Clinico-morphological parameters affecting survival of patients with advanced cervical cancer

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The aim of the study was to investigate the prognostic significance of selected clinico-morphological parameters including Ki-67 antigen expression and microvessel density. The data of 122 patients with squamous cell carcinoma, FIGO stages IB-IIIB and treated with radiochemotherapy and brachytherapy were studied. Significant prognostic factors for disease-free survival in univariate analysis were the FIGO stage and the presence of atypical mitoses in carcinoma cells. Multivariate Cox analysis confirmed prognostic significance of the FIGO stage and Ki-67 expression with regard to disease-free survival. With regard to overall survival, the most important prognostic factor was Ki-67 antigen expression. The data concerning the pretreatment status of these parameters may be helpful in clinical practice.

Key words: cervical cancer, radiochemotherapy, histopathology, Ki-67, MVD.

Introduction

The diagnostic work-up for cervical cancer includes general and gynaecological examination, cystoscopy, rectosigmoidoscopy, chest x-ray, ultrasonography, computed tomography or magnetic resonance imaging and optional positron emission tomography. Histopathological confirmation of cancer remains the fundamental part of the diagnostic process. However, in the case of an inoperable patient with cervical cancer, the usual histopathological report on the biopsy specimen includes only basic information concerning the histological type of tumour and its grade.

Many studies have dealt with the subject of the prognostic factors in patients with cervical carcinoma. One of the most important prognostic factors is the FIGO stage: in stage IB, five-year survival ranges from 80 to 90%, in stage IIA it reaches 75%, in stage IIB – 60-65% and in stage IIIB survival rates range from 28 to 45%. Patients' age, histological texture, grade, and tumour size, haemoglobin level, and selected biomarkers (like expression of VEGFR, bax, bcl-2, EGFR, p53) have been also the subject of intensive investigations. Their results are not coherent.

The purpose of this study was to analyze the prognostic value of several clinico-morphological parameters and to estimate their influence on survival of cervical cancer patients treated with radiochemotherapy and their usefulness in daily clinical practice.

Material and methods

The data of 122 patients with squamous cell carcinoma, FIGO stages IB-IIIB, and treated with radiochemotherapy and brachytherapy were included in this retrospective analysis. Six patients (4.9%) were in stage IB, 33 (27.0%) were in stage IIA, 52 (42.6%) in stage IIB, 3 (2.5%) in stage IIIA and 28 patients

(23.0%) in stage IIIB. The mean age of the patients was 53.3 years (range 29-80). All patients were treated with a combination of 3D conformal external beam radiotherapy (EBRT) and high-dose-rate brachytherapy with Ir-192 (HDR ICT). External beam radiotherapy was performed with 6-12 MV photon beams. The dose per fraction was 2 Gy given five times per week to a total dose of 50 Gy and "box" technique was used. All patients received concurrent chemotherapy with weekly cisplatin in the dose of 40 mg/m^2. High-dose-rate brachytherapy with Ir-192 was delivered once a week in four fractions with a fractional dose of 7 Gy at point A.

The histopathological slides were reviewed by two experienced pathologists. The following morphological parameters have been assessed: histological subtype and grade according to the WHO 2003 classification, keratin pearl formation, the presence of confluent sheets of low-differentiated carcinoma cells, the presence of atypical mitoses, Ki-67 (MiB1) proliferation index, the type of tumour border, cervical glands involvement or invasion of vascular spaces as well as several determinants of stroma reaction, that is desmoplasia, lymphoplasmacytic infiltration of the tumour neighbourhood, tumour infiltrating lymphocytes (TILs) and microvessel density.

Immunohistochemistry

Anti-CD34 antibody was chosen as a marker of endothelial cells. CD34 class II, Clone OBEnd, Code M7165, DAKO, in a dilution of 1 : 25 and streptavidin-biotin method were used. The mean value of the total number of microvessels was calculated in 5 fields at magnification 100× (~1.43 mm^2) and recorded. Every single cross-section of vessel stained with CD34 was treated separately. In the fields fulfilled with necrosis and in healthy tissues vessels were not counted. CD 34 staining reactivity was graded as low – mean value of the total number of microvessels in 1.43 mm^2 less than 43 vascular elements and high – mean value of the total number of microvessels in 1.43 mm^2 equal or greater than 43 vascular elements.

Immunostaining for Ki-67 was performed using antibodies to human Ki-67 (mouse anti-human M7240; DAKO, Glostrup, Denmark) and streptavidin-biotin method. For each case, we counted the total number of Ki-67 positive nuclei per 500 cancer cells.

Statistical analyses

Disease-free and overall survival was calculated using the Kaplan-Meier method. Survival differences were assessed by the log-rank test. Multivariate analyses were performed using Cox proportional hazard regression models. A significance level of $p = 0.05$ was used for statistical analyses.

Results

The stage of the disease as well as histological and immunohistochemical parameters studied are depicted in Table I.

Most of the patients presented advanced cervical cancer, stage IIB-IIIB (68.1%). Keratinizing and nonkeratinizing squamous cell carcinoma was diagnosed in 50% and 43.4% of cases, respectively. The majority of squamous cell carcinomas (57.4%) were moderately differentiated (grade 2), followed by poorly differentiated (grade 3) and well differentiated (grade 1) (9.8%). Keratin pearl formation was noticed in 59 of 122 (47.5%) tumours studied. Among 40 tumours diagnosed as low-differentiated (G3) squamous cell carcinoma, 27 were characterized by the presence of solid/confluent sheets of low-differentiated cells. Mitotic activity of cancer cells ranged from 3 to 180 (mean 41.4, median 40.0). Atypical mitotic figures were observed in 73.6% of tumours.

Carcinomas invaded the cervical stroma in three different patterns. The pushing pattern of invasion (macrofocal invasion), and spray-like one (microfocal invasion) were observed in 27.3% and 20.7% of cases, respectively. However, most frequently both patterns of stroma invasion (the so-called mixed texture) were seen. In 16.4% of cases cervical glands involvement was additionally noticed.

In the response to the spread of carcinomatous cells, two different types of cervical stroma reaction appeared. Desmoplastic reaction of the stroma occurred in 32.2% of tumours, and lymphoplasmacytic infiltration in the adjacent tissues were visible in nearly all tumours studied. Intensive and moderate lymphoplasmacytic reaction in the stroma occurred in 73.6% of cases. However, tumour infiltrating lymphocytes (TILs) were noticed in 11.6% of cases only.

Ki-67 antigen index ranged from 14.8 to 93.4 (mean 53.3, median 52.1).

Microvessel density varied from 14.3 to 146.6.

Clinico-morphological correlations

The follow-up study of the patients revealed 11 (9%) loco-regional recurrences, 7 (5.7%) cases with distant failures and one case with distant and local failure (0.8%). Overall 5-year survival for 122 patients was 54.6% and 5-year disease-free survival rate was 53.8%.

The mean Ki-67 values for tumours with complete remission and those with no or partial response to the treatment were similar (52.7 and 55.5, respectively, $p = 0.4281$). The mean number of microvessels in tumours with complete remission after treatment and in persistent tumours were also similar (49.2 and 45.7, respectively, $p = 0.5025$). Atypical mitoses were observed in 89 patients and 24 of them (26.97%) had persistent tumour after treatment, compared with 3 pa-

Table I. The stage of disease as well as all histological and immunohistochemical parameters studied

Parameter	Variables	No. (%) of patients or tumours
FIGO stage	IB	6 (4.9)
	IIA	33 (27.0)
	IIB	52 (42.6)
	IIIA	3 (2.5)
	IIIB	28 (23.0)
Histological subtype of squamous carcinoma	Keratinizing squamous cell type	61 (50.0)
	Nonkeratinizing squamous cell type	53 (43.4)
	Basaloid (small cell nonkeratinizing type)	5 (4.1)
	Squamotransitional and/or warty carcinoma	3 (2.5)
Grade	I	12 (9.8)
	II	70 (57.4)
	III	40 (32.8)
Keratin pearl formation	Distinct	36 (29.5)
	Sparse	22 (18.0)
	None (absent)	64 (52.5)
Confluent sheets of low-differentiated cells	Present	27 (22.2)
	Absent	95 (77.9)
Cervical glands involvement	Present	20 (16.4)
	Absent	102 (83.6)
Atypical mitoses	Present	89 (73.6)
	Absent	32 (26.4)
Tumour necrosis	Extensive	25 (20.7)
	Focal	29 (24.0)
	Trace	2 (1.7)
	Absent	65 (53.7)
Invasion of the cervical stroma	Macrofocal	33 (27.3)
	Microfocal	25 (20.7)
	Mixed	61 (50.4)
	Other	2 (1.7)
Desmoplasia	Extensive	39 (32.2)
	Absent	82 (67.8)
Vascular invasion	Present	23 (19.0)
	Absent	98 (81.0)
Lymphoplasmacytic infiltration	Intensive	63 (52.1)
	Moderate	26 (21.5)
	Trace	20 (16.5)
	Absent	12 (9.9)
Tumour infiltrating lymphocytes (TILs)	Present	14 (11.6)
	Absent	107 (88.4)
Ki-67 (MiB1) index	Mean	53.3
	Minimum	14.8
	Maximum	93.4
Microvascular density (MVD)	Mean	48.5
	Minimum	14.3
	Maximum	146.6

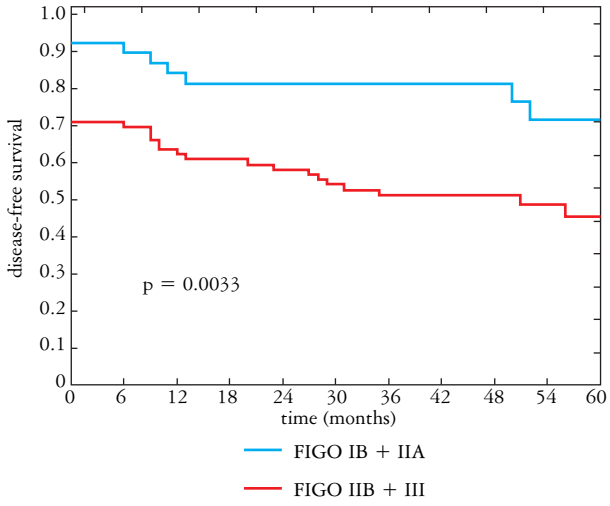

Fig. 1. Disease-free survival curves according to the FIGO stage

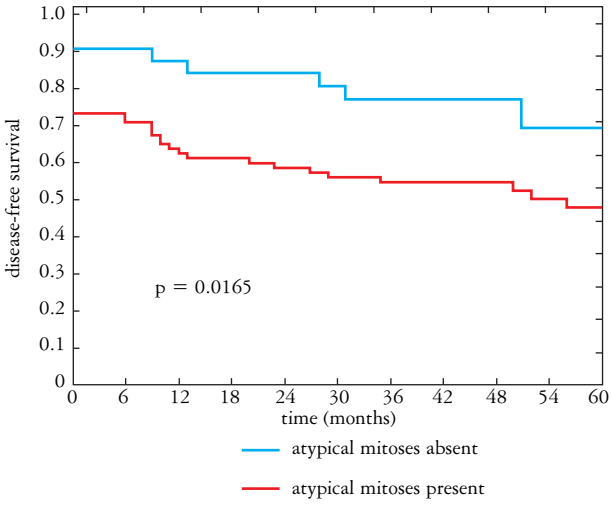

Fig. 2. Disease-free survival curves according to atypical mitoses

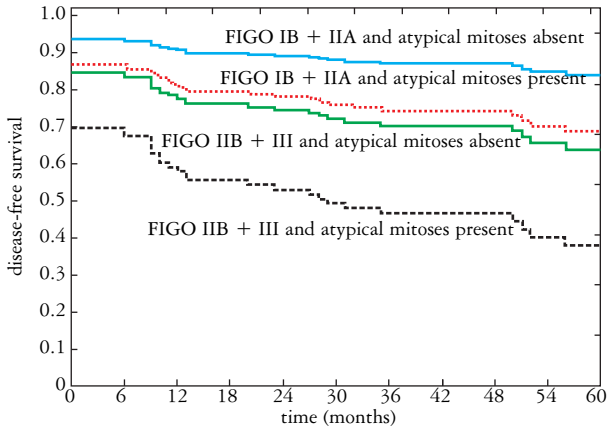

Fig. 3. Disease-free survival curves according to the FIGO stage and atypical mitoses

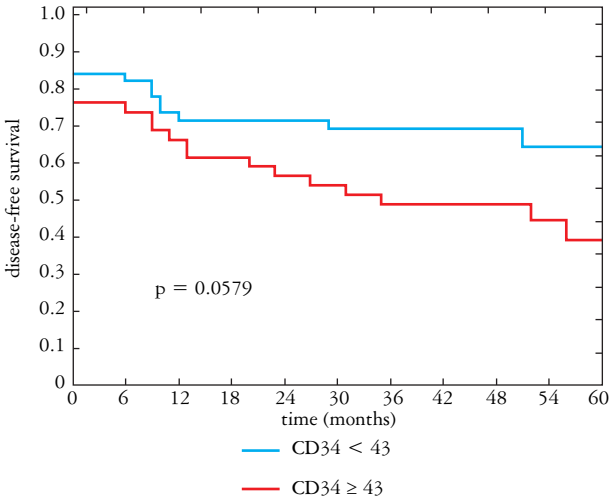

Fig. 4. Disease-free survival curves according to microvascular density (MVD)

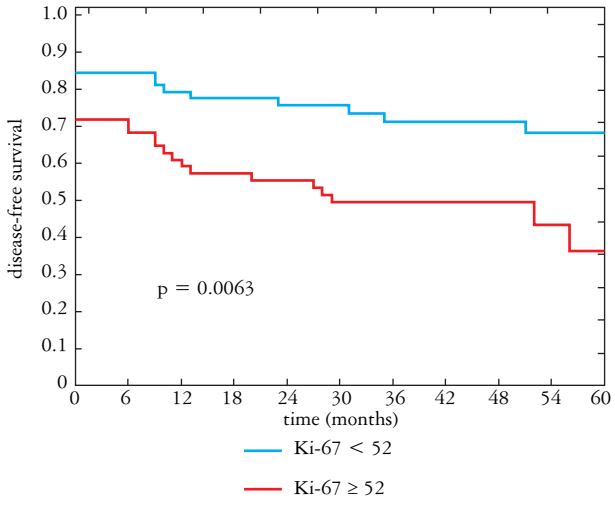

Fig. 5. Disease-free survival curves according to Ki-67 proliferation index

tients (9.38%) in the group without atypical mitoses ($p = 0.0286$).

Univariate log rank analysis for disease-free survival pointed to the FIGO stage, atypical mitoses and Ki-67 index as important prognostic factors ($p = 0.0033$, $p = 0.0165$ and $p = 0.0063$, respectively). Microvessel density was a prognostic factor for borderline significance ($p = 0.0579$). Figures 1, 2 and 3 show disease-free survival curves according to the FIGO stage of the disease and the presence of atypical mitoses in carcinoma cells. Figure 4 presents disease-free survival rates of patients depending on the microvessel density of tumour stroma. Figure 5 shows disease-free survival curves according to the proliferation index measured by Ki-67 antigen expression. The remaining histological or immunohistochemical parameters analyzed in this study did not influence the survival of patients.

Table II. Multivariate proportional hazard regression for disease-free survival (n = 117)

VARIABLE	CLASS	RR	95% CI	P-VALUE
FIGO	IB + IIA, IIB + IIIB	1.000	1.090-5.070	0.0293
Ki-67 index	< 52	1.000	1.046-3.498	0.0351
	≥ 52	1.913		

Table III. Univariate proportional hazard regression for overall survival (n = 117)

VARIABLE	CLASS	RR	95% CI	P-VALUE
Ki-67 index	< 52	1.000	1.268-4.106	0.0059
	≥ 52	2.282		

Multivariate analysis (Table II) shows prognostic significance of the FIGO stage (p = 0.0293) and Ki-67 proliferation index (p = 0.0351) for disease-free survival.

Univariate log rank analysis for overall survival indicated that the FIGO stage and Ki-67 expression were important prognostic factors (p = 0.0297 and p = 0.0227, respectively).

In regard to overall survival, univariate proportional hazard regression revealed Ki-67 index as the only one important prognostic factor (Table III).

Discussion

A histopathological report, apart from giving confirmation of invasive cancer, should include information about a variety of tumour characteristics. Whereas for advanced cervical cancer, a histopathological report on the biopsy specimen often includes only basic information about the type and grade of the tumour. Although progress in modern immunohistochemical techniques has made morphological techniques partly insufficient, undoubtedly, histopathology still plays an important role in the assessment of the prognostic factors in cervical carcinoma. To date, many molecular biomarkers have been the subject of intensive investigation, but they are not recommended for standard diagnostic work-up in the clinical practice in patients with cervical carcinoma.

Most authors suggest at the most little prognostic value of grade of histological differentiation of squamous cell cervical carcinoma [1-4]. Stock *et al*. [5] in a group of 445 patients with advanced cervical carcinoma treated with radiotherapy did not find any correlation between histopathological grading and prognosis.

Chung *et al*. [6] observed a higher rate of lymph node metastases and recurrence in patients with less differentiated tumours. In the presented series of 122 patients with different stage cervical carcinoma we did not find any correlation between grade and prognosis (p = 0.7372). Also other histological parameters, i.e. cell type, keratin pearl formation, confluent sheets of low-differentiated cells, cervical glands involvement, tumour necrosis, invasion of the cervical stroma, desmoplasia, vascular space invasion, lymphoplasmacytic infiltration, tumour infiltrating lymphocytes (TILs), were not prognostic factors with regard to overall and disease-free survival in the presented material.

Regarding the cell type it is important to distinguish between small cell and usual squamous cell carcinoma. Aggressive clinical course and early metastases are typical of small cell carcinoma [7, 8]. Correct diagnosis enables more aggressive treatment and careful follow-up. In our material this cell type occurred in 5 patients (4.1%), 3 of them are alive without signs of cancer.

Vascular space invasion is an important prognostic factor in all histological types of cervical cancer [9, 10] and correlates with lymph node metastases and recurrence. Matthews *et al*. [11] suggest an alternative treatment (radiotherapy) for patients whose preoperative biopsies demonstrate lymph-vascular space invasion. In our series, 23 patients (19%) presented vascular space infiltration and this factor was not correlated with prognosis deterioration (p > 0.05 for disease-free and overall survival).

Lymphoplasmacytic reaction of the cells located around the tumour is one of the parameters evaluating the tumour-host relationship. In our material, 89 out of 122 (73.6%) tumours were characterized by intensive or moderate inflammatory cell reaction, but it was not a prognostic factor. Graflund *et al.* [9] noticed borderline prognostic significance of this factor.

The prognostic significance of tumour infiltrating lymphocytes is controversial. In the cases where TILs have improved patients' outcome, lymphocytes acted as effectors of antitumour immune response [12-14]. In 14 patients (11.6%) in whom we recognized TILs

this parameter was not associated with prognosis ($p > 0.05$ for overall and disease-free survival).

The local connective tissue reactions in cervical cancer showed changes during carcinogenesis. Treyvaud and Gloor [15] suggest that prognosis for patients with cervical carcinoma characterized by fibrous reaction of the stoma is less favourable. Sano and Ueki [16] concluded that an increased number of reticular fibres and plasma cells are an example of protection against invasiveness of cervical carcinoma. In our material, desmoplasia (connective tissue proliferation) occurred in 39 (32.2%) of patients but without influence on survival.

For disease-free survival, important prognostic factors are atypical mitoses and the FIGO stage in univariate analysis, and the FIGO stage and Ki-67 proliferation index in multivariate analysis.

For overall survival, important prognostic factors are the FIGO stage and Ki-67 expression in univariate analysis, and Ki-67 in multivariate analysis.

Atypical mitoses frequently occur throughout epithelium in cervical carcinoma, but until now, the studies were mainly directed towards factor involvement in cervical dysplasia [17]. In our material this factor was a statistically significant prognostic factor.

Tumour angiogenesis is fundamental for tumour growth and further invasion and metastases. Some studies have shown a relationship between increased microvessel density and decreased survival in early-stage patients with cervical carcinoma [18, 19]. However, other reports did not demonstrate any prognostic influence [20, 21]. The majority of our patients (68.1%) have advanced cervical cancer and we found borderline influence of microvessel density on disease-free survival.

Uncontrolled tumour proliferation translated its aggressiveness. Ki67 antigen is a well-known proliferation marker and has been recognized as a biomarker in cervical intraepithelial neoplasia [22, 23]. A few researchers have studied prognostic significance of Ki-67 expression in invasive cervical carcinoma and their results are not coherent. Some authors suggested that a high Ki-67 expression correlated with poor survival [24, 25]. Others did not find correlation between Ki-67 expression and prognosis. In the presented group of patients with invasive cervical carcinoma, Ki-67 expression was a statistically significant prognostic factor in regard to disease-free and overall survival.

In conclusion, Ki-67 expression and atypical mitoses are of prognostic importance. Information about these markers pretreatment status may be helpful in clinical practice.

Our results also confirmed the well-documented role of the FIGO stage as an important clinical prognostic factor.

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