EGFR, HER2/neu and Ki67 immunoexpression in serous ovarian tumors

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Abstract
In this study, we analyzed EGFR, HER2/neu and Ki67 immunoexpression for 26 benign, borderline and malignant serous ovarian tumors. EGFR and HER2/neu immunoreactions were present in some benign/borderline tumors with high/low intensity of immunostain. In poorly differentiated adenocarcinomas, the EGFR/HER2/neu reaction was intense compared to well-differentiated ones. The Ki67 medium proliferation index was 2.1% for benign tumors, 6% in the borderline and 47.7% in malignant tumors. EGFR, HER2/neu and Ki67 can be used to identify benign/borderline tumors with progression potential and the malignant aggressive tumors.

Keywords: ovarian serous tumor, EGFR, HER2/neu, Ki67, immunohistochemistry.

Introduction
Serous ovarian tumors represent over 30% of ovarian surface neoplasms while serous carcinomas are about 50% of malignant tumors with this location [1]. Serous ovarian adenocarcinomas represent over 30% of genital malignant tumors and provide the greatest number of deaths, the survival rate at 5 years being estimated at 20% [1, 2]. The age, tumor size, degree of differentiation and FIGO stage are the most important clinicopathological prognostic factors for ovarian serous tumors [3, 4]. Detection of malignant lesions in early stages and the identification of biomarkers with prognostic significance are the major concerns of recent studies. Structural similarity of EGFR and HER2/neu growth factors, have led to the hypothesis that overexpression of both tyrosine kinase proteins are involved in signal transduction for the corresponding growth factors and activation of pathogenic pathways that ultimately lead to ovarian cancer [2]. The immunoexpression significance of epidermal growth factor receptor (EGF) is controversial regards malignant serous ovarian tumors, and are relatively few studies which have analyzed the expression of these proteins in the serous benign and borderline tumors [5, 6].

In this study, we analyzed EGFR, HER2/neu and Ki67 immunoexpression in benign, borderline and malignant ovarian serous tumors, and their association with clinicopathological prognostic parameters.

Materials and Methods
The study included a total of 26 selected ovarian tumors and diagnosed between 2007–2011 in the Pathology Laboratory of C.F.R. (Romanian Railways) Hospital of Craiova.

The biological material was represented by total and partial hysterectomy pieces, which were processed by common histopathological technique using 10% formalin fixation, paraffin embedding and Hematoxylin–Eosin stain. Clinicopathological data were analyzed and the histopathological diagnosis was done in conformity with criteria established in 2003 by IARC nominated work group for female genital tract tumors within World Health Organization [7].

The immunohistochemical processing was made on serial sections, antibodies being presented in Table 1.

Table 1 – The panel of antibodies used

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone/Source</th>
<th>Dilution</th>
<th>Antigen retrieval</th>
<th>External positive control</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR</td>
<td>Polyclonal/Sigma</td>
<td>1:1000</td>
<td>Citrate buffer, pH 6</td>
<td>Placenta</td>
</tr>
<tr>
<td>HER2/neu</td>
<td>Polyclonal/Dako</td>
<td>1:300</td>
<td>Citrate buffer, pH 6</td>
<td>Breast carcinoma</td>
</tr>
<tr>
<td>Ki67</td>
<td>MIB-1/Dako</td>
<td>1:100</td>
<td>Citrate buffer, pH 6</td>
<td>Breast carcinoma</td>
</tr>
</tbody>
</table>

Immunohistochemical reactions were performed with the CSA II, Biotin-Free Catalyzed Amplification System (code K197, Dako) for EGFR, respectively LSAB+ System-HRP (DAKO) for HER2/neu and Ki67. For the visualization we used DAB (3,3’-diaminobenzidine, Dako), followed by counterstained with Hematoxylin.

For the interpretation of immunohistochemical reactions, we used an adapted system [8], which was
reported the number of positive cells to total cells counted in 10 microscopic 40x fields obtaining an index of positivity for EGFR and HER2/neu respectively for proliferation in case of Ki67. For receptors, the intensity of reaction was assessed as weak or strong.

Statistical analysis of the results was performed in SPSS 10 software using the chi-square test for dependence assessment. The acquisition of the images was done with Nikon Eclipse E600 microscope and Lucia 5 software.

Results

Histopathological analysis included 26 of serous ovarian tumors of which 57.7% were benign, 7.7% borderline and 34.6% malignant.

Benign tumors were represented by cystadenofibromas (nine cases), adenofibromas (two cases) and papillary cystadenomas (four cases). These were unilateral, being identified in 60% of cases in young patients, average age of diagnosis was 47 years and tumor size was below 10 cm in 86.6% of cases (Table 2).

Table 2 – Clinicopathological parameters

<table>
<thead>
<tr>
<th>Clinicopathological parameters</th>
<th>Range</th>
<th>Benign tumors (No.)</th>
<th>Borderline/Malignant tumors (No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [years]</td>
<td>&lt;50</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>&gt;50</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Tumor size [cm]</td>
<td>&lt;10</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>&gt;10</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Histopathological appearance</td>
<td>–</td>
<td>CAF/AF (11)</td>
<td>WD (5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CAP (4)</td>
<td>MP (2)</td>
</tr>
<tr>
<td>FIGO stage</td>
<td>IA</td>
<td>–</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>IB</td>
<td>–</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>IIA</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td>EGF expression No./marked cells</td>
<td>weak</td>
<td>3 (10%)</td>
<td>1 (15%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 (15%)</td>
<td>3 (22.3%)</td>
</tr>
<tr>
<td>HER2 expression No./marked cells</td>
<td>negative</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>positive</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(12.5%)</td>
<td>3 (72%)</td>
</tr>
<tr>
<td>Ki67 expression (marked cells)</td>
<td>–</td>
<td>2.1%</td>
<td>6%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>47.7%</td>
<td></td>
</tr>
</tbody>
</table>

CAF: Cystadenofibroma; AF: Adenofibroma; CAP: Papillary cystadenoma; MP: Micropapillary; WD: Well-differentiated; PD: Poorly differentiated; PI: Medium positivity index.

Tumors with low malignant potential (borderline) were unilateral, with a micropapillary growth pattern; average age of diagnosis was 52 years and the size of tumors over 10 cm.

Serous ovarian malignancies were represented by well-differentiated (five cases) or poorly differentiated carcinomas (four cases). The average age of diagnosis of patients with malignant ovarian tumors was 55.4 years. In 66.6% of cases, tumors were unilateral, over 10 cm in size (Table 2).

Immunohistochemical analysis of EGFR revealed membrane positivity in 20% (three cases) of benign tumors (CAF, CAP), with weak, incomplete apical and basal membrane stain and medium positivity index of 10% (Figure 1a).

In case of borderline tumors, the immunoreaction was present in 50% (one case) of cases, with apical and basal, incomplete and heterogeneous in intensity immunostain and the medium positivity index was 15%.

In this case, positive cells particularly were located distal to the micropapilla tips (Figure 1b).

Adenocarcinomas were EGFR positive in 77.7% of cases, with low intensity, heterogeneous stain and PI 22.3% for well-differentiated tumors and with increased intensity, and PI 23.5% for the poorly differentiated tumors. Positive cells were located in solid areas in cases of well-differentiated adenocarcinoma and cystic areas or to the surface for the poorly differentiated ones (Figure 1, c and d).

HER2/neu immunostain was identified in 20% (three cases) of examined benign tumors (CAF, CAP), the reaction being weak positive and PI 10% in one case of CAP and intense in two cases of CAP, respectively one case of CAP, with PI 12.5%. In these cases, the stain was incomplete and heterogeneous (Figure 2a). The three cases of benign serous tumors EGFR positive were also HER2/neu positive.

Borderline tumors were HER2/neu positive in one case, with heterogeneous, incomplete stain and IP 20% (Figure 2b). Adenocarcinomas were positive in 55.5% of the cases, the immunostain being weak, with IP 30% for well-differentiated forms and intense, with IP 72% for poorly differentiated tumors. In all these cases, the HER2/neu immunostain was incomplete and heterogeneous (Figure 2c and d).

Ki67 nuclear immunostain was present in 53.3% of benign tumors and the medium proliferation index was 2.1% (Figure 3a). In case of borderline tumors and adenocarcinomas, the medium proliferation index was 6%, respectively 47.7% (Figure 3b). Well-differentiated adenocarcinomas showed a Ki67 proliferation index below 50%, all poorly differentiated tumors having more than 50% marked cells (Figure 3, c and d).

Negative or weak EGFR/HER2/neu immunostain and the low index of positivity/proliferation were associated to benign/borderline serous ovarian tumors, and intense stain and high positivity/proliferation index were observed in adenocarcinomas (chi-square, p<0.05).

Also, there were statistically significant correlations between the degree of differentiation of adenocarcinomas and intensity of EGFR/HER2/neu expression respectively Ki67 proliferation index (chi-square, p<0.05).

There were no differences in EGFR/HER2/neu positivity index and the degree of tumor differentiation (chi-square, p>0.05). Also, we observed statistical association of clinical factors and tumor stage with immunoeexpression markers analyzed (chi-square, p>0.05).

In addition, we did not observed statistical association of clinical factors and tumor stage with immunoeexpression of the analyzed markers (chi-square, p>0.05).
Figure 1 – EGFR immunostain, ×200: (a) Adenofibroma; (b) Borderline tumor; (c) Low-grade adenocarcinoma; (d) High-grade adenocarcinoma.

Figure 2 – HER2/neu immunostain, ×200: (a) Adenofibroma; (b) Borderline tumor; (c) Low-grade adenocarcinoma; (d) High-grade adenocarcinoma.
Discussion

Pathogenesis of malignant serous ovarian tumors has been intensively studied over the past decade, resulting in the appearance of dual concept of ovarian carcinogenesis, whereby low-grade adenocarcinomas are the result of progression of a serous cystadenoma to borderline and then malignant tumor, while high-grade adenocarcinomas occur most frequently de novo [9]. Serous ovarian carcinomas are aggressive lesions that have a poor prognosis and the amplification/overexpression of EGFR and HER2/neu may have therapeutic implications [2, 10].

In this study, the expression of EGFR, HER2/neu and Ki67 was correlated with tumor type and grade of tumors differentiation, but no other associations with clinicopathological parameters. In addition, we observed benign/borderline stained tumors for all used biomarkers.

Literature data on EGFR and HER2/neu immunexpression are controversial as regards association with clinical and histopathological prognostic factors. In 2001, Skúrisdóttir I et al. analyzes the expression of EGFR and HER2/neu in ovarian carcinomas in early stages and found that their expression is not correlated with clinicopathological prognostic parameters [11]. The authors concluded that EGFR and tumor grade are independent parameters and EGFR/Her2/neu coexpression is more common in serous ovarian carcinoma [11]. The same authors analyzed the expression of EGFR and p53 on a group of 226 surface ovarian carcinomas and proposed their stratification into three groups – low risk (well-differentiated, and negative for p53 and EGFR), intermediate risk (well-differentiated, p53/EGFR positive or poorly differentiated and p53/EGFR negative) and high-risk (poorly differentiated and p53/EGFR positive) [12].

In a large study that included 783 ovarian malignant surface tumors, Nielsen JS et al. found HER2/neu and EGFR overexpression in 35%, respectively 62% of lesions. The authors indicated the association of HER2/neu expression with tumor grade, and no other correlation with clinical stage or prognostic factors (age, size, FIGO stage) [3]. Further, notes that the panel consists of EGFR, HER2/neu and p53 tumors may provide prognostic information for borderline tumors [3].

In a communication from 2007, Sueblinvong T et al. indicated no correlation between HER2/neu and clinicopathological analyzed factors for 74 cases of surface malignant ovarian tumors and the protein overexpression in 10.2% of lesions [2]. In 2008, Nofech-Mozes S et al. did not identify any cases of malignant ovarian tumor that shows HER2/neu overexpression [1, 8].

By contrast, in 2006, Lassus H et al. indicate EGFR protein overexpression in 17% of serous ovarian adenocarcinomas and association with tumor grade, residual tumor size and patient age [8]. In a study conducted in 2008 on a cohort of 50 serous ovarian carcinomas, found that 64% of lesions were positive to EGFR, in correlation with tumor grade and survival [5]. In another study conducted in 2004, Suo Z et al. analyzed the
EGF receptor expression and indicate that in poorly differentiated carcinomas the intensity of EGFR and HER2/neu immunostain was strong, compared with well-differentiated lesions [13].

Ovarian surface epithelium is weakly positive for EGFR/Her2/neu and Wang DP et al. indicates in 1992 the presence of HER2/neu positive and EGFR negative benign/borderline ovarian tumors [6]. Heinrich JK et al. found in 2004 HER2/neu overexpression or amplification in ovarian borderline tumors [14].

Transmembrane receptor encoded by the gene c-ErbB2 is overexpressed in 25% of ovarian cancers and may represent a therapeutic target for these patients [10]. Villella JA et al. identified, in 2006, 19 patients with papillary serous ovarian carcinoma and HER2/neu overexpression, being candidates for FISH and Herceptin therapy [10]. Stadlmann S et al. found a significant association between EGFR amplification and intense immunohistochemical expression of protein in the ovarian serous malignant cells [15]. Moreover, Rasppolini MR et al. noted the amplification of HER2/neu in all cases with 3+ protein overexpression, suggesting the therapeutic potential of gene [4].

In this study, Ki67 proliferation index presented values above 50% in all poorly differentiated adenocarcinoma and below 50% in the well-differentiated ones. The results are similar to those of other studies. Thus, O’Neill CJ et al. Ki67 registers a proliferation index of 55.4% in poorly differentiated adenocarcinomas and 23% in the well-differentiated ones [9]. In another study, 73% of high-grade adenocarcinomas and 11% of the low-grade revealed over 50% Ki67-positive cells [13].

Conclusions

Ovarian serous tumors presents distinct immunostain for the analyzed biomarkers, frequency of positive cases increases progressively from benign to borderline and malignant tumors and the latter from the well to poorly differentiated adenocarcinomas. EGFR, HER2/neu and Ki67 can be used to identify benign/borderline tumors with potential for progression. EGFR may be considered a predictive marker for the occurrence of peritoneal implants, the immunostain being identified in tumor compartments with mobilization and metastatic potential.

References


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