Immunohistochemical evaluation of a new epithelial antigen, Ber-EP4, in ovarian cancer: preliminary results

P. L. Cherchi[1], *M.D., Prof.*; **V. Marras**[2], *M.D., Research Fellow*; **G. Capobianco**[1], *M.D., Research Fellow*; **G. Ambrosini**[1], *M.D., Research Fellow*; **M. Piga**[1], *Midwife*; **G. M. Fadda**[1], *M.D., Research Fellow*; **S. Dessole**[1], *M.D., Prof.*

[1]*Department of Pharmacology, Gynaecology and Obstetrics;* [2]*Institute of Pathology*
University of Sassari (Italy)

Summary

Objective: To assess the immunohistochemical expression of Ber-EP4, a new epithelial antigen in ovarian cancer.

Methods: We studied 25 cases of ovarian cancer in which Ber-EP4, CEA and CA 125 were investigated by an immunohistochemical method. We evaluated the correlations between immunohistochemical positivity and grading, histotype and stage of disease.

Results: CEA was positive in 5 out of 25 cases (20%), CA-125 in 17 out of 25 cases (68%) and Ber-EP4 in 14 out of 25 cases (56%). Ber-EP4 was mainly present in mucinous tumors in comparison to serous tumors (78.6% vs. 50%). Ber-EP4, as well as CA-125, were directly proportional to tumor differentation (70% of positivity in G1 vs 37.5% in G3 for the former and 80% in G1 vs 50% in G3 for the latter, respectively), whereas CEA showed no relevant difference regarding the grading. There were no differences among the three antigens studied with regard to clinical stage.

Conclusions: In our study Ber-EP4 was positive in 14 out of 22 cases (63.6%) of the primary epithelial ovarian cancers studied. The presence of this antigen seems to be related to histotype and grading but not to clinical stage.

Key words: Epithelial antigen; Ber-EP4; 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 secondary gastrointestinal tumors (Krukenberg) and primary ovarian tumors which is done by immunohistochemistry [13].

Immunohistochemical evaluation of some tumor-associated antigens has shown a higher sensibility than in serologic studies of this tumor, most likely because in the first stages of disease, where immunohistochemistry is often positive, the number of tumor cells producing antigens is too low to determine measurable quantities of their intake in peripheral blood. Many are tested antigens 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.

Ber-EP4, an epithelial antigen recently introduced into clinical practice, consists of two glycoproteins of 34 and 39 KD, respectively, and is located both on the surface and in the cytoplasm of the epithelium, with the exception of squamous epithelium and mesothelium [14].

To our knowledge there is little data on the BerEP4 antigen in ovarian cancer and in other gynecological tumors in the literature.

The aim of our study was to evaluate immunohistochemical positivity of Ber-EP4, 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 25 cases of malignant ovarian tumors and investigated the presence of Ber-EP4 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 59.5 years (range 26-75). The 25 cases were represented by 22 ovarian cancers: six serous, 14 mucinous, two malignant mixed mesodermal, two yolk sac tumors and one metastatic tumor. Tumor grades were: 1) ten patients (40%); 2) seven patients (28%); and 3) eight patients (32%). With regard to the FIGO stage, five had IA, six IC and 14 III.

The immunohistochemical 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; then 4 micron sections were colored by hematoxylin-eosin. Some sections were expelled onto glass slides before being treated with 0.1% poly-L-Lysin in order to increase their adhesiveness.

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Antigens were investigated in neoplastic tissue by an immunohistochemical method, using polyclonal antibodies for CEA and monoclonal antibody (Mab) for CA-125 and Ber-EP4. The immunodetermination was performed using immunoperoxidase Avidin-Biotin Complex (ABC method). Endogenous peroxidase was inhibited by the Heyderman and Neville procedure. Diaminobenzidine was the chromogen. Non-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 tumoral cells. The 2+ positivity was assumed as the cut-off of the method.

Results

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

CEA was positive (at least 2+) in five out of 25 cases (20%) with 15 cases completely negative. CA-125 resulted positive (at least 2+) in 17 out of 25 cases (68%) with six cases completely negative. Immunohistochemical positivity was observed only in glandular cells, especially on the surface, in some cases with a thin granular staining. Ber-EP4 was positive (at least 2+) in 14 out of 25 cases (56%), with five cases completely negative. If we consider only the cases of primary epithelial ovarian tumors, Ber-EP4 was positive in 14 out of 22 cases (63.6%) whereas it was positive in 31 out of 45 cases of endometrial carcinoma (68.9%) in our previous study [15].

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

With regard to a positive correlation between the three antigens and histotype and grading (Table 2), Ber-EP4 was mainly present in mucinous tumors in comparison to serous tumors (78.6% vs 50%). We observed no positivity for Ber-EP4 in either metastatic ovarian tumors or germinal tumors.

Ber-EP4, as well as CA-125, was directly proportional to tumor differentation (70% of positivity in G1 vs 37.5% in G3 for the former and 80% in G1 vs 50% in G3 for the latter, respectively), whereas CEA showed no revelant difference regarding grading. There were no differences in the three antigens studied with regard to clinical stage.

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

	Negative	+	++	+++	Positive %
CEA	15/25	5	4	1	20
CA-125	6/25	2	10	7	68
Ber-EP4	5/25	6	9	5	56

Table 2. — *Correlation between the 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	6	3 (50)	5 (83.3)	1 (16.6)
Mucinous	14	11 (78.6)	10 (71.4)	3 (21.4)
Mixed mesodermal	2	–	1 (50)	–
Yolk sac tumor	2	–	–	–
Metastatic tumor	1	–	1 (100)	1 (100)
Total	25	14	17	5
Grading				
G1	10	7 (70)	8 (80)	2 (20)
G2	7	4 (57.1)	5 (71.4)	2 (28.6)
G3	8	3 (37.5)	4 (50)	1 (12.5)
Total	25	14	17	5

Discussion and conclusions

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

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

With regard to the 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 mesothelium. This data could suggest its use in the immunocytochemical study of cells recovered from the peritoneal cavity.

In the literature there is little data on the study of Ber-EP4 in ovarian cancer. Davidson *et al.* [17] evaluated Ber-EP4 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 gynaecological malignancies. These authors reported that Ber-EP4 had a sensitivity in detecting malignant cells (immunocytochemical positivity) in 78% of cases which is only somewhat lower than that of CA-125 (88%). Furthermore Ber-EP4 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 a limited role in the detection of metastases from gynaecological tumors owing to the low sensitivity of the former and the low specificity of the latter [17].

In our study Ber-EP4 was positive in 63.6% of the primary epithelial ovarian cancers studied, with a prevalent membranous staining but with no characteristic topographic distribution. The presence of the antigen seems related to histotype and grading but not to clinical stage.

Further studies on a larger series are necessary in order to get definitive conclusions on the expression of this antigen from more differentiated tumoral tissue.

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Address reprint requests to:
S. DESSOLE, M.D.
Viale San Pietro, 12
07100 Sassari (Italy)