PhD THESIS

ABSTRACT

Clinical-epidemiological, imagistic, histological and immunohistochemical study of ovarian mucinous tumors

Scientific coordinator
Prof. Univ. Dr. Brăila Mihai

Doctoral Candidate
Kamal Kamal Constantin

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INTRODUCTION

The ovary is an organ with a complex embryology and steroidogenesis, which is always changing, undergoing more structural changes than any other organ. It has a default (pre-established) development, for a given period of time, between puberty and menopause, which is genetically determined. Woman's biological clock runs on the interdependence of secreted hormones (which regulates the reproductive function) and their receptors, relationship that transforms the ovary in a target organ. In the first part of a woman's reproductive life the ovary is predisposed mainly for developing active inflammatory phenomena, starting with pre-menopause and menopause, it becomes the target of neoplastic processes.

The ovarian pathology is currently among the widest and most complex problems in modern gynecology mainly through ovarian tumors. Despite its small size, the ovary is an organ that requires the attention of several specialties like gynecology, endocrinology and pathology. Ovarian tumor pathology is a medical problem which frequently echoes in the family and social sphere. The advancements in science and modern diagnostic methods made possible the thorough investigation of this organ but there are still unsolved questions.

Ovarian cancer has an unknown natural evolution, starting often insidiously, without specific symptoms; the diagnosis is put during a routine exam. Although it was tried to associate precursor lesions to the disease, the results were not conclusive, cellular changes can be incriminated also in other non-tumor pathologies.

Structurally, the ovary presents on the surface, an epithelial tissue, with mesothelium cells, being a germinal epithelium. Ovarian functional or dysfunctional cysts may occur, due to a hypothalamic-pituitary-ovarian dysfunction. They can reduce spontaneously or after medical treatment.

Our attention will be focused, in this paper, on ovarian epithelial tumors. I have chosen this topic because of the alarming increase in the number of cases in the last 20 years, this type of tumor becoming the main cause of death from malignancy in gynecology. Of all diagnosed tumors, a percentage of 90% is due to epithelial ovarian carcinomas.

KEY WORDS: mucinous ovarian tumors, prognostic factors, p53, ki67, EGFR.

OVARIAN TUMORS EPIDEMIOLOGY

Ovarian cancer is responsible for the most deaths in the gynecological sphere. Death rate caused by this type of malignancy is continuously rising, the values increasing from year to year. An overview of statistical data shows that the number of deaths of women born in the mid-century was growing compared with those born at the beginning of the century. This variation is probably due to the improvement in diagnosis methods but also highlights a change of lifestyle. Proof for this theory is the fact that people migrating from areas with low incidence to a higher incidence area will be exposed to the same chances of developing the disease as the indigenous population.

The quest to finding a cause of ovarian cancer, from an epidemiologic standpoint has been hampered by the high number of histological subtypes and the lack of consensus between
It would be surprising for all the ovarian tumors to have the same cause, even though they have similar characteristics.

The risk for developing the disease increases with age and it’s more common in white women compared to the risk of the black women. A high risk of disease can be found in women who have never been married. This can be explained through the argument that unmarried women carry the same risk as married women who have never procreated.

Another possible cause is nutrition, cancer mortality having a correlation with eating habits in different countries and these habits change when people move to another country. Though it is impossible to prove a connection between nutrition and cancer this idea is worth investigating.

Worldwide, the ovarian cancer incidence varies with each continent, the highest can be found in America and the lowest in Africa and Japan. In Europe, the highest number of cases can be found in Lithuania, Ireland or Estonia and the lowest number in Cyprus, Spain or Portugal. Romania is situated in the middle of this list, the 12.8/100.000 women rate being the european average. This rate can be a little lower than reality because of the lack of national monitoring programs, early detection seminars.

Worldwide age distribution shows that most cases affect women between the ages of 45 and 74, this being an argument for the high risk in women who have reached menopause.

**OVARIAN TUMORS PATHOGENESIS**

Ovarian cancer is the most lethal of the gynecological malignancies and despite all the efforts made for improving the existing treatment methods and the early diagnosis, no progress has been achieved. Kurman and his colleagues investigated the pathogenesis “de novo” theory and concluded that the arguments supporting it were invalid. Their studies demonstrated that ovarian tumors don’t come from a single epithelial cell but a multitude of cancer cells with different morphological, genetic and molecular characteristics.

**DIAGNOSTIC METHODS IN OVARIAN TUMORS**

The positive diagnosis of the ovarian tumors represents the setting stone of all therapeutic actions. The ovarian pathology is a complex matter which baffles even the most experimented clinicians. One of the main problems of the protocol you need to follow in case of an ovarian tumor is a correct pre-surgery diagnosis. This leads to a precise and exact evaluation of the pelvic mass that in turn leads to a surprise-free surgical intervention.

**CLINICAL EXAMINATION**

Ovarian cancer is considered one of the hardest to diagnose diseases in gynecological pathology, often being referred as the silent killer. This “name” was attributed because of the lack of precursor lesions and a specific set of symptoms, the disease being diagnosed in most cases during a routine examination and even then in a late stage. The medical history is very important, and we must be careful not to exclude any risk factors. Family history is in most cases very meaningful, women being more exposed to ovarian cancer if a blood relative had ovarian or breast cancer.
IMAGING EXAMINATION

The clinical examination and the family history are the first building blocks to a correct diagnosis but cannot alone confirm the presence of an ovarian tumor. The next step in investigating ovarian tumor pathology is the imaging examination. Whether we are talking about standard abdominal echography, 3D or contrast echography or CT and MRI, imaging examinations are indispensable to ovarian cancer diagnosis. With the help of trans-vaginal echography we can assess characteristics like: ovarian size, tumor size, unilateral/bilateral, tumor morphology (content, wall, and vegetation) and solid/liquid/mixt consistency.

OBJECTIVES AND GOAL

The objective we followed was a complex one: coming up with a screening protocol in the general population along with a diagnostic protocol which can lead to a more precise evaluation of the tumor stage, without having to perform surgery. Also, we set off to find the correlation between the histological and immunohistochemistry findings with the imaging and clinical data in order to create a diagnostic model useful either before or after the surgery with the main goal of limiting the over or undervaluation of this type of cancer.

MATERIAL AND METHOD

The clinical study was performed on human subjects, patients admitted in the Obstetrics and Gynecology Department of the Clinical and Emergency County Hospital Craiova.

The patients in the study underwent hysterectomy surgery with unilateral or bilateral adnexectomy. The surgical pieces were processed using the histological and immunohistochemical techniques in the Pathology Department in the University of Medicine and Pharmacy Craiova.

We also used the patient charts, from which we extracted their history, symptoms, clinical examination and the imaging findings along with the results from the blood workup.

The histopathological and immunohistochemistry study used the following antibodies

<table>
<thead>
<tr>
<th>ANTIBODY</th>
<th>CLONE</th>
<th>DILUTION</th>
<th>ANTIGENIC DEMASQUATION</th>
<th>POSITIVE CONTROL</th>
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</thead>
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<tr>
<td>Ki67</td>
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<td>1:100</td>
<td>Citrat, pH 6</td>
<td>Tonsil</td>
</tr>
<tr>
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<td>Breast Cancer</td>
</tr>
<tr>
<td>EGFR</td>
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<td>1:1000</td>
<td>fără demascare</td>
<td>Oral Mucosa</td>
</tr>
<tr>
<td>p53</td>
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<td>1:50</td>
<td>EDTA, pH 9</td>
<td>Tonsil</td>
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<tr>
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<td>Citrat buffer, pH 6</td>
<td>Ovary</td>
</tr>
<tr>
<td>CXCR4</td>
<td>policlonal</td>
<td>1:1000</td>
<td>Citrat buffer, pH 6</td>
<td>Skin</td>
</tr>
</tbody>
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RESULTS AND DISCUSSIONS

CLINICAL-EPIEMIOLOGIC STUDY

We performed a retrospective study, unrolled from 2008 to 2012 which included 189 patients, diagnosed with mucinous ovarian tumors after the pathology exam. The patients in our study were questioned and examined in order to gather the data included in our clinical study. We also used the patient’s charts to have a more complete overview of the case.

An important factor in following the patients was the Body Mass Index (BMI), a high index being correlated with the presence of the disease. In our study only 12.65% of the patients had a normal BMI, the rest presenting with values over 25 kg/m$^2$. A 48.68% percentage of our group were overweight and 39% were obese. Other studies have shown that a high BMI is often associated with the disease.

An argument in favor of using Ovarian Cancer Symptom Index (OCSI) is the low cost compared to the immunological explorations and most of all the ease with which it is implemented. This screening method is underappreciated but its screening potential is close to that of CA125 and HE4. The test’s sensitivity is close to 60% and it’s specific in 70% of the cases. We can find similar values in the CA125 testing where false positive results lead to overvaluation and unnecessary surgery.

CA125 marker was tested in all 189 cases included in our study and the results were grouped into intervals which represent the most common values for this marker. A value under 35 U/ml is considered risk free. In our study, 121 women (64%) had values between 0-35 U/ml for the CA125 tumor marker indicating a low or inexistent risk of malignancy. Some authors plead that in studies of large groups of patients there were cases with CA125 values under 35 U/ml that developed malignancies. Values between 35 and 65 U/ml corresponded to 24 patients (12%) while values between 65 and 100 U/ml were found in 18 patients (9.5%). These values represent that patients have a medium to high risk of malignancy. It must be said that the value of CA125 can be high in associated illnesses like ascites and peritonitis.

Values over 100 U/ml are considered of high malignancy risk and in this group we found 26 patients (13%). Out of the 26 patients found with values over 100 U/ml, only 17 cases (65.38%) were confirmed by the pathology examination, the rest (34.61%) were false positive cases. Using CA125 as an indicator in evaluating malignancies was until recently the only viable option. The discovery of HE4 tumor marker opened the door to a completely new way of investigating ovarian cancer.

HE4 values kept for the most part the same pattern as CA125, even though they evaluate distinct components. For the HE4 marker, the normal values are included in the 0-150 pM interval. In our study, 118 patients representing 62% of the total presented with values in this interval. Montagnana appreciated that 80% of HE4 values are in concordance with the pathological reality. In order to have a high risk of malignancy, the HE4 value must exceed 500 pM. In our study, 12 patients (6.5%) presented values greater than 500 pM. Even if our study found 17 malignant tumors, the HE4 examination indicated only 12 cases, out of which, only 9 were later confirmed during the pathology examination.
IMAGING STUDY

All the 189 patients were examined before the surgery, using the echography technique, in order to define a preliminary diagnosis but also to provide the surgeon with a wide perspective of the pelvic cavity and the tumor size.

For proposing an echography diagnosis we investigated tumor characteristics like size, septal structure, ecogenity and the presence of septs. The standard method of examination is the transvaginal approach. Even though the histological variants are many, echography cannot discriminate tumor type. Some authors claim that even though ultrasonography is an indispensable tool in diagnosing ovarian cancer, its limits are obvious.

We also evaluated the cystic component of the tumors and the type of vascularization. Malignant tumor had in most of the cases central vascularization in contrast with benign and borderline tumors in which case the vascularization was only peripheral. These results are in accordance with the literature where many authors have proven that Doppler sonography is extremely useful in determining tumor vascularization and correlate the results with the tumor type.

HISTOLOGICAL STUDY

In our study we found a number of 144 benign mucinous tumors which represented 76.19% of all analyzed tumors. Depending on the architectural pattern, in 97 cases they corresponded to cystadenomas, in 38 cases cystadenofibroma and 9 cases were represented by adenofibroma. Most of the benign mucinous tumors presented endocervical differentiation, meaning 58 cases (40.27%), the rest being of the intestinal type (39 cases – 26.80%).

Borderline tumors were also present in our study and constituted 14.81 % (28 cases) of all the analyzed tumors. Out of the 28 cases, 23 were of the intestinal type and 5 cases of endocervical differentiation. In our study we also reported 13 cases of mucinous carcinomas with an expansive, confluent development pattern and 4 cases of ovarian carcinomas with an infiltrative growth pattern. In a study including 34 tumors, the authors found that clinical stage and stromal invasion represent the most important prognostic interdependent variables. In contrast, there are no differences regarding nuclear grade, mitotic activity, stratifying on more than 3 layers and invasion. Borderline tumors, noninvasive carcinomas, microinvasive and invasive carcinomas with an expansive growth pattern are generally stage I tumors, with an excellent prognosis and rare metastasis. Infiltrative growth pattern carcinomas and invasive carcinomas are usually more aggressive, representing almost all mucinous tumors in advanced stages and are responsible for most of the deaths caused by the tumor.

IMMUNOHISTOCHEMISTRY STUDY

In our study, the immunoexpression for p53 was positive in a small number for benign tumors (2 cases – 6.25%) but for most of the borderline carcinomas and malignant tumors, regardless of the differentiation degree. The score analysis for p53 indicated a maximal value (+3) only in carcinomas. Moreover, in case of ovarian mucinous carcinomas we found positive response not only in the malignant tumor itself but also in the adjacent benign and borderline areas, suggesting that these are not typical cystadenomas, but can have a genetic carcinogenic predisposition which is rarely present in common cystadenomas. Also, the distribution of the
immunostaining was heterogeneous in different areas of the same tumor (benign, borderline and malignant) sustaining thus the heterogeneity theory in ovarian mucinous carcinogenesis.

The study returned positive results for EGFR in all three tumor groups, which corresponded in 14 cases (18.18%) to benign tumors, 11 cases (14.28%) to borderline tumors and 12 cases (15.57%) to malignant ones. The immunostaining score was higher in carcinomas than the other two groups.

The immunoexpression study for Her2 for all 77 analyzed tumors indicated positivity in 9 cases (11.68%), predominantly in mucinous carcinomas which were positive in 37.5% of the cases. Benign tumors were negative across the board for this marker while borderline tumors were positive in an reduced amount and with a low immunostaining score.

Ki67 and p53 analysis reported the correlation between the two with tumor type and grade, the overexpression of p53 and ki67 being absent in borderline tumors and present only in carcinomas. We can asses that p53 and ki67 can be used successfully as markers for the evaluation of aggressive tumor behavior but also for differentiating borderline tumors from carcinomas.

In our study we also noticed that the expression of SMAD4 was positive in 23 cases (12.16%) with 100% of mucinous carcinomas showing positive results. Some borderline tumors were also positive while the benign ones were all negative. For the investigated carcinomas we concluded that the highest intensity and score were associated with high grade tumors while low grade carcinomas and borderline tumors indicated a low or average immunostaining score.

CXCR4 was the last antibody whose expression we analyzed and we found that its presence in the cytoplasm was indicated in all three tumor groups. For the benign tumors we found positivity for 42 cases (54.5%), borderline tumors indicated a 50% positivity score and malignant tumors were all positive, regardless of their degree of differentiation.
FINAL CONCLUSIONS

- Age over 45 years represented a risk factor in ovarian mucinous tumors, over 55% of the patients included in our study were aged between 45-65 years.
- Obesity has been associated with this disease in over 70% of cases and is one of the causes of ovarian pathology development.
- OCS1 is a sufficiently effective method of early diagnosis of ovarian cancer, primarily because of low cost, high addressability, sensitivity and specificity presented well by providing the constant ability to compare results with the anterior ones, by organizing an international data base.
- The determination of CA125 and HE4 levels, is a good method for assessing malignancy of ovarian tumors, helping clinicians to better preoperative classification and establishment of optimal treatment protocol.
- The ROMA scoring (CA125 + HE4 is an additional way to determine the degree of tumor malignancy but its use is currently restricted.
- The 189 histologically analyzed mucinous ovarian tumors corresponded in 144 cases of benign tumors, in 28 borderline tumors, 30 cases of cystadenofibromas, and in 9 cases of adenofibromas.
- Mucinous borderline tumors accounted for 14.81% of mucinous tumors, presenting in most of the cases (23 cases) with intestinal type differentiation, only rarely (5 cases) endocervical type; their association with areas of mucinous cyst adenoma was quite common that in 14 cases (50%).
- Mucinous carcinomas representing 8.99% of mucinous tumors, analyzed according to tumor grade corresponded to 11 cases of low-grade tumors, and in 6 cases high-grade tumors.
- Depending on the stromal invasion pattern of mucinous carcinomas, we noted the presence of 2 architectural models: infiltrative in 4 cases and expansive in 13 cases.
- Tumor necrosis was present in 15 of the 17 cases of mucinous carcinomas investigated (88.23%) most often moderately represented.
- Regarding the classification of malignant tumors in one of pTNM stage of the FIGO system, I noticed that most corresponded to tumor stage I, with 43 cases (95.5%) of which -26 cases of mucinous borderline tumors (60.46%) and 17 malignant tumors (39.53%) ; in stage II were rated only 2 cases (4.44%) of mucinous carcinomas.
- The immunohistochemical study was performed on a total of 77 cases of ovarian mucinous tumors, of which 32 cases of benign mucinous tumors, 28 cases of mucinous borderline tumors and 17 cases of mucinous carcinomas for which we investigated the imunoexpression of p53, Ki67, EGFR, Her2, SMAD4, and CXCR4.
- The study of p53 oncoprotein immunoexpression showed positivity for 18 cases of mucinous tumors (23.37%), respectively for a total of two benign tumors (2.59%), for 7 mucinous borderline tumors (9.09%) and 9 mucinous carcinomas (11.68%).
- The Her2 immunoexpression investigation for the analyzed mucinous tumors, showed positivity in 9 cases (11.68%) of which borderline mucinous tumors were positive only in a small number of cases, respectively 3 cases (3.89%) compared with mucinous carcinoma who presented positivity in 6 cases (7.78%); all benign tumors were negative for this marker of the cases reviewed.
- The EGFR immunoexpression investigation revealed positivity in 37 of the cases, which corresponded in 14 cases (18.18%) of benign mucinous tumors, in 11 cases (14.28%) of mucinous borderline tumors and in 12 cases (15.58%) of mucinous carcinomas.
- The medium IP Ki67 analysis showed the highest values of mucinous carcinoma group especially for the high-grade tumors (IP Ki67 28%) compared to low grade tumors (IP Ki67 14%) or borderline tumors (IP Ki67 11%) and benign tumors (IP Ki67 4%).
- The analysis of Ki67 and p53 indicates correlation of the expression of p53 and Ki67 with the degree and type of tumor in mucinous carcinomas, overexpression of p53 and Ki67 was absent in mucinous borderline tumors and present in mucinous carcinomas.
- p53 and Ki-67 can be used as markers to assess the aggressive behavior of mucinous carcinomas and for differentiating them from mucinous borderline tumors.
- Highest score of immunostaining for SMAD4 was associated with high-grade tumors, while low-grade carcinomas and borderline tumors showed a low or medium immunostaining score.
- The presence of CXCR4 in all the phases of the mucinous ovarian tumorigenesis indicate it’s intervention since the early stages of carcinogenesis for this group of malignancies.
SELECTIVE REFERENCES


