Original Articles

Relationship between angiogenesis and grade of histologic differentiation in endometrial adenocarcinoma

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Summary

The objective of the study was to quantify vessels and to relate them to the degree of histologic differentiation in endometrial adenocarcinoma. We studied 35 cases of which ten were G1, 13 G2 and 12 G3 adenocarcinomas. The control group consisted of 11 atrophic and 10 proliferative endometria. From each case two histologic sections were obtained: one for hematoxylin-eosin staining and the other for immunohistochemical study with anti-CD34. Vessel count was performed by morphometric study. Mean vessel count was 15.3 for G1; 19 for G2 and 22.7 for G3 adenocarcinomas; in the control group it was 11.6 for atrophic and 13.2 for proliferative endometria. Slightly differentiated adenocarcinoma presented greater angiogenesis than normal and well-differentiated carcinoma. In contrast, moderately differentiated carcinoma showed greater angiogenicity as related to normal endometrium, but did not differ from other tumoral endometria.

Key words: Angiogenesis; Adenocarcinoma; Endometrium.

Introduction

In the United States of America endometrial carcinoma is the most common malignant neoplasia of the female genital organs and is responsible for 45% of genital tract neoplams. In Brazil, according to Torloni and Brunini, 11% of the genital tumors affect the uterus [1]. In 1998, according to data of the Ministry of Health, 7.6 cases occurred per 100,000 women [2]. In a two-year (2001-2002) evaluation by the "Fundação Oncocentro de São Paulo, SP" the mortality rate of uterine tumors was 4.4% [3].

Endometrial carcinoma represents 80% of the tumors of the uterus. The study of prognostic factors helps in choosing the best therapeutic management for each case.

Gynecology Oncology Groups have been of great help with extensive staging protocols which evaluate multiple prognostic factors that enable the determination of the most effective treatment lines. These factors are the key elements for the evaluation of tumor behavior [4].

Among the prognostic factors, those usually described in Brazil are: grade of histologic differentiation, myometrial infiltration, histologic type, peritoneal cytology, lymphonodal and adnexal metastasis staging and invasion of the lymphonodular space. The latter is considered a sensitive and independent prognostic factor in endometrial adenocarcinoma, since it occurs in 35.3% of metastatic cases and happens only when there is infiltration of the myometrium. In tumors limited to the endometrium and grade 1 differentiation (EC Stage IA, G1) there is no impairment of the lymphovascular space [5].

At present other prognostic means have been considered. Among them the following should be noted: study of hormonal receptors, tumoral ploidy, karyotype, flow cytometry, plasma tumor markers and oncogene amplification and expression. Howerover, they are difficult to perform, have a high cost and are difficult to access in daily practice.

Angiogenesis is an important prognostic marker for most solid tumors and has been valued in endometrial neoplasias. A worse evolution has been demonstrated when it was determined in prostate tumors [6], cutaneous melanoma [7] and breast cancer [8]. In the genital tract, tumoral angiogenesis is well-described in the uterine cervix [9]. In the endometrium, angiogenesis is well-established in physiological conditions, as the initial proliferative phase of the menstrual cycle and in the implantation of the fertilized ovum [10]. In pathologic endometrium, Abulafia *et al.* [11] reported that complex hyperplasia and adenocarcinoma are angiogenic compared to normal endometrium and simple hyperplasia, mainly in tumors with myometrial infiltration, as well as grade 2 differentiation carcinomas when compared to grade 1. More recently, Salvesen *et al.* [12] related high angiogenesis to metastatic tumors, high Ki 67 proliferation index and shorter survival.

Histologic grade is an important marker in the prognosis and is presently included in staging (FIGO) [14] whereas neovascularization is predictive of better or worse evolution. Thus, we attempted to relate these two important factors, quantitatively evaluating the angiogenic activity of endometriotic-type endometrial adenocarcinoma at histologic differentiation grades (G) 1, 2 and 3. Immunohistochemical study with the CD34 endothelial marker was used for this purpose.

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Material and Methods

The material for the study was collected at the Pathological Anatomy Service of the Brazilian Institute of Cancer Control. For the study groups we selected paraffin blocks of surgical samples containing endometriotic adenocarcinoma of the endometrium of patients who were treated with total abdominal hysterectomy, bilateral salpingo-oophorectomy, segmental or selective pelvic and paraaortic lymphadenectomy and partial omentectomy. For the control group we used blocks with material from abdominal or vaginal hysterectomy indicated due to benign disease and whose endometrium was atrophic or proliferative.

Fifty-six cases were studied and distributed as follows:
11 atrophic endometria
10 proliferative endometria
10 G1 adenocarcinomas
13 G2 adenocarcinomas
12 G3 adenocarcinomas

Histopathologic specimens were submitted to histopathologic revision by three pathologists. In order to be included in the study, the tumor had to be of the endometriotic type and histologic grade had to be concordant. The patient should not have been submitted to any previous treatment with radiotherapy, chemotherapy or hormone therapy. Grade 3 tumor with irregularities at the tumor-stroma interface and marked necrosis were also excluded.

Two histologic sections were obtained for each case: one was sent to be stained with hematoxylin-eosin and the other for immunohistochemical study. The latter was treated with anti-CD34 whose antigen is present in the cytoplasmic membrane of endothelial cells, thus being a good vascular marker and staining of endothelial cells brown [15].

The histometric method employed was vessel count by an the image digital analysis computerized system (Kontron Imaging System - KS300). In the study group the vessels of the interface between tumor growth and adjacent stroma, while in the control group, those in the interface between endometrial glands and stroma, were studied. Counts were performed by two observers with no knowledge of histologic grade, who counted vessels in 10 fields at 100 x magnification. In all, we obtained 560 analyzed fields with 8,180 vessels.

Statistical analysis was performed by comparing the means of the three groups using analysis of variance (Neter). Bonferroni's multiple comparisons was used to test the equality of the means (2 by 2). Since vessels counts were performed with a constant number of fields (10 per slide), means resulted in being distributed according to Gauss' curve.

Results

Distribution of vascular quantification means per group of the 56 women with normal endometrium and (G1, G2 and G3) adenocarcinoma of the endometrium is shown in Table 1.

The mean number of vessels of G2 and G3 adenocarcinomas was statistically higher than that of control group cases. G3 carcinoma also presented a higher number of vessels than G1 carcinoma (Table 2).

Discussion

In the endometrium angiogenesis is well-established in physiological conditions, being a phenomenon mediated by ovarian steroids [10].

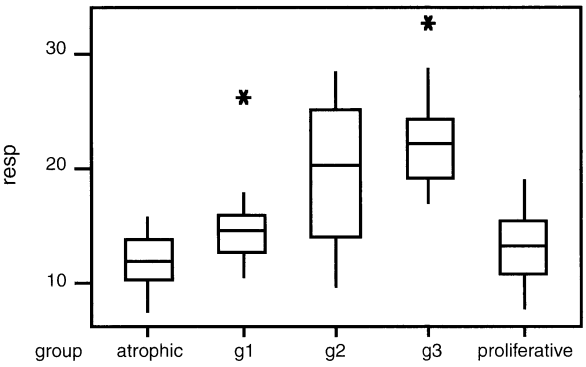
Peek *et al.* showed there is a significant decrease in angiogenic activity in the late secretory phase of the menstrual cycle, suggesting that angiogenic factors are produced in the endometrium during the menstrual cycle.

In endometrial carcinoma, angiogenesis is related to tumor progression, metastatic potential and tumor suppressor gene expression [17]. Abulafia *et al.* [11] in evaluating vascularization, demonstrated that complex hyperplasia and adenocarcinoma are angiogenic. In addition, in Stage I adenocarcinoma, deeper invasion and higher grade of differentiation were directly related to the intensity of angiogenesis.

Table 1. — *Means of vessel numbers for 10 fields according to the groups: atrophic, proliferative, G1, G2, G3 adenocarcinomas of the endometrium - mean of means and results of statistical analysis.*

Case	Atrophic	Proliferative	G1 Adenoca	C2 Adenoca	G3 Adenoca
1	10.3	11.2	14.6	19.2	28.6
2	12.2	7.9	17.9	13.9	24.5
3	10.8	15.2	14.9	22.8	23.7
4	14.9	14.8	26.2	23.0	23.8
5	11.9	19.0	14.8	28.4	17.6
6	15.7	10.4	11.8	21.5	19.9
7	11.9	11.7	12.8	17.0	32.8
8	7.8	15.1	16.0	9.7	19.9
9	10.3	11.1	13.0	27.6	19.1
10	7.5	15.7	10.5	25.9	17.0
11	13.8			11.3	24.0
12				14.3	20.9
13				12.9	
Mean	11.6	13.2	15.3	19.0	22.7

Box plots of the mean number of vessels per group



We reject the equalness of the means in the five groups ($p < 0.0001$).

Table 2. — *Descriptive levels of the comparisons of the groups, 2 by 2.*

Group 1	Group 2	p
G1	G2	0.5305
	G3	**0.0039**
	atrophic	0.6869
	proliferative	1.0000
G2	GIII	0.5268
	atrophic	**0.0019**
	proliferative	**0.0366**
G3	atrophic	**< 0.0001**
	proliferative	**0.0001**
atrophic	proliferative	1.0000

There were no significant differences between the means of G1 and G2 ($p = 0.5305$); G1 and atrophic ($p = 0.6869$); G1 and proliferative ($p = 1.0000$); G2 and G3 ($p = 0.5268$); atrophic and proliferative ($p = 1.0000$).
The differences between the following groups were significant: G2 and atrophic ($p = 0.0019$); G2 and proliferative ($p = 0.0366$); G3 and G1 ($p = 0.0039$); G3 and atrophic ($p < 0.0001$); G3 and proliferative ($p = 0.0001$).

Salvesen *et al.* [18] measured microvessel density and pointed out that when the number was higher than 68 mm^2, there was a decrease in five-year survival and that the density was increased in Stages III and IV. They did not observe differences regarding type and histologic grade. Szymanski *et al.* [19] noted that preinvasive disease presented less angiogenesis and that the histologic grade of differentiation in carcinoma was not subject to its influence. Increased vascular density was an adverse factor regarding survival.

Wagatsuma *et al.* [13] found a relationship between angiogenesis of little differentiated Stage III and IV endometrial adenocarcinoma and positive lymph nodes and less survival.

In our study, the vessel number in G3 endometrial carcinoma was statistically higher than that in atrophic, proliferative endometria and G1 carcinoma. This leads us to suppose that the higher angiogenesis intensity is *per se* a factor of poor prognosis.

The number of vessels in G2 endometrial carcinoma was significantly higher that that in atrophic and proliferative endometrium and equal to G1 and G3 carcinoma. We see that G2 tumor has a well-established angiogenic behavior in normal endometrium whereas in pathologic endometrium, its behavior is uncertain. It may be similar to that of the G1 tumor, with good prognosis, as observed in cases 2, 8, 11 and 13, or be similar to the G3 tumor (cases 5, 9 and 10). Probably other factors should be considered in these cases (Table 1).

The angiogenic behavior of G1 carcinoma in proliferative and atrophic endometria was similar, which, as is known, confers a good prognosis for G1 tumors.

We believe that solid, growth of G2 and G3 tumors is an important factor in the production of angiogenic molecules. G1 tumors produce few angiogenic molecules. Consequently, the less differentiation the more angiogenesis and vice-versa.

These results show that angiogenesis is an important prognostic marker for carcinoma of the endometrium. It could be included in pathological anatomy reports, which is feasible in Brazil. Thus, more radical management as well as use of anti-angiogenic drugs should be adopted for strongly angiogenic tumors.

Folkman [20], already in 1971, speculated about "anti-angiogenesis" and its therapeutic implications; he considered them as a form cancer therapy. Thus the start of anti-angiogenesis could occur by the production of tumor angiogenesis anti-factor antibodies.

Careful investigation of this issue could reveal fundamental facts about tumor cell growth and its inhibition. As stated by Folkman, who thoroughly studied angiogenesis, we could act with the purpose of letting the tumor die of hunger.

Conclusion

We conclude that slighly differentiated tumors present greater angiogenesis than normal endometrium and well-differentiated carcinoma. Moderately differentiated adenocarcinoma has been shown to be more angiogenic than atrophic and proliferative endometrium.

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