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TUMOUR ANGIOGENESIS, AS A PREDICTOR IN CERVIX CARCINOMA

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Abstract
Cervical carcinoma is one of the leading causes of female death worldwide due to cancer. The aim of this study is to correlate tumour angiogenesis expressed as microvessels density (MD) with proliferative activity. Clinical data were obtained from case notes. Materials and methods: Paraffin-embedded samples from 36 patients with cervical carcinoma were assessed for pattern, Ki 67 and CD34 expression in immunohistochemical analyse. Biotinylated secondary antibodies and the streptavidin-biotin peroxidase complex were applied according to the manufacturer`s instructions ($LSAB_2$ kit, DAKO, Denmark). Target retrieval solution was used . Results and Conclusions: Microvessels density (MD) is an important parameter predicting lymph node metastasis (LNM) and mean survival time.MD was significantly correlated with proliferation index (KI67) and offers the possibility to individualize treatment in patients with highly vascularised tumours in early clinical stages. Key words: Cervical carcinoma, microvessel density, proliferating index.

Rezumat
Carcinomul cervical este una din principalele cause ale mortalitatii provocate de cancer in randul femeilor din lumea intreaga. Scopul acestui studiu este de a corela angiogeneza tumorala cuantificata prin densitatea microvasculara (MD) cu activitatea proliferativa. Datele clinice sunt obtinute din observatiile obtinute din urmarirea fiecarui caz. Material si metoda: Blocuri de parafina de la 36 de paciente cu carcinom cervical au fost evaluate imunohistochimic pentru Ki67 and CD34 . S-a aplicat anticorpul secundar biotinilat si complexul streptavidin-biotin peroxidaza (LSAB2) conform instructiunilor manufacturierului (DAKO,Danemarca). A fost folosita solutie de demascare antigenica. Rezultate si concluzii: Densitatea microvasculara (MD) este un important parametru predictiv pentru metastazele in ganglionii limfatici si a supravietuirii medii in timp.MD este semnificativ corelata cu indicele de proliferare (Ki67) si ofera posibilitatea de a inividualiza tratamentul in cazul pacientilor cu tumori bine vascularizate in stadii clinice neavansate.

Cuvinte cheie:carcinom cervical,densitate microvasculara,index proliferativ.

Sfârşit de secţiune (Pagina următoare)

INTRODUCTION

The molecular mechanisms of tumor aggressiveness are usually dependent on the proliferative stimuli induced by various tumor promoters; numerous proto-oncogenes and oncogenes regulating tumor cell growth, differentiation, and motility have been investigated to identify molecular targets that might be used as potential predictors of survival in the management of cancer [1, 2]. Studies on prognostic factors should not only aim to identify factors statistically associated to prognosis, but they should also investigate whether this factor provides improved means of dividing patients in high and low risk groups, taking into account already established prognostic factors. Pelvic lymph node metastasis, tumor diameter, deep stromal invasion, capillary lymphatic space tumor invasion, parametrial invasion and positive resection margins have most frequently been identified as prognostic factors [3,4]. Metastatic spread of the solid tumor depends on a critical cascade of events that includes tumor cell adhesion, migration, invasion, proliferation and ultimately neovascularization [5]. Tumors promote angiogenesis by secreting various angiogenic factors, and newly formed blood vessels induce tumor cell proliferation and invasiveness [6,7]. The aim of the present study was to evaluate the potential of the prognostic information gained by analyzing the coexpression of proliferative activity (Ki67 immunoreactivity) in patients with squamous cell carcinoma of the uterine cervix.tumour and angiogenesis expressed as microvessels density (MD).

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MATERIALS AND METHODS:

Patients

Thirty-six cervical cancer patients, aged between thirty and sixty-two, were enrolled into this study. They underwent type III radical hysterectomy and bilateral pelvic lymphadenectomy at “Cuza Vodă” Gynecology and Obstetrics Hospital, Iaşi. Post-treatment evaluation consisted of history taking, physical and pelvic examination, cervical cytology, biopsy and other imaging studies performed when clinically indicated.

Immunohistochemistry

Formalin-fixed, paraffin-embedded 4-Am sections were stained using standard immunohistochemical methods. Each section was deparaffinized then antigen was retrieved using sodium citrate, pH 6.0 and boiled in microwave oven. Endogenous peroxidase activity was blocked with 3% H2 O2 for 5 min. Sections have been incubated with Mouse-anti-Human CD34 antibody Class II , clone QBEnd 10, code No M7165, DAKO, Denmark , dilution 1:25 over night and KI-67 Clone

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Sfârşit de secţiune (Pagina următoare)

MIB , N1633, DAKO, Denmark , RTU, over night.
Diaminobenzidine was used as the chromogen and Meyer's hematoxylin was used as the counterstain. Negative control was the section which was performed without primary antibody . The regions of greatest immunostaining were selected and 500 cells in each section were counted for estimation of the percentage of immunoreactive cells for Ki67 positive immunostaining.

Evaluation of immunostained slides
For assessment of Ki67 and CD34 expression levels, staining intensity and percentage of stained cells were analyzed. The intensity of staining was marked as strong (+++), medium (++) and weak (+). The number of tumor cells with positive expression in highpower field was used to calculate the percentage of Ki67 positive nuclei. MD (microvessel density) was scored as 1 (low), 2- 3 (medium) and 4 (high), respectively.
Statistical analysis
The associations between Ki67 and CD34 expression and clinicopathologic variables were analyzed by Chi-square test. A P value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSIONS:
Tissue samples from 36 patients with cervical carcinoma were analyzed by hematoxilin and eosin and immunohistochemical Ki67 and CD34 antibodies. Patients age was between thirty and sixty-two years. According to the histological classification 6 (17%) were adenocarcinomas and 30 (83%) were squamous cell carcinoma. According to the size, 14 (39%) were less than 4 cm in diameter and 22 (61%) were more than 4 cm. The tissues were divided into 3 groups of cervical carcinoma following the stage of FIGO in 1995, including 10 at stage I (28%), 18 at stage II (50%), and 8 at stage III (22%). The high-risk factors such as lymph node involvement (15 out of 36), and lymph vascular space invasion (17 out of 36) were recorded. The clinicopathological parameters of the patients are listed in Table 1.

Table 1. The clinico-pathological parameters of the patients

Clinicopathological Parameters	Total n (%)
The size	
< 4 cm ≥	14 (39%)
4 cm	22 (61%)
Differentiation	
High Mediate	16 (44%)
Low	14 (39%)
	6 (17%)
Histologic	
Adenocarcinoma	6 (17%)
Squamous cancer	30 (83%)
State I	
II III-	10 (28%)
IV	18 (50%)
	8 (22%)

Cervical squamous cell carcinoma (SCC) has high risk of progression depending on the presence of unfavourable classic prognostic factors such as advanced FIGO-stage and LN involvement.

Other classical histopathologic prognostic factors as venous invasion, perineural invasion, endometrial invasion, myometrial

invasion and parametrial invasion were also assessed. Tumor diameter, deep stromal invasion and capillary lymphatic space tumor invasion are the only independent prognostic factors for 3 years disease-free survival.

In our study, no correlation was demonstrated between CD34 expression and other clinicopathologic

variables such as age, tumor size and depth of stromal invasion.

Elevated Ki67 expression was associated with lymph node metastasis in cervical cancer patients who underwent radical surgery.

Cases FIGO stage I and low differentiation (fig. 1) had not lymph node involvement; MD immunohistochemical assessment was encoded 1, Ki67 proliferating index was between 25-35% (table 2).

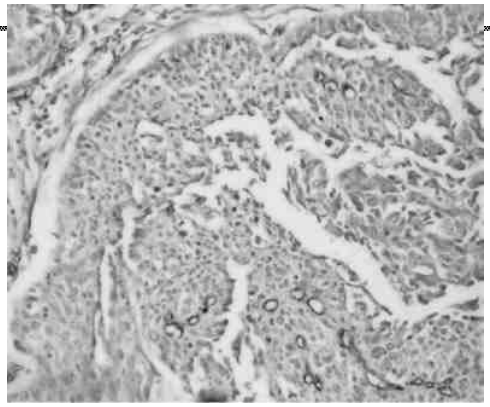

Fig. 1 Invasive carcinoma – low

Differentiation	KI67	MD
Low	25-35%	1+ - 2+
Median	30-45-50%	2+
High	40-65%	3+

Table 2. Index of proliferation

High differeentiation tumors, that produce a high level of MD, have a more aggressive behavior in the process of invasion and metastasis than tumors negative for this neoangiogenic expression; high MD is closely associated with invasive phenotype of the cells assessed by Ki67 proliferating index.

CONCLUSIONS:

- combination of classic prognostic factors and immunohistochemical assessement of CD34 and Ki67

proliferating index may be more effective in the management of cervical cancer patients. ♦ Coexpression of increased MD and Ki67 reflects an aggressive fenotipe in cervical carcinoma.

- MD was significantly correlated with proliferation index (KI67) and offers the possibility to individualize treatment in patients with highly vascularised tumours in early clinical stages.
- Treatment decisions based on this risk division leads to better survival and/or decreased morbidity

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