Clinical and molecular comparison between borderline serous ovarian tumors and advanced serous papillary ovarian carcinomas

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Summary

The aim of this study was to characterize the clinical and molecular markers of borderline serous ovarian tumors (BSOT), and to study their expression in the progression from benign lesions to advanced serous papillary ovarian carcinomas (SPOC).

The clinical records of 20 patients with BSOT and 22 patients with SPOC were reviewed. Specimens from all these cases and from six benign ovarian serous cystadenomas were evaluated for expression of estrogen receptors (ER), progesterone receptors (PR), p53, HER-2/neu and Ki-67 by immunohistochemical techniques.

The mean patient age and the age at menarche differed significantly between the compared groups of BSOT and SPOC (p=0.0006 and p=0.0014, respectively). No difference was observed comparing the other clinical parameters.

The immunohistochemical analysis demonstrated a significant increase in the expression of ER (100% vs 72.7%), and a significant decrease in the immunoreactivity for p53 (0% vs 45.4%) and Ki-67 (2% vs 26.8%) in cases of BSOT compared with those of SPOC (p=0.007, p=0.0003 and p=0.012, respectively). No significant difference was demonstrated comparing the expression of PR and HER-2/neu.

The immunostaining of benign ovarian serous cystadenoma specimens did not differ significantly from immunoreactivity observed in cases of BSOT. According to immunohistochemical analysis, BSOT had much more in common with benign serous tumors than with SPOC. The main difference between BSOT and SPOC was regarding the overexpression of p53 and Ki-67.

Key words: Borderline serous ovarian tumor; Serous papillary ovarian carcinoma; Benign serous ovarian tumor; Immunohistochemistry; Clinical parameters; Molecular markers.

Introduction

Epithelial neoplasms of the ovary comprise a spectrum that includes benign, borderline and invasive tumors. It has been proposed that these pathologic entities may represent sequential stages in the evolution of ovarian cancer [1]. Borderline tumors account for approximately 15% of malignant tumors of the ovary [2]. Although a large proportion of patients with borderline tumors experience prolonged survival, some patients die of disease [3].

Attempts made to identify a poor prognostic group by clinicopathological criteria, such as stage, histologic type or grade have not been successful. The search for prognostic factors could help in deciding for or against adjuvant therapy.

Development of a malignant neoplasm requires sequential damage to several genes. This might imply activation of oncogenes due to amplification or mutation, and inactivation of tumor suppressor genes due to deletion or mutation. Most of the previous studies have described the role of oncogenes [4, 5] or tumor suppressor genes [6-11] in the pathogenesis of borderline ovarian tumors; yet, several studies [12, 13] have focused on the coexpression of both the proto-oncogene HER-2/neu and the tumor suppressor gene p53.

The aim of the current study was to extend the array of molecular parameters and to investigate their possible coexpression in the progression of borderline tumors from benign lesions to invasive ovarian carcinomas. The examined molecular markers included estrogen receptors (ER), progesterone receptors (PR), tumor suppressor gene p53, HER-2/neu proto-oncogene and Ki-67 proliferative index. We focused on the serous histologic subtype, encompassing the whole spectrum of increasingly aggressive neoplasms, and studied clinical parameters for further comparison of borderline serous ovarian tumors (BSOT) with advanced serous papillary ovarian carcinomas (SPOC).

Materials and Methods

The clinical records and pathology reports of women, operated on between January 1995 and December 1999 and diagnosed with serous ovarian tumors, were reviewed. The slides from all tumor specimens were reviewed by a single pathologist (S.Z.). All patients with a confirmed diagnosis of BSOT (n=20) and advanced SPOC (n=22) were included in the study. Staging and grading were determined using the FIGO criteria [14]. The

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group of BSOT included 14 cases in stage IA, two cases in stage IB, one case in stage IC, one case in stage II and two cases in stage III. All cases in the SPOC group were staged as either IIIB, IIIC, or IV and appropriately graded. Sections from benign ovarian serous cystadenomas (n=6) were also examined for all molecular parameters.

Immunohistochemistry

Two paraffin-embedded blocks from each case were selected for staining. Three-micron tissue sections, placed on positive ion-charged slides, were stained. The slides were then deparaffinized, treated with 3% hydrogen peroxide for 20 min to block endogenous peroxidases, and then washed in distilled water. A microwave antigen retrieval procedure using citrate buffer, pH=6, was performed on all slides, as well as those used for immunostaining with anti HER-2/neu. All slides were incubated with primary antibody in Ventana autoimmunostainer ES (Ventana Medical Systems, S.A., Strasbourg, Cedex, France). For ER, the monoclonal mouse antibody-clone 6F11, prediluted, from Zymed (Zymed Laboratories, Inc. San Francisco, CA) was used. The mouse monoclonal antibody-clone PR-2C5, prediluted (Zymed), was used for PR immunostaining. For demonstration of Ki-67 and p53, the slides were incubated with monoclonal mouse antibodies: anti Ki-67 – clone 7B11 (Zymed) (dilution 1:40) and anti p53-clone D07 (Dako A/S, Glostrup, Denmark) (dilution 1:100), respectively. For HER-2/neu immunostaining, the slides were incubated with mouse monoclonal anti HER-2/neu antibody-clone TAB 250 (Zymed) (dilution 1:100) and with Protease I in Ventana autoimmunostainer ES for 8 min. Slides were then developed with diaminobenzidine chromogen, lightly counterstained with Mayer's hematoxylin, and mounted.

A hematoxylin and eosin stained section was examined for each block, and a negative control slide, using nonspecific mouse IgG substituted for the primary antibody, was performed on all blocks. Background staining was negligible. The immunostained slides were compared with positive controls.

Immunostaining Scoring

The immunostaining score was based on the percentage of stained cells out of 500 cells counted (0=<10%, 1=10-25%, 2=26-50% and 3=>50%), intensity (1=weak, 2=moderate, 3=strong) and heterogeneity (1=marked, 2=intermediate, 3=mild). Heterogeneity was defined as non-uniform or sporadic immunostaining patterns in tumor sections. The final score was calculated by adding the three parameters, as described by Zheng *et al.* [15]. The staining was defined positive when the final score was ≥7. Ki-67 index was expressed as percentage of positively stained cells per total 500 cells counted.

Statistical Analysis

Statistical comparison between the two groups regarding the different clinical and molecular parameters, was performed using the chi-square test and the two-tailed Student's t test; $p<0.05$ was considered statistically significant.

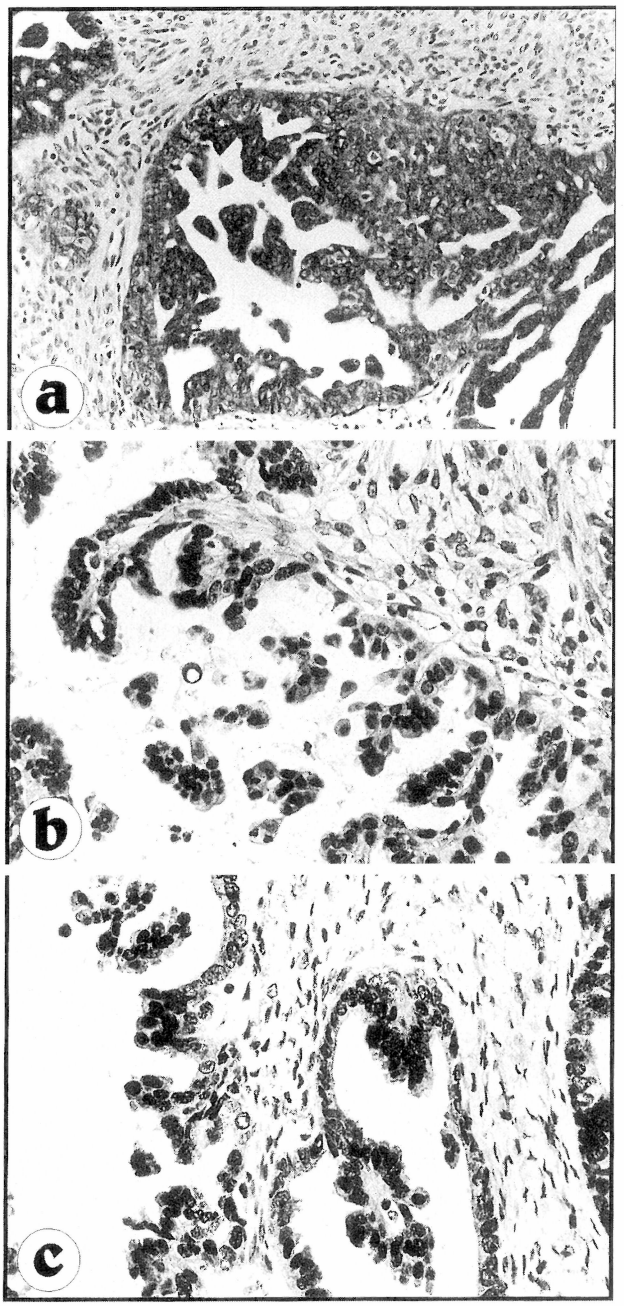

Figure 1. — Immunostaining in borderline serous ovarian tumor (BSOT). A) Hematoxylin and eosin stain; B) Nuclear staining for estrogen receptor (ER); C) Nuclear staining for progesterone receptor (PR) (Original magnification, x200).

Results

Clinical parameters compared between patients with BSOT and those with SPOC are presented in Table 1. The patients' age and the age at menarche differed significantly (p=0.0006 and p-0.0014, respectively). No difference between the two groups was observed comparing the number of pregnancies and births, the use of contraceptives, history of infertility in the past and smoking. The level of CA-125 was significantly lower in the group of BSOT (157 U/ml) than in the SPOC group (1,075 U/ml) (p=0.03).

Table 2 presents a comparison of immunohistochemical analysis between the study groups BSOT and SPOC. There was a significant difference in immunostaining for ER (p=0.007), being more predominant in BSOT (100%) than in SPOC (72.7%), but there was no significant difference in the immunopositivity for PR (Figure 1).

Table 1. — *Comparison of clinical parameters in borderline serous ovarian tumors (BSOT) versus serous papillary ovarian carcinomas (SPOC)*

Clinical Parameters	BSOT (n=20)	SPOC (n=22)	Significance (p)
Median age	42.8	59.0	0.0006
Age at menarche	12.8	14.5	0.001
No. of pregnancies	3.6	5.1	NS[a]
No. of births	2.5	2.5	NS
Use of contraceptives	40%	28%	NS
OC[b] only	15%	4%	NS
IUD[c] only	25%	24%	NS
Inferlitity	5.5%	4.2%	NS
Smoking	14.3%	14.3%	NS
Mean CA-125 (U/ml)	157	1075	0.03

[a] NS = non significant; [b] OC = Oral contraceptives; [c] IUD = intrauterine device.

Table 2. — *Comparison of immunostaining scores for different molecular parameters in borderline serous ovarian tumors (BSOT) versus serous papillary ovarian carcinomas (SPOC)*

Molecular parameters	BSOT (n=20)	SPOC (n=22)	Significance (p)
ER	20 (100%)	16 (72.7%)	0.007
PR	20 (100%)	20 (90.9%)	NS
p53	0 (0%)	10 (45.4%)	0.0003
HER-2/neu	1 (5%)	2 (9.1%)	NS
Ki-67	2%	26.8%	0.012

Nuclear specific staining for p53 was negative in the group of BSOT, whereas 45.4% of cases in the SPOC group stained positively for p53 protein (p=0.0003) (Figure 2). There was no difference in expression of HER-2/neu in the BSOT (5%) versus SPOC group (9.1%). On the other hand, the Ki-67 proliferation index, expressed as percent of stained nuclei, was significantly more prevalent in the SPOC (26.8%) than in the BSOT group (2%) (p=0.012) (Figure 2).

The immunostaining of sections from benign ovarian cystadenomas demonstrated 100% immunopositivity for ER and PR, negative immunoreactivity for p53 and HER-2/neu and immunopositivity for Ki-67 equal to 1% of tumor cells.

Discussion

As the rate of detection of ovarian neoplasms increases, the distinction between borderline tumors and carcinomas is an important problem in ovarian pathology. The main criterion for differentiating ovarian carcinoma from borderline tumor is based on stomal invasion. The obvious limitations of microscopical procedures in diagnosis and in predicting the behaviour of borderline

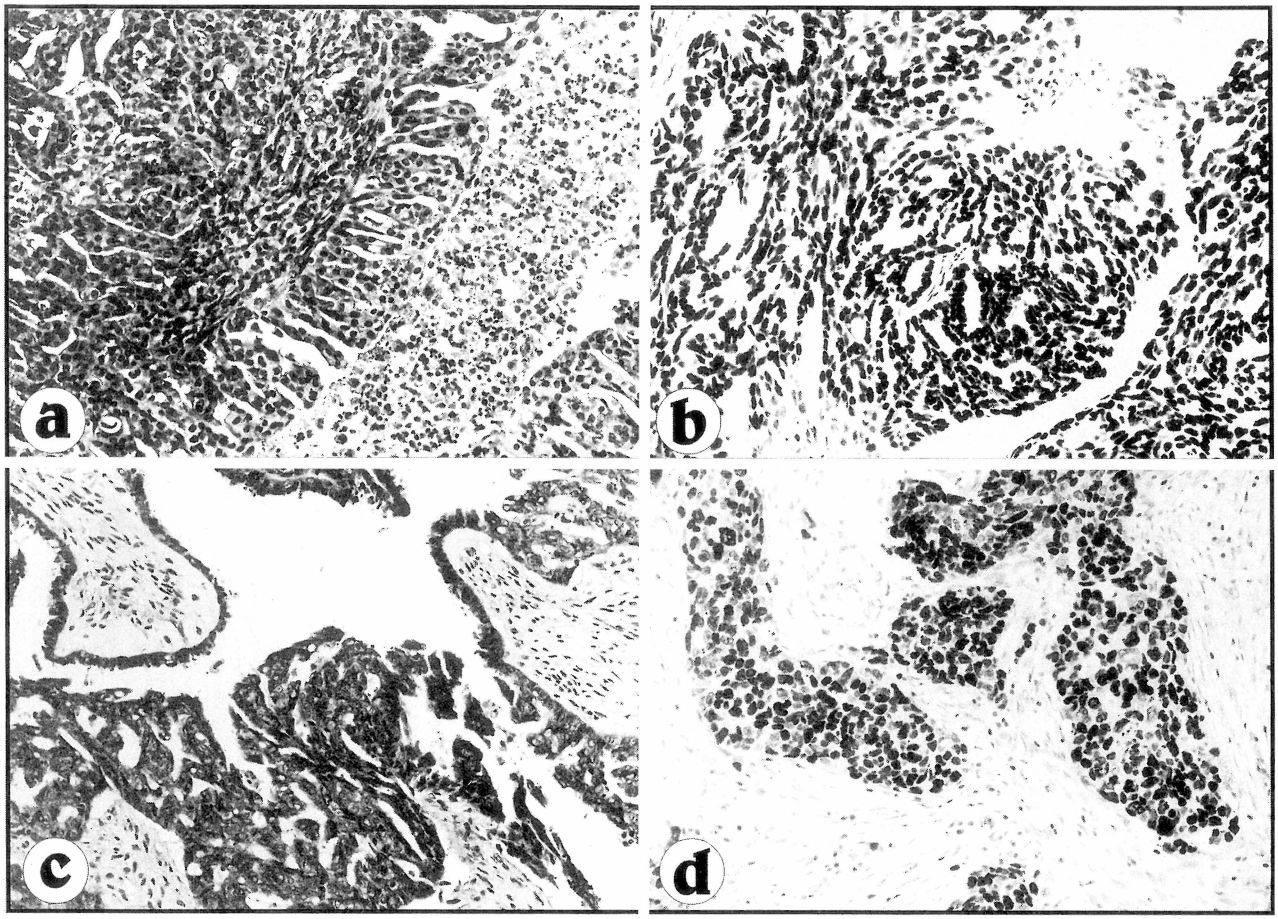

Figure 2. — Immunostaining in advanced serous papillary ovarian carcinoma (OSPC). A) Hematoxylin and eosin stain B) Nuclear staining for p53 C) Membrane staining for HER-2/neu; D) Nuclear staining for Ki-67 (Original magnification, x200).

tumors led us to investigate whether different molecular parameters could help to identify neoplasms with aggressive potential.

The results of this study support the fact that benign, borderline and malignant serous tumors of the ovary form a continuum of increasing aggressive neoplasms. The patients with BSOT were significantly younger, and they had a significantly earlier menarche compared with the SPOC patients. The level of CA-125 was also significantly lower in cases of BSOT compared with SPOC. All cases of benign and borderline serous tumors showed positive immunoreactivity in all specimens for both ER and PR. The immunostaining for ER and also PR decreased in cases of SPOC. The expression of ER and PR in borderline tumors was examined by Koshiyama *et al.* [16], but their series included only two cases of BSOT.

The most significant difference between BSOT and SPOC was demonstrated in immunostaining for p53 protein. Studies investigating p53 overexpression and mutation in BSOT have produced conflicting results [6-8, 10-13, 16, 17]. While the negative immunostaining for p53 described in the current study is in agreement with previous studies reported by Klemi *et al.* [6], Kiyokawa [7] and van Haaften-Day *et al.* [12], other authors [10, 11, 13, 16] presented higher rates of p53 overexpression in borderline tumors. The higher rate of p53 immunopositivity reported in the other studies could be attributed to an increased number of borderline advanced-stage cases with invasive implants [8]. Probably, overexpression of p53 is not a feature of benign epithelial ovarian tumors or early-stage borderline ovarian tumors, thus supporting the association of p53 overexpression with evolution of invasive ovarian cancer. Therefore, p53 immunostaining may have diagnostic value in discriminating between borderline and malignant serous ovarian tumors. Immunostaining for HER-2/neu in BSOT has previously been reported [13, 18-20]. HER-2/neu expression can be found in a number of normal human tissues, including the epithelial cells of the ovaries [21]. Only cells that show positive staining beyond that seen in the normal ovaries should be considered to exhibit overexpression of HER-2/neu. According to our results, there was no significant difference in HER-2/neu immunoreactivity comparing the BSOT and SPOC cases. Rubin *et al.* [22] suggested that HER-2/neu overexpression does not appear to be a common early event in the development of ovarian cancer. Moreover, overexpression of HER-2/neu was found to be associated with resistance to conventional chemotherapy in breast [23] and ovarian [21] cancers.

Proliferative activity of the ovarian tumor, expressed by immunopositivity for Ki-67, differed significantly ($p=0.012$) in cases of BSOT and those of SPOC.

The monoclonal antibody Ki-67 reacts with human nuclear antigen expressed only in cycling cells, but not in quiescent cells [24]. It has been shown that the Ki-67 labeling index corresponds to the growth fraction of tumors. In ovarian carcinomas high Ki-67 immunostaining has been correlated with advanced-disease stage and reduction of patient survival [25]. Signs of proliferative activity have been found also in benign tumors [26]. The significance of this proliferative activity remains to be elucidated. Powell *et al.* [27] have postulated that a certain percentage of benign ovarian tumors may eventually undergo neoplastic transformation. Our results are in agreement with this data and demonstrate very low proliferative activity in benign and borderline serous ovarian tumors. Ki-67 immunoreactivity expresses a continuum from benign to borderline and to invasive ovarian cancers.

The results of this study support the concept that a marked difference exists between borderline and malignant serous tumors. According to immunohistochemical analysis, BSOT has much more in common with benign serous tumors than with SPOC. The same conclusion was demonstrated in an in vitro study by van Haaften-Day *et al.* [28].

In summary, the group of BSOT and SPOC differed mainly in patient age and in overexpression of p53 and Ki-67, namely that no borderline serous tumor overexpressed p53 nor Ki-67. Further immunohistochemical studies are needed for differentiation of advanced borderline tumors, presenting with either invasive or noninvasive implants.

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