

NJSC " West Kazakhstan Marat Ospanov Medical University "

Abstract for dissertation work
for a degree
Doctor of Philosophy (Ph.D.)

**Study of morphological indicators of ovarian malignancy in
women with neoplasms**

Specialty "6D110100-Medicine".

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The Republic of Kazakhstan
Aktobe – 2022y

The relevance of research:

Worldwide, according to Globocan (2021), ovarian cancer is the most common pathology, tends to increase in morbidity and mortality. The number of cases of malignancy of benign ovarian formations in 2020 amounted to 1106 cases. Kazakhstan, unfortunately, according to world statistics, has a coefficient standardized by age ASR-9.5, which emphasizes the global nature of this pathology [Sung H., Ferlay J. et al., 2021].

According to the International Agency for Research on Cancer, more than 250 thousand new cases of ovarian tumors have been registered in the world [Bray F., Ferlay J. et al., 2018]. In developed countries, the standardized incidence rate is 9.1; in developing countries - 5.0 per 100 thousand female population. In the CIS countries, standardized morbidity rates range from 3.0 to 7.0 per 100 thousand female population [Axel E.M., Vinogradova N.N., 2018]. In Kazakhstan, the standardized mortality rate was 4.9 per 100 thousand women and tends to increase [Kuanyshkalieva A.N., Iginov N.S., Turganbayeva A.K., 2016]. This is due to the high prevalence of this pathology and the high potential of some of the histological variants of benign tumors to malignant degeneration [Flaum N., Crosbie E.J., Edmondson R.J. et al., 2020].

Ovarian cancer is the most common, the second most common gynecological malignancy with high mortality [Siegel, R.L.; Miller, K.D. et al., 2019]. Epithelial tumors of the ovaries are characterized by a poor prognosis, including a decrease in survival or 5-year survival is not achieved [Khalid H. Sait, Mohammad Z. Alam et al., 2022]. Although, the conditional 3- and 5-year survival with advanced epithelial tumors of the ovaries was 57.2 and 37.8%, respectively, and increased over time [Zheng P, Zheng P and Chen G., 2021; Shin DW, Bae J. И др., 2021].

In the strategy of reducing the incidence of ovarian cancer and knowing where the early precursors of the lesion begin can help in the development of new methods of visualization and detection of predictors. In particular, the study of epithelial biomarkers of epithelial tumors of the ovaries has a special purpose, since this histological type accounts for 90% of all epithelial tumors of the ovaries [Elorriaga M.Á., Neyro J.L., Mieza J. et al., 2021]. In most patients, this pathology is diagnosed at a late stage due to the latent course of the early stages of development and the lack of an effective screening strategy [Hamidi F, Gilani N, Belaghi RA., 2021]. Although over time, numerous biomarkers have been studied and used to assess the condition, progression and effectiveness of treatment [Radu M.R.; Prãdatu A.; Duicã F. и др., 2021].

The aggressive course of this pathology is due to the biological behavior of the malignant process and a variety of histological variants. The holistic prognosis is determined by the morphological type of benign neoplasm, but still detected at the early stages of maturation [Chen F., Jiang K. Giant, 2020]. Definitely, the expediency and prospects of introducing the immunohistochemical method into everyday practice and the possibility of combining it with other methods of molecular biology makes it possible to detail the proliferative and apoptotic

activity of cells. It is important to make the most correct morphological diagnosis, which is basic. At the same time, the results of immunohistochemical reactions are significantly supplemented with the use of an antibody panel and are the basis for accurate verification [Beisenaeva A.R., 2015].

At the moment, in order to diagnose and predict the malignancy of ovarian neoplasms, there is a need to know potential biomarkers that determine the role of modern and more accurate predictors. At the same time, preoperative examination of the activity of the tumor process and early diagnosis using new algorithms and complexes of long-term methods can significantly affect the prognosis and favorable outcome of the disease [Rojas V. et al., 2016]. One of the studied indicators is currently the calculation of the malignancy risk index, which is disclosed in detail in our work.

Purpose of the study: To investigate the morphofunctional state of the ovaries in women with neoplasms and to assess the risk of early malignancy.

Research objectives:

1. To determine the prognostic significance of the risk index of malignancy (RMI) in women with ovarian neoplasms in comparison with the histological conclusion;
2. To study biological markers of the state of cellular structures of ovarian tissue in women with neoplasms;
3. Give diagnostic and morphological criteria for the risk of early malignancy of ovarian neoplasm in women.

The scientific novelty of the research:

1. The threshold indicators for calculating the risk of malignancy index of benign ovarian neoplasms in different age groups of women were determined, with the specification of specificity, sensitivity, positive and negative predictive values.
2. The obtained results of the risk of malignancy index supplemented scientific data on the frequency of malignancy of benign ovarian neoplasms in different age groups, which in general determines the tactics of therapeutic measures and favorable outcomes of surgical treatment.
3. The conducted study of the risk of malignancy index expands the diagnostic ability at the stage of treatment planning and selection of surgical intervention tactics in line with the "gold standard" - the histological diagnostic method.

Theoretical and practical significance:

1. The introduction of a diagnostic method for calculating the risk of malignancy index will increase the diagnostic accuracy at the diagnostic stage and reduce the ineffectiveness of surgical intervention in the postoperative period.
2. The calculation of the risk index of malignancy is presented in the clinical guidelines for practicing physicians.

3. The present study is of practical interest for predicting groups at increased risk of progression, requiring additional treatment or more careful follow-up.

Basic provisions or protection

Prediction of the risk of malignancy of benign ovarian neoplasms should be carried out comprehensively: determination of the level of CA125, ultrasound characteristics of the localization and structure of the cyst, the woman's age. The study of these indicators separately does not allow diagnosing and reliably predicting the outcome of a neoplasm.

Morphological study of the nature of a benign neoplasm showed age-dependent morphological changes, the greatest risks of malignancy appear in premenopausal and postmenopausal ages. The expression of genes for proliferation (Ki67) and apoptosis (P53) is not the main indicator in assessing the risk of malignancy among benign neoplasms.

Approbation of work. The main provisions of the dissertation work were reported at an expanded meeting of the scientific and problem commission of the NJSC "ZKMU named after M. Ospanova ". The results of the study were reported at international congresses and conferences:

XXVII International Symposium of Morphological Sciences "ISMS 2020".
XXVII International Symposium on Morphological science "Cell, tissue, organs - experience, innovation and progress" May 27-31, 2021, Almaty.

International practical conference - The Second International Scientific - Practical Virtual Conference In Modern Medicine "Women's Health & Reproductive Endocrinology: Prognosis, Achievement & Challenges" July 30-31, 2021, Tallinn, Estonia.

International Congress of Morphological Sciences of Anatomy "16th Congress of the European Association of Clinical Anatomy (EACA) held jointly with the XII Meeting of the International Symposium of Clinical and Applied Anatomy (ISCAA)" September 14-16, 2021, Padova, Italy.

Publication details

On the topic of the dissertation research, 7 scientific works have been published, of which:

1 - publication in an international scientific publication that has a non-zero impact factor, in a publication indexed in the Scopus information base.

3- in scientific publications recommended by the Committee for Control in the Sphere of Education and Science of the Ministry of Education and Science of the Republic of Kazakhstan; 3 - in materials of international scientific congresses (2021-Almaty, Kazakhstan; 2021- Padova, Italy) and conferences (2021-Tallinn, Estonia).

1- Methodical recommendation "Features of the study of the ovarian reserve of women and early predictors of malignancy of ovarian cysts" Zhurabekova, A.S. Adilgereeva, I.A. Abdelazim, A.D. Balmagambetova. Methodical recommendation Almaty, 2021.

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Object name: "Clinical and pathological features of women with adnexal masses admitted as emergency cases to the Gynecology Department of West Kazakhstan University". Object creation date: 12/17/2019

Copyright certificate No. 24336 dated march 14, 2022

Object name: «Interrelation of risk indicators of malignancy index and morphological determinants of ovarian tumor». Object creation date: 11/01/2021

The volume and structure of the thesis

The dissertation work is presented in Russian on 123 pages of computer text and consists of an introduction, an analytical review of the literature, materials and research methods, the results of their own research, discussion of the results, conclusions, practical recommendations, a list of references and an attachment. The work is illustrated with 23 tables, 44 figures. The list of references contains 229 scientific publications, 43 of them in Russian and 186 in English.

MATERIALS AND RESEARCH METHODS

General characteristics of the study

Design: Stage 1: Retrospective analysis.

Stage 2: a prospective comparative study.

Stage I of the study - a retrospective analysis of the case histories of 318 patients who received treatment in the gynecological departments of Aktobe for three years (2016-2018) was carried out after obtaining the consent of the head of the clinic for retrospective analysis. According to the history of the disease, the following were studied: age, parity (reproductive history: number of pregnancies, number of births), menarche and menstrual status, heredity, past gynecological and extragenital diseases, data of histological findings.

The principles of group formation. All patients were divided into four age groups according to the reproductive age of women according to WHO (15-49 years) and, taking into account the impact of possible factors affecting the health of the mother and child, the age group was composed as follows: Group I - 18-40 years (n = 62); II group - 41-44 (n = 17); III group - 45-49 (n = 11); Group IV \geq 50 years (n = 21).

Inclusion criterion: Age \geq 18 years; No history of malignant diseases and severe somatic pathology of other organs and systems; Ultrasound results confirmed by the presence of a neoplasm; Patients who have been subject to surgical treatment since the day and have a further postoperative histological report; The presence of the result of Ca 125 in the blood serum.

Exclusion criteria: Women who are not sexually active (in case of impossibility of performing TV ultrasound); Accessory formations during pregnancy; Suspected endometriosis, adenomyosis; Suspected PID; Confirmed cases of treatment of malignant neoplasms of the ovaries; Pelvic formations arising from the urinary tract and / or gastrointestinal tract.

Stage II - a prospective study of n = 264 patients with benign ovarian neoplasms (BP) was carried out. The work was carried out in the departments of gynecology in Aktobe (Kazakhstan) in the period from 2019 to 2021. The studied women were divided into groups by age: group I - reproductive age (\geq 18-40 years), group II premenopause ($>$ 40-50 years) and group III postmenopause ($>$ 50 years) to determine morphological parameters of ovarian tumors in the three study groups (especially women of reproductive age) and to determine the accuracy of RMI versus histological results in distinguishing between benign and malignant ovarian neoplasms. In a prospective study of collecting information about anamnesis from medical records (extracts from outpatient cards, case histories, epicrisis), additional material was also collected directly from the patient, the following data: age, menarche, the nature of the menstrual function, data on menopause, the onset of sexual activity, gynecological and extragenital diseases, features of reproductive function, the number and volume of surgical interventions in the anamnesis.

The Quetelet body mass index (BMI) was calculated by dividing the body weight in kilograms by the person's height, expressed in meters and squared (kg / m²). BMI values from 18.5 to 24.9 kg / m² were taken as normal values. With BMI

values of 18.5 kg / m² or less, a body weight deficit was diagnosed; from 25.0 to 29.9 kg / m² - excess body weight [Stevens GA et al., 2012].

Laboratory and instrumental methods

Ultrasound examination of benign ovarian neoplasms. During the assessment, attention was paid to the following parameters: ovarian volume, size of cystic formation, the cyst wall thickness in mm, internal structure, echogenicity. Five ultrasound signs of malignancy were taken as the basis for calculating the U criterion in the formula for the risk index of malignancy: 1) multilocularity (binocularity); 2) the presence of hard areas, 3) two-sidedness; 4) the presence of ascites; 5) the presence of metastases with a score for calculating the formula.

The determination of the marker CA125 was carried out in the laboratory by immunoassay using enzyme-linked immunosorbent assay (CA125 ELISA) for quantitative diagnostic measurement of CA 125 in vitro in serum and plasma. When determining the concentration of CA125 in the serum of patients, all manufacturer's recommendations were followed. The material for the study of CA 125 was venous blood.

The calculation of the risk index of malignancy (RMI) was calculated at the preoperative stage using the results of ultrasound (U), the state of menopause (M) and serum level CA-125: $RMI = U \times M \times CA-125$ [Jacobs I, 1990]. The U score is 0 if no ultrasound parameters are detected, 1 if one ultrasound parameter is detected, and 3 if ≥ 2 ultrasound parameters are detected.

Morphological conclusion

The histological examination was carried out in the histological department of the medical center of the West Kazakhstan Marat Ospanov Medical University and included staining of glasses with hematoxylin-eosin and carrying out immunohistochemical stains. After cutting out the material, fixation was carried out; a 10% solution of buffered formaldehyde was used as a fixative. Then, after paraffinization, sections with a thickness of 4-5 μm were cut from paraffin blocks. To assess the morphological manifestation, one section from each sample was stained with hematoxylin-eosin according to the standard technique.

Immunohistochemical methods: For Ki-67, P53 staining, the sections were dewaxed with xylene and alcohol solutions and rehydrated in 96% ethanol. Thermal incubation in citrate buffer solution with pH = 6.0 in a water bath and blocking at room temperature by incubation for 5-10 minutes in 3% H₂O₂. Material incubated with primary antibodies: MAC047Hu22 Monoclonal antibody to Ki protein -67 (Ki67P) 100 μg ; MAA928Hu22 Monoclonal antibody to p53 protein (P53), 100 μl (Cloud-Clone Corp., Wuhan, China). Then incubation with a working solution streptavidin - HRP 100 μl (reagent kit - IS086-2) for 30 minutes. Microscopic examination of histological preparations was carried out in the morphological laboratory of the West Kazakhstan Marat Ospanov Medical University using an Axio Lab A1 video microscope, RK-MT-7 No. 009046.

Quantitative microscopic analysis was carried out at different levels of magnification of a light microscope $\times 10$, $\times 40$ by the stereometric method using a

screw eyepiece micrometer and an object-micrometer to determine the degree of pathological changes (G.G. Avtandilova, 1990).

The analysis of the results of images of immunohistochemical reactions was carried out using the the GenASIs program.

Statistical analysis was performed using the SPSS application, version 20 (Chicago, IL, USA). According to our data, the nominal measuring scale is mainly used (qualitative variables that cannot be directly measured). Indicators were calculated in (%). An analysis of variance was carried out using the χ^2 test (Pearson's chi-square, differences between qualitative characteristics were assessed using the χ^2 test). Differences were considered statistically significant at $p < 0.05$. - The arithmetic mean values of quantitative indicators were calculated, $M \pm SD$, where M is the arithmetic mean, SD is the standard deviation. Validation of RMI was carried out based on the construction of predictive models of a binary classifier using ROC curves (Receiver Operator Characteristic).

Main research findings

At the 1st stage, according to the analysis of data from a retrospective study, benign neoplasms were more common in the reproductive age group is 77.04%. When evaluating the histological conclusion according to the International Classification of Tumors (2014) of the ovaries, it was found that in the studied material, tumor-like ovarian formations predominated in 36.2% and true benign ovarian tumors in 18.55% of cases. According to the results of histological examination, the most common histological subtypes were follicular and serous cysts. The main reason for surgical intervention in the studied women was apoplexy of the ovarian cyst - 27.1%, torsion of the epididymis - 9.7%. When analyzing menstrual function, it was found that the average age of menarche corresponded to 13-14 years in more than 70% of patients in the 1st group and more than 60% of the 2nd and 3rd groups. In a comparative study of the age of onset of menarche, there were no significant differences between the study groups ($p = 0.54$). Violations of the ovarian-menstrual cycle of the studied groups are with the same frequency ($p = 0.20$). In 44.34% of the women studied, benign neoplasms were associated with pregnancy, which ended unfavorably in 34.28% of cases. However, it was not possible to identify alarming risk factors for the development of an oncological process in the ovaries, both according to the data of parity, menstrual function, and gynecological pathology of the anamnesis.

At stage 2, as a result of a prospective study, the characteristics of the three study groups showed that most often women are brought to examination by arising pains in the lower abdomen. When analyzing the circumstances of detecting tumors and tumor-like formations of the ovaries (OO) in the majority of patients with ovarian neoplasms, the most common complaint was pain in the lower abdomen, which was detected in more than half of patients in the reproductive group - 71.5%, in the premenopausal and postmenopausal groups in 1.5 and 1, 0 times lower than 45.45% and 67.04%, respectively ($P = 0.4$). According to the age characteristics of the three studied groups: reproductive 30.04 ± 5.7 and premenopausal 45.3 ± 2.6 years, respectively, there was no significant difference

between them ($P_1 = 0.0$). While women in the reproductive group were significantly younger than in the postmenopausal group (30.04 ± 5.7 and 57.6 ± 4.3 years, respectively) ($P_2 = 0.004$). The age of the participants in the malignant group was 1.26 times older than in the benign neoplasm groups and was 52.3 ± 9.1 and 41.5 ± 11.7 years, respectively, ($P = 0.9$). The analysis of the correlation coefficient showed a significant positive correlation between the age of women and RMI ($r = 0.38$, $P = 0.001$, 95% CI: 0.164-0.568), in the group of ovarian malignancies. On average, the parity indicator is in three studied groups: reproductive 3.05 ± 1.02 , premenopausal 2.4 ± 1.65 and postmenopausal 2.24 ± 1.4 , ($P_1 = 0.9$, $P_2 = 0.06$ and $P_3 = 0.9$ respectively). In the premenopausal and postmenopausal groups, the parity index is 0.78 ± 0.8 and 3.2 ± 0.9 ($P = 0.8$), respectively, in the three studied groups. The reproductive, premenopausal, and postmenopausal groups were similar, with no significant differences in BMI ($P_1 = 0.2$, $P_2 = 0.7$, and $P_3 = 0.9$, respectively). Although there was a significant difference in BMI in the postmenopausal group between malignant and benign tumors ($P = 0.03$).

Ultrasound. In the reproductive group, the ultrasound scores were significantly lower by 1.5 and 1.75 times compared with the premenopausal ($P_1 = 0.01$) and postmenopausal ($P_2 = 0.0$) groups. The assessment of the ultrasound index in the premenopausal group was 1.5 times higher than in the reproductive group ($P_1 = 0.01$) and 1.1 times lower than in the postmenopausal group ($P_3 = 1.0$). Ultrasound indicators for malignant ovarian tumors are 1.4 times higher than for benign ovarian tumors ($P = 1.0$). Indicators of ultrasound assessment in the postmenopausal group are significantly higher by 1.75 times compared to the reproductive group ($P_2 = 0.0$) and 1.1 times higher than in the premenopausal group ($P_3 = 1.0$). In those examined, in relation to the assessment of ultrasound in malignant ovarian tumors, it was 1.5 times higher than in benign ovarian tumors ($P = 1.0$). Although the USG score in this study was significantly higher compared to the reproductive group ($P = 0.01$), the analysis showed no significant correlation between the USG score and RMI in the ovarian malignancy group ($r = 0.16$, $P = 0.1$, 95% CI: -0.075-0.383). Significant differences in the assessment of ultrasound parameters were detected between the groups of malignant (1.5 times higher, $P = 1.0$) and benign ovarian neoplasms.

CA 125. As a result of the study, the concentration levels of CA125 had an average value of 25.5 ± 27.9 IU / ml, ($P_1 = 1.0$). Whereas in the premenopausal and postmenopausal groups they tended to increase by 2.1 and 1.5 times, respectively, than in the reproductive group ($P = 1.0$). The levels of Ca125 of the reproductive group in cases of a malignant tumor are significantly higher by 4.9 times than that of a benign tumor and amounted to, ($P = 0.0$). In the premenopausal group, Ca125 levels were with an average value of 54.1 ± 60.7 IU / ml, $P_2 = 1.0$, except for some cases. Among premenopausal patients in the malignant neoplasm group, CA125 levels were 4.6 times significantly higher than in benign tumors ($P = 0.0$). In the postmenopausal group with an average value of 40.1 ± 106.3 U / ml, ($P_3 = 1.0$). Ca125 levels were 6 times higher in the malignant tumor group than in

the benign tumor group ($P = 0.0$). It should be noted that there was a positive correlation between CA-125 examined and RMI ($r = 0.55$, $P < 0.0001$) in the group of ovarian malignant neoplasms.

Thus, among the histologically confirmed benign neoplasms of the ovary in the reproductive, premenopausal and postmenopausal groups, CA125 tended to increase, but in benign tumors it remained within the normal range. In cases of histological confirmation of the malignancy of the process, we found that there is a tendency for a uniform increase in the level of CA 125, regardless of the woman's age.

RMI values in the reproductive group are within the normal range (56.2 ± 89.6), while in premenopause and postmenopause, respectively, 2.7 and 4.2 times are significantly higher ($P_1 = 1.0$ and $P_2 = 1.0$). In the group of malignant cases, RMI values were significantly higher by 8.2 times than in benign cases. In the premenopausal group, RMI was significantly 2.7 times higher than in the reproductive group ($P_1 = 1.0$), but did not go beyond < 200 , while compared to the postmenopausal group, it was 1.5 times lower ($P_2 = 0.9$). In malignant processes in the premenopausal group, RMI values are 6.7 times higher than in benign and postmenopausal processes ($P = 0.0$). In the postmenopausal group, the RMI value is > 200 and significantly exceeds the reproductive group by 4.2 times ($P_2 = 1.0$), the premenopausal group by 1.5 times ($P_3 = 0.9$). In the postmenopausal group between benign and malignant ovarian tumors, the RMI values averaged 94.7 ± 58.5 and 497.1 ± 240.7 , respectively ($P = 0.0$).

According to the histological conclusion, 81 cases with RMI < 200 were negative; 2 were confirmed as false negative ((FN) = 2) and 79 were confirmed as true negative ((TN) = 79). RMI at a cut-off value > 200 6 were confirmed as a true positive ((TP) = 6) and 1 was confirmed as a false positive ((FP) = 1). RMI at a cut-off value > 200 had a sensitivity of 75%, a specificity of 98.75%, a PPV of 85.7% and an NPV of 97.5% in distinguishing benign and malignant ovarian tumors in the reproductive group. ROC showed that RMI at a cut-off value > 231.6 at reproductive age had a sensitivity of 75%, a specificity of 100%, a PPV of 100% and an NPV of 97.3% (area under the ROC curve (AUC) 0.950, 95% CI: 0.88 - 0.98, $P = < 0.001$). In the premenopausal RMI group at a cut-off value of > 200 at histological conclusion, 25 were confirmed as true positive ((TP) = 25) and 5 were confirmed as false positive ((FP) = 5). In addition, RMI with a cut-off < 200 were confirmed as false negative in 6 cases ((FN) = 6), and 52 were confirmed as true negative ((TN) = 52). RMI with a cut-off value > 200 had 80.6% sensitivity, 91.2% specificity, 83% PPV and 89.7% NPV in differentiating malignant and benign ovarian tumors in the premenopausal group. ROC showed that RMI at cut-off value > 247.5 in the premenopausal group had a sensitivity of 80.65%, a specificity of 100%, 100% PPV and 97.9% NPV (AUC 0.96, 95% CI: 0.89-0.99, $P = < 0.001$). In the postmenopausal RMI group with a cut-off value of > 200 , 27 cases were confirmed as true positive ((TP) = 27), in 6 cases were confirmed as false positive ((FP) = 6) based on histological examination. At the same time, < 200 in 4 cases were confirmed false negative ((FN) = 4), 51 were confirmed true negative

((TN) = 51). When distinguishing benign and malignant ovarian tumors in the postmenopausal group, RMI at a threshold value of > 200 had a sensitivity of 87.1%, a specificity of 89.5%, PPV 81.8% and NPV 92.7%. ROC showed that RMI at cut-off value > 245.7 in the postmenopausal group had 87.1% sensitivity, 100% specificity, 100% PPV and 98.6% NPV (AUC 0.960, 95% CI: 0.89-0.99 , P = <0.001).

In the three study groups, RMI accuracy at a cut-off value of > 200 was false positive in 12 cases, 182 were confirmed as true positive. With RMI <200, false negatives were found in 12 cases, in 58 they were confirmed as true negative based on histological examination. The RMI accuracy at a cut-off value of > 200 in the three study groups had a sensitivity of 82.9%, a specificity of 93.8%, a PPV of 82.9% and an NPV of 93.8% when distinguishing between benign and malignant ovarian lesions. The ROC showed that RMI at a cut-off value > 247.5 in the three subjects was 82.9% sensitivity, 100% specificity, 100% PPV and 98.1% NPV.

Thus, the RMI index exceeded 200 in women with malignant tumors, including those with benign ovarian neoplasms, which morphologically corresponded to an epithelial tumor. The incidence of malignant tumors was significantly higher in the studied premenopausal and postmenopausal groups compared to the reproductive group (P = 0.0008 and 0.0008, respectively). RMI at a cut-off value of > 200 in the three study groups, ROC had a sensitivity of 82.9%, a specificity of 93.8%, a PPV of 82.9% and an NPV of 93.8%.

Morphological features of benign ovarian neoplasms according to age groups.

In the reproductive group, the frequency of benign ovarian tumors was 90.9%. Among the benign ovarian neoplasms, the serous (simple) ovarian cyst was high in 38.75%. It is followed by a follicular cyst in 23.75%, a dermoid cyst in 16.25%, cystadenoma in 13.75%, and a luteal cyst in 7.5% of cases. In the reproductive age group, the incidence of ovarian malignant tumors was 9.1%. The most common benign ovarian tumor was simple serous ovarian cyst (38.75%), and the frequency of which was significantly higher in the reproductive group compared with the premenopausal and postmenopausal groups ($p_2 = 0.001$). Also, in the same group, the follicular cyst was more common than in the premenopausal and postmenopausal groups ($P_1 = 0.09$, $P_2 = 0.1$, respectively). The frequency of indicators of the dermoid cyst ($P_1 = 0.6$, $P_2 = 0.5$) is less than in the premenopausal and postmenopausal groups, respectively.

In the premenopausal group, the incidence of benign ovarian tumors is 64.8%. The incidence of malignant ovarian tumors was 35.2% (31/88). Among benign ovarian tumors, cystadenoma prevailed - 31.6%, followed by serous ovarian cyst - 24.6%, dermoid cyst - 19.3%, follicular cyst - 10.5%, luteal cyst theca - 7.0%, ovarian fibroma - 5.3% and fibrothecoma - 1.7%. In the premenopausal group, the incidence of ovarian cystadenoma was significantly higher in the premenopausal group ($P_1 = 0.04$) more often than compared with the reproductive group and then in the postmenopausal group ($P_3 = 0.8$). A serous

ovarian cyst ($P1 = 0.2$) is less common than in the reproductive group and ($P3 = 0.2$) more often detected than in the postmenopausal group. Dermoid cyst ($P1 = 0.6$) was more common than in the reproductive group, less frequently ($P3 = 0.8$) than in the postmenopausal group. A follicular cyst ($P1 = 0.09$ and $P3 = 0.8$, respectively) is less common than in the reproductive group and in the postmenopausal group.

In the postmenopausal group, the incidence of benign ovarian tumors is 64.8%. Among the DAY, cystadenoma prevails - 29.8%, then dermoid cyst - 21.0%, simple serous ovarian cyst - 14.0%, then follicular cyst - 12.3%, fibrothecoma in 8.8%, luteal cyst in 5, 3%, ovarian fibroma in 5.3% and thecoma in 3.5%. The incidence of malignant ovarian tumors was 35.2%. The incidence of cystadenoma is significantly higher in the postmenopausal group ($P2 = 0.06$) than in the reproductive group and ($P3 = 0.8$) than in the premenopausal group. Dermoid cyst ($P2 = 0.5$ and $P3 = 0.8$, respectively) was more common than in the reproductive group and in the premenopausal group. A serous ovarian cyst ($P2 = 0.001$, $P3 = 0.2$, respectively) is less common than in the reproductive group and in the premenopausal group, respectively.

Comparative analysis of the results shows that the incidence of benign tumors is higher in the reproductive age group than in the premenopausal and postmenopausal groups ($P1 = 0.1$, $P2 = 0.1$, respectively). The frequency of malignant ovarian tumors in the reproductive group ($P1 = 0.0008$ and $P2 = 0.0008$, respectively) is less frequent than in the premenopausal and postmenopausal groups.

Morphologically, the epithelium of the serous cyst is represented by a single-layer flattened epithelium, $18.3 \pm 0.12 \mu\text{m}$ thick ($P=0,01$). Dystrophic changes and necrosis of epithelial cells (or desquamation) are observed in places. The diameter of flattened epithelial cells is on average $16.5 \pm 0.73 \mu\text{m}$ ($P = 0.01$). The nuclear-cytoplasmic ratio is 0.46 ± 0.14 . The inner wall of the follicular cyst is lined with multilayer follicular epithelium, consisting of granulosa cells. The basis of the epithelium is loose connective tissue, on the border of which the plethora of the vessels of the microvasculature is expressed.

The incidence of benign ($P3 = 1.0$) and malignant ($P3 = 0.8$) ovarian tumors did not differ significantly in premenopausal and postmenopausal patients. Among benign tumors in the reproductive group, in the first place are serous and follicular cysts with a high frequency of occurrence, followed by dermoid cysts and cystadenomas, which were less common. While cystadenomas were in the lead in the premenopausal and postmenopausal groups, serous cysts were located behind them. There is a close relationship between the increase in the frequency of dermoid cysts with age (19.3% and 21.0%), but the follicular type is adherent to a younger age - 2.2 and 1.9 times less common than the reproductive group. Morphologically, in the premenopausal group, cystadenoma prevails among benign ovarian neoplasms. The inner wall of serous cystadenoma is lined with prismatic epithelium, $22,5 \pm 0,37 \mu\text{m}$ thick ($P = 0.001$). The diameter of the epithelial cells is $18.1 \pm 0.29 \mu\text{m}$. The nuclear-cytoplasmic ratio is $0.62 \pm$

0.25(P=0,002). Ovarian cystadenomas are also represented by the following subtypes: serous papillary and mucinous types. With papillary cystadenoma of the ovary, on the inner surface, there are multiple branching high papillary outgrowths of a complex structure, in the formation of which the stroma is involved. Lining epithelium with signs of dysplasia, with stratification. The thickness of the epithelium in the places of stratification with a diameter ranging from 25.4 ± 0.13 microns to 29.3 ± 0.11 microns (P = 0,001). The inner wall of the mucinous cystadenoma is lined with a single-row high columnar epithelium, 23.7 ± 0.38 μm thick (P = 0.01). The cytoplasm is eosinophilic with a basal arrangement of the nuclei. The cell diameter is 22.9 ± 0.16 μm . The nuclear-cytoplasmic ratio was 0.48 ± 0.28 (P=0,03).

The morphological characteristics of the ovaries of women of the 3rd group, as well as in the second, in the first place, are the morphological types of cystadenoma of the BON.

Serous cystadenoma is lined with a single-layer flattened epithelium, 15.5 ± 0.70 μm thick (P = 0.01). Epithelial cells with an average diameter of 14.8 ± 0.27 μm (P = 0.002). The nuclear cytoplasmic ratio was 0.61 ± 0.13 .

Papillary serous cystadenomas are represented by papillary outgrowths covered with cubic epithelium. In multi-chambered mucinous cystadenoma, the inner lining is represented by high cylindrical ones, which consist of cuboidal cells with a basal arrangement of nuclei.

Cell diameter is 18.0 ± 0.49 μm (P = 0.02), nuclear-cytoplasmic ratio equal to 0.42 ± 0.15 (P = 0.03).

Immunohistochemical results.

In the reproductive group, the results of those immunopositive to Ki-67 had an expression level of $51.4 \pm 2.29\%$. The intensity of expression was $2.0 \pm 0.76\%$ (P <0.001). The expression level of the oncosuppressor P53 was $19.2 \pm 0.33\%$ (P <0.001), mainly moderate or weak expression.

In the premenopausal group with serous cystadenoma the expression level of the Ki-67 were $2.02 \pm 0.33\%$. The intensity of expression compared with the reproductive age group is reduced by 1.7 ± 0.69 (P <0.001), but higher than in the postmenopausal group. The expression level of the tumor-suppressing protein p53 was $21.5\% \pm 3.19\%$ with a staining intensity of 2.14 ± 0.12 , (P <0.001) higher than in the other groups. In the postmenopausal group with serous cystadenoma, the expression of the Ki 67 protein in the cyst wall averaged $16.1 \pm 2.34\%$ with a staining intensity of 1.3 ± 0.09 . The expression level of the tumor suppressing protein p53 in the epithelial layer is $43.2 \pm 3.15\%$ (p <0.001). The intensity of manifestation was 1.8 ± 0.09 .

So, the process of active proliferation of the epithelium of cysts, among women of fertile age from 18 to 40 years, increased to 51% and revealed a pronounced suppression of the P53 protein suppressor gene activity. This fact is very alarming and can be attributed to early predictors of cellular degeneration. The protein in this case does not recognize the genotoxic stress of the cell and thus does not block

proliferation in the group of young women. Perhaps the start of the formation of a malignant process begins at the reproductive age and in the youngest women, and towards the approach of menopausal age reaches its peak - a detailed picture of ovarian malignancy. According to the results obtained, among women aged 41 and older, despite the low rate of proliferative activity in the second and third groups, there is an increase in apoptosis to 21% and 43%, respectively. But gene suppression in this age group is due to many factors such as age and concomitant chronic diseases. Therefore, consideration of the predictor of malignancy is well expressed among women under 41 years of age.

Thus, the results of a retrospective study based on the study of the influence of gynecological diseases, history, and parity, we concluded that these parameters do not reveal a holistic view of the risks of malignancy in women of different age groups. At the same time, a close correlation was revealed between age and the morphological subtype of the neoplasm. The susceptibility of women with chronic inflammatory diseases of the pelvic organs to the development of neoplasms was revealed, and a high impact of benign neoplasms in women on the quality of gestation and its outcomes was noted.

The results of a prospective study showed that RMI is a method for early diagnosis of neoplasms, which depends directly on the initial state of the female body, morphological type of tumor, size, and growth rate. The parameters that make up the calculation of the risk of the index of malignancy have their significance only when confirmed by morphological research. We also obtained a significant positive correlation between the age of women and RMI, as well as between CA125 and RMI in the group with a high risk of malignancy of ovarian neoplasms, while our results are confirmed by the data of other researchers.

The applied "gold standard" - morphological research allowed to make weighty conclusions. The frequency of diagnostics of various types of benign ovarian neoplasms has an adherence to the woman's age and a tendency to malignancy in a later period. The high incidence of cystadenomas in pre- and postmenopausal women causes great concern, which is an alarming indicator that is reflected in the works of many other researchers. Immunohistochemical study showed several interesting facts that we classified as early predictors of malignancy among women of reproductive age.

CONCLUSION

Based on the results obtained, the following conclusions were drawn:

1. Calculation of RMI ($RMI > 200$) showed high efficiency in predicting the type of ovarian neoplasm in women of different age groups. The malignant type of formations had a higher RMI. Analysis of the ROC curve showed that RMI has a sensitivity of 82.9%, specificity of 100%, PV of 100%, NPV of 98.1% in all the studied groups.

2. Cancer marker Ca 125 has a positive correlation with RMI in all studied groups with malignant neoplasms. With benign formations, these indicators remained within the normal range. The cell structures of neoplasms with a low

positive value of P53 protein expression had a pronounced expression of Ki-67. Indirect predictors of malignancy of benign ovarian neoplasms may be increased cell proliferation and suppression of the activity of apoptosis processes.

3. The morphological criterion for the malignancy of benign ovarian neoplasms is the serous subtype of the neoplasm, chronic diseases of the pelvic organs, and the woman's age, the diagnostic criterion is the malignancy risk index above 200, confirmed by the "gold standard" - morphological examination.