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ORIGINAL ARTICLE

Expression of HER2/neu, estrogen and progesterone receptors, CA 125 and CA19-9 on cancer cell membrane in patients with serous and mucinous carcinoma of the ovary

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Summary

Purpose: *To examine the expression of the membrane markers of estrogen (ER) and progesterone receptors (PR), CA-125, CA 19-9 and HER2/neu in ovarian cancer tissues.*

Methods: *Fifty-four samples of ovarian cancer tissues originating from 55 patients were examined by immunohistochemistry. Forty-three had serous papillary ovarian cancer, 9 of which were grade I, 12 grade II and 2 grade III. Twelve patients had a classic mucinous ovarian cancer, 5 of which were grade I, 4 grade II and 0 grade III.*

Results: *Out of 43 patients with serous ovarian cancer, 7 expressed both steroid receptors, 22 had only one (10 ER and 12 PR), while 14 were negative. Only 2/12 patients with classic mucinous ovarian cancer expressed of both receptors. CA-125 was expressed in 37/43 patients with serous ovarian cancer and in 4/12 patients with classic mucinous ovarian cancer. CA 19-9 was expressed in 3/43 patients with serous ovarian cancer, and coexpressed with CA-125 in 2/3 patients. In patients with classic mucinous ovarian cancer, 4/12 had expression of CA 19-9 without coexpression with CA-125. HER2/neu positivity (3+) was proven in only one case with classic mucinous ovarian cancer, and any other expression (1+) in 7 additional patients (1 mucinous and 6 serous ovarian cancers).*

Conclusion: *Positive HER2/neu expression in the cells of ovarian cancer is very rare and HER2/neu overexpression is even rarer. Expression of ER and PR does not depend on tumor grade and/or at least not in grade I and II. Positive CA 19-9 expression may be present not only in cases of classic mucinous ovarian cancer but also in typical serous ovarian cancer. However, in the classic mucinous ovarian cancer, CA-125 may be expressed, though in relatively low percentage.*

Key words: CA-125, CA 19-9, classic mucinous ovarian cancer, estrogen/progesterone receptors, HER2/neu, serous papillary ovarian cancer

Introduction

Ovarian tumors originating from the surface epithelium (epithelial tumors) make about 60% of all ovarian tumors, and about 90% of all malignant ovarian tumors. Serous ovarian cancer is the most frequent malignant ovarian tumor [1-6]. Ovarian tumor cells may express tumor markers and hormone receptors on their membranes, but their amount may vary.

The relevant literature shows contradictory results of immunohistochemical examinations of ER [7-10], PR [11], CA-125, CA 19-9 [12,13] and HER2/neu [14] in ovarian cancers. In this study we examined the expression of the membrane markers HER2/neu, ER, PR, CA-125 and CA 19-9 in cases of mucinous and serous ovarian cancers, in order to determine whether a difference exists between serous and mucinous ovarian cancers regarding the expression of these receptors [15].

Methods

Patients

Fifty-five samples of ovarian cancer tissues originating from 55 women who had undergone operation

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at the Institute for Oncology and Radiology of Serbia (IORS), Belgrade, and in the Gynaecological-Obstetrics clinic (GAK) "Narodni front" from 2003 to 2006, were examined. Forty-three had serous papillary ovarian cancer and 12 classic mucinous ovarian cancer. Tumors' grading is shown on Table 1.

Methods

Paraffin moulds of the tumors were used for immunohistochemical staining with antibodies to HER2/neu (Polyclonal Rabit Anti-Human c-erb B2 oncoprotein; DACO antibody), CA-125 (Monoclonal Mouse Anti-human CA 125 clone OC 125; DAKO antibody), CA 19-9 (Monoclonal Mouse Anti-Human with 19-9; DAKO antibody), ER (Monoclonal Mouse Anti-Human Estrogen receptor IgG1 kappa; DAKO antibody), and PR (Monoclonal Mouse Anti-Human Progesterone receptor IgGl kappa; DAKO antibody).

For determination of HER2/neu receptor a semiquantitative method was used, the scoring system of which is based on the proportion between the number of the stained and unstained cells, and the intensity of the cell membrane staining (Table 2).

Results of CA-125 and CA 19-9 determination were expressed as: no staining; microfoci of cytoplasmic and membrane staining <50%; cytoplasmic granular staining <50%; membrane staining >50%; cytoplasmic granular staining >50%; membrane staining >50%; membrane and cytoplasmic staining >50%; membrane and cytoplasmic epithelial component, focal cytoplasmic and focal membrane staining. All cases with >50% of membrane and cytoplasmic staining as well as >50% membrane staining were regarded as positive.

Semiquantitative assessment of ER and PR expression was performed according with the so-called "simple point system" recommended by Leake at al. (Table 3), which is based on the determination of the percentage of immunoreactive nuclei of tumor cells, and on the determination of the intensity of immunoreactive staining as well. The maximal score for assessment of either ER or PR, calculated by adding the points to the percentage of immunoreactive nuclei and intensity of immunoreactive staining was 8. Expression of ER and PR >3 of the point system was regarded as positive (Table 3).

Statistical analyses

For testing the differences between the parameters and depending on their nature, the Mann-Whitney exact test, Kruskal-Wallis test and Fisher's exact test were used. Statistical significance was put at $p < 0.05$. Statistical processing of the data was performed with the statistical packages SPSS 10.0 for Windows, and "open source" statistical package R (V. 2.8.1/2008-12-22, Copyright 2008; the R Foundation for Statistical Computing; ISBN 3-900051-07-0).

Table 1. Serous and mucinous ovarian cancer grading

Grade	*Serous*		*Mucinous*	
	n	*%*	*n*	*%*
I	9	20.94	5	41.67
II	12	27.88	4	33.33
III	2	4.66	0	0
No data	20	46.51	3	25
Total	43	100	12	100

Fisher exact test, p=0.844

Results

Table 1 shows the distribution of patients of both histological tumor types according to histological grade. The exact grade in a certain number of patients was not possible due to inadequate sample. In serous ovarian cancer grade I had 9 patients, grade II 12 patients and grade III 2 patients. Twenty of them did not have a verified tumor grade.

Table 2. HER2/neu scoring system

Score	*Membrane staining*
0	Not existing or less than 10% of tumor cells are stained
1+	Partial membrane staining in more than 10% of tumor cells
2+	Completely weak or moderate membrane staining in more than 10% of tumor cells
3+	Complete, striking membrane staining in more than 30% of tumor cells

Table 3. Semiquantitative system for ER and PR expression assessment in breast cancer

Percents of immunoreactive staining of nuclei of tumor cells	*Intensity of immunoreactive staining of nuclei of tumor cells*
0 = no stained nuclei	0 = no nuclear staining
1 = < 1% stained nuclei	1 = weak staining intensity
2 = 1-10% stained nuclei	2 = moderate staining intensity
3 = 11-33% stained nuclei	3 = very intensive staining
4 = 34-66% stained nuclei	
5 = 67-100% stained nuclei	

In mucinous ovarian cancer grade I had 5 patients, grade II 4, and in the remaining 3 patients grade was undetermined (Table 1).

In grade I serous cancer, only one of 9 patients showed HER2/neu(1+), whereas no HER2 /neu expression was found in grade II and III cases. In grade I mucinous cancer only one out of 5 patients showed HER2/neu (3+), whereas no expression was seen in grade II.

In grade I serous ovarian cancer all 9 patients were CA-125-positive and in grade II, 11 out of 12 were CA-125-positive. In grade III 2/2 patients were CA-125-positive. In grade I mucinous cancer 2/5 patients were CA 19-9-positive and 2 were CA-125-positive. In grade II, 4 patients were CA 19-9-negative, and only 2 were CA-125-positive.

In grade I serous ovarian cancer, one or both hormone receptors were expressed in 7/9 patients, and in 9/12 patients with grade II disease. In grade I, 4/9 women were ER-positive and 3/9 PR-positive, while 1 patient exhibited ER and PR coexpression.

In grade II serous ovarian cancer, 4/12 women were ER-positive, 6/12 were PR-positive, 2 coexpressed both receptors and in 3 patients both receptors were negative.

In grade III serous ovarian cancer, 2 patients were both ER- and PR-positive.

In grade I mucinous ovarian cancer, ER and PR were negative in all 5 patients, and in grade II 2/4 were both ER- and PR-positive.

HER2/neu overexpression was noted only in one mucinous ovarian cancer patient, while a small percentage (13.95%) with serous ovarian cancers showed any kind of HER2/neu positivity (Figure 1).

In mucinous ovarian tumors, expression of CA-125 existed in a relatively small percentage (33.3%; Figure 2).

Figure 3 depicts the frequency of positivity of both tumor types to CA 19-9. In regard with CA 19-9 no significant difference between serous and mucinous ovarian cancer was noted. However, CA 19-9 expression was seen in both types.

Coexpression of CA-125 and CA 19-9 was found only in serous ovarian cancer cases (Table 4).

ER were present in less than 50% of ovarian cancers of either serous or mucinous type, but significantly rarer in mucinous cases (Figure 4). PR were also positive in less than 50% of both types, but considerably more rare in mucinous types (Figure 5).

Coexpression of ER and PR was equal in both tumor types (Table 5).

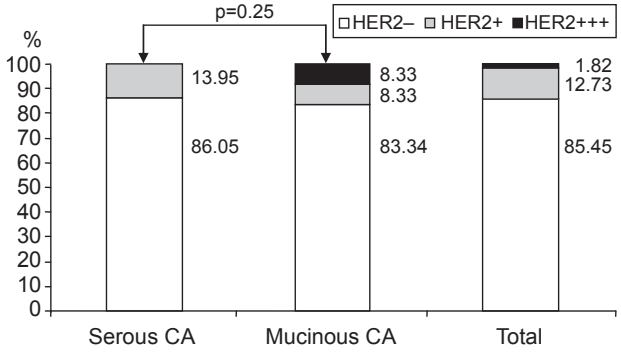

Figure 1. HER2/neu in patients with serous and mucinous ovarian cancer.

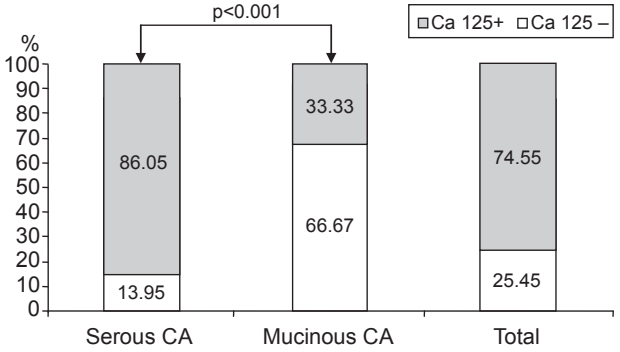

Figure 2. CA-125 in patients with serous and mucinous ovarian cancer.

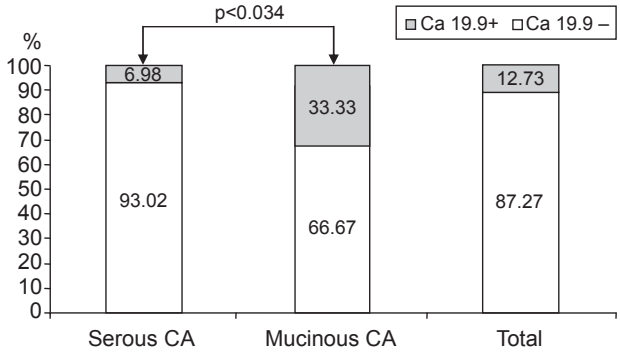

Figure 3. CA 19-9 in patients with serous and mucinous ovarian cancer.

Table 4. CA 19-9 and CA-125 according to ovarian cancer type

CA 19-9 CA-125	*Serous*		*Mucinous*	
	n	*%*	*n*	*%*
Both negative	5	11.63	4	33.33
Negative/positive	35	81.4	4	33.33
Positive/negative	1	2.33	4	33.33
Both positive	2	4.65	0	0
Total	43	100	12	100

Fisher exact test, p <0.001

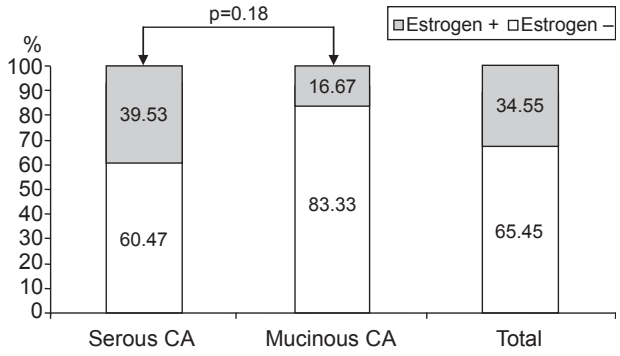

Figure 4. Estrogen receptors in patients with serous and mucinous ovarian cancer.

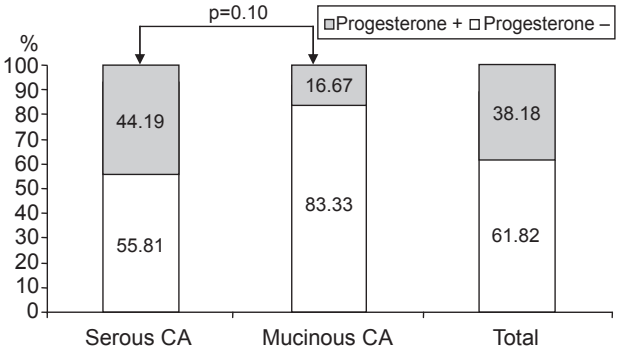

Figure 5. Progesterone receptors in patients with serous and mucinous ovarian cancer.

Table 5. ER and PR receptors parameters according to ovarian cancer type

ER PR	*Serous*		*Mucinous*	
	n	*%*	*n*	*%*
Both negative	14	32.56	10	83.33
Negative/positive	12	27.91	0	0
Positive/negative	10	23.26	0	0
Both positive	7	16.28	2	16.67
Total	43	100	12	100

Fisher exact test, p=0.0049

Discussion

A number of contradictory results were noticed in regard to the expression of ER, PR, HER2/neu, as well as CA-125 and CA 19-9 in serous and mucinous ovarian tumors. Discrepancies of results depend to a great extent on the method for expression determination.

In most cases, mucinous tumors showed a reduced expression for CA-125 and no expression of the ER and PR. Results in a few published studies are not uniform [16-18]. In a study the seromucinous type of ovarian cancer showed expression of ER, PR and CA-125 [19]; this subtype was not included in our study.

HER2/neu in ovarian tumors is rarely overexpressed. Tumors with intense expression of this marker have a greater probability for relapse. The existing few and contradictory results gave the idea to carry on this work [20].

There are numerous studies on ER and PR in breast cancer, but, although ovarian cancer is the second most frequent malignancy in women, there are only few reports, which, however, point to the importance of determining steroid receptors in ovarian cancer, both for immunohistochemical differentiation and for treatment decision-making [21-24].

The significance of serum determination of CA-125 and CA 19-9, is well-known; however, for immunohistochemical analysis there are not enough data as yet concerning the level of both membrane and cytoplasmic staining [9,12].

HER2/neu is being examined in breast cancer, and even in gastrointestinal cancers, but there are not many data about HER2/neu status in ovarian cancer.

In conclusion, the results point out to the following:

Steroid receptors are present in both histological types of ovarian cancer, but considerably more rarely in the mucinous type. Coexpression of both steroid receptors in both serous and mucinous ovarian cancer is rarely seen. In fact, most of the positive samples are positive for only one receptor. This finding could imply that the relationship between these two receptors in ovarian cancer is immunohistochemically different from that observed in breast cancer. There is no significant difference in the frequency of positive steroid receptors between grade I and II serous ovarian cancer; it seems that grade III tumors may also possess both receptors, although the number of cases in our series was small.

HER2/neu positivity in ovarian cancer is rare, and HER2/neu overexpression is even more rare.

Although significantly more frequent in the serous type, positive staining of CA-125 is not so rare even in mucinous ovarian cancer; it is not clear whether the membrane positivity of CA-125 is always correlated with the serum CA-125 level.

CA 19-9 may be positive on the membrane of the mucinous ovarian cancer cells, although membrane expression of this marker can be observed even in certain cases of serous ovarian cancer. However, it is found more frequently in the mucinous type of tumor.

Taken altogether, these results could have therapeutic implications for women with progressive ovarian cancer.

Acknowledgements

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