Staining characterization by immunohistochemistry of tumor cancer antigen in patients with endometrial cancer

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Summary

Objective: The aim of the present study was to evaluate the correlation between the pattern of cancer antigen (CA-125) expression by immunohistochemistry and pathologic parameters in endometrial carcinoma. *Methods:* Seventy-two cases of primary uterine carcinomas, 66 endometrioid carcinoma and six non-endometrioid, were analyzed by immunohistochemistry for CA-125 expression. Myometrial invasion was evaluated by assessing the percentage of myometrial thickness involved at the site of deepest tumor extension. Presence or absence of vascular invasion, cervical stromal invasion, lymph node metastasis, and ovarian metastasis from endometrial cancer was assessed. Tumor size was measured by the maximum diameter. Peritoneal washings were examined for the presence or absence of cancer cells. The extent and location of immunohistochemical staining for CA-125 was assessed according to the immunoreactive score (IRS) that evaluated the proportion of cells expressing CA-125 and the intensity of staining. Percentage of the cancer area stained in high-power fields was examined. Staining intensity was graded as 0 (negative), 1 (weak), 2 (modarate), and 3 (strong); percentage of positive cells examined was scored as 0 (negative), 1 (< 10%), 2 (11-50%), 3 (51-80%), and 4 (> 80%). The two scores were multiplied and the IRS (values from 0-12) was determined: 0 as negative, values 1-3 as weak, values 4,6 as positive, and multiplication values 8, 9, 12 as strongly positive. *Results:* Of the 72 patients, 66 (91.7%) had endometrioid carcinoma and six (8.3%) had non-endometrioid carcinoma. Of the seventy-two patients, 38 (52.7%) had surgical Stage I disease, 12 (16.7%) had Stage II, 16 (22.2%) had Stage III disease, and six (8.4%) had Stage IV disease. Ten (14.7%) of the 68 patients who underwent lymphadenectomy had positive nodes. Nine (12.5%) of 72 patients had positive peritoneal cytologic findings. Forty-eight (66.7%) patients had deep myometrial invasion, 29 (40.3%) had lymphovascular invasion, 25 (34.7%) had cervical stromal involvement, and 12 (16.7%) had ovarian metastasis. Twenty-eight (38.9%) patients had grade 1, 25 (34.7%) had grade 2, and 19 (26.4%) had grade 3 disease. Fifty-nine (81.9%) patients had a tumor size greater than 2 cm. Negative staining was noted in ten (13.9%) tumors, weakly positive in 23 (31.9%), positive in 16 (22.3%) and strongly positive in 23 (31.9%). Grade 0 intensity was found in nine (12.5%) tumors, grade 1 in 16 (22.3%), grade 2 in 21 (29.16), and grade 3 in 26 (36.11). Negative percentage of positive cells examined was found in nine (12.5%) tumors, < 10% in 19 (26.38%), 11-50% in 18(25%), 51-80% in 13 (18.05%), > 80 in 13 (18.05%). We found that intensity, percentage of positive stained cells, and IRS correlated with deep myometrial invasion ($p < 0.05$). *Conclusions:* Intensity, percentage of positive stained cells for CA-125, and IRS can be used to determine the need for abdominal hysterectomy and lymphadenectomy for staging in endometrial cancer.

Key words: Endometrial carcinoma; CA-125; Immunohistochemistry.

Introduction

The tumor antigen CA-125 is expressed in the derivates of epithelium of müllerian origin (fallopian tube, cervix, and endometrium) and mesothelial cells lining (coelomic epithelium derivates) the peritoneum, pleura, and pericardium [1]. The frequency and tissue distribution of CA-125 expression in normal, hyperplastic, and neoplastic endometrium has been reported [2-4]. The endometrium has been shown to express high levels of CA-125 with positive immunostaining [5] and high cytosolic tissue concentrations that are approximately 20-fold higher than normal ovarian tissue [6]. Weintraub *et al.* reported that CA-125 is an exocrine product of endometrial epithelial cells and is normally prevented from entry into the circulation. The plasma levels may be of endometrial origin only when the membrane barriers are damaged [7]. The use of CA-125 as a single diagnostic and prognostic tool for endometrial carcinoma has been restricted by the fact that it is produced by the peritoneum, normal cycle endometrium, and gestational endometrium. However, the pattern of CA-125 staining may be useful in distinguishing between low- and high-risk endometrial malignancies. Our study was designed to assess the intensity and distribution of staining correlation between pathologic parameters in endometrial cancer.

Materials and Methods

The study population consisted of 72 patients who were primarily treated by total abdominal hysterectomy and salpingo-oophorectomy, and bilateral pelvic and paraaortic lymphadenectomy for International Federation of Gynecology and Obstetrics (FIGO) Stage I-IV endometrial carcinoma between January 2002 and December 2005 at Ankara Oncology Education and Research Hospital.

Surgical procedures for endometrial cancers in our institution are defined as extended surgical staging consisting of washing cytology, total abdominal hysterectomy and bilateral salpingo-oophorectomy, with full pelvic and paraaortic lymphadenectomy. The tumors were surgically staged according to the FIGO staging system [8]. Endometrioid adenocarcinomas were graded according to FIGO classification; undifferentiated carcinoma, clear cell carcinoma, and papillary serous carcinoma were classified as grade 3 because the prognosis in these histo-

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logic types is reported to be poor [9]. The histologic classification recommended by the World Health Organization Classification of Tumors was used [10]. Histologic types of endometrial cancer in the present study included endometrioid carcinoma, undifferentiated carcinoma, clear cell carcinoma, and papillary serous carcinoma; they were categorized as endometrioid and non-endometrioid (clear cell carcinoma, papillary serous carcinoma, undifferentiated carcinoma) groups. Formalin-fixed hematoxylin and eosin-stained 5-µm slides of tumor tissue from the same patients were performed again and revised by two senior pathologists to verify the diagnosis. Myometrial invasion was evaluated by assessing the percentage of myometrial thickness involved at the site of deepest tumor extension. Presence or absence of vascular invasion, cervical stromal invasion, lymph node metastasis, and ovarian metastasis from endometrial cancer was assessed. Tumor size was measured by its maximum diameter. Peritoneal washings were examined for the presence or absence of cancer cells.

Immunohistochemistry of CA-125

Briefly, 4-µm unstained sections from each of all 72 patients were prepared for immunohistochemical staining. After deparaffinization and rehydration, sections were placed in 3% hydrogen peroxide for 15 minutes to inactivate endogenous peroxidase, and then autoclaved at 121ºC in citrate buffer (10 mM, pH 6.0) for six minutes for antigen activation. After cooling at room temperature for 30 minutes the specimens were nonspecifically blocked by incubation with UltraV block for five minutes and endogenous avidin/biotin blocking kit for ten minutes each at room temperature. Sections were then incubated with anti CA-125 mouse monoclonal antibody (NeoMarkers, 1/20, Ab-1, Clone OV 185:1) for two hours at room temperature. Immunohistochemical staining was performed using a Standard avidin- biotin- peroxidase (Lab Vision); 3,3´-diaminobenzidine was used as the chromogen. All sections were counterstained with Mayer's hematoxylin. Sections of ovarian serous carcinoma were used as positive controls.

The extent and location of immunohistochemical staining for CA-125 were assessed according to the IRS that evaluated the proportion of cells expressing CA-125 and intensity of staining [11]. The percentage of the cancer areas stained in high-power fields was examined. Staining intensity was graded as 0 (negative), 1 (weak), 2 (moderate), and 3 (strong); percentage of positive cells examined was scored as 0 (negative), 1 (< 10%), 2 (11-50%), 3 (51-80%), and 4 (> 80%). The two scores were multiplied and the IRS (value from 0-12) was determined: 0 as negative, 1-3 values as weak, 4,6 as positive, and values 8,9,12 as strongly positive.

Statistical Analyses

Statistical analyses were performed using the "SPSS 10.05 for Windows" computer program. All variables were analyzed statistically as categorical covariates. Several risk factors were evaluated in univariate analysis for any association with intensity, distribution, and IRS of CA-125 immunohistochemical staining; p values less than 0.05 derived from two-tailed tests were considered significant.

Results

Seventy-two women underwent surgery for endometrial adenocarcinoma during the 48-month period. Ages ranged from 35 to 87 years, with a mean age of 58.59 ± 9.87 years.

Four patients (5.6%) with grade 1 or grade 2 tumors underwent total abdominal hysterectomy and bilateral salpingo-oophorectomy with collection of peritoneal fluid for cytologic testing. Sixty-eight (94.4%) patients underwent total abdominal hysterectomy and bilateral salpingo-oophorectomy and bilateral pelvic together with periaortic lymphadenectomy, biopsies or debulking, or different combinations of these. All patients had peritoneal fluid collected for cytologic testing.

Table 1 shows the clinicopathologic profile of patients with endometrial carcinoma. Of the 72 patients, 66 (91.7%) had endometrioid carcinoma and six (8.3%) had non-endometrioid carcinoma. Of the seventy-two patients, 38 (52.7%) had surgical Stage I disease, 12 (16.7%) had Stage II, 16 (22.2%) had Stage III disease, six (8.4%) had Stage IV disease. Among patients with Stage I disease, one had Stage IA, 15 had Stage IB, and 22 had Stage IC disease. Ten (14.7%) of the 68 patients who underwent lypmhadenectomy had positive nodes. Nine (12.5%) of the total 72 patients had positive peritoneal cytologic findings. Forty-eight (66.7%) patients

Table 1. — *Clinicopathologic profile of patients with endometrial carcinoma.*

Factor	No. of cases (%) n: 72
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Age (years)	58.59 ± 9.87 (35-87)
FIGO Stage	
I	38 (52.7)
II	12 (16.7)
III	16 (22.2)
IV	6 (8.4)
Tumor grade	
Well differentiated	28 (38.9)
Moderately differentiated	25 (34.7)
Poorly differentiated	19 (26.4)
Myometrial ınvasion	
None	1 (1.4)
< 1/2	23 (31.9)
≥ 1/2	48 (66.7)
Lymphovascular invasion	
No	43 (59.7)
Yes	29 (40.3)
Histologic subtype	
Endometrioid	66 (91.7)
Non-endometrioid	6 (8.3)
Ovarian metastasis	
No	60 (83.3)
Yes	12 (16.7)
Lymph-node metastasis*	
No	58 (85.3)
Yes	10 (14.7)
Washing cytology	
Negative	63 (87.5)
Positive	9 (12.5)
Cervical involvement	
No	47 (65.3)
Yes	25 (34.7)
Tumor diameter (cm)	
≤ 2	13 (18.1)
> 2	59 (81.9)

* No. of cases: 68.

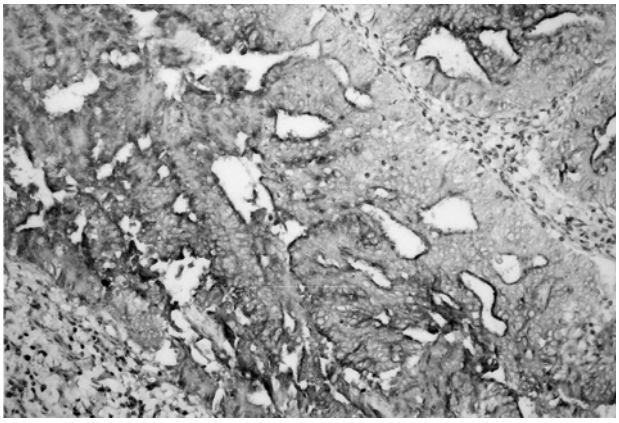

Figure 1. — Positive staining for CA-125 is prominent at the apical cell surface (x 200).

had deep myometrial invasion, 29 (40.3%) had lymphovascular invasion, 25 (34.7%) had cervical stromal involvement, and 12 (16.7%) had ovarian metastasis. Twenty-eight (38.9%) patients had grade 1, 25 (34.7%) had grade 2, and 19 (26.4%) had grade 3 disease. Fifty-nine (81.9%) patients had a tumor with size greater than 2 cm.

Intensity varied from trace to very strong. Immunostaining was confined to the glandular epithelial cells and was present in the lumen, apical border (Figure 1). No staining of the basal border was observed in any case of endometrioid carcinoma.

Negative staining was noted in ten (13.9%) tumors, weakly positive in 23 (31.9%), positive in 16 (22.3%) and strongly positive in 23 (31.9%).

Grade 0 intensity was found in nine (12.5%) tumors, grade 1 in 16 (22.3%), grade 2 in 21 (29.16), and grade 3 in 26 (36.11).

A negative percentage of positive cells examined were found in nine (12.5%) tumors, < 10% in 19 (26.38%), 11-50% in 18 (25%), 51-80% in 13 (18.05%), and > 80 in 13 (18.05%).

We found that intensity, percentage of positive stained cells and IRS correlated with deep myometrial invasion ($p < 0.05$) (Table 2).

Table 2. — *Relationship between intensity, distribution, and immunoreactivescore (IRS) of immunohistochemical CA-125 staining and pathologic variables of prognostic significance in patients with endometrial carcinoma.*

Factor	Intensity of staining (≥ 1 grade) No. (%)	p value	Distribution of staining (1-100%) No. (%)	p value	IRS (≥ 1) No. (%)	p value
Age						
≥ 50	10 (90.9)		10 (90.9)		10 (90.9)	
> 50	53 (86.9)	0.584	53 (86.9)	0.584	52 (85.2)	0.524
Stage						
I-II	46 (90.2)		46 (90.2)		45 (88.2)	
III-IV	17 (81)	0.240	17 (81)	0.240	17 (81)	320
Tumor Size						
≤ 2 cm	11 (84.6)		11 (84.6)		11 (84.6)	
> 2 cm	52 (88.1)	0.514	52 (88.1)	0.514	51 (86.4)	0.578
Histology						
Endometrioid	57 (86.4)		57 (86.4)		56 (84.8)	
Non-endometr.	6 (100)	0.435	6 (100)	0.435	6 (100)	0.393
Grade						
1	25 (89.3)		25 (89.3)		25 (89.3)	
2, 3	38 (86.4)	0.509	38 (86.4)	0.509	37 (84.1)	
Myometrial invasion						
< 1/2	18 (75)		18 (75)		17 (70.8)	
≥ 1/2	45 (93.8)	0.032	45 (93.8)	0.032	45 (93.8)	0.013
Cervical involvement						
No	41 (87.2)		41 (87.2)		40 (85.1)	
Yes	22 (88)	0.620	22 (88)	0.620	22 (88)	0.519
Ovarian metastasis						
No	53 (88.3)		53 (88.3)		52 (86.7)	
Yes	10 (83.3)	0.466	10 (83.3)	0.466	10 (83.3)	0.529
Washing cytology						
Negative	55 (87.3)		55 (87.3)		54 (85.7)	
Positive	8 (88.9)	0.688	8 (88.9)	0.688	8 (88.9)	0.636
LVI						
No	37 (86)		37 (86)		36 (83.7)	
Yes	26 (89.7)	0.471	26 (89.7)	0.471	26 (89.7)	0.363
Lymph-node metastasis						
No	51 (87.9)		51 (87.9)		50 (86.2)	
Yes	8 (80)	0.395	8 (80)	0.395	8 (80)	0.454

Discussion

CA-125 has been localized by immunohistochemical techniques to the apical surface of secretory endometrial glands. Secretions in the endometrial gland lumina are intensely positive [12]. No staining of normal stromal or decidual cells has been demonstrated. CA-125 immunostaining in endometrial cancers has been reported in few studies [4].

The distribution and expression of CA-125 suggests that the antigen was a secretory product of normal endometrium [3]. CA-125 was expressed by the glandular tissue in the endometrial carcinomas and accumulated on the apical cell surface and in cytoplasm. Tumors with solid features had a lesser glandular component and had less CA-125 expression. Endometrioid grade 1 adenocarcinomas were more likely to express CA-125. Tumors of the clear cell and papillary serous histologic types contributed disproportionately to the positive staining seen in grade 2 and grade 3 tumors [3]. In our study, 25 of 28 (89.2%) endometrioid grade 1 tumors were CA-125 positive and the CA-125 immunostaining was intense in low-grade and early-stage endometrial carcinoma.

Berchuck *et al.* evaluated CA-125 expression in endometrial adenocarcinomas using a histologic score that evaluated the proportion of cells expressing CA-125 and the intensity of staining [13]. A high CA-125 score correlated with the presence of lymph-node metastases and increased metastatic potential. In our study, positive CA-125 staining (IRS > 0) significantly correlated with deep myometrial invasion ($p < 0.05$).

Nu*r et al.* [14] showed that patients with endometrial carcinoma and solitary metastases to the ovaries with lymph-node involvement had lower IRS. Thus in cases of endometrial carcinoma as the tumor becomes aggressive, it loses its functional capabilities. The source of elevated serum CA-125 levels in such patients may be due to secretions by mesothelial cells rather than neoplastic endometrial cells.

The prognosis of endometrial carcinoma has major implications on patient management. In our study, intensity and distribution of staining correlated with deep myometrial invasion ($p < 0.05$). Intensity, percentage of positive stained cells, and IRS can be used to determine the need for abdominal hysterectomy and lymphadenectomy for staging in endometrial cancer. Further studies are required to correlate this hypothesis.

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