Immunohistochemical evaluation of a new epithelial antigen, BER-EP4 in ovarian cancer: a propos of 62 cases

G. Capobianco[1], V. Marras[2], G. Battista Meloni[3], S. Dessole[1], N. Ashqar[1], C. Cherchi[1], F. Dessole[1], P.L. Cherchi[1]

[1]Gynecologic and Obstetric Clinic, [2]Institute of Pathology, [3]Institute of Radiology, University of Sassari, Sassari (Italy)

Summary

Objective: To assess the immunohistochemical expression of BerEP4, a new epithelial antigen in ovarian cancer. *Methods:* We studied 62 cases of ovarian cancer in which BerEP4, CEA and CA-125 were investigated by an immunohistochemical method. We evaluated the correlations among immunohistochemical positivity and the grading, histotype and stage of disease. *Results:* BerEP4 was positive in 45 out of 62 cases (72.58%), CA-125 in 36 out of 62 cases (58.06%) and CEA in ten out of 62 cases (16.13%). BerEP4 was present both in serous and in mucinous tumors (80.96% vs 80.77%). CA-125 was mainly expressed in serous vs mucinous tumors (66.67% vs 57.69%). CEA was more prevalent in mucinous vs serous tumors. Ber-EP4 was mainly expressed in G1 (75%) and G2 (77.27%). CA-125 was more present in G1 and G3 (both 62.50%) than G2 (50%), whereas CEA showed positivity in G1: 12.50%, G2: 22.73% and G3: 12.50%. There were no differences among the three antigens studied with regard to clinical stage. *Conclusions:* In our study Ber-EP4 was positive in 45 out of 62 cases (72.58%) of primary epithelial ovarian cancers. The presence of this antigen seemed to be related to the histotype and grading but not to clinical stage.

Key words: Epithelial antigen; BerEP4; Ovarian cancer.

Introduction

Several studies have been performed on the impact of CA-125 both for cancer screening [1-3] and follow-up [4-7] of patients treated for ovarian cancer. In Western and Northern Europe, as well as in the USA, ovarian cancer is the third most frequent cancer of the genital tract with an estimated 191,000 newly diagnosed cases per year worldwide. Because of its insidious onset, the disease is diagnosed in 70% of cases in an advanced stage. Thus ovarian cancer is the fifth leading cause of cancer-related deaths in women [8]. Furthermore the development of an effective technique for detection of early-stage ovarian cancer is an unrealized goal [9].

The clinical application of tumor markers in ovarian cancer is used also for the follow-up of women at risk of familial ovarian cancer [10, 11] as well as the diagnosis on serous peritoneal spilling of malignant potential (immunohistochemistry) [12] or for the differential diagnosis between the secundary gastrointestinal tumors (Krukenberg) and primary ovarian tumors which is done by immunohistochemistry [13].

Immunohistochemical evaluation of some tumor-associated antigens has pointed out a higher sensibility than in serologic studies of this tumor, probably because in the first stages of disease, in which immunohistochemistry is often positive, the amount of tumoral cells producing antigens is not enough to determine their intake in peripheral blood in measurable quantities. Many of the antigens are tested with immunohistochemical methods by monoclonal antibodies in ovarian cancer, in order to make a correct histopathologic diagnosis and to discriminate, in dubious cases, a primary or secondary ovarian origin.

BerEP4, an epithelial antigen recently introduced into clinical practice, which consists of two glycoproteins of 34 and 39 KD, respectively, is located both on the surface and in cytoplasm of the epithelia, with the exception of those of squamous and mesothelial origin [14]

To our knowledge, in the literature there are few data on BerEP4 antigens in ovarian cancer and in other gynecological tumors.

The aim of our study was to evaluate immunohistochemical positivity of BerEP4, CEA and CA-125 in ovarian cancer and the correlation of these antigens with grading, histotype and stage of disease.

Material and Methods

We studied 62 cases of malignant ovarian tumors and investigated the presence of BerEP4 and simultaneously of CEA and CA-125 antigens by immunohistochemistry. The study was approved by our local ethical Committee.

The mean age of the women was 57.5 years (range 19-90). The 62 cases were represented by 55 ovarian cancers: 21 serous, 26 mucinous, four clear cell and four endometrioid tumors. Then three mixed mesodermal, two yolk sac tumor, one granular and one metastatic. Tumor grades were: 1) 24 patients (38.71%); 2) 22 patients (35.48%); and 3) 16 patients (25.81%). With regard to FIGO stage, 15 had IA, four IB, seven IC, 32 III and four IV.

The immnunohistochemical study was performed at the Institute of Pathology of the University of Sassari.

The surgical specimens were fixed in 10% neutral buffered formalin and paraffin-embedded to carry out 4 μm sections; they then were stained by hematoxylin-eosin. Some sections were expelled onto glass slides before being treated with 0.1% poly-L-Lysine in order to increase their adhesiveness.

Revised manuscript accepted for publication July 28, 2011

Antigens were studied in neoplastic tissue by immunohistochemisty, using polyclonal antibodies for CEA and monoclonal antibody (Mab) for CA-125 and BerEP4. The immunodetermination was performed using the immunoperoxidase avidin-biotin complex (ABC method). Endogenous peroxidase was inhibited by the Heyderman and Neville procedure. Diaminobenzidine was the chromogen. No immune rabbit serum was used as a negative control.

With regard to the score used to quantify the positivity of the method we marked 1+ in case of weak intensity of staining, 2+ in case of stronger intensity, and 3+ in case of high intensity related to over 50% of the tumoral cells. The 2+ positivity was assumed as the cut-off of the method.

Results

The positivity for the three tested antigens with indication of the score is shown in Table 1.

CEA was positive (at least 2+) in ten out of 62 cases (16.13%) with 34 cases completely negative. CA-125 resulted positive (at least 2+) in 36 out of 62 cases (58.06%) with 14 cases completely negative. Immunohistochemical positivity was observed only in glandular cells, especially on the top, in some cases with a thin granular staining. BerEP4 was positive (at least 2+) in 45 out of 62 cases (72.58%), with six cases completely negative whereas it was positive in 31 out of 45 cases of endometrial carcinoma (68.9%) in our previous study [15].

As with the other two antigens, Ber-EP4 is mainly located on the top of cells and did not have any particular location in the neoplastic tissue. In some cases there was a spread of membranous staining that showed a marked limit between a glandular cell and the one beside.

With regard to the correlation between positivity for the three antigens and histotype and grading (Table 2), BerEP4 was slightly present in mucinous tumors in comparison to the serous (21/26 80.77% vs 17/21 80.96%). We observed no positivity for BerEP4 in either metastatic ovarian tumors or yolk sac tumors.

Ber-EP4 was directly proportional to tumor differentation (75% of positivity in G1 vs 62.50% in G3), whereas CA-125 and CEA showed no revelant difference regarding grading. Also there were no differences among the three antigens studied with regard to clinical stage.

Table 1. — *Immunohistochemical positivity for the three tested antigens (Ber-EP4, CA-125 and CEA) in ovarian cancer.*

	Negative	+	++	+++	Positive (%)
Ber-EP4	6/62	11	25	20	45/62 (72.58)
CA-125	14/62	12	24	12	36/62 (58.06)
CEA	34/62	18	7	3	10/62 (16.13)

Table 2. — *Correlation between positivity of the three tested antigens (Ber-EP4, CA-125 and CEA) and histotype and grading of ovarian cancer.*

	No cases	BerEP4 +	CA 125 +	CEA +
Histotype				
Serous	21	17 (80.96%)	14 (66.67%)	2 (9.52%)
Mucinous	26	21 (80.77%)	15 (57.69%)	7 (26.92%)
Mixed mesodermal	3	1 (33.34%)	2 (66.66%)	–
Yolk sac tumor	2	–	–	–
Endometrioid	4	3 (75%)	2 (50%)	–
Clear cell carcinoma	4	3 (75%)	2 (50%)	–
Granular	1	1 (100%)	–	–
Metastatic tumor	1	–	1 (100)	1 (100)
Total	62	46	36	10
Grading				
G1	24	18 (75%)	15 (62.50%)	3 (12.50%)
G2	22	17 (77.27%)	11 (50%)	5 (22.73%)
G3	16	10 (62.50%)	10 (62.50%)	2 (12.50%)
Total	62	45	36	10

Discussion and Conclusions

Early diagnosis of malignant ovarian tumors represents an important issue for social medicine, especially towards future projection (progress in imaging techniques, availability of specific markers) so that the patient may benefit from primary therapy with adequate staging and optimal debulking.

The risk of malignancy index, which is a simple scoring system based on menopausal status, ultrasound and serum concentration of CA-125, was able to differentiate malignant and benign pelvic masses efficiently to optimize therapy [16].

With regard to new markers studied in the literature, the multitude of antigens and several biological factors tested do not seem to be useful in early biochemical diagnosis, especially when serum levels are determined.

Ber-EP4, a recently introduced epithelial antigen in clinical practice, is not present on mesothelial cells. The data can suggest its use in the immunocytochemical study of cells recovered from the peritoneal cavity.

There are few data regarding the study of BerEP4 in ovarian cancer. Davidson *et al*. [17] evaluated BerEP4 in association with four antigens (CA-125, CEA, BG8 and B72.3) in 94 samples of fresh pleural, peritoneal and pericardial effusions from patients diagnosed with gynecological malignancies. These authors reported that BerEP4 had a sensitivity in detecting malignant cells (immunocytochemical positivity) in 78% of cases which is only a little lower than that of CA-125 (88%). Furthermore BerEP4 and B72.3 appeared to be the best markers when both sensitivity and specificity were considered, followed by BG8, while CEA and CA-125 had limited roles in the detection of metastases from gynecological tumors owing to the low sensitivity of the former and the low specificity of the latter [17].

Comin *et al*. [18] suggested Ber-EP4 and estrogen receptor (ER) as the markers with the greatest discriminatory power in differentiating epithelioid mesothelioma of the peritoneum from serous papillary carcinoma of the ovary. Okamoto *et al*. [19] concluded that combined immunostaining for Ber-EP4 and the anti-calretinin antibody was helpful for the differential diagnosis between mesothelial cells and not only serous type, but also mucinous, endometrioid and clear cell types of ovarian cancer cells in cytologic specimens.

In our study Ber-EP4 was positive in 72.58% of primary epithelial ovarian cancers studied, with a prevalent membranous staining but with no characteristic topographic distribution. The presence of the antigen seemed related to the histotype and grading but not to clinical stage.

Further studies on larger series are necessary to have definitive conclusions on the expression of this antigen by more differentiated tumoral tissue.

References

[1] Taylor K.J., Schwartz P.E.: "Cancer screening in a high risk population: a clinical trial". *Ultrasound Med. Biol.,* 2001, *27,* 461.

[2] Woolas R.P., Oram D.H., Jeyarajah A.R., Bast R.C., Jacobs I.J.: "Ovarian cancer identified through screening with serum markers but not by pelvic imaging". *Int. J. Gynecol. Cancer,* 1999, *9*, 497.

[3] Partridge E.E., Barnes M.N.: "Epithelial ovarian cancer: prevention, diagnosis and treatment". *C.A. Cancer J. Clin.,* 1999, *49,* 297.

[4] Van Dalen A., Favier J., Baumgartner L., Hasholzner U., De Bruijn H., Dobbler D. *et al*.: "Serum levels of CA 125 and TPS during treatment of ovarian cancer". *Anticancer Res.,* 2000, *20,* 5107.

[5] Zurawski V.R., Knapp R.C., Einhorn N., Kenemans P., Mortel R., Ohmi K. *et al*.: "An initial analysis of preoperative serum CA 125 levels in patients with early stage ovarian carcinoma". *Gynecol. Oncol.,* 1988, *30*, 7.

[6] Tuxen M.K., Soletormos G., Rustin G.J., Nelstrop A.E., Dombernowsky P.: "Biological variation and analytical imprecision of CA 125 in patients with ovarian cancer". *Scand. J. Clin. Lab. Invest.,* 2000, *60*, 713.

[7] Nyvang G.B., Mogensen O., Bichel P., Jakobsen A.: "Combined prognostic importance of CA 125, histopathologic grade and DNA-index in advanced ovarian cancer". *Eur. J. Gynaecol. Oncol.,* 2000, *21,* 569.

[8] Runnebaum I.B., Stickeler E.: "Epidemiological and molecular aspects of ovarian cancer risk". *J. Cancer Res. Clin. Oncol.,* 2001, *127,* 73.

[9] Morrow C.P., Curtin J.P. (eds.): "Surgery for ovarian neoplasia". In: Gynecologic Cancer Surgery. New York, Churchill Livingstone, 1996, 627.

[10] Elit L.: "Familial ovarian cancer". *Can. Fam. Physician,* 2001, *47,* 778.

[11] Werness B.A., Eltabbakh G.H.: "Familial ovarian cancer and early ovarian cancer: biologic, pathologic and clinical features". *Int. J. Gynecol. Pathol.,* 2001, *20,* 48.

[12] Gitsch G., Tabery U., Feigl W., Breitenecker G.: "The differential diagnosis of primary peritoneal papillary tumors". *Arch. Gynecol. Obstet.,* 1992, *251*, 139.

[13] Baker P.M., Oliva E., Young R.H., Talerman A., Scully R.E.: "Ovarian mucinous carcinoids including some with a carcinomatous component: a report of 17 cases". *Am. J. Surg. Pathol.,* 2001, *25,* 557.

[14] Latza U., Niedobitek G., Schwarting R., Nekarda H., Stein H.: "Ber-EP4. New monoclonal antibody which distinguishes epithelia from mesothelia". *J. Clin. Pathol.,* 1990, *43,* 213.

[15] Cherchi P.L., Bosincu L., Dessole S., Ruiu G.A., Cossu Rocca P., Capobianco G. *et al*: "Immunohistochemical expression of BerEP4, a new epithelial antigen, in endometrial carcinoma: correlation with clinical parameters". *Eur. J. Gynaecol. Oncol.,* 1999, *20,* 393.

[16] Manjunath A.P., Pratapkumar, Sujatha K., Vani R.: "Comparison of three risk of malignancy indices in evaluation of pelvic masses". *Gynecol. Oncol.,* 2001, *81,* 225.

[17] Davidson B., Risberg B., Kristensen G., Kvalheim G., Emilsen E., Bjamer A. *et al*.: "Detection of cancer cells in effusions from patients diagnosed with gynaecological malignancies. Evaluation of five epithelial markers". *Virchows Arch.,* 1999, *435,* 43.

[18] Comin C.E., Saieva C., Messerini L: "h-Caldesomn, Calretinin, Estrogen receptor, and Ber-EP4: a useful combination of immunohistochemical markers for differentiating epithelioid peritoneal mesothelioma from serous papillary carcinoma of the ovary". *Am. J. Pathol.*, 2007, *31*, 1139.

[19] Okamoto S., Ito K., Sasano H., Moriya T., Niikura H., Terada Y.: "Ber-EP4 and anti-calretinin antibodies: a useful combination for differentiating diagnosis of various histological types of ovarian cancer cells and mesothelial cells". *Tohoku J. Exp. Med.*, 2005, *206,* 31.

Address reprint requests to:
P.L. CHERCHI, M.D.
Viale San Pietro 12
07100 Sassari (Italy)
e-mail: capobia@uniss.it