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The immunohistochemical expression of p53 and Ki67 in ovarian epithelial borderline tumors. Correlation with clinicopathological factors

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Abstract

Background: Borderline tumor of the ovary is an epithelial tumor with a low rate of growth and a low malignant potential to invade or metastasize. This tumor often is associated with a significantly better prognosis than epithelial ovarian cancer. Most of these tumors are either serous or mucinous in histology. *Aim*: Assessment of p53 and Ki67 immunohistochemical expression in 52 epithelial ovarian tumors, correlation with clinicopathological factors, and comparison between results in benign, borderline, and malignant tumors. *Materials and Methods*: From the total number of 125 patients diagnosed with epithelial ovarian neoplasms in the period 2002–2010, 52 operated patients were selected, with serous and mucinous tumors. There were 26 (50%) malignant cases, 15 (28.8%) borderline and 11 (21.15%) benign. We used the monoclonal antibody DO7 and Ki67–MM1. *Results*: P53 immunoreactions were positive in 41.66% of malignant serous tumors, most of them (90%) high-grade carcinomas; 6.66% of borderline and none benign tumors were positive. Ki67 was positive in 61.53% of malignant cases, with higher percents in advanced clinical stages. Ki67 immunoreactions were also positive in borderline and benign tumors, with lower percents, 13.3% respectively 9.09%. *Conclusions*: We found almost similar frequency of immunostaining in borderline tumors and low-grade invasive serous carcinomas in contrast to the significantly higher frequency of p53 mutations in high-grade serous carcinomas. Proliferative activity as assessed by Ki67 staining does not explain any possible relationship of serous borderline tumors to epithelial ovarian cancer.

Keywords: ovarian tumor, borderline tumor, p53, Ki67.

Introduction

Ovarian borderline (low malignant potential) tumors are a puzzling group of neoplasms that do not fall neatly into benign or malignant categories [1]. Their behavior is enigmatic, their pathogenesis unclear, and their clinical management controversial, especially for serous borderline tumors the most common type of ovarian borderline tumor [2]. Clarifying the nature of borderline tumors and their relationship to invasive carcinoma has confused investigators since the category was created over 30 years ago. Much of the controversy concerning these tumors is due to a lack of understanding of their pathogenesis and an absence of a model for the development of ovarian carcinoma [3].

P53 suppressor gene has an essential role in controlling cell cycle and initiating carcinogenesis. The tumor-suppressor gene p53, located on the short arm of the 17 chromosome, has an essential role in controlling cell cycle and initiating carcinogenesis. Unlike normal p53 protein, rapidly removed from the nucleus, mutant forms have a prolonged half-life, which favors intranuclear accumulation, becoming detectable immuno-histochemically [4].

Ki67 protein is a cellular marker for proliferation. It is strictly associated with cell proliferation. Ki67 is an excellent marker to determine the growth fraction of a given cell population. The fraction of Ki67-positive tumor cells is often correlated with the clinical course of cancers. MIB1 is a commonly used monoclonal antibody that detects the Ki67 antigen. It is used in clinical applications to determine the Ki67 labelling index. One of its primary advantages over the original Ki67 antibody (and the reason why it has essentially supplanted the original antibody for clinical use) is that it can be used on formalin-fixed paraffin-embedded sections, after heat-mediated antigen retrieval [5].

The purpose of this study was to evaluate the biological significance of reactivity of p53 and also Ki67 antigen expression in cystadenomas, borderline tumors, and invasive cystadenocarcinomas; the correlation between this cell proliferation and clinicopathologic parameters (FIGO stage and grade) was also investigated in invasive cystadenocarcinomas.

Materials and Methods

From the total number of 125 patients diagnosed with epithelial ovarian neoplasms in the period 2002–2010 in the Obstetric and Gynecological Hospital in

Botoşani, 52 operated patients were selected, with serous and mucinous tumors.

We used extensive samples of tumoral ovarian tissue and Hematoxylin–Eosin (HE) classical technique to diagnose these neoplasms.

Immunohistochemical staining for Ki67 and p53 was performed on the representative samples of ovarian tissue.

For p53 immunoexpression, we used the DO7 monoclonal antibody (Novocastra, Leica Biosystems, Newcastle, United Kingdom), with pre-treating in citrate solution (pH 6), for 20 minutes and incubation with the primary prediluted antibody for 30 minutes, visualization with DAB and counterstained with Hematoxylin. For negative control, buffer replaced the primary antibody. Positive control was represented by a breast carcinoma, with strong nuclear reaction for p53 in carcinomatous cells.

The expression of primary tumor proliferation related to Ki67 antigen was immunohistochemically evaluated by monoclonal MIB1 antibody (Novocastra, Leica Bio-systems, Newcastle, United Kingdom) on microwave oven-processed formalin-fixed paraffin-embedded tissue, and citrate pH 6 as antigen retrieval. Development was performed with DAB (3,3'-diaminobenzidine dihydro-chloride) solution, which was applied for 3–5 minutes. Nuclei were stained using Mayer's Hematoxylin. We considered a positive reaction only in the presence of immunostained nuclei in brown shades.

On the sections examined, we observed different patterns of positivity of tumoral cells:

▪ a focal model, which express a small number of stained neoplastic cells;

▪ a heterogeneous pattern, with islet of strong positive reaction alternating with areas with low positive reaction;

▪ a diffuse model presenting uniform diffuse positivity.

We also used a quantitative assessment in our analysis, according to the number of stained cells:

▪ 0: score 0;
▪ 1–10%: score 1;
▪ 10–50%: score 2;
▪ 50–100%: score 3.

When the expression of p53 was analyzed in relation with Ki67 expression status, the staining pattern was divided in four groups:

▪ Ki67 negative/p53 negative (Ki67-/p53-);
▪ Ki67 positive/p53 negative (Ki67+/p53-);
▪ Ki67 positive/p53 positive (Ki67+/p53+);
▪ Ki67 negative/p53 positive (Ki67-/p53+).

Also, a statistical study was applied.

Results

Histopathological results (HE staining)

In our study, we identified 26 malignant tumors, 15 borderline, and 11 benign tumors. The average age for malignant tumors was 59.64 years (ranging between 42 and 78 years). According with FIGO stages, there were seven tumors stage I, eight – stage II, eight – stage III, three – stage IV. Twenty-four (92.3%) of malignant tumors were serous type and two (7.7%) cases were mucinous.

Mucinous malignant tumors were intestinal type, with expansile architectural pattern of invasion.

The serous malignant tumors were the most frequent in our study: the high-grade carcinomas were represented in 66.6% and the low-grade carcinomas in 33.3% from the chosen cases. The diagnosis of serous carcinomas was established as low and high grade according to nuclear atypia (uniform *vs.* pleomorphic) and mitotic activity.

Age for borderline tumors ranged between 30 and 68 years with an average of 43.36 years. All borderline cases in our study were stage FIGO I. Borderline tumors were represented by 15 cases: 13 (86.6%) of serous type and two (13.3%) of mucinous epithelium. All the serous borderline tumors from our group which were immuno-histochemically investigated were of classic type, with hierarchical branching, irregular papillae, detached tufts, cuboidal to columnar eosinophilic cells, low nuclear-cytoplasm ratio for at least of 10% of tumor (Figures 1 and 2).

From the entire group of benign tumors from our study, 72.72% were serous and 27.27% were mucinous intestinal type.

Benign tumors were represented by serous cyst-adenomas and cystadenofibromas and mucinous cyst-adenomas intestinal type.
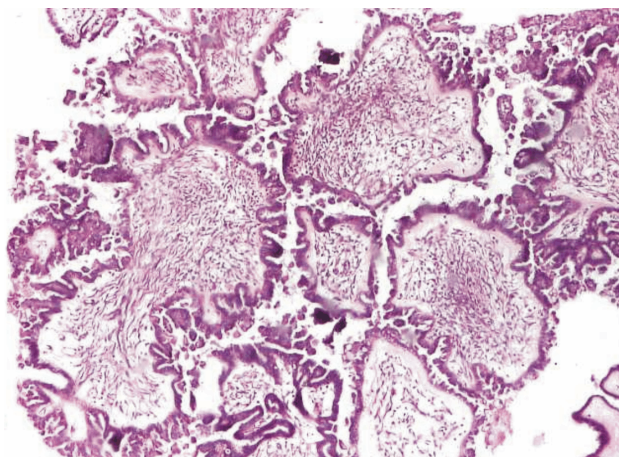

Figure 1 – *Borderline serous tumor with papillary epithelial proliferation, detached isolated cells and tufts, ob. 10×.*
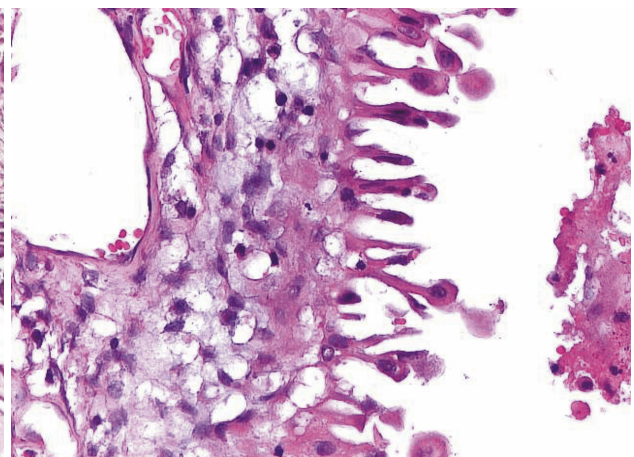

Figure 2 – *Eosinophilic cells with moderate atypia, ob. 20×.*

P53 immunoexpression

For the entire study group, p53-immunoreactions became positive in 10 cases, all serous type, representing 38.46% of malignant tumors and 41.66% of investigated serous malignancies. Nuclear staining was in general intense and moderate and was limited only to neoplastic cells, without interesting the stromal nuclei.

From these p53 positive cases, 10% were low-grade serous carcinomas and 90% were high-grade carcinomas, representing 12.5% (1/8) and 56.25% (9/16) from low-grade, respectively high-grade carcinomas.

In serous carcinomas category, we have observed p53 positivity for 20% (1/5) in stage I, 37.5% (3/8) stage II, 50% (4/8) positive reaction in stage III and 66.6% (2/3) in stage IV carcinomas (Figure 3).
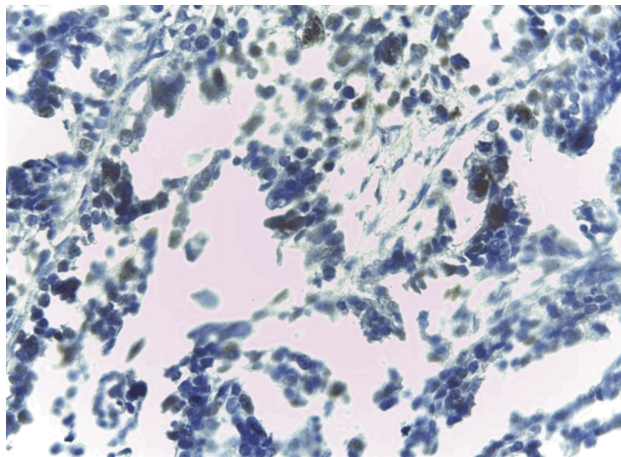

Figure 3 – *P53 positivity in different clinical stages in serous carcinomas.*

The prevalence of p53 immunostaining was 31% (score 2) for low-grade carcinomas and 75% (score 3) for high-grade carcinomas (Figures 4 and 5).

Figure 4 – *P53 staining in low-grade serous carcinomas: focal pattern and isolated cells, ob. 20×.*

Figure 5 – *P53 staining in high-grade serous carcinomas: diffuse, strong immunostained cells, ob. 20×.*

For the borderline tumors, we noted just a small number of p53 immunopositive reaction in serous neoplasms: 1/15 cases p53+ (6.66%); a small number of isolated stained cells and heterogeneous pattern were observed (21% – score 2) (Figure 6).
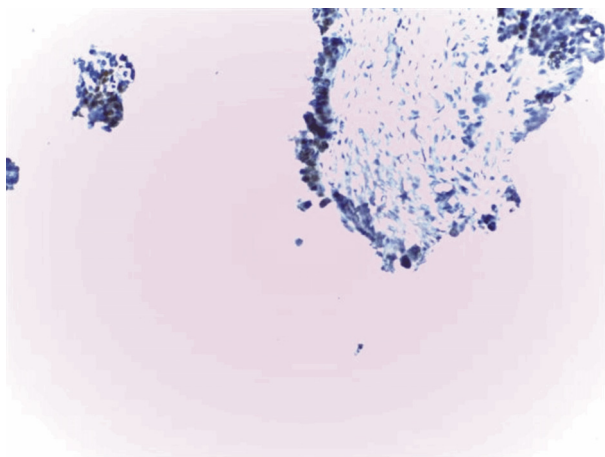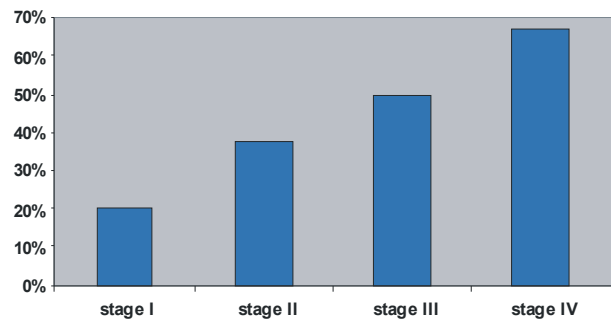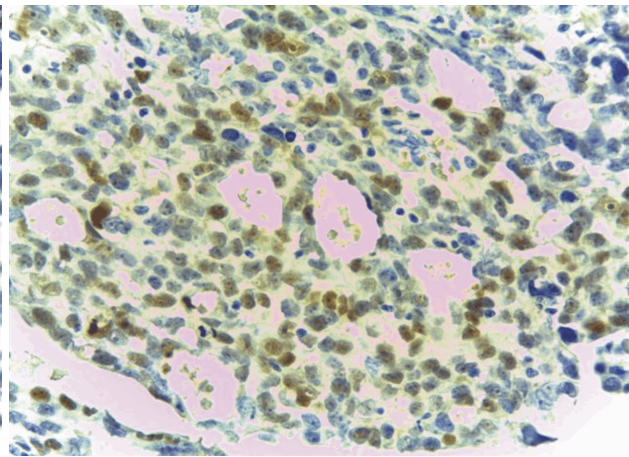

Figure 6 – *P53 immunopositive reaction in borderline tumor, ob. 20×.*

In the benign category of ovarian tumors we did not identify any positive reaction for p53 immunostaining (score 0).

Ki67 immunoexpression

Ki67 positive stained sections were observed in 16 cases (61.53%) of malignancies: two mucinous type and 14 of serous type.

According with FIGO stages, the Ki67 positivity has the following distribution in serous type tumors: 4.16% (1/24) stage I, 16.66% (4/24) stage II, 25% (6/24) stage III and 12.5% (3/24) stage IV carcinomas, from the total number of carcinomas immunohistochemical analyzed. This fact corresponded with the following percents of corresponding stage cases: 14.98% of cases chosen in stage I, 50% of stage II, 75% stage III, 100% stage IV.

In our study, Ki67 immunoreactions were noticed in four (13.3%) cases of low-grade serous carcinoma and 10 (41.6%) cases of high-grade carcinoma (Figure 7).
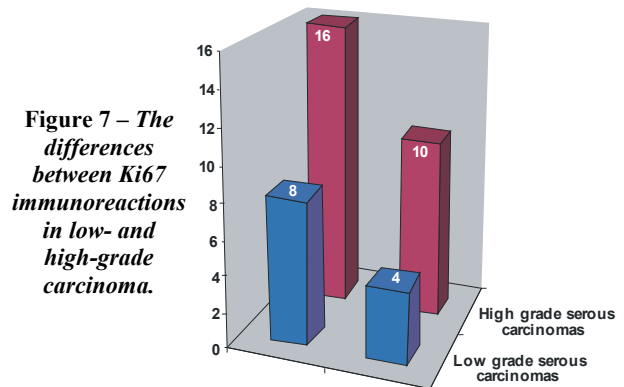

Figure 7 – *The differences between Ki67 immunoreactions in low- and high-grade carcinoma.*

We noticed a strong, diffuse pattern in high grade serous carcinomas, with a prevalence of immunostained

cells of 75% (score 3) in 41.66% of cases, more than low grade carcinomas and borderline tumors, which both exhibited a lower prevalence, respective 29% and

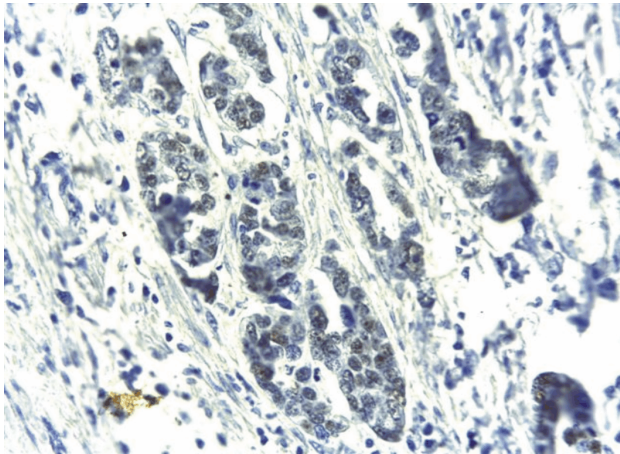21% (score 2), with heterogeneous aspects and also isolated stained cells (Figures 8 and 9).

Figure 8 – *Ki67 immunoexpression in low-grade serous carcinomas with heterogeneous pattern, ob. 20×.*

Figure 9 – *Ki67 positive reaction in high-grade serous carcinoma, strong and diffusely positive immuno-staining can be observed, ob. 20×.*

We identified two cases of stage II low-grade carcinomas Ki67 positive immunoreactions and two cases stage III low-grade carcinomas, corresponding with 66.6% of low-grade carcinomas of same stage.

In the high-grade carcinomas category, we noted 10 cases of Ki67 positivity: one case stage I, two cases stage II, four cases of stage III, three cases of stage IV. This corresponded with following percents of represen-tation in the similar stages: 33.3% of stage I, 50% of stage II, 80% of stage III and 100% of stage IV (Figure 10).

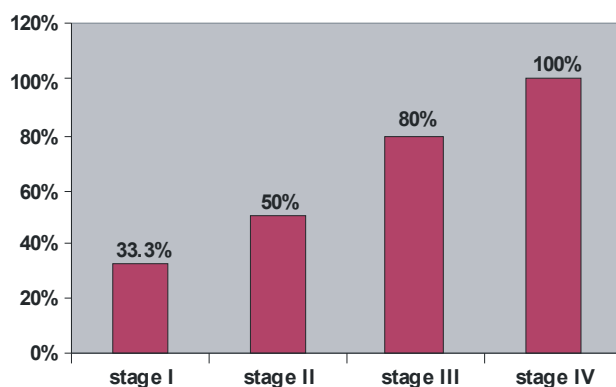Both mucinous tumors from our study were stage I

and expressed Ki67 moderate stained, with heterogeneous pattern, score 2.

Borderline tumors we have analyzed expressed 2/15 cases Ki67+ (13.33%); both were serous type, with 8% immunostained cells (score 1). The staining had a heterogeneous pattern and isolated cells were also observed (Figure 11).

In the benign category, we identify only one serous tumor that presented Ki67 positive reaction (9.09%). The positive Ki67 reaction was represented by focal, isolated brown-stained nuclei, less than 5% of immuno-stained cells (score 1).

Figure 10 – *Ki67 positivity in different clinical stages for high-grade serous carcinomas.*
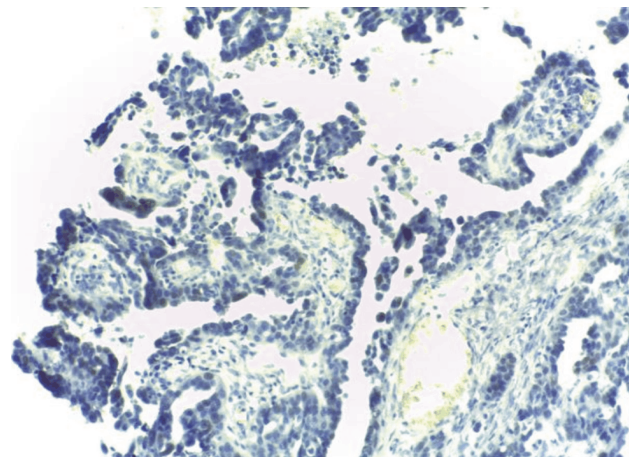
Figure 11 – *Ki67 reaction in borderline tumor. The staining had a heterogeneous pattern and isolated cells were also observed, ob. 20×.*

⊟ Discussion

The division of the various epithelial subtypes into benign, borderline, and malignant forms is based on the premise that tumors with architectural and cytologic features that are intermediate between those of clinically benign and malignant tumors of the same epithelial cell type have a significantly better prognosis, stage for stage, than their malignant counterpart. By definition, borderline tumors lack destructive stromal invasion, but
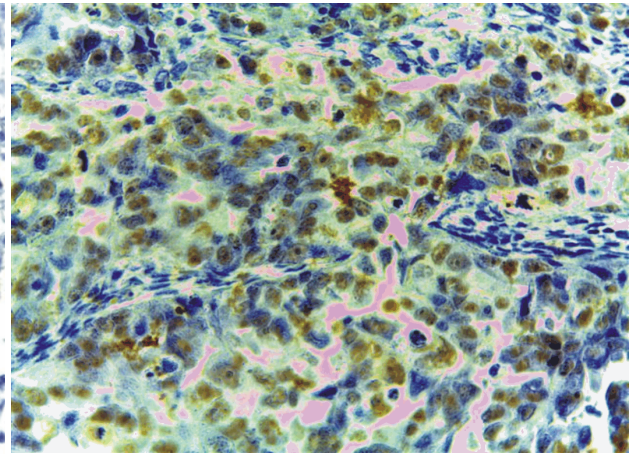
have other histologic features of malignancy (*e.g.*, nuclear atypia, cellular stratification, mitotic activity) [6, 7].

For the most part, the clinical behavior of these histologically intermediate or borderline tumors is benign, with the exception of serous borderline surface epithelial stromal tumors, which are frequently associated with extraovarian disease and exhibit a clinical behavior intermediate between benign serous tumors and invasive serous carcinomas [8].

In contrast to invasive cancers, borderline tumors occur in younger age groups, with lower disease stage and better prognosis.

A dual oncogenic pathway has been described in which serous benign and borderline ovarian tumors undergo stepwise transformation to low-grade serous carcinomas and high-grade serous carcinomas possibly arise *de novo* from surface epithelia due to p53 [4, 9–11].

In our study, we checked if there is a correspondence between p53 and Ki67 positivity and the pathogenic model described before.

We noticed the next distribution according with Ki67 and p53 immunoexpression:

▪ Ki67-/p53-: seven cases;
▪ Ki67+/p53-: eight cases;
▪ Ki67+/p53+: eight cases;
▪ Ki67-/p53+: three cases.

Assessment of p53 immunoexpression

We observed in our research none p53 positive reaction in benign, low immunoreaction in borderline tumors, and 42.3% positive reactions in malignancies, all of them of serous type.

In the study of Morita K *et al.*, p53 overexpression was observed more frequently in serous adenocarcinomas (5/8, 63%) than in mucinous adenocarcinomas (2/9, 22%) and was correlated with the malignant potential of serous tumors [12]. The small number of mucinous type tumors may be a reason for the lack of p53 immunoreaction in mucinous type tumors in our study.

The results of p53 positivity are relatively different in diverse studies. Some reviews presented p53 mutation prevalence estimated as 45% (42–47%), 5% (2–9%), and 1% (0–5%), respectively, for invasive, borderline and benign tumors. The prevalence of these p53 abnormalities was found to be associated positively with increasing tumor grade and stage. Differences based on histologic subtype also were found [4, 13].

Studies of Caduff RF *et al.* confirmed that borderline tumors present a high positivity for Ki-RAS (41%) comparing with malignant high-grade serous tumors (11%), since p53 is overexpressed in high-grade serous carcinomas (44%) comparing with low malignant neoplasms (8%) [14], these results were similar in different researches [13, 15].

In the study of Berchuck A *et al.*, the authors followed if expression of p53 protein is a common feature of invasive epithelial ovarian cancers and they investigated whether immunoreaction of the p53 tumor-suppressor gene product occurs in benign and borderline epithelial ovarian tumors [16]. Immunoexpression of p53 was observed in 0/17 (0%) benign ovarian tumors and 2/49 (4%) borderline tumors (only seen in advanced stage cases: expression was seen in 2/8 (25%) stage III cases, but not in any of 41 stage I/II cases). P53 expression was not a feature of benign epithelial ovarian tumors or early-stage borderline ovarian tumors. Other authors confirmed later this fact [17].

The rarity of p53 abnormalities among benign ovarian tumors compared with the increasing prevalence of these abnormalities among tumors of borderline and carcinomas might be taken as evidence that ovarian carcinogenesis follows a multistep model [4, 18].

In some studies, in contrast to the carcinomas, for which the prevalence of p53 expression in ≥50% of the cells was 29%, the prevalence among the tumors of borderline category was only 5% [4, 19].

The prevalence of p53 overexpression appeared to differ by the antibodies used. The overall pooled estimates were determined largely by the most frequently used antibodies (DO7, PAb-1801, and DO1). Although these three antibodies yielded similar estimates of the prevalence of p53 overexpression among ovarian carcinomas (51–56%), less commonly used antibodies such as CM1 were found to yield lower estimates (32%). This difference persisted when the carcinomas were evaluated by histology, grade, and stage [4, 20, 21].

Assessment of Ki67 immunoexpresion

Our study was conducted to evaluate the diagnostic accuracy of histopathology in ovarian neoplasms, and to investigate the biological significance of Ki67 antigen expression in benign and malignant ovarian tumors and correlate it with histological type, grade, and stage of malignant tumor.

In our present study, 61.53% Ki67 immunopositive reactions we observed in investigated malignancies, most of them in higher stages; borderline and benign cases presented low positive reactions (13.3%, respectively 9.09%).

In low-grade serous neoplasms, we observed moderate heterogeneous immunostained cells; in high-grade carcinomas, there was a strong diffuse pattern.

In our study, staining pattern in borderline tumors was heterogeneous, with low positivity in less than 10% of cells; malignancies exhibited immunoreactions in >50% of cells; benign tumors presented only focal, isolate cellular staining.

In different studies and also in our research, the tissular Ki67 antigen immunostaining was significantly higher in cystadenocarcinomas, compared to cystadenomas and borderline tumors, with the highest values in architectural high grade neoplasms. Proliferative activity as assessed by Ki67 staining does not explain any possible relationship of serous borderline tumors to epithelial ovarian cancer.

In studies of Henriksen R *et al.* (1994), Ki67 expression was present in tumor cells in the main part of borderline and malignant tumors and even in small number of benign counterparts, which might indicate an active state in these commonly believed dormant neoplasms. In the malignant group, the Ki67 correlation to survival seemed to be independent of staining intensity, although the observed decay in survival seemed to be faster in the strongly stained group [21, 22].

In the study of Palazzo JP *et al.*, Ki67 was only weakly expressed in 42% benign cystadenomas, all borderline tumors showed Ki67 staining in less than

50% of the cells, and 55% of serous carcinomas stained in more than 50% of tumor cells [23].

Some original articles we have examined, presented low Ki67 positivity in ovarian benign tumors (7.5–12%), higher positivity levels in borderline neoplasms (22.6–40%) and ranged between 55–70% in carcinomas [24, 25]. The most studies indicated the same results: low Ki67 positivity in benign tumors is increasing in borderline and malignant tumors [26, 27].

A significant difference in Ki67 immunostaining was found between carcinomas and benign tumors, and between borderline and carcinomas but not between benign and borderline tumors, similar results we obtained in our study [28].

⯐ Conclusions

In our study, all tumors with p53 positive immunostaining were serous type, which supports the involvement of this protein in the pathogenesis of serous borderline and malignant tumors. We have found similar frequencies of p53 positive immunoreactions in serous borderline tumors and low-grade invasive serous carcinomas, in contrast to the significantly higher frequency of p53 mutations in high-grade serous carcinomas. This fact suggests a common pathogenesis for serous borderline tumors and low-grade invasive serous carcinomas and supports the view that borderline neoplasms are unrelated to the high-grade neoplasms.

Nuclear Ki67 immunoexpression was more obvious in malignant tumors compared to benign and borderline tumors. This highlights the role of nuclear factor in tumor growth. The low Ki67 immunoreaction in borderline tumors suggests that increased expression occurs later in the development of carcinoma.

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