THE ASSESSMENT OF THE ANGIOGENESIS IN THE UTERINE CERVIX CARCINOGENESIS

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Abstract: The uterine carcinoma represents one of the most frequent malignant tumors in female. Great progresses were made in the understanding of the mechanisms of the appearance and evolution of the tumors, and also in the early diagnosis of them. In the present time, the discussions are centered on the angiogenesis and the neovascularisation. During the researches regarding the new factors of prognosis in cervical carcinomas, we are interesting firstly in the study of neoangiogenesis and its predictive role. The microanatomic quantitative study was made on fragments which were processed from uterine cervix dysplasias, preinvasive and invasive tumors. The sections were stained specific immunohistochemically (CD34) and examined with an interactive digital program. We have quantified the density of the microvessels/mm^2 tumor stroma and the density of the microvessels/mm^2 tumor. The preliminary results regarding the study of the microvessels in uterine cervix carcinomas show a positive correlation between the density and the evolution of the tumor, the angiogenetic process rises simultaneously with the risk of metastasis. This study shows a significant correlation between the density of vessels and the evolution of the uterine cervix carcinomas, the appearance of recurrences and metastasis. The quantification of the angiogenesis is an indicator with an high degree of precision, with is used for the detection of the aggressiveness and malignancy of the uterine cervix tumors.

INTRODUCTION

The processes of spread, growth and tumoral metastasis are dependent on the tumoral angiogenesis which simulates the normal angiogenesis (1, 2, 3).

The cells of the primary tumour must have access to the blood vessels, survive in the blood stream and locate the target organs in which they will initiate a neoangiogenesis process that determines the appearance of metastases. This mechanism of angiogenesis isn't perfectly known, but it is mediated by specific molecules released by the tumoral cells and/or the host cells in the tumoral stroma (4, 2). The new formed vessels are structurally different from the normal ones by the platting of the basal membrane with the appearance of fenestrations or complete disappearance of them. Moreover, the microvessels wall can be made up by endothelial cells beside the tumoral cells.

The data concerning cervical cancer are rather conflicting. Some authors had proved the predictive significance of the microvascular density (5, 6), while other research had obtained opposite results, especially in carcinoma *in situ* (7).

To the level of the feminine genital system, the tumoral angiogenesis is considered to be an essential factor which controls the malignancy degree of some tumors and consequently, the prognosis (6, 8). For this reason, we have proposed to study the predictive importance of the neoangiogenesis in the carcinogenesis of the uterine cervix.

MATERIALS AND METHODS

The studies were made on a material made up by uterine cervix fragments without pathological changes and fragments from cervix dysplasias, preinvasive lesions and tumors: 25 intraepithelial lesions and 36 cervical carcinomas (6 adenocarcinomas and 30 squamous carcinomas). The age of the patients is between 30 and 62 years.

All of the fragments were processed through paraffin technique and stained through current methods for histopathologic diagnosis and after, the significant cases were selected, and the necrotic areas weren't included in the analysis.

In the second stage, biopsy specimens were investigated using immunochemical staining for the vascular endothelium. The sections of biopsy specimen were deparaffinized in xylene and dehydrated through graded concentrations of alcohol. CD-34 staining was performed using biotin-avidin immunoperoxidase technique. Specimens were incubated with the CD-34 mouse monoclonal antibody 2nd class, M7165 code, Mo a Hu (Dako). Then, they were incubated with biotin-labeled antimouse secondary antibodies and next were treated with avidin biotin complex reagent.

After the qualitative examination and the acquisition of the significant images with the 200x magnification, the microvascular density was quantified in the area with the most active angiogenesis, evaluating the sensibility and the specificity to the endothelial marker CD34. Filters were used for the color segmentation and for the obtaining of an intense dark color of the blood vessels in contrast with the image background.

The quantitative measurements were made by using a digital interactive program (PRODIT 5.0.), that use the grid with Weibel parallels and the distance between two point is d = 15.60 μm. The vessels and the stroma of tumor were quantified on a rectangular surface with 0.023244 mm^2 area, on a 50 consecutive fields five cases for each lesional type.

The microvessels were counted in the area with the highest density ("hot spot"), after the identification of the respective areas with a smaller magnification.

A brown-staining endothelial cell clearly separated from adjacent microvessels, tumor cells and other connective tissue elements was considered a single quantifiable microvessel. Counting of microvessels was performed by one pathologist without any knowledge of possible prognostic factors or other clinical data. The report of the stereological quantification had calculated automatically the microvessels density per mm^2 of tumoral stroma (MD/TS) and the microvessels density per mm^2 of tumor (MD/T).

RESULTS AND DISSCUSIONS

The qualitative examination of the specimens had permitted the study of the normal appearance of the uterine cervix and the identification of the tumoral types.
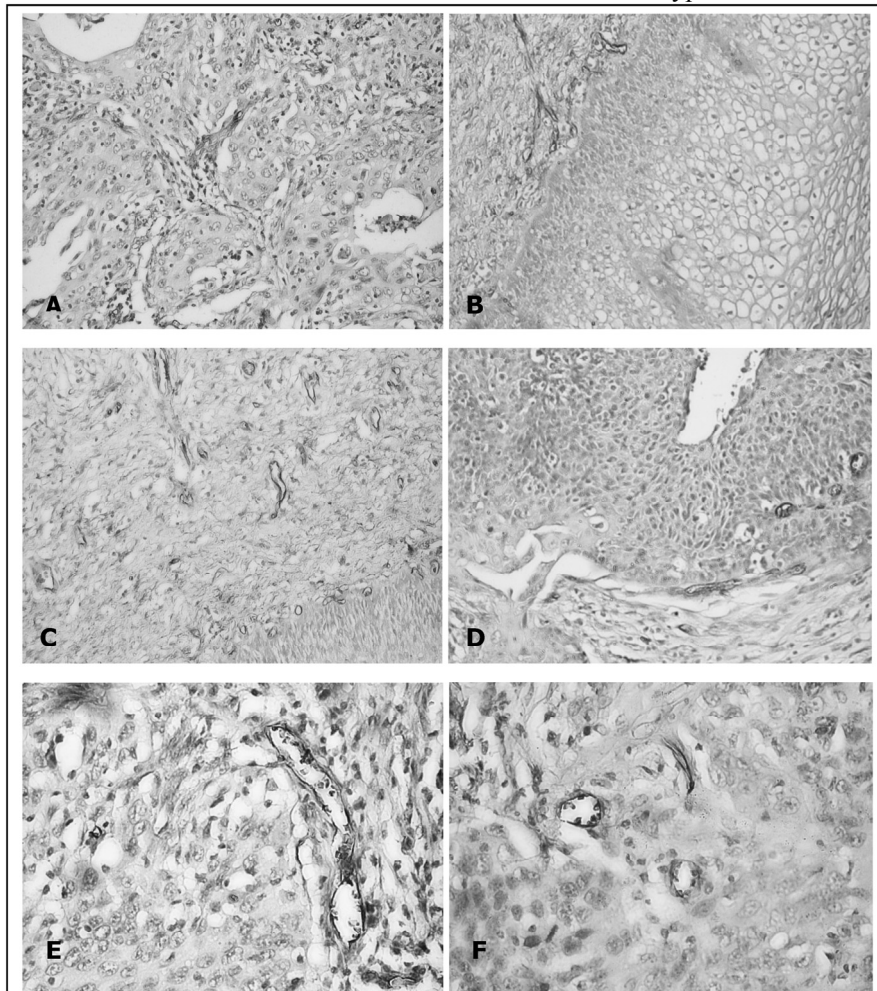

Fig. 1.   A. Intratumoral microvessels in squamous invasive carcinoma. B. Carcinoma in situ. C. peritumoral microvessels in squamous carcinoma. D. Microinvazive carcinoma. E. Peri-islands microvessels in squamous carcinoma. F. Intratumoral microvessels squamous carcinoma.

The examination of the immunohistochemical stained slides had pointed out the presence of the vessels in the stromal connective tissue of the tumor, without being observed any reaction with the tumoral cells. The existence of varied densities of the microvessels in different tumoral degrees of the cervix was observed depending on the differentiation degree (fig. 1).

The microvessels density per mm^2 of tumoral stroma (MD/TS) and the microvessels density per mm^2 of tumor (MD/T) are presented in the Table I.

TABLE I
Results of the quantification of the microvessels densities
(CIN - cervical intraepithelial neoplasias, CIS – carcinoma in situ, MIC – microinvasive carcinoma, IC – invasive carcinoma, AC – adenocarcinoma).

Parameter	TYPE OF LESION						
	CIN1	*CIN2*	*CIN3*	*CIS*	*MIC*	*IC*	*AC*
MD/TS	367.65	377.48	467.80	479.55	519.63	627.51	613.58
MD/T	9605.87	9741.32	12716.98	12727.49	14264.31	16344.42	12869.00

Depending on the differentiation degree, from the forms considered precancerous to the invasive carcinoma and adenocarcinoma, the density of the microvessels varied thus: the microvessels density per mm^2 of tumoral stroma presents a relatively constant density in CIN 1 (367.65) and CIN 2 (377.48), after presents a marked increase in CIN 3 (467.80), but in CIS (479.55) it maintain to an appropriate value. The MD/TS increases significantly in the microinvasive carcinoma (519.63), touches the maximum value in the invasive carcinoma (627.51) and in adenocarcinoma has a reduced value (613.58), firstly because of the high/density of the differentiated tumoral structures.

The density of microvessels per mm^2 of tumor (MD/T) had presented the same sense of change, the differences presenting a higher significance.

The graphical representations of the observed changes (fig. 2) highlight the significant increase of the tumoral microvessels density in the same time with the increase of the risk/degree. The marked increase of the density in CIN 3 and CIS is direct balanced with the invasive risk and metastasis, the highest density in the invasive carcinoma supports the its dissemination capacity. In adenocarcinoma, the reduced density of the microvessels in the tumoral islands, but increased in the tumoral stroma, leads finally to an increased density in the whole tumor, but more appropriate to that from CIN 3 and CIS, than that from invasive carcinoma.

Generally, in the tumor with metastases, the microvascular density is higher than in those without metastases. Moreover, great variations of the vascular density exist among the different areas of the same tumor in the poor-differentiated forms. In the in situ carcinomas and adenocarcinomas, the topographic arrangement is more constant. In the perinecrotic central area and extratumoral areas at distance, the densities of microvessels are likewise.

Anti-CD34 antibody is a highly sensitive marker for endothelial cell differentiation and has also been studied as a marker for vascular tumors. However, there are few studies relating it

to cervical carcinoma. Vierira' studies (9) suggested that a high anti-CD34 antibody reactivity in cervical carcinoma is associated with histopathological features of poorer prognosis.
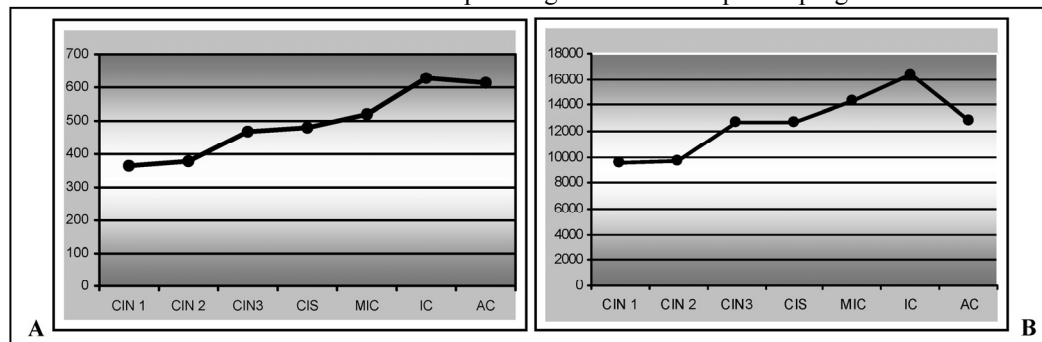

**Fig. 2. Graphical representations of the microvessels per mm^2
of tumoral stroma (A) and per mm^2 of tumor (B).**

In the specialty literature, the majority of data is referred to the microvessels density on a conventional field (10, 7, 11). Calculating per mm^2 of stroma, the results are analogous with those from our researches regarding the in situ carcinoma and the microinvasive squamous carcinoma. The forms with a low microvascular density (fewer than 40 microvessels per field) have a reduced capacity of recurrence, presenting a long free interval (10). Abulafia and co-workers (7) consider that the microinvasive forms of the squamous carcinoma are angiogenic, but the profoundness of invasion doesn't determine an increase of the angiogenic capacity. Squamous cell CIS is not angiogenic.

It had demonstrated the role of the development of the tumoral microvessels as a predictive factor of recurrence in squamous carcinoma in the IB stage (12, 11) and IIA (5). In the cervical tumors stage IB that have a microvessels density fewer than or equal to 20 / field, 89.7% of patients have a good prognosis (11).

The in vivo indicator of angiogenic activity (VI), performed before surgery through transvaginal power Doppler ultrasound, is well correlated with the conventional indicator of tumor angiogenic activity (MVD) assessed immunohistochemically using a monoclonal antibody against CD34 (6).

Cooper and co-workers (13) were investigated the relationship between intrinsic radiosensitivity and microvessels in carcinoma of the cervix given radiotherapy and they were observed that tumor angiogenesis and cellular radiosensitivity are independent prognostic factors for cervix carcinoma treated with radiotherapy. Allowing for tumor radiosensitivity increases the prognostic significance of microvascular quantification in cervix tumors.

CONCLUSIONS

High intensity of tumor angiogenesis has been correlated with an increased potential of metastasis and poor prognosis in human malignancies.

Significanty differences exist between the degrees of differentiation of carcinomas, spatial variations being predictive. In the low-risk lesions, in situ carcinoma and microinvasive carcinoma, the vessels appearance is uniform and steady in comparison with the high-risk forms where the vessels are irregular, dilated and sinuous.

A significant increse of the density of the tumoral vessels is found at the same time with the increase of risk in a direct balanced ratio. The highest microvascular density is found in the invasive and undifferentiate carcinoma.

The intratumoral variation of the vascular density was recorded: in „in situ" carcinoma and in the well differentiated carcinoma the topographic arrangement is more constant. In the poor-differentiated carcinomas exist bigger variations among the different areas of the same tumor, the densities of the microvessels are likewise in the perinecrotic central area and extratumoral, at distance from tumor.

The quantification of the neoangiogenesis can allow the division of the patients by the type of the treatment and the selection of the long time management for the patients with a low tumoral microvascular density.

REFERENCES

Weiss, L. 1976. *Biophysical aspects of the metastatic cascade.* Ed. Amsterdam: Holland, N.

Blood, C.H., Zetter, B.R. 1990. Tumor interactions with the vasculature: Angiogenesis and tumor metastasis. Biochim. Biophysica Acta. 1032:89-118.

Folkman, J. 1990. What is the evidence that tumors are angiogenesis-dependent? Natl. Cancer Inst. 82:4-6.

Folkman, J., Klagsbrun, M. 1987. Angiogenic factors. Science. 235:442-447.

Bremer, G. L., Tiebosch, A. T., van der Putten, H. W., Schouten, H. J. , de Haan, J., Arends, J. W. 1996. Tumor angiogenesis: an independent prognostic parameter in cervical cancer. Am.. J. Obstet. Gynecol. 174(1 Pt 1):126-131.

Cheng, W. F., Lee, C. N., Chu, J. S., Chen, C. A., Chen, T. M., Shau, W.Y., Hsieh, C. Y., Hsieh, F.J. 1999. Vascularity index as a novel parameter for the in vivo assessment of angiogenesis in patients with cervical carcinoma**.** Cancer. 85(3):651-657.

Abulafia, O., Triest, W. E., Sherer, D. M. 1996. Angiogenesis in squamous cell carcinoma in situ and microinvasive carcinoma of the uterine cervix. Obstet. Gynecol. 88(6):927-932.

Pilch, H., Schaffer, U., Schlenger, K., Hawighorst, H., Tanner, B., Bahlmann, F., Hockel, M., Kanpstein, P. G. 2000. Angiogenesis in cervical cancer. Gynecol. Obstet. Fertil. 28(1):29-37.

Vieira, S. C., Silva, B.B., Pinto, G. A., Vassallo, J., Moraes, N. G., Santana, J. O., Santos, L. G., Carvasan, G. A., Zeferino, L. C. 2005. CD34 as a marker for evaluating angiogenesis in cervical cancer. Pathol. Res. Pract. 201(4):313-831.

Kainz, C., Speiser, P., Wanner, C., Obermair, A., Tempfer, C., Sliutz, G., Reinthaller, A., Breitenecker, G. 1995. Prognostic value of tumour microvessel density in cancer of the uterine cervix stage IB to IIB. Anticancer Res., 15(4):1549-1551.

Obermair, A., Wanner, C., Bilgi, S., Speiser, P., Kaider, A., Reinthaller, A., Leodolter, S., Gitsch, G. 1998. Tumor angiogenesis in stage IB cervical cancer: correlation of microvessel density with survival. Am. J. Obstet. Gynecol. 178(2):314-319.

Dinh, T. V., Hannigan, E. V., Smith, E. R., Hove, M. J., Chopra, V., To, T. 1996. Tumor angiogenesis as a predictor of recurrence in stage Ib squamous cell carcinoma of the cervix. Obstet. Gynecol. 87(5 Pt 1):751-754.

Cooper, R. A., West, C. M., Wilks, D. P., Logue, J. P., Davidson, S.E., Roberts, S. A., Hunter, R. D. 1999. Tumour vascularity is a significant prognostic factor for cervix carcinoma treated with radiotherapy: independence from tumour radiosensitivity. Br. J. Cancer. 81(2):354-358.

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