [22]. The interobserver reliability found in our study with a three-category classification slightly exceeds the lower threshold value for κ and is comparable to those reported by others. A scoring system for a histological invasive front grading with four features according to Bryne et al. (degree of keratinisation, nuclear polymorphism, pattern of invasion and host response) was evaluated in 125 surgically treated patients with squamous cell cervical carcinoma FIGO stage IB [23,24]. For interobserver agreement κ coefficients of 0.47 and 0.66 were reported.

In a recent study the reliability of an invasive front grading with five histological components (additional: host response) was assessed in 102 cases of intraoral squamous cell carcinoma [21]. In order to strengthen the degree of agreement, the pattern of invasion was categorized into either four or two categories. The interobserver agreements using the κ statistic were 0.193 and 0.449, respectively.

The total agreement in the determination of various histopathologic factors for grading of squamous cell carcinomas was also examined in a study from the gynecologic oncology group (GOG) [10]. All 195 patients had been surgically treated for FIGO stage IB cervical cancer. Data from primary evaluation in the participating institutions were compared with those generated by two reviewers. The pattern of tumor invasion at the stromal interface was examined at a magnification of 40× and 400× and classified as “pushing or infiltrating” and “smooth or rough”. In accordance with our result of 72%, an agreement in the determination between reviewer 1 and 2 of 81% was reported.

Many factors may influence the degree and quality of agreement between different assessors in the evaluation of individual histological components, including differences in experience of the assessors, varying interpretations of the definition of the categories in each histological parameter, subconscious ‘baseline shift’, fatigue, memory of specific cases and tumor heterogeneity [21]. One possibility to decisively improve the quality of agreement is provided by using a metrically defined criterion which adequately represents the morphological feature. The significant difference of both types of compactness in relation to the assignment of invasion front patterns to three groups by both pathologists confirms that, in principle, the same feature was regarded. The wide overlap of compactness between the group with a *finger-like* and a *diffuse* pattern highlights the subjective nature of histological assessment while missing precise criteria.

The herewith introduced approach of a computer assisted quantification of invasion front patterns has several advantages. The labeling of the tumor area is checkable and the values are influenced by the pattern alone. Furthermore, the values are dimensionless and independent of the magnification provided that the tumor area is representative. The disadvantages are the necessity of digitization and the availability of image editing software for labeling the tumor area. Various labeling tools of this software, such as brushes, free-hand masks, color-based magic wand masks and others, are helpful and save time.

The computation of discrete compactness C_D is practically not available in commercial software packages utilized for digitization and analyzing of microscopic images. Therefore, the ROC analysis and the variation coefficient of repeated examinations were used to verify the theoretically higher robustness of C_D in comparison with C_C and to evaluate its practical importance. The area under the ROC curve represents a summary of test performance (the higher the area, the better the test’s performance). The group with a *diffuse* and a *finger-like* pattern showed a pronounced overlap in C_D and C_C, but the former had a 9% better ability for discriminating both categories assessed

by the pathologists. Furthermore, intraindividual analysis of a subset of tumors revealed a mean of variation coefficient of more than 60 times lower for C_D in comparison with C_C , whereas the computations were based on the same binary template images. It must be emphasized that for examination of intraindividual variation of compactness sometimes the tumor structures cover only 20–30% of the whole image area. However, the detected low variation for C_D is more in accordance with the experience of pathologists, who have typically observed only minor changes of the invasion pattern along the whole front. Extra high variation coefficients were found for C_C and tumors with a more *closed* invasion front pattern (relative low values of C_C and relative high values of C_D). This result demonstrates that, in practice, non-realistic variations of C_C are especially caused by slight irregularities of *closed* tumor boundaries, in consequence of image noise. In summary, C_D is more suitable for practical use due to the better discrimination ability and the higher robustness.

On one hand, the influence of image noise on C_C also finds its expression in the right-skewed histogram. On the other hand, the Gaussian distribution of C_D may explain the high frequency above 50% of the middle one, if the patterns are divided into three categories. In a previous study the frequency distribution was similar to our results (24.2%, 51.5% and 24.3%) [5].

A further interesting point of discussion is the reason for our choice to use compactness as criterion for quantification. Approaches which might appear simpler or less laborious at a first glance are expected to have more restrictions and a lower robustness. For instance, region activity (variation rate) or the rate black (non-tumor) versus white (tumor) pixels of the binary template image are less appropriate, since these cannot reasonably be applied globally onto whole images, because of the strong dependence on the respective ROI position with respect to the covered tumor/non-tumor portion. However, with the compactness measure, the portion of non-tumor pixels does hardly affect the morphometric result. To avoid the latter, e.g. with usual textural measures (as could be determined from co-occurrence matrices) one would face analogue problems. Fractal dimension was not considered here, since just about two orders of magnitude in spatial resolution which could be analyzed are considered far too few. All these problems can be circumvented using discrete compactness.

According to a Medline® enquiry, there is only one molecular-biological study which has attempted to relate their findings to different patterns of the tumor invasion front. Thus, the rate of human papillomavirus (HPV) type 18 DNA positivity detected by polymerase chain reaction has been found more frequently in “tentacular” than “broad front” tumors [25].

The analysis of compactness in relation to other histopathologic factors revealed a statistically significant correlation with the detection of a microscopic invasion into the parametria and the lymph-vascular space invasion. Although, there was no decisive influence of the relative depth of cervical stroma invasion, from a biological point of view, it suggests that tumors with a low C_D and a high C_C , representing a *diffuse* invasion front pattern, infiltrate the cervical stroma faster and invade lymph vessels more frequently so that they represent an aggressive cohort. In analogy to the term “angiogenic phenotype” which

is histologically defined by the microvessel density, the discrete compactness C_D defines now the “motile phenotype”.

In an earlier study comprising 100 randomly selected patients with FIGO stage IB squamous cell carcinoma of the cervix the absolute depth of cervical invasion was significantly correlated with a “pushing” or “spreading” tumor-stromal border [3]. Tumors with a maximal depth of stromal invasion ≤ 1.5 cm measured from the surface of the epithelium showed in 49% a “spreading” (= *diffuse*) invasion front pattern in comparison with a depth > 1.5 cm in 71% ($p = 0.0315$). With regard to the significance level and the above discussed reproducibility, a difference of 22% points in that study could just be considered as a general trend. This result has not been confirmed by others.

Another previous investigation of 95 low-risk patients with FIGO stage IB squamous cell cervical carcinoma (negative nodes and clear margins) noted an association between the presence of lymphovascular space involvement (LVSI) and a “spreading” tumor-stroma border ($p = 0.03$), however, the exact proportions were not shown in the paper [2]. This result is confirmed by our study based on a morphometric parameter. In spite of these facts, evaluation of LVSI is dependent on special stains and better histological and immunohistochemical parameters need to be established that can be universally used to standardize the identification of LVSI [26].

In general, more effort must be done working out exact definitions for several histologic parameters. Our ongoing studies are concentrated on the comparison of manual and automatic segmentation of the tumor regions, since the latter decisively could extend the applicability of computer assisted morphometric analyses. Concerning the evaluation of the prognostic value of C_D , no difference in compactness was found in patient with and without a recurrence; however, for exact analysis we essentially need a larger population with a longer follow-up period.

In conclusion, discrete compactness is a useful and reproducible parameter for a computer assisted assessment of the invasion front pattern of squamous cell carcinoma of the uterine cervix which allows a metric quantification. Compactness correlates with the invasion in lymph vessels and the parametrium and, thus, defines the motile phenotype.

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