Immunohistochemical Expression of CA 125 in Endometrial Adenocarcinoma:
Correlation of Antigen Expression with Metastatic Potential


ABSTRACT

Immunohistochemical localization of CA 125 using murine monoclonal antibody OC 125 was performed on fresh frozen tissue from 44 endometrial adenocarcinomas and 26 benign endometria. Immunohistochemical evaluation incorporated both intensity and distribution of staining (CA 125 HSCORE). Thirty-seven cancers (84%) and 23 benign endometria (88%) expressed immunohistochemically detectable CA 125. Staining was confined to epithelial cells and was present both on the cell membrane and in the cytoplasm. Among the 44 endometrial cancers, CA 125 HSCORE did not correlate with histological grade, depth of myometrial invasion or estrogen/progesterone receptor levels. Following surgical staging, 13 patients (30%) were found to have extraperitoneal metastasis. The median CA 125 HSCORE of patients with metastatic disease (2.25) was significantly higher than that of patients with disease confined to the uterus (0.6) \( (P < 0.001) \). In addition, high CA 125 HSCORE also correlated with the presence of lymph node metastasis \( (P < 0.001) \). The results of this study suggest that high CA 125 expression by endometrial adenocarcinomas is associated with increased metastatic potential.

INTRODUCTION

OC 125 is a murine monoclonal IgGl antibody that was raised against a human ovarian serous cystadenocarcinoma cell line (1). CA 125, the antigenic determinant defined by OC 125, is expressed by most human epithelial ovarian cancers (1). It also is shed from the cell surface and CA 125 can be measured in serum using an immunoradiometric assay that employs OC 125 (2). Greater than 80% of patients with untreated nonmucinous epithelial ovarian cancer have elevated serum CA 125 levels, and serial serum CA 125 levels reflect the response of residual tumor to chemotherapy more accurately than physical examination or radiographic studies (3).

Although CA 125 is not detectable in normal ovarian epithelial cells, CA 125 can be demonstrated in the epithelial lining of the endocervix, endometrium and fallopian tubes of adult women (4). In addition, both cancers that arise in these sites and endometriosis often are associated with elevated serum CA 125 levels (5, 6). Adenocarcinoma of the endometrium is the most prevalent gynecologic malignancy and several studies of serum CA 125 levels in patients with this disease have been performed (7, 8). These studies have shown that, although serum CA 125 levels usually are normal when tumor is confined to the uterus, most patients with metastatic disease have elevated serum CA 125 levels. Elevation of serum CA 125 in patients with metastatic disease is thought to be due to the presence of a relatively larger volume of tumor or increased access of antigen to the circulation due to vascular invasion.

In this study, we have used an immunohistochemical technique to examine directly the expression of CA 125 by normal endometrium and endometrial adenocarcinomas. All of the patients whose tumors were studied underwent hysterectomy, and in most cases complete surgical staging also was performed. In addition, estrogen and progesterone receptor levels were determined in all of the primary tumors. We have utilized this clinical information to study the relationship between CA 125 expression and known prognostic factors in endometrial cancer.

MATERIALS AND METHODS

Patients. All of the patients in this study with endometrial cancer underwent primary surgical treatment at Duke University between 1980 and 1986. During these years, fresh tissue was frozen in liquid nitrogen when material still was available after an adequate amount of tumor was submitted for histological examination and for biochemical steroid receptor measurement. When this study was initiated, tissue from 54 tumors was available. In 10 cases, however, the amount of tissue remaining was inadequate to allow assessment of CA 125 expression.

All histological material from each case was reviewed by a single pathologist (K. S. M.); and the histological type, histological grade, and depth of myometrial invasion of each cancer was determined. Histological grade was specified as either Grade 1, well differentiated; grade 2, moderately differentiated with partly solid areas; or Grade 3, predominantly solid or undifferentiated, according to International Federation of Gynecology and Obstetrics (F.I.G.O.) staging criteria (9). The depth of myometrial invasion was specified as either: 1, noninvasive tumors and those tumors invading the inner one-third of the uterine wall; 2, tumors with a maximal depth of invasion in the middle one-third of the uterine wall; or 3, tumors invading into the outer one-third of the uterine wall.

The estrogen receptor and progesterone receptor status of each tumor was determined using immunohistochemical techniques that employ murine monoclonal antibodies raised against these receptors (H222 and B39). These techniques have been described previously (10, 11) and they have been shown to correlate closely with biochemical estrogen receptor and progesterone receptor assays. The threshold for positivity in the immunohistochemical assays has been set at a level that corresponds to greater than or equal to 10 femtomole of specific binding per milligram of tissue protein in the biochemical assays.

Normal uterine tissues, including endometrium and myometrium, from 26 women were frozen following hysterectomy. All of these uteri were removed at Duke University in 1987 for the treatment of benign gynecological diseases. All 21 women who were of reproductive age were having regular cyclic menses. Endometrial dating was assessed using histological criteria of Noyes et al. (12). Five women were postmenopausal. None of these women had received hormonal therapy in the 2 months prior to hysterectomy.

Immunohistochemical Localization of CA 125. Tissue samples were removed from uteri immediately after surgical excision and stored in phosphate buffered saline (PBS) at \(-70^\circ\)C. Tissue samples were thawed and refrozen in Tissue Tek OCT compound (Ames division, Miles laboratories, Elkhart, Indiana) and 6-µm thick cryosections were mounted on plain glass slides. Slides were air dried for 1 h and then incubated for 10 min in phosphate buffered saline with 0.3% hydrogen peroxide to neutralize endogenous peroxidase. The slides were fixed in acetone for 10 min at room temperature and then rehydrated in phosphate buffered saline.

Immunohistochemical staining was performed with the Vectastain...
CA 125 AND METASTASIS OF ENDOMETRIAL ADENOCARCINOMA

Immunohistochemical localization of CA 125 was evaluated using serial sections. First, a section stained with hematoxylin & eosin was examined to evaluate the histology. Then, a negative control slide stained with JO, a murine monoclonal antibody raised against a hepatitis virus, was examined to assess nonspecific staining. Finally, the slide in which OC 125 was used as the primary antibody was examined. The proportion \( P_i \) of tumor cells expressing detectable CA 125 was estimated after taking into account the percentage of tumor cells present. In cases in which staining was greater than control, the intensity \( i \) was judged as 1+ (light staining) or 2+ (heavy staining). These two indices of CA 125 expression were used to calculate the CA 125 HSCORE (histological score). The CA 125 HSCORE was derived by summing the proportion of cells staining at each intensity multiplied by the intensity of staining.

\[
CA 125 \text{ HSCORE} = \sum P_i (i + 1)
\]

where \( i = 1, 2 \) and \( P_i \) varies from 0.0 to 1.0. CA 125 HSCOREs ranged from a minimum of zero in cases with no staining to a maximum of 3.0 in cases in which all of tumor cells stained with maximal intensity. The CA 125 HSCORE was determined by two sets of independent observers. Differences of greater than 10% occurred in 18% of cases and were resolved by consensus.

Statistics. The statistical analysis of data in this study was performed using either the \( x^2 \) or Mann-Whitney tests (13).

RESULTS

Immunohistochemically detectable CA 125 was present in 88% of 26 benign endometria. CA 125 expression was seen only in endometrial epithelial cells. Among the 21 endometria obtained from premenopausal patients, 13 were proliferative and eight were secretory. Staining greater than control was observed using OC 125 in all but one specimen. The percentage of glandular cells expressing CA 125 was 100% in 14 cases, 90% in two cases, 75% in one case, and 50% in three cases. The median CA 125 HSCORE in these 21 cases was 2.7.

Although staining usually was most intense on the luminal surface of cells, staining often was seen in the basal cytoplasm as well. In Fig. 1, a normal secretory endometrium stained with OC 125 is shown along with its negative control. No quantitative or qualitative difference in CA 125 expression could be appreciated between samples from various phases of the menstrual cycle. Among five postmenopausal patients with atrophic endometrium, CA 125 expression was observed in three cases.

The median age of the 44 women with endometrial cancer was 66 years. All of the patients underwent clinical staging preoperatively including fractional D&C. The clinical stage of disease was assigned prior to surgery according to criteria outlined in the F.I.G.O. staging system (9). Thirty-five patients had Stage I (corpus only), seven patients had Stage II disease (corpus and cervix), one patient had Stage III disease (metastatic disease confined to pelvis), and one patient had Stage IV disease (metastatic disease outside the pelvis). All 44 patients underwent exploratory laparotomy, total abdominal hysterectomy, and bilateral salpingo-oophorectomy. Pelvic peritoneal cytology was performed in 42 cases and selective pelvic and paraaortic lymphadenectomy was performed in 31 cases. Eleven patients with clinical Stage I and II disease (confined to the uterus) were found to have occult extrauterine disease (surgical Stage III/IV) after surgical staging. Patients with malignant peritoneal cytology were not considered surgical Stage III or IV unless there also was histological evidence of metastatic disease.

Thirty-seven endometrial cancers (84%) were found to express immunohistochemically detectable CA 125 while in seven tumors (16%) staining was not greater than control. Five of seven CA 125 negative tumors were reactive with either or both the antiestrogen receptor (H222) or antiprogesterone receptor (B39) monoclonal antibodies. The preservation of these heat-labile antigens is suggestive that failure of these seven tumors to react with OC 125 was not due to poor preservation of tissues. In endometrial cancer specimens, like normal endometrium, immunohistochemically detectable CA 125 was observed only in glandular cells. CA 125 expression was strongest at the apices of malignant glandular cells on the luminal surface when tumor cells were forming glands. Cytoplasmic staining was also noted in most cases as well. In Fig. 2, a CA 125-positive tumor is demonstrated along its negative control. Several tumor specimens contained small areas of benign endometrium that usually stained intensely with OC 125. Areas of benign endometrium were not included in the calculation of the CA 125 HSCORE.

All of the cancers studied were endometrioid adenocarcinomas. None were adenosquamous, clear cell, or papillary serous
type. Table 1 demonstrates the relationship between histological grade and depth of myometrial invasion and the presence of metastatic disease (surgical Stage III and IV). Although Grade 3 tumors had the highest incidence of metastatic disease, the relationship between grade and metastatic disease was not statistically significant in this study ($\chi^2 = 1.45; P = 0.5$). There was, however, a significant relationship between the depth of myometrial invasion and the presence of metastatic ($\chi^2 = 6.06; P < 0.05$). There was no significant correlation between CA 125 HSCORE and histological grade or depth of invasion.

In Table 2; the relationship between CA 125 HSCORE and lymph node metastases is examined. Thirty-one of 44 patients underwent lymph node sampling. In two patients, lymph node sampling was not performed because of the presence of extrauterine disease at other sites. In eleven other patients, node sampling was omitted either because the patient was at increased risk of surgical complications due to concomitant medical problems or because the tumor was found to be well differentiated and only superficially invasive at the time of surgery. Among these 31 patients, eight (26%) were found to have nodal metastases. There was a significant correlation between high CA 125 HSCORE and the presence of lymph node metastases ($P < 0.001$). The median CA 125 HSCORE of patients with nodal metastases was 2.03 while the median CA 125 HSCORE of patients with negative nodes was 1.0. Eight of sixteen (50%) patients with a CA 125 HSCORE greater than 1.0 had nodal metastases while none of 15 patients with a CA 125 HSCORE less than or equal to 1.0 had nodal metastases.

In Fig. 3, the relationship between CA 125 HSCORE and the presence of extraterine metastasis (surgical Stage III and IV disease) is shown. Among the 44 patients in this study, 13 (30%) had extraterine disease. There was a significant correlation between high CA 125 HSCORE and the presence of metastatic disease ($P < 0.001$). The median CA 125 HSCORE of the 13 patients with extraterine disease was 2.25 while the median CA 125 HSCORE of the 31 patients without extraterine disease was 0.6. If the 11 patients with surgical Stage I or II disease who did not undergo lymphadenectomy are excluded, the median CA 125 HSCORE of the patients with disease confined to the uterus is 0.70. Fifty-six % of 23 patients with a CA 125 HSCORE greater than 1.0 had extraterine disease while none of 21 patients with a CA 125 HSCORE less than or equal to 1.0 had extraterine disease.

The relationship between CA 125 HSCORE and the findings of pelvic peritoneal cytology is examined in Table 3. Pelvic peritoneal cytology was not performed in two cases. Among the 42 patients in whom cytology was performed, 13 (31%) were found to have malignant cells present. There was a significant correlation between high CA 125 HSCORE and positive pelvic peritoneal cytology ($P < 0.01$). The median CA 125 HSCORE of patients with distant metastases was 2.25 while the median CA 125 HSCORE of patients without distant metastases was 0.6. Fifty-seven % of 22 patients with a CA 125 HSCORE greater than 1.0 had distant metastases while none of 21 patients with a CA 125 HSCORE less than or equal to 1.0 had distant metastases.
of patients with positive cytology was 1.8 while the median CA 125 HSCORE of patients with negative cytology was 0.8. The relationship between pelvic peritoneal cytology and CA 125 HSCORE is not significant, however, if the six patients in whom positive cytology was the only evidence of extratumoral disease are examined separately from the other seven patients who had histological evidence of extratumoral disease in addition to positive cytology. Among the six patients with positive cytology only, three had a CA 125 HSCORE less than 1.0. These data are consistent with the widely held belief that tumor cells which reach the peritoneal cavity have been shed through the fallopian tube and will not necessarily establish metastases.

Among the 44 patients in this study, 50% were found to have estrogen receptor positive tumors and 49% were found to have progesterone receptor positive tumors. Both estrogen receptor and progesterone receptor positivity were found to be related inversely to histologic tumor grade. For estrogen receptor ($\chi^2 = 6.86; P < 0.05$) and for progesterone receptor ($\chi^2 = 13.1: P < 0.005$). Among Grade 1 tumors, 79% were estrogen receptor positive and 86% were progesterone receptor positive. Among Grade 2 tumors, 40% were estrogen receptor positive and 40% were progesterone receptor positive. Among Grade 3 tumors, 33% were estrogen receptor positive and 20% were progesterone receptor positive. In this study, estrogen receptor status was not predictive of the presence of metastatic disease. Thirty-five % of patients with estrogen receptor-positive tumors had metastatic disease compared to 23% of patients with estrogen receptor negative tumors. Although patients with progesterone receptor-negative tumors had a higher incidence of metastatic disease (39%) than patients with progesterone receptor-positive tumors (19%), the difference was not statistically significant. In addition, the combined estrogen receptor/progesterone receptor status also was not predictive of the presence of metastatic disease. Finally, there was no correlation between estrogen and progesterone receptor status and CA 125 HSCORE.

**DISCUSSION**

CA 125 was the first human epithelial ovarian cancer tumor-associated antigen to be defined by a murine monoclonal antibody (1). Although CA 125 has not been completely characterized, it is known to be a determinant expressed on a high molecular weight glycoprotein. Several immunoreactive forms of CA 125 have been found that range from $\text{Mr} = 200,000$ to greater than 1,000,000 (14). Immunohistochemical studies have revealed that CA 125 is detectable on the apical surface of normal epithelial cells of the pleura, pericardium, peritoneum, fallopian tube, endometrium, and endocervix (4). In contrast, normal ovarian epithelium, although also derived from coelomic epithelium, does not express immunohistochemically detectable CA 125. CA 125 has been shown to be a valuable tumor marker for monitoring the status of disease in patients with epithelial ovarian cancer. Greater than 80% of patients with epithelial ovarian cancer have elevated serum CA 125 levels at diagnosis and serial serum CA 125 levels correlate with changes in disease status in most cases (2).

More recently, studies have been performed to assess the utility of CA 125 as a tumor marker in endometrial cancer (7, 8). Approximately 20-25% of patients with disease that clinically appears to be confined to the uterus (Stage I and II) have elevated serum CA 125 levels. In addition, almost 75% of patients with advanced stage disease or recurrent disease have elevated levels. Complete surgical staging of patients with clinical Stage I and II disease reveals that most patients with elevated serum CA 125 levels actually have occult metastatic disease. In one recent study (8), 57 of 58 patients with clinical Stage I and II disease who had normal serum CA 125 levels did not have evidence of occult extratumoral disease after complete surgical staging. In contrast, 20 of 23 patients with clinical Stage I and II disease who had elevated serum CA 125 levels were found to have occult extratumoral disease. Although elevation of serum CA 125 has been found to be predictive of the presence of metastatic disease, it has not been found to correlate with histological features that historically have been predictive of the presence of extratumoral disease—namely histological grade and depth of myometrial invasion (7, 8).

The patients in the present study are not a random sample of all patients with adenocarcinoma of the endometrium. The study population is drawn mostly from patients with large tumors because we only were able to save frozen tumor tissue in cases in which there was residual tissue available after a prior uterine curettage and after adequate material had been submitted for routine histological examination and biochemical steroid receptor determination. Due to this selection process, the distribution of patients with respect to histological grade and depth of myometrial invasion is not normal. Recently, a large cooperative study designed to evaluate surgical staging of endometrial cancer reported that 19% of patients had poorly differentiated tumors and that 15% of patients had tumors that invaded the outer one-third of the myometrium (15). In the present study, 32% of patients had poorly differentiated tumors and 41% had outer one-third invasion.

Due to the large proportion of patients in this study with poorly differentiated or deeply invasive tumors, the percentage of patients with metastatic disease (30%) also was higher than ordinarily would be expected in a randomly selected group of patients with endometrial cancer. As in prior studies, we found a significant relationship between the depth of myometrial invasion and the presence of metastatic disease. Although in this study, poorly differentiated cancers had the highest incidence of metastases, the relationship between histological grade and the presence of metastatic disease was not statistically significant due to the small number of patients in this study. Similarly, steroid receptor status also was less predictive of the presence of metastatic disease than has been reported previously, although receptor status did correlate with histological grade (16).

In the only prior immunohistochemical study of CA 125 expression in adenocarcinoma of the endometrium, Duk et al. reported reactivity in 100% of 20 cases (7). Our results do not confirm the findings of this previous report. Although we too initially observed reactivity with OC 125 in all cases, staining also was seen on the negative control slides that had been exposed to JO rather than OC 125. When tissue sections were preincubated in 0.3% hydrogen peroxide to neutralize endogenous peroxidase, the slides that were exposed to JO were nonreactive as were 16% of the slides exposed to OC 125. Since five of the seven tumors that failed to react with OC 125 did react with either the antiestrogen or anti-progesterone receptor monoclonal antibodies, it is unlikely that failure to react with OC 125 was due to poor preservation of tissues. Reactivity of
100% of cases with OC 125 in the study of Duk et al. may have been due to the presence of endogenous peroxidase that was not neutralized. This probably was not noted because a negative control antibody apparently was not used.

Previously, it has been thought that elevated serum CA 125 levels are present only in patients with metastatic endometrial cancer because of the presence of a larger volume of tumor in these patients. Alternatively, it has been suggested that elevation of serum CA 125 levels is due to increased access of CA 125 to the circulation as a result of vascular invasion by metastatic tumors. In contrast, the data presented in this study is suggestive that elevated serum CA 125 levels in patients with metastatic disease may be due at least in part to increased expression of CA 125 by individual tumor cells. The regulation of CA 125 production and its route of entry into the circulation remain poorly understood, however, in both normal individuals and those with cancer. Since most normal individuals have small amounts of CA 125 in serum (2), it has been proposed that CA 125 produced by normal glandular cells probably reaches the circulation via lymphatics. Additional studies are needed to clarify further the factors that regulate serum CA 125 levels.

Although in this study a high CA 125 HSCORE correlated with the presence of metastatic disease, it remains unclear whether expression of CA 125 is involved directly in the complex biologic events that culminate in tumor metastasis or whether these are merely coincident biological phenomenon. Evidence does exist, however, that is suggestive that changes in the composition of cell surface glycoproteins are related to the ability of tumors to metastasize. It has been shown, for example, that the metastatic potential of B16 mouse melanoma variants is related to the amount of glycosylation of cell surface glycoproteins (17). Since not all tumors with the highest CA 125 HSCORE had metastatic disease, high CA 125 expression may represent one of a number of biological phenomenon that may correlate with the development of tumor metastases.

Adenocarcinoma of the endometrium is the most common gynecological malignancy, and approximately 85% of patients have clinical Stage I or II disease at diagnosis (9). The results of this study are suggestive that direct evaluation of CA 125 expression by endometrial adenocarcinomas may allow more accurate assessment of the risk of occult metastatic disease than can be achieved using traditional histological prognostic factors. In this preliminary study, CA 125 HSCORE was more highly predictive of the presence or absence of metastatic disease than histological grade, depth of myometrial invasion or steroid receptor status. Fifty-six % of the patients with a CA 125 HSCORE greater than 1.0 had metastatic disease while none of the patients with a CA 125 HSCORE less than or equal to 1.0 had metastases. In this study, as in prior studies of serum CA 125 levels, CA 125 HSCORE was independent of other traditional prognostic factors.

These preliminary data suggest that CA 125 HSCORE determination might permit more accurate assessment of the metastatic potential of endometrial cancers prior to surgery if this technique was performed on tissue samples obtained at D&C. More accurate identification of high risk patients, who could be considered candidates for surgical staging, would facilitate individualization of surgical treatment. Alternatively, CA 125 HSCORE determination might be used in place of surgical staging to select patients who may benefit from adjuvant therapy following hysterectomy. Presently, we are studying prospectively CA 125 HSCORE in tumor obtained at D&C, as well as serum CA 125 levels, to define further the utility of this tumor marker in the management of patients with endometrial cancer.

REFERENCES

Immunohistochemical Expression of CA 125 in Endometrial Adenocarcinoma: Correlation of Antigen Expression with Metastatic Potential

Andrew Berchuck, Andrew P. Soisson, Daniel L. Clarke-Pearson, et al.