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Clinical factors and biomarkers in ovarian tumors development

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Abstract

Ovarian cancer is a disease difficult to detect in early stages due to nonspecific symptoms and has a rapid progression with frequent relapses after radical surgical procedure. For these reasons, ovarian cancer generally represents the fourth cause of death through cancer in females, while in our country it is surpassed only by cervix cancer. The reduced survival is associated with the absence of symptoms, especially in early stages. Therefore, the diagnosis is delayed, when the metastases are already present and the prognosis is poor. While the etiology of the ovarian cancer is less understood, the histopathological studies and experiments regarding ovarian cancer development suggest that the majority of the tumors refined to the surface epithelium, a cuboidal layer that lays the ovary. It is still unclear if the molecular changes in this layer generate a neoplastic precursor that can be used for establishing an early diagnosis. None of the changes of the involved genes (p53, k-Ras, Her-2/neu, c-Myc, etc.) does seem to follow certain steps. We analyzed histological and immunohistochemical a group of 60 female patients admitted during January 2004 and January 2005 in Surgery Clinic of "Sfantul Ioan" Emergency Hospital, Bucharest. Our study reveals that a high percent (68.33%) of females had a correct diagnosis at admission, only five patients (8.33%) being diagnosed with other diseases. In 86.66% of cases, total hysterectomy with bilateral anexectomy has been made, in two cases (3.33%) tumor resection was the only needed therapy and in 19 cases (31.66%) peritoneal implants were found. More than 75% were serous tumors, 20% mucinous carcinoma and 5% borderline ovarian tumors. We found three cases of borderline tumors (5%) that histopathological proved to be serous tumors. The analysis of hormone receptors showed estrogen receptors in 32 cases (71.1%) of serous ovarian adenocarcinoma, in seven cases (58.33%) of mucinous adenocarcinoma, all three cases (100%) of borderline tumors and in four cases (21.05%) of the 19 with peritoneal implants. Progesterone receptors were found in 27 cases (60%) of serous carcinoma, five cases (41.66%) of mucinous carcinoma, one case (33.33%) of borderline tumors and five cases (26.31%) with peritoneal metastases. Immunohistochemical study of CerbB-2 showed positively only in 37 cases (82.22%) of serous carcinomas, five cases (41.66%) of mucinous carcinomas, one case (33.33%) of borderline tumor and eight cases (42.10%) with peritoneal metastases. All tumor types presented positives for CA125 (91.1% in serous tumors, 83.33% in mucinous tumors, 33.33% in borderline tumors and 73.68% in tumors with peritoneal implants. The investigated proliferative factors p53 and Ki-67 demonstrated correlation with tumor aggressiveness. Lack of positivity in borderline tumors and strong positivity in serous and mucinous carcinomas shows correlations with literature. This study outlines that an immunohistochemical analysis of certain antibodies cannot offer useful data regarding the prognosis or the screening for ovarian cancer.

***Keywords*:** ovarian carcinomas, immunohistochemistry, prognosis and screening markers.

Introduction

Ovarian cancer is one of the most frequent cancers in female patients. Among genital malignancies, epithelial ovarian cancer has the most increased mortality rate. The low survival is due to the lack of symptoms in early stages. Therefore, the diagnosis is delayed, when the metastases are already present and the prognosis is poor. While the etiology of the ovarian cancer is not fully understood, the conclusions of the histopathological studies and the experiments concerning ovarian cancer evolution suggested that the majority of the tumors originate in the surface epithelium, a cuboid layer that wraps the ovary. It is still unclear if the molecular changes in this layer generate a neoplastic precursor that can be used for establishing an early diagnosis. None of the changes of the involved genes (p53, k-Ras, Her-2/neu, c-Myc, etc.) does seem to follow certain steps [1, 2]. The most challenging ovarian tumors from diagnostic and therapeutic point of view are borderline tumors and invasive carcinomas.

Ovarian cancer represents 30% from all female genital cancers. According to the latest WHO statistics, ovarian cancer is as frequent as corpus uteri cancer (35%) and invasive cervix cancer (27%) [3].

Ovarian cancer incidence in accordance with the age varies from less than two new cases per 100 000 females in South-East Asia and Africa, to more than 15 cases in Eastern and Northern Europe. The highest rates are registered in economically advanced countries; in USA, it is considered that more female patients die because of ovarian cancer than because of the other genital cancers [2, 3].

Literature outlines two factors that may reduce the risk of ovarian cancer development: multi-parity and oral contraceptive pills [4, 5]. Three recent studies show

that postmenopausal women, treated with high estrogen doses over 10 years, have an increased risk of ovarian cancer development [6].

Scarce data is available upon the etiology of non-familial cases [7]. The protective role of pregnancy and of the contraceptive pills suggests that ovulation is a trigger of the disease, although no mechanism was ascribed to correlate the risk factors with malignant transformation [4, 6].

Concerning the diet, the studies showed the possible role of obesity in carcinogenesis [8].

The most important risk factor for ovarian cancer remains the positive family history [9–11].

Numerous epidemiological studies assessed the risks associated with a positive family history of ovarian cancer [12, 13]. The studies were not as comprehensive as those for breast cancer were, but it was established that the relative risk in one-degree relatives varies between 1.94 until 25.5 the highest values being encountered if both the mother and one sister were affected [14–16].

Designing models in order to predict BRCA1 and BRCA2 gene mutations permitted to discover certain factors associated with these mutations [17–20]. Personal characteristics include [5, 11, 21]:

- breast cancer diagnosed at an early age;
- bilateral breast cancer;
- history of breast and ovary cancer;
- breast cancer in family.

The characteristics of family history associated with increased risk for mutation development are [22–24]:

- multiple breast cancers in the family;
- breast and ovary cancer in the family;
- one or more family members with two primary cancers;
- Ashkenazi Jews.

Considering the age, epithelial ovarian cancer is absent before the age of 20. The incidence increases linear between 30–50 years and continues to increase after 50 years. The highest incidence is encountered in the eight decade of life (57 cases/100 000) [10, 25].

Not neglected, the surgical procedures like bilateral tubal ligation and hysterectomy are associated with a low risk for ovarian cancer. The lack of methods able to detect the disease in early stages, prophylactic oophorectomy was proposed for ovarian cancer prevention [26].

Genetic counseling provided by multidisciplinary centers has increased the number of females with gene mutations who choose this method of prevention. Recent studies showed that the risk for peritoneal carcinomatosis persists, although the oophorectomy reduces the risk for ovarian cancer and also for breast cancer development [12, 27, 28].

The histological classification of ovarian tumors established by WHO in 2003 comprises serous, mucinous, endometroid, clear cell, transitional cell tumors, squamous cell, mixed tumors and undifferentiated/unclassified tumors [2, 4].

Serous ovarian tumors classification is shown in Table 1:

Table 1 – ***Serous ovarian tumors classification (codes ICD–0)***

Serous adenocarcinoma	8441/3
Borderline serous tumor	8442/1
Benign serous tumor	
Papillary serous cystadenoma	8460/0
Serous cystadenoma	8441/0
Surface serous papiloma	8461/0
Serous adenofibroma / cystadenofibroma	9014/0

WHO classification of mucinous tumor includes (Table 2):

Table 1 – ***Mucinous ovarian tumor classification (codes ICD–0)***

Mucinous adenocarcinoma	8480/3
Mucinous cystadenocarcinofibroma	9015/3
Borderline mucinous tumor	8472/1
Mucinous cystadenoma	8470/0
Mucinos adenofibroma	9015/0

Histological criteria for borderline serous tumor diagnosis were established as follows (Table 3):

Table 2 – ***Histological criteria for borderline serous tumor diagnosis were established (after WHO [72])***

- Epithelial hyperplasia: stratification, tufting, cribriform, micropapilar;
- Atypia (usually slight/moderate);
- Detached cell groups;
- Variable mitotic activity, usually minimal;
- Absence of destructive stromal invasion.

Borderline serous tumors are considered to have low malignancy, with an atypical epithelial proliferation greater than in benign tumors [29, 30].

Terms like **serous tumor with low malignant potential** or **serous tumor with borderline malignancy** are still used. Literature did not recommend the term proliferative atypical serous tumor because this histological type of tumor did not always have a benign course [31].

Recently, this tumor type comprises the subtype called "**microinvasive borderline serous tumor**", characterized by single cells or cell groups similar to those of non-invasive tumor. These foci should not represent more than 10 mm^2. Borderline serous tumors are divided into typical and atypical tumors. The last studies evidenced that more than 30% of borderline serous tumors (SBT) are associated with a tumor on external surface of the ovary and more than 2/3 are associated with peritoneal implants [32, 33].

Morphological aspect of the peritoneal implants is extremely important, and is considered as a prognostic factor in patients with SBT in stages II–III.

First defined as intermediary or semi-malignant tumors, borderline serous tumors are characterized by epithelial proliferation, lack of destructive ovarian stromal invasion and a better prognosis compared to serous carcinomas, even if peritoneal metastases are present. Implants classification in invasive and non-invasive implants seems to have a real prognosis importance. Recently, Kurman RJ *et al.* proposed that a small subset of borderline serous tumors with micropapillary architecture and with a high incidence of

peritoneal invasive implants should be named "micropapillary serous carcinoma" (MPSC) [34–36].

We should outline that borderline mucinous tumors are divided in two types, depending on the epithelium type: intestinal type and endocervical type [37].

Tumor stage remained the most important prognostic determinant. Recently, new molecular factors have been proposed to have prognostic importance in ovarian cancer [28, 38, 39]. Most of them were identified in retrospective studies without multivariate analysis or confirmation on extensive studies. We outline that these factors emerged from experimental studies in ovarian cancer biology but none of them is routinely used in selection of the patients with ovarian cancer [40–42] (Table 4):

Table 4 – ***Experimental prognosis factors in ovarian cancer***

Morphometry
DNA-ploidy and phase fraction S
Markers for drugs resistance:
▪ Glycoprotein P immmunoreactivity;
▪ Glutathion S-transferase pi;
▪ CerbB-2;
▪ Pluridrug resistance proteins;
▪ DNA repair genes through nucleotide excision (ERCC1 and XPAC).
Oncogenes:
▪ p53 mutant expression;
▪ AKT-2.
Proliferation markers:
▪ Ki-67;
▪ Proliferating Cell Nuclear Antigen.
Tumor dissemination markers:
▪ Metastasis genes (nm-23-H1);
▪ Cathepsine D;
▪ Plasminogen activators like urokinase;
▪ CSF-1;
▪ CD44 molecules.
Cytokines and other active proteins:
▪ Caloric shock protein;
▪ Interleukin 6;
▪ Platelet-derived growth factor.

The aim of our study is to analyze clinical, histopathological and immunohistochemical a group of patients from a general surgery clinic, but not a gynecological clinic. Immunohistochemical markers were classified depending on the gathered data: for hormone receptors analysis and specific ovary antibodies: ER, PGR, CA125 and CerbB-2, for proliferation factors analysis: Ki-67 and p53, for exclusion diagnosis: S100 protein and CK7.

Material and methods

A group of 60 patients from "Sfantul Ioan" Hospital General Surgery Clinic was analyzed clinical, macroscopy, microscopy and immunohistochemical during January 2004–January 2005. The diagnosis at admission was variable, appropriate for a surgery department. None of the cases was sent to another department. The lab tests have included routine tests (cell blood count, ESR, urine analysis), also abdominal ultrasound, and blood level of CA125.

The surgical procedures have been elaborated before or during the surgical intervention, none of the cases needed a second intervention. The main surgical techniques were:

- total hysterectomy with bilateral anexectomy;
- bilateral anexectomy;
- tumorectomy.

We did not perform for the cases examination on ice. We excluded the cases that were possible metastasis. A single case proved to be a gastric cancer metastasis after the histopathological examination, and was excluded too. The surgical samples have been analyzed macroscopically, outlining the following:

- cystic or nodular tumor pattern;
- uni-/bilateral disease;
- cyst multilocularity;
- macroscopic calcifications;
- mucinous aspect of the tumor;
- ovarian capsule involvement;
- peritoneal implants;
- surgical procedure;
- another resected organs;
- primary surgical intervention / second intervention.

The tumor samples and those from the other resected organs have been histopathologically examined. We have performed the histopathologic exam on the 60 cases, using Hematoxylin–Eosin staining in order to define tumor stage and grade. The tumor tissues have been fixed in 10% formalin and embedded in paraffin. Histological sections were cut at 3 μm thickness and stained with Hematoxylin–Eosin.

The immunohistochemistry (IHC) was performed on 3 μm thick sections from 10% formalin fixed paraffin embedded tissues, according to the indirect tristadial Avidin–Biotin–Complex method of Hsu SM *et al.* [43]. Briefly, the procedure was: deparaffinization in xylene and alcohol series, rehydration, washing in phosphate saline buffer (PBS), incubation with normal serum, for 20 minutes, incubation with primary antibody overnight, standard labeled streptavidine-antibody biotin (LSAB) kit (DAKO), washing in carbonate buffer and development in 3,3'-DAB hydrochloride/H_2O_2; microwave antigen retrieval in M-citrate buffer pH 6.0 was performed for it. To ensure the reliability of the experimental study, internal quality control of immunohistochemical techniques was performed as a part of an implemented and certified quality assurance system (ISO 9001/2001). The selected cases were tested by immunohistochemistry by means of (using) the following antibodies (Table 5).

Table 5 – ***The panel of antibodies used for immunohistochemical examination of malignant ovarian tumors***

Antibody	Specificity	Dilution	Source
ER	Estrogen receptor	1/40	NOVOCASTRA
PR	Progesterone receptor	1/100	NOVOCASTRA
EGFR	Epidermal growth factor receptor	1/1000	SIGMA
CerbB-2	Gene protein Her-2/neu / erbB-2	1/250	DAKO
Ki-67	Proliferating Cell Nuclear Antigen (PCNA)	1/50	DAKO
CA125	Epithelial ovarian marker	1/100	NOVOCASTRA
S100	Dendritic cell marker	1/500	DAKO
p53	p53 gene protein	1/50	DAKO
CK7	Markers for other epithelium than ovary	1/50	DAKO

The samples immunohistochemical stained were examined using optical microscopy. A panel of antibodies for ovarian tumors investigation and for metastases detection was analyzed.

Results

The majority of the 60 female patients had the diagnosis of ovarian tumor at admission, but also were encountered other similar diagnostics (Figure 1).

We noticed that the most affected age groups were those between 41–60 years, as the following table shows (Figure 2).

Due to the lack of an ice examination, the surgical procedures used were (Table 6).

Table 3 – *Surgical procedures used*

	Patients (n = 60)	%
Total hysterectomy with bilateral adnexectomy	52	86.66
Bilateral anexectomy	6	10.00
Tumorectomy	2	3.33

It has been noticed within the surgery and macroscopically, the bilateral and peritoneal involvement (Figure 3).

The histopathological examination revealed the following: more than half of the cases were serous tumors (Figure 4) and less were borderline tumors (Table 7, Figure 5).

Table 4 – *The distribution of cases regarding the histological type of tumor*

Histological tumor type	Patients (n = 60)	%
Serous carcinomas	45	75
Mucinous carcinomas	12	20
Borderline tumors	3	5

Peritoneal involvement was histopathological confirmed in all 19 examined cases and we noticed calcifications in 10 cases of serous carcinomas. We considered that peritoneal implants were invasive, due to ovarian capsule involvement.

The immunohistochemical examination associated with the antibodies panel allowed us a useful classification in different groups: for *hormone receptors* analysis and *specific ovary antibodies*: ER (Figure 6), PGR (Figure 7), CA125 (Figure 8) and CerbB-2 (Figures 9 and 10), for *proliferation factors* analysis: Ki-67 (Figure 11) and p53 (Figure 12), and for *differential diagnosis*: S100 protein and CK7.

The statistical results regarding the analysis of the hormone receptors compared to histological type of the malignant ovarian tumor were presented in Table 8 and Figure 13.

The antibodies that investigated proliferation factors (Figures 11, 14 and 15) and p53 gene protein (Figure 12) have provided data showed in Table 9 and Figure 16.

Table 5 – *The distribution of hormones receptors in ovarian tumors (n = 60)*

Markers	Serous ovarian adenocarcinomas (n = 45)	Mucinous ovarian adenocarcinomas (n = 12)	Serous borderline tumor (n = 3)	Ovarian tumors with peritoneal involvement (n = 19)
ER	32 (71.10%)	7 (58.33%)	3 (100%)	4 (21.05%)
PGR	27 (60.00%)	5 (41.66%)	1 (33.33%)	5 (26.31%)
CerbB-2	37 (82.22%)	8 (66.66%)	1 (33.33%)	8 (42.10%)
CA125	41 (91.10%)	10 (83.33%)	1 (33.33%)	14 (73.68%)

Table 6 – *The distribution of proliferative factors in ovarian tumors (n = 60)*

Proliferative markers	Serous adenocarcinomas (n = 45)	Mucinous adenocarcinomas (n = 12)	Borderline tumors (n = 3)	Ovarian tumors with peritoneal involvement (n = 19)
p53				
▪ *<50%*	35 (77.77%)	7 (58.33%)	0	13 (68.42%)
▪ *>50%*	9 (20.00%)	5 (41.66%)		
Ki-67				
▪ *<50%*	38 (84.44%)	5 (41.66%)	0	11 (57.90%)
▪ *>50%*	7 (15.50%)	6 (50.00%)		

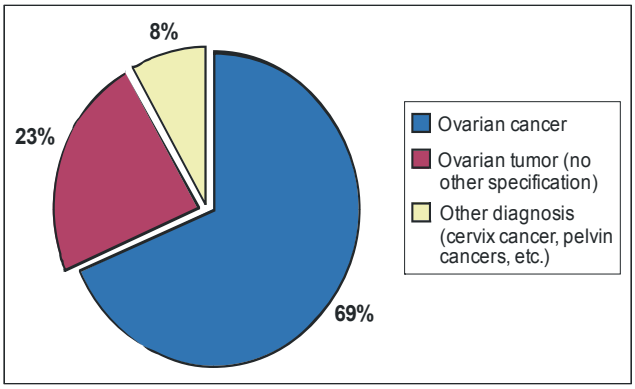

Figure 1 – *The distribution of patients regarding the diagnosis at admission*

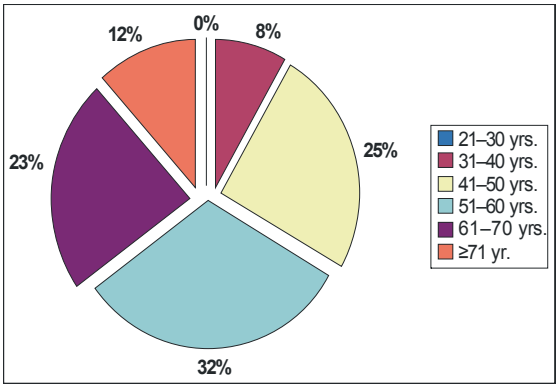

Figure 2 – *Distribution of age of patients with ovarian cancer*

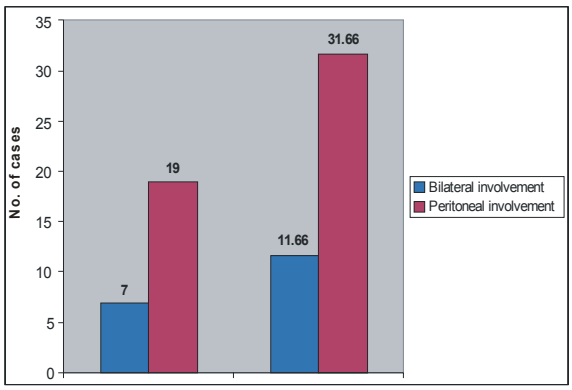

Figure 3 – ***The distribution of cases with peritoneal metastasis***

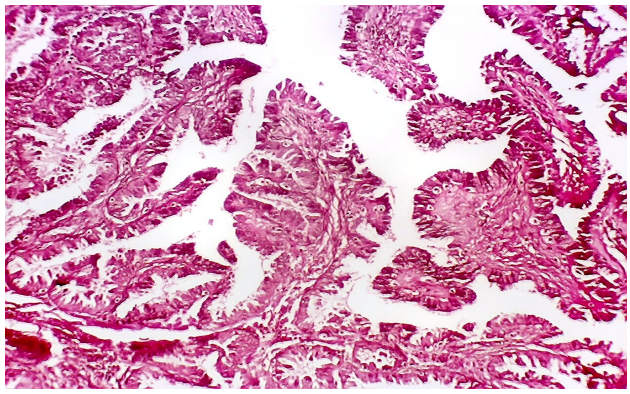

Figure 4 – ***Serous adenocarcinoma (HE staining, 100×)***

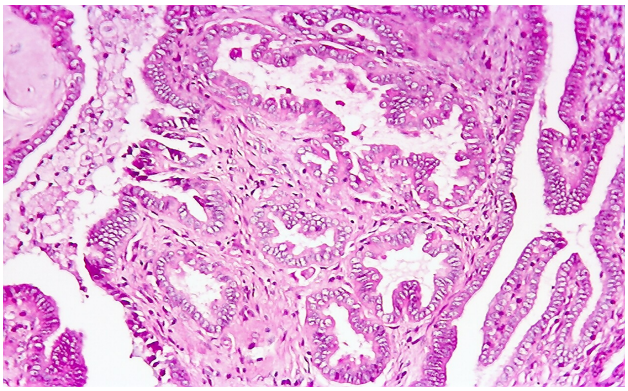

Figure 5 – ***Serous borderline tumor (HE staining, 100×)***

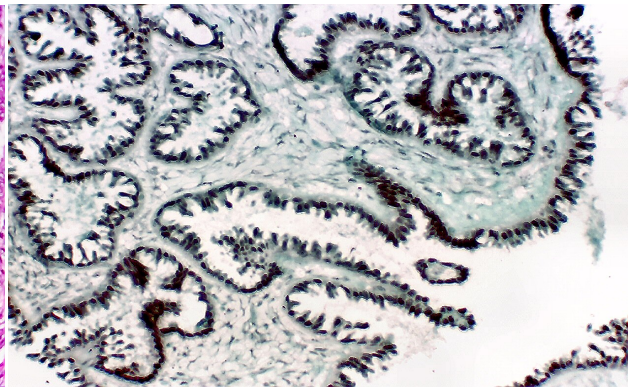

Figure 6 – ***Immunoreactivity for estrogen receptors, serous adenocarcinoma (200×)***

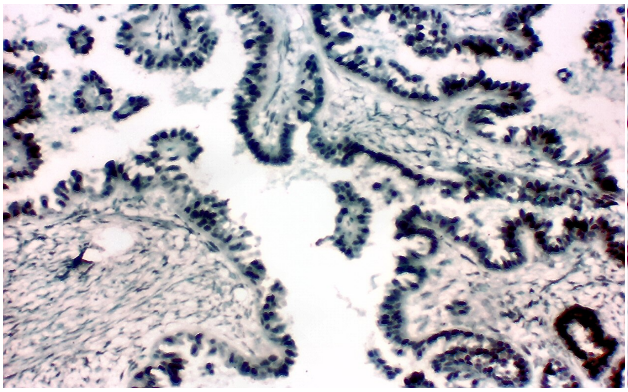

Figure 7 – ***Immunoreactivity for progesterone receptors, serous adenocarcinoma (200×)***

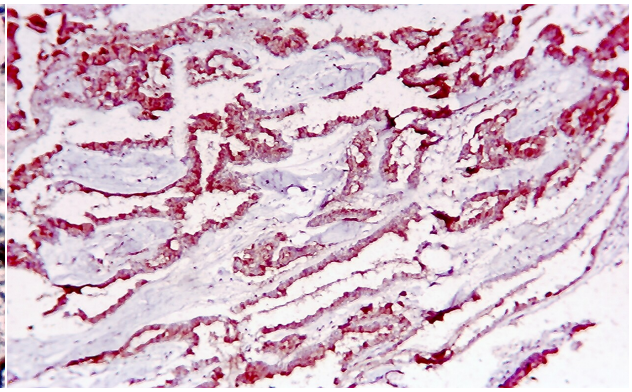

Figure 8 – ***Immunoreactivity for CA125, mucinous carcinoma (100×)***

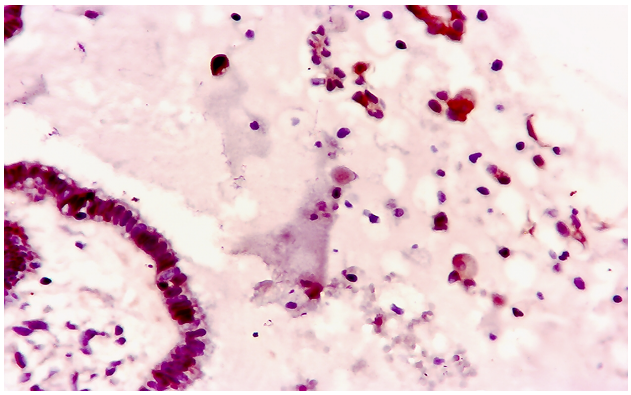

Figure 9 – ***Immunoreactivity for CerbB-2, serous borderline tumor (200×)***

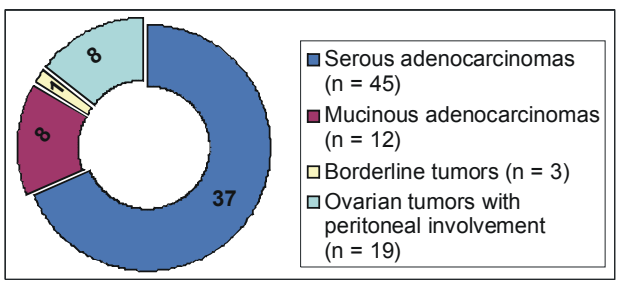

Figure 10 – ***The distribution of CerbB-2 in ovarian tumors***

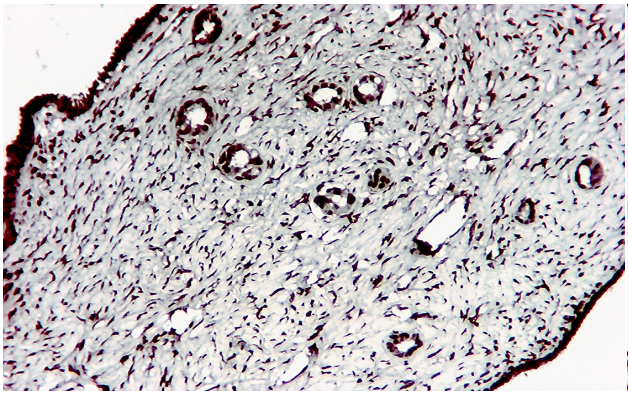

Figure 11 – ***Immunoreactivity for Ki-67, serous adenocarcinoma (100×)***

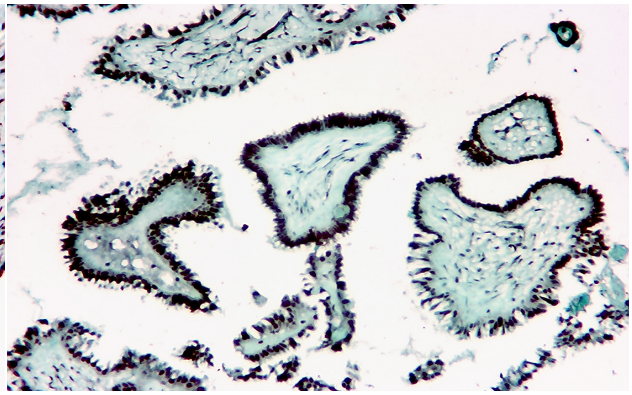

Figure 12 – ***Immunostaining for p53, serous adenocarcinoma (200×)***

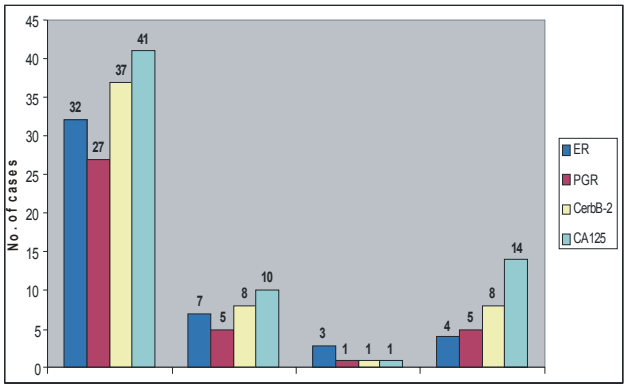

Figure 13 – ***The distribution of hormones receptors in ovarian tumors (n = 60)***

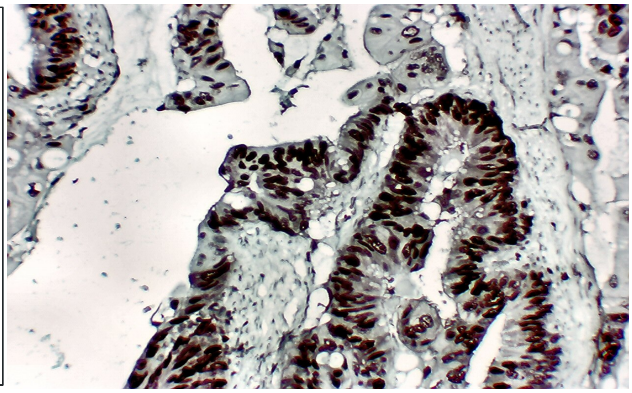

Figure 14 – ***Immunoreactivity for proliferative cell nucleolar antigen (PCNA), serous adenocarcinoma (200×)***

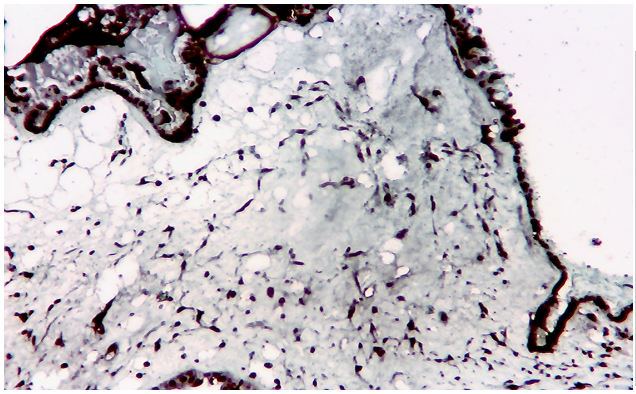

Figure 15 – ***Immunoreactivity for PCNA, serous borderline tumor (200×)***

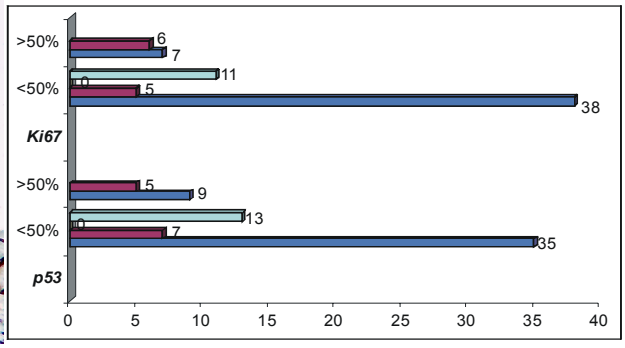

Figure 16 – ***The distribution of proliferative factors in ovarian tumors (n = 60)***

Discussion

Ovarian cancer represents 30% from all female genital cancers. According to the latest WHO statistics [4] ovarian cancer is as frequent as corpus uteri cancer (35%) and invasive cervix cancer (27%) [3].

Ovarian cancer incidence in accordance with the age vary from less than two new cases/100 000 females in South-East Asia and Africa, to more than 15 cases in East and North Europe. The highest rates are registered in economically advanced countries; in US female die from ovarian cancer than from all other pelvic gynecological cancers sites combined [2, 3].

The most challenging ovarian tumors from diagnostic and therapeutic point of view are borderline tumors and invasive carcinomas [35].

The aim of our study is to analyze clinical, macroscopy and microscopy a group of 60 patients from a General Surgery Clinic of "Sfantul Ioan" Hospital, Bucharest. The 60 patients were admitted based upon a variable surgical diagnosis. None of the cases was transferred from other non-surgical departments.

We concluded that a high percent of patients (68.33%) had a correct diagnosis at admission that superposed subsequently on the surgical diagnosis. However, we have had also five cases (68.33%) with other diagnostics, which did not suggest ovarian diseases (like cervix tumor).

We considered that a correct diagnosis at admission is linked to a detailed preoperative examination performed by the general practitioner, that is helpful both for the preoperative monitoring of the patient and for establishing the targeted investigations before surgery [27]. We outlined here that none of the patients had a positive family history for ovarian cancer. We considered that is very important to know the potential hereditary diseases, in order to elaborate files for each patient and perhaps to perform genetic testing [44–46].

The researchers suggested that the presence of several risk factors in ovarian cancer (age, demographics, and surgical history) requires a more detailed anamnesis to discover a positive familial history [12, 47–49].

The studies outlined that the type and the duration of the symptoms in patients with borderline ovarian tumors versus invasive carcinoma is not significant different [34, 50–52]. The tests suggested that borderline tumors had no symptoms, and that the duration of symptoms and the mean time from the first examination until the diagnosis is made, did not significantly differ comparing to invasive carcinomas (six months *vs.* four months). Besides this, borderline tumors are two times more easily diagnosed on a routine examination, compared to invasive carcinomas, which are diagnosed when they became symptomatic [34].

As our study outlined, the most affected age group has been 44–60 years (56.66%). Literature confirms that the incidence increases at 40–44 years, with a peak around 70 years. We considered that the predominance on certain age groups was correlated with other epidemiological factors, mentioned earlier, which our study did not take into account.

Imaging and lab exams have comprised abdominal ultrasound, computed tomography (with or without contrast dye) and serological determination of CA125 [53].

The studies suggested that the discovery of cyst lesions of 1–3 cm did not raise surgical problems, the resection being not necessary if the level of CA125 is normal. However, in menopausal women, large pelvic masses and high level of CA125 (>35 U/mL) require surgical therapy [25, 54, 55].

Otherwise, serum values of CA125 correlate with disease progression or regression; it suggests a lack of specificity of this test that makes impossible its use as a routine test in ovarian cancer screening. CA125 is extremely useful in the disease follow-up; high values are associated with peritoneal invasion, with serous carcinoma and with local relapse [56]. In the same time, a good chemotherapy response is associated with normal values of CA125. Only the patients suspected of ovarian cancer were tested for CA125 and the values did not always correlate with cancer invasion. It seems that transvaginal ultrasound has great diagnosis specificity. In 1994, *National Health Institute* established a *Consensus Statement* regarding routine screening in ovarian cancer. Concerning ovarian cancer, sonography (three-dimensional, transvaginal, trans-abdominal, Color Doppler and Power Doppler) is a complementary examination because of a large accessibility and of the techniques improvement. It has been recommended that women at risk for ovarian cancer should be tested for serum CA125 and should perform a transvaginal ultrasound annually/biannually [1, 53, 57].

Surgical procedures were suggested by the preoperative diagnosis and also by the intra operative local findings. Only two cases were treated with tumorectomy, in patients older than 71 year who also had peritoneal involvement [58]. The other patients were treated completely, irrespective the age group. We outlined that ice examination was not done to establish the intraoperative diagnosis.

The literature data confirmed an effort on behalf of the surgeons to preserve the procreative potential especially in young women. The group of women with tumorectomy for borderline tumors had normal fertility and pregnancy, the relapses being not influenced by the surgical procedure [16, 54, 59, 60].

As our study revealed, seven cases presented bilateral involvement (11.66%) and 19 cases presented peritoneal involvement (31.66%). Ovarian tumors staging showed that bilateral involvement is an important criteria. All seven cases with bilateral involvement presented the same type of lesion on both ovaries (carcinoma), with involvement of ovarian capsule in two cases. Peritoneal implants raised several problems. First was the development of peritoneal implants in noninvasive tumors, a very important aspect from prognostic point of view [34]. An attempt has been made to distinguish between invasive and noninvasive peritoneal implants using histopathological criteria, a quite difficult task but with prognostic importance. We did not use the classification of peritoneal implants because of the reliability of the histological examination. We did not found peritoneal invasion in none of the borderline tumors (n = 3).

Histopathological examination of the 60 ovarian tumors allowed identifying a predominance of serous tumors (75%), 20% mucinous tumors and 5% borderline tumors. In most of the cases, we noticed glandular and papillary aspects. A few cases presented psammomatous bodies.

All three borderline cases were serous tumors that mean this tumor type presents early stromal invasion, with foci under 10 mm^2 [3].

Recently, the term *serous ovarian tumor with low malignant potential* (SLMP) was abandoned and the entity was divided into two categories: serous micropapillary carcinoma and proliferative atypical serous tumor [51]. The relapses and the progression to invasive carcinoma are frequent in serous micropapillary carcinoma, with peritoneal involvement and evolution similarly to malignant tumors.

The 12 mucinous tumors that represented 20% from our cases were unilaterally, with mucoid, viscous content. We often encountered hemorrhages and necrosis. The most important issue is metastases development. The characteristic features for primary ovarian mucinous carcinoma are extensive pattern of invasion and the complex papillary aspect. Borderline

mucinous ovarian tumors are divided into two types: intestinal and endocervical type [2]. In intestinal type of tumors, epithelial component is similar to intestinal epithelium with goblet cells, neuroendocrine cells and rare Paneth cells. Borderline mucinous ovarian tumors, endocervical type, does not present destructive stromal invasion and it seems to originate especially from endometriosis foci.

Now, serous ovarian carcinoma is regarded as an homogenous group of tumors [61, 62]. Although these neoplasms are graded into well-, moderate- and poor-differentiated, it is considered that the differentiation spectrum reflects the progression from a low to a high malignancy grade. Unlike colorectal cancer, where the model of tumor progression with sequential accumulation of genetic molecular abnormalities towards recognizable morphological stages is well established, in serous ovarian cancer it cannot be established a similar model, because the precursor lesions are not well defined. The researchers from Pathology Department from John Hopkins University, Baltimore, Maryland, proposed a model concerning ovarian tumorigenesis. In their opinion, the epithelial surface tumors are divided into two main categories that correspond to the two tumorigenesis pathways: type I represent low malignancy tumors that originate from borderline tumors, while type II encompasses high malignancy tumors, where a precursor lesion is hard to recognize [16, 61, 63].

Type I includes: low grade serous carcinoma, mucinous carcinoma, endometrioid carcinoma, malignant Brenner tumor and clear cell carcinoma. Type II includes: moderate and poor differentiated serous cacinomas, mixed mezodermal tumors and undifferentiated carcinoma. Type I is similar to adenoma–carcinoma sequence from colorectal cancer and is characterized through easy recognizable precursor lesions (cystadenoma, proliferative atypical serous tumor, noninvasive carcinoma).

Type II originates from surface epithelium or from inclusion cysts, has a rapid evolution and presents early metastases. The definition of molecular changes associated with ovarian carcinogenesis will facilitate the development of diagnostic tests for early detection of ovarian cancer and also of new strategies to block the transmission pathways of growth signals.

Immunohistochemical analysis [43] provided several conclusions concerning the studied ovarian carcinoma types: serous and mucinous [56, 64]. First, it was thought that the presence of estrogen/progesterone receptors could be proportional to hormone responsiveness of the tissues, but the literature expresses contradictory data.

Although some authors believed that ER/PGR are found only in subgroups of breast, ovary and endometrial cancers, others noticed ER also in lung, stomach and thyroid gland. This evidence has complicated the hypothesis more, so the positivity for ER in a lung adenocarcinoma did not necessarily means metastases from breast cancer in the lung [26, 37].

Therefore, immunohistochemical tests for ER/PGR are disputed because there is no standard regarding the significance of a positive test. The receptors linked the hormones that exerted their effects in the nucleus, and the immunostaining could be demonstrated on a normal tissue, too. One of the estrogen effects is to induce PGR, so the coordinate expression of the two hormones in the same cell reflects the functionality of the ER/PGR axis in that cell [65]. Issues that induced hormone imbalance lead to controversial data regarding the risk for ovarian cancer development [66]. It has been suggested that ovulation cessation, with repeated trauma and repairs of the ovarian epithelium may increase the neoplasia risk. At the same time, it has been suggested that chronic ovarian stimulation due to luteinizing hormone (LH) could influence the ovarian cancer pathology [67].

At the same time, it has been suggested that multiparity reduced the risk of cancer development. The data are limited to women with genetic predisposition [49, 68, 69].

There is scarce data upon the role of contraceptive pills in women with high risk mutations of BRCA-1 gene. The case-reports suggested that the use of contraceptive pills for more than 6 months reduced the risk for ovarian cancer development with 60%. Other studies did observe any protective role for contraceptive pills [4].

Some studies showed that borderline ovarian tumors presented a significant increase of ER, without significant differences for PGR, compared to carcinomas [27, 35].

As our study shows, both serous and borderline tumors significantly express hormone receptors (>50%). Some cases showed lack of positivity for hormone receptors, but the results obtained concluded that immunostaining for hormone receptors is significant in borderline tumors (ER = 100%) and in serous carcinomas (ER = 71.10%; PGR = 60%), and high in mucinous carcinomas (ER = 58.33%; PGR = 41.66%). We considered that the two types of receptors can confirm a primary site for the tumor or could evidence the hormone sensitivity of the tumor. Unlike breast cancer, where the treatment response is influenced by the presence of ER/PGR, in ovarian cancer the surgical treatment should not be changed depending on the hormone profile but can be adjusted according to the age or the request to preserve fertility [22].

HER-2/neu oncoprotein belongs to the family of tirosinkinase receptors, and is a growth factor receptor, 50% homologous to epidermal growth factor receptor; it presents a transmembrane and a cytoplasmic domain. The cytoplasmic domain exhibits functions of phosphorylation activation and transcription initiation. Activation ligand for HER-2 receptor is unknown. In breast cancers, the role of HER-2 receptor is well known: (1) predictor of therapy response and (2) determination of the group of patients who may benefit from monoclonal therapy. However, in ovarian tumors its significance and importance has not yet been established. We investigated CerbB-2 in order to discover some connections with BRCA1 mutations in sporadic ovarian cancers. It is well known that ERBB2 gene is amplified and overexpressed in 20–25% of breast cancers; tumors that show ERBB2 amplification

have lost ER expression. Paradoxally, in breast cancer, although amplification and superexpression of ERBB2 suggests a worse progression of the disease, it does not represent an independent prognostic factor; more, CerbB-2 protein, a transmembranar receptor with a reduced expression in normal tissues, became an interesting target in therapeutic management [68, 70, 71].

We have considered positive those cases with continuous perimembrane staining and in large groups of cells that can be included in the scoring system we used. We also mentioned that all positive cases had only 1+ and only one case had 2+.

Numerous serous carcinomas were positive (82.22%) and also the mucinous ones (66.66%). Nevertheless, from borderline tumors, a single case was positive (33.33%) and less than half of those with peritoneal involvement were positive (42.10%). The studies of certain molecular markers, among which CerbB-2, established there were no significant differences of this protein in borderline and serous tumors [34, 35, 52].

CA125 assay had a limited relevance, due to its positivity in other malignancies: metastatic extragenital carcinomas, mesothelial proliferations. Its value becomes important when the metastases origin is not known; but the ovarian origin should also suggested by other elements that should confirm the presence of this marker.

Among tumor markers, CA125 is considered the gold standard for pelvic masses assessment. The serum level is useful in 80% cases of the advanced tumors. The analysis is easy to perform, either pre-/post-operative or after therapy, but has a low specificity; for this reason CA125 cannot be a useful marker for routine screening of ovarian cancer [3].

Some studies stressed that the increase of CA125 levels in premenopasal women is associated with ultrasound evidence of endometriosis, adenomiosis and leiomyoma [4].

Taking into account the lack of specificity of CA125, *Cancer Genetic Studies Consortium* recommended that women at risk for ovarian cancer development, with BRCA1 gene mutations, to perform biannually or annually screening – transvaginal sonography and serum CA125, beginning with the age of 25 [27, 29].

The proliferation factors we used, p53 and Ki-67, demonstrated their correlation with tumor aggressiveness [72].

Lack of positivity in borderline tumors and the high positivity in serous and mucinous carcinomas is sustained by the literature data. Large studies revealed important differences in p53 and Ki-67 expression in ovarian carcinomas *versus* borderline tumors. We divided the cases in accordance with the percent of the cells immunostained with the two markers. From the 45 serous carcinomas, 43 were Ki-67+ and 44 p53+, from the 12 mucinous adenocarcinomas we found 11 Ki-67+ and 12 p53+. On the other side, not all samples of peritoneal implants were Ki-67 (11 cases) and p53 (13 cases) positive.

Conclusions

Our study confirms that surface epithelial tumors are the most frequent ovarian neoplasia (n = 60).

The high percent of patients (68.33%) with a correct diagnosis at admission, comparing with a number of five cases (8.33%) where the diagnosis suggested other diseases, sustains the important role of the GP in establishing an accurate diagnosis at admission – helpful both for the preoperative monitoring of the patient and for establishing the targeted investigations before surgery.

Among the serum markers easily to detect preoperatively, but with an important role in postoperative monitoring of the patients, we remarked that CA125 assay was performed only in patients with suspicion for ovarian tumor. CA125 is considered the gold standard serum test for pelvic masses assessment. The serum level is useful in 80% cases of the advanced tumors. The analysis is easy to perform, either pre-/postoperative or after surgery, but has a low specificity; for this reason CA125 cannot be a useful marker for routine screening of ovarian cancer.

We remarked that in 86.66% of cases, radical hysterectomy with bilateral anexectomy was performed, only in two cases (3.33%) simple tumorectomy was necessary. Bilateral disease was found in 11.66% (seven cases), with the same histological type. The ovarian capsule involvement of the opposite ovary was found in two cases.

Peritoneal implants were present in 19 cases (31.66%).We did found peritoneal invasion in none of the borderline tumors (n = 3).

We identified a significant number of serous tumors (75%), 20% mucinous tumors and 5% borderline tumors. The 12 mucinous tumors represented 20% (n = 60) from the studied group; the most important problem was the differential diagnosis with the digestive metastases.

The immunohistochemical tests for ER/PGR are controversial because there is no standard regarding the definition of a positive test.

Serous and borderline tumors significantly expressed hormone receptors.

We remarked the positivity for ER in 32 cases of serous ovarian adenocarcinomas (71.1%), in seven cases of mucinous adenocarcinoma (58.33%), all three cases of borderline tumors (100%) and in four from 19 peritoneal implants. In the same time, PGR were immunohistochemically positive in 27 cases of serous ovarian adenocarcinoma (60%), five cases of mucinous adenocarcinoma (41.66%), one case of borderline tumor and five cases of peritoneal metastases (26.31%).

The studies of certain molecular markers, as CerbB-2, established there were no significant differences of this protein in borderline and serous tumors. Immunohistochemical examination of CerbB-2 evidenced positivity for 37 serous carcinomas (82.22%), five mucinous carcinomas (41.66%), a single case of borderline tumor (33.33%) and eight cases with peritoneal involvement (42.10%). We considered positive only the cases with 2+ and 3+.

Our study, which analyzed a group of patients with sporadic ovarian tumors, highlights that an immunohistochemical examination of a certain panel of antibodies could not offer prognostic data or screening aspects. Besides this, the most important risk factor for ovarian cancer development remains the positive family history and the tumor stage.

All the molecular factors with prognostic significance in ovarian cancer, studied in the last decade, were identified through retrospective studies without multivariate analysis or confirmation on large studies. We outline here that these factors resulted from experimental studies in ovarian cancer biology; none of them is routinely used for the selection of the patients with ovarian cancer.

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