Exp Oncol 2014
36, 2, 125–133

Experimental ONCOLOGY

# CLINICAL SIGNIFICANCE OF HORMONAL RECEPTOR STATUS OF MALIGNANT OVARIAN TUMORS

*I.G. Tkalia[1*], L.I. Vorobyova[1], V.S. Svintsitsky[1], S.V. Nespryadko[1], I.V. Goncharuk[1], N.Y. Lukyanova[2], V.F. Chekhun[2]*

[1]*National Cancer Institute, Kyiv 03022, Ukraine*

[2]*R.E. Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine, Kyiv 03022, Ukraine*

***Objectives*: To study hormonal receptor status (HRS) of malignant ovarian tumors (MOT) and determine its clinical significance. *Patients and Methods*: Retrospective analysis of case histories of 284 patients with MOT of different genesis of I–IV stages was carried out; immunohistochemical study of paraffin-embedded tissues. The HRS for serous, mucinous ovarian cancer (OC) and sex cord-stromal tumors (SCST) was studied. The phenotype of tumors by HRS in patients with serous OC was determined; overall and relapse-free survival in these patients was evaluated depending on the tumor HRS. *Results*: Positive expression of ER has been registered in 66.4% of patients with serous OC, PR — in 63.4%, TR — in 53.0%; in patients with mucinous OC — 88.0; 84.0; 60.0%, respectively. Positive staining of cells of stroma-cellular tumors has been observed in 74.1% of patients for ER and 77.8% — for PR and TR. The highest number of patients with tumor phenotype ER+PR+TR+ has been observed in postmenopause — 52.4%, especially in late postmenopausal period — 39.0%. The lowest percentage of patients with mentioned phenotype has been marked in reproductive age — 20.7%. Most patients of reproductive period had phenotype of tumor ER-PR-TR- (35.1%), in late postmenopause this phenotype has been observed only in 16.2%. The patients with serous OC with the positive tumor HRS demonstrated the low indices of overall and relapse-free survival compared to the patients with receptor-negative tumors concerning all steroid hormones ($p < 0.05$). *Conclusions*: Positive HRS was registered in serous, mucinous OC and in SCST, high percentage of tumors with expression of all receptors of steroid hormones was observed at that. The highest frequency of tumors with positive HRS was recorded in patients with serous OC of late postmenopausal period. The patients with serous OC with receptor-positive tumor phenotype showed the rates of overall and relapse-free survival significantly lower compared to the patients with receptor-negative phenotype of OC. Positive HRS, the same as strong expression of TR in patients with serous OC, is a predictive factor of unfavorable course of tumor process. HRS of MOT can be regarded as the additional criterion for solution of a question concerning application of hormonal therapy as a component of complex treatment for the patients.**

***Key Words*: malignant ovarian tumors, serous ovarian cancer, hormonal receptor status, estrogen, progesterone, testosterone receptors, phenotype of tumor.**

Malignant ovarian tumors (MOT) are one of the most complicated problems in oncogynecology [1, 2]. Complexity of the problem lies in uniqueness of this organ and its role in a woman organism [3, 4]. Multicomponent structure of gonad, variety of structures with different functions provides a wide range of histological forms of ovarian tumors and complexity of etiopathogenesis of this disease [5]. Hormonal factors play the most important role in pathogenesis of MOT at present time. Ceaseless ovulation owing to pregnancies and childbirths declination in some countries, sterility of different genesis and application of drugs stimulating ovulation belong to them [6–8]. According to the data of literature, ovaries are not only producing sex steroid hormones, but also act as a target tissue for them, the same as other hormone-dependent organs [9]. Estrogens, progesterone and testosterone, the same as receptors to them, are promoters of all hormone-dependent tumors, in particular, of breast cancer (BC), endometrial and ovarian cancer (OC) [10].

Therapy strategy for patients with MOT includes surgical component and chemotherapy. Despite improvement of surgical treatment strategies and application of modern chemotherapy schemes [2–14], long-term results of treatment in patients with disseminated MOT remain unsatisfactory [15, 16].

For many years, hormonal therapy for patients with progressing and chemo-resistant MOT has been empirically prescribed as "despair therapy" demonstrating the low rate of efficacy [17]. Clinical interest in hormonal treatment of OC patients was increased due to development of molecular-biological technologies and possibility to determine receptors to estrogens (ER), progesterone (PR) and androgens in tumor tissue [18]. However, criteria of hormone-dependency of MOT and application of hormonal therapy in combined treatment of patients, the same as prognosis depending on expression of steroid receptors are remaining disputable.

Prognostic value of hormonal receptor status (HRS) of MOT has been studied for many years. Certain studies have determined high survival of OC patients at expression of ER and PR in tumor [19–21]. Other studies shown that expression of PR and receptors to testosterone (TR) are favorable prognostic factors, and expression of ER is associated with progression of disease and short relapse-free period [22–26].

Interrelation between HRS and degree of tumor differentiation as well as impact of chemotherapy on ER, PR, TR in tumor tissue of ovary has not been determined yet [9, 27].

Submitted: March 24, 2014.

*Correspondence: E-mail: j.tkalya@gmail.com

*Abbreviations used*: BC – breast cancer; ER – receptors to estrogens; HRS – hormonal receptor status; MOT – malignant ovarian tumors; OC – ovarian cancer; PR – receptors to progesterone; SCST – sex cord-stromal tumors; TR – receptors to testosterone.

Thus, the question concerning the mechanisms for realization of systemic-local dyshormonal disorders causing MOT and clinical significance of ovarian tumors receptor status till this day remains open. Solution of this question will allow us to specify MOT pathogenesis, prognosis of disease, but also to substantiate indications for hormone therapy carrying out as a component of the patients' combined treatment.

The aim of the research was to study HRS of MOT of different genesis and to determine its clinical significance in patients with serous OC.

## MATERIAL AND METHODS

The results of retrospective analysis of case histories of 284 patients with MOT of different genesis of I–IV stages and paraffin blocks of postsurgical material have been taken as material for study. Patients were treated in National Cancer Institute within 2001–2009. All patients underwent treatment by radical program: surgical, combined and complex (according to the standards of cancer patients' diagnostics and treatment approved by orders of MH of Ukraine № 140 from 27.07.1998 and № 554 from 17.09.2007). Combined treatment consisted in cytoreductive surgery followed by adjuvant chemotherapy or in combination with neoadjuvant chemotherapy. Complex treatment included surgical intervention, chemotherapy and radiation. Informed consent was received from all of the patients participating in the study.

MOT diagnosis has been verified using morphological study of post-surgical material according to the histological classification of ovarian tumors (2002), clinical staging of MOT — by international FIGO classification (2009) [28].

Immunohistochemical study of ER, PR, and TR in ovarian tumor cells has been carried out on paraffin sections 4–5 micron thickness, which were placed on glasses processed with poly-L-lysine. As primary antibodies, monoclonal antibodies specific to ER, PR and TR have been used (anti-Human Estrogen Receptor alfa Clone 1D5 (DakoCytomation, Denmark), anti-Human Progesterone Receptor Clone PgR 636 (DakoCytomation, Denmark), testosterone antibody Clone GTX72779 (GeneTex, USA)). For visualization of the results of reaction, kit of reagents En Vision system (DakoLSAB2system, Denmark) has been used in accordance with recommendations of the manufacturer, sections were stained by Meyer's hematoxylin. For evaluation of immunohistochemical expression of ER, PR and TR, semi-quantitative method has been applied. At presence of specific nuclear staining, the quantity of immune-positive and immune-negative cells in percentage has been determined. In each histological specimen, expression of steroid receptors in 1000 tumor cells has been analyzed. Since till this day no conventional semi-quantitative methods of total assessment of HRS for MOT exist, method, which is mostly found in data of literature [29], has been taken as a model. Degree of steroid receptor expression has been taken in scores: 0 scores — no staining of nuclei of tumor cells; 1 score — poor staining ⩽ 10%; 2 scores — moderate staining — 11–50%; 3 scores — high staining — 51–80%; 4 scores — hyperexpression — >81% of cells. The quantity of stained nuclei with more than 10% of moderate and high level of staining has been regarded as a positive expression of steroid receptors. As positive control, monoclonal antibodies against pan-cytokeratins have been used; as negative control — buffered physiological solution has been applied to histological sections, instead of monoclonal antibodies.

Results of studies have been evaluated depending on women age periods: reproductive (up to 45), perimenopause (46–55), early postmenopause (56–60) and late postmenopause (61 and more) according to the WHO classification (1980) [30]. Overall and relapse-free survival of patients with serous OC depending on tumor HRS has been determined. Correlation between HRS and stage, tumor differentiation grade has been assessed. Influence of neoadjuvant chemotherapy on expression of steroid hormone receptors in serous OC has been analyzed.

Statistical analysis of obtained data consisted in use of nonparametric statistics. For comparison of studied groups, *U*-criterion of Whitney — Mann has been applied. Correlation has been evaluated using Gamma rank correlation. Survival of patient has been analyzed by Kaplan — Meier method and *log-rank* criterion. Statistically significant were considered data at $p < 0.05$. Processing of results of study has been carried out using program package STATISTICA 6.0.

## RESULTS AND DISCUSSION

Analysis of case histories has demonstrated that age of patients with MOT (n = 284) of different genesis varied from 16 to 82, mean age 52.8 ± 2.2. By histological structure of tumors, patients were allocated (Table 1) into serous OC (232/81.7%), mucinous OC (25/8.8%), sex cord-stromal tumors (SCST) (27/9.5%). Mean age of patients was equal (51.7, 52.8 and 53.9, respectively).

**Table 1.** Distribution of patients by age, MOT histology, grade of tumor differentiation (n = 284)

| Histological type of tumor | Number of patients | | Mean age/ range, years | Tumor differentiation grade | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | high | | moderate | | low | |
| | n | % | | n | % | n | % | n | % |
| Serous OC | 232 | 100.0 | 51.7±0.8/18–82 | 45 | 19.4 | 73 | 31.5 | 114 | 49.1 |
| Mucinous OC | 25 | 100.0 | 52.8±3.2/16–69 | 7 | 28.0 | 10 | 40.0 | 8 | 32.0 |
| SCST | 27 | 100.0 | 53.9±2.5/27–70 | 3 | 11.1 | 8 | 29.6 | 16 | 59.3 |

Patients with MOT, who were prescribed neoadjuvant chemotherapy, depending on genesis of tumors, have been divided as follows: serous OC has been diagnosed in 84 (36.2%) patients, mucinous OC — in 2 (7.4%) and SCST — in 4 (16.0%). Grade of differentiation of ovarian tumors is represented in Table 1. Patients with serous OC and SCST had high percentage of tumors with low differentiation (49.1 and 59.3%, respectively). In patients with mucinous OC, most tumors were moderately differentiated (40.0%).

Analysis of MOT spreading has showed that number of patients with III stage was the highest —

73.6% (n = 209). Data on distribution of patients by stages depending on histological MOT type are represented in Table 2. As seen from Table 2, expression of steroid hormones in MOT was variable. Positive expression of ER was observed in 66.4% of patients with serous OC, PR — in 63.4%, TR — in 53.0% (Fig. 1–3 and see Table 2).

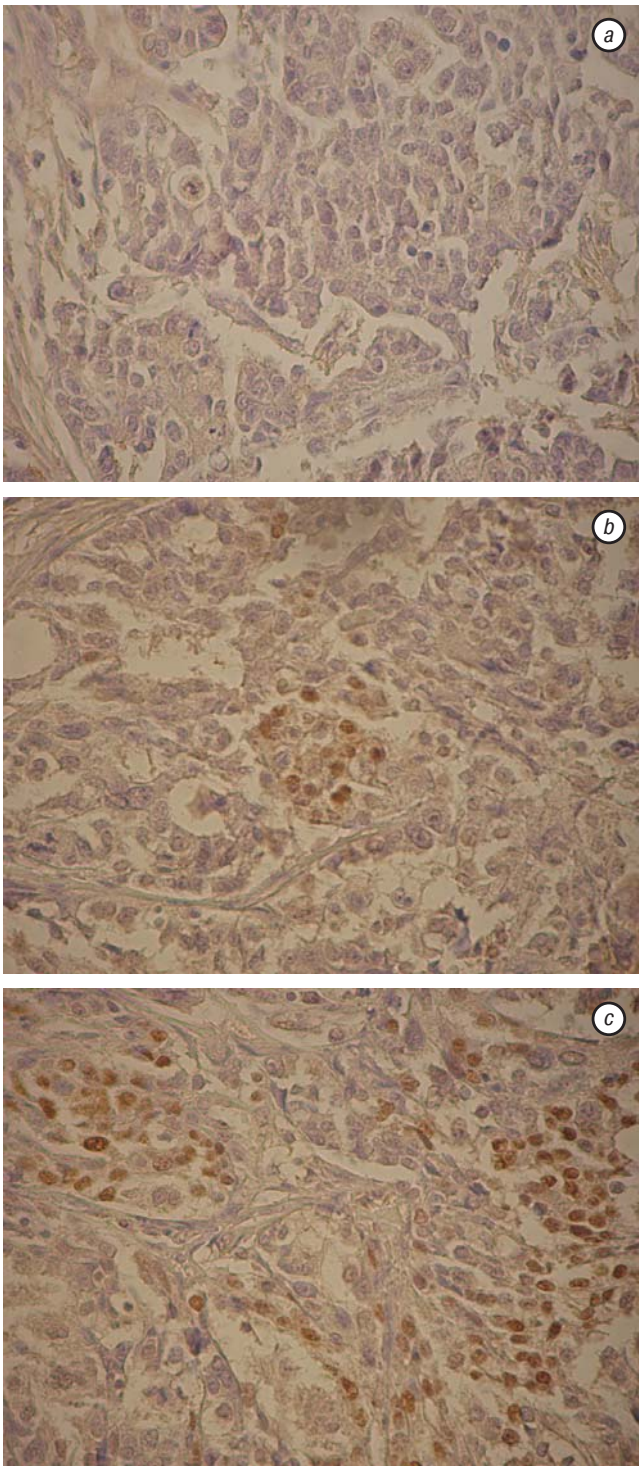


**Fig. 1.** Serous OC. Immunohistochemical expression of ER in tumor cells (× 400): *a* — no expression; *b* — low expression of ER; *c* — high expression of ER

Positive expression of the same markers has been detected in patients with mucinous OC — 88.0; 84.0; 60.0%, respectively. Positive staining of cells of stromacell tumors has been observed in 74.1% of patients for ER and 77.8% — for RP and TR. It should be mentioned that the highest percentage of receptor-positive tumors has been determined in patients with mucinous OC and SCST. Statistical analysis has demonstrated significant rank correlation between morphological structure of tumors and expression of steroid hormone receptors: for ER — r = 0.364 (p = 0.002), RP — r = 0.408 (p < 0.001) and TR — r = 0.289 (p = 0.0048) (Table 3). When comparing frequencies of expression of steroid hormone receptors in serous OC and SCST, reliable differences have been established only for TR (p = 0.016). When comparing frequency of expression of steroid hormone receptors in serous and mucinous OC, differences for ER and PR have been marked (p = 0.027 and p = 0.039, respectively), but when comparing frequencies of expression of ER, PR and TR in mucinous OC and SCST, no significant differences were found.

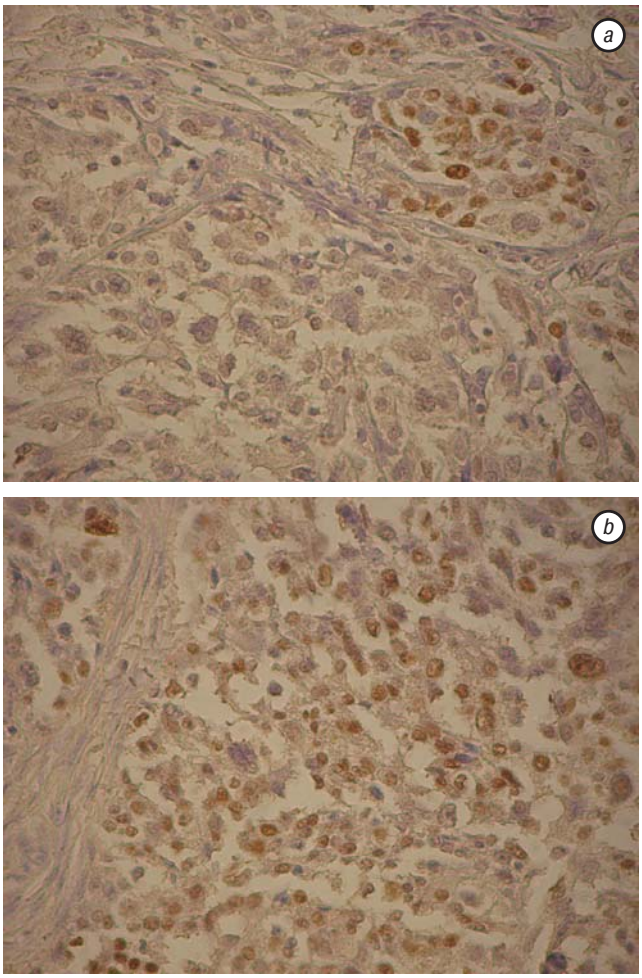


**Fig. 2.** Serous OC. Immunohistochemical expression of PR in tumor cells (× 400): *a* — low expression of PR; *b* — high expression of PR

**Table 2.** Frequency of receptor-positive MOT of different genesis depending on stage of disease (by ER, PR and TR) (n = 284)

| MOT histology/stages | Number of patients with receptor-positive tumor | | | | | |
|---|---|---|---|---|---|---|
| | ER + | | PR + | | TR + | |
| | n | % | n | % | n | % |
| Serous OC n = 232 (100.0%) | 154 | 66.4 | 147 | 63.4 | 123 | 53.0 |
| I stage, n = 23 (100.0%) | 20 | 87.0 | 17 | 73.9 | 13 | 56.5 |
| II stage, n = 13 (100.0%) | 11 | 84.6 | 11 | 84.6 | 2 | 15.4 |
| III stage, n = 185 (100.0%) | 116 | 62.7 | 113 | 61.1 | 103 | 55.7 |
| IV stage, n = 11 (100.0%) | 7 | 63.6 | 6 | 54.5 | 5 | 45.5 |
| Mucinous OC, n = 25 (100.0%) | 22 | 88.0 | 21 | 84.0 | 15 | 60.0 |
| I–II stage, n = 10 (100.0%) | 9 | 90.0 | 8 | 80.0 | 6 | 60.0 |
| III stage, n = 15 (100.0%) | 13 | 86.7 | 13 | 86.7 | 9 | 60.0 |
| SCST n = 27 (100.0%) | 20 | 74.1 | 21 | 77.8 | 21 | 77.8 |
| I–II stage, n = 17 (100.0%) | 11 | 64.7 | 11 | 64.7 | 15 | 88.2 |
| III stage, n = 10 (100.0%) | 9 | 90.0 | 10 | 100.0 | 6 | 60.0 |

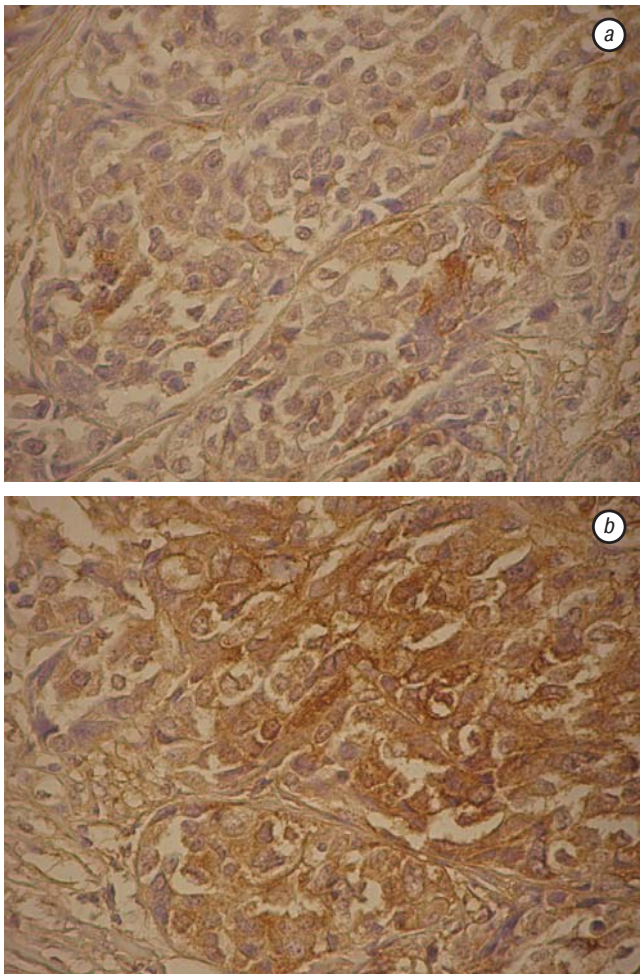


**Fig. 3.** Serous OC. Immunohistochemical expression of TR in tumor cells (× 400): *a* — low expression of TR; *b* — high expression of TR

**Table 3.** Gamma rank correlations between steroid hormone receptors, phenotype of tumor, its grade of differentiation, stage of disease, neoadjuvant chemotherapy, age period of patients with serous OC (n = 232)

| Rate | Correlation coefficient | p |
|---|---|---|
| ER & stage | −0.420 | 0.0005 |
| ER & tumor differentiation grade | −0.118 | 0.1470 |
| ER & neoadjuvant chemotherapy | −0.154 | 0.1056 |
| ER & PR | 0.913 | 0.0001 |
| ER & TR | 0.362 | 0.0001 |
| ER & age period | −0.253 | 0.0009 |
| PR & stage | −0.310 | 0.0062 |
| PR & tumor differentiation grade | −0.060 | 0.4565 |
| PR & neoadjuvant chemotherapy | −0.316 | 0.0005 |
| PR & ER | 0.913 | 0.0001 |
| PR & TR | 0.374 | 0.0001 |
| PR & age period | −0.173 | 0.0205 |
| TR & stage | 0.176 | 0.0902 |
| TR & tumor differentiation grade | 0.045 | 0.5632 |
| TR & neoadjuvant chemotherapy | 0.041 | 0.6528 |
| TR & ER | 0.362 | 0.0001 |
| TR & PR | 0.374 | 0.0001 |
| TR & age period | −0.076 | 0.2968 |
| Tumor phenotype & stage | 0.118 | 0.1531 |
| Tumor phenotype & tumor differentiation grade | 0.031 | 0.6120 |
| Tumor phenotype & neoadjuvant chemotherapy | 0.117 | 0.1063 |
| Tumor phenotype & age period | 0.180 | 0.0017 |

Weak correlation between frequency of expression of ER, PR in tumors of different genesis and stage of disease has been established: r = −0.304 (p = 0.002) and r = −0.207 (p = 0.027), respectively (see Table 2, Table 4). In patients with serous OC, at increase of disease stage, tendency to decline of expression frequency for ER and PR has been determined, while in patients with mucinous OC and SCST, tendency to elevation of expression frequency for these receptors has been observed. No connection between TR expression in tumors of different genesis and stage of tumor process has been noticed (r = −0.053, p = 0.538).

**Table 4.** Gamma rank correlations between expression of steroid hormone receptors, histological type and tumor differentiation grade, stage of disease in patients with MOT (n = 284)

| Index | Stage | Histological type of tumor | Differentiation of tumor | ER | PR | TR |
|---|---|---|---|---|---|---|
| Stage | 1.000 | −0.587* | 0.128 | −0.304* | −0.207* | −0.053 |
| Histological type of tumor | −0.587* | 1.000 | −0.069 | 0.364* | 0.408* | 0.289* |
| Differentiation of tumor | 0.128 | −0.069 | 1.000 | −0.066 | 0.020 | 0.054 |
| ER | −0.304* | 0.364* | −0.066 | 1.000 | 0.928* | 0.398* |
| PR | −0.207* | 0.408* | 0.020 | 0.928* | 1.000 | 0.354* |
| TR | −0.053 | 0.289* | 0.054 | 0.398* | 0.354* | 1.000 |

*Notes*: *correlation coefficient significant at the level of p < 0.05.

Statistical analysis has not detected significant correlation between frequency of receptor-positive MOT of different genesis (see Table 4) and grade of MOT differentiation: for ER — p = 0.382, PR — p = 0.789 and TR– p = 0.444. However, significant correlation of influence of chemotherapy on expression of PR (p = 0.001) and lack of influence on expression of ER and TR (p = 0.091 and p = 0.787, respectively) has been determined. When analyzing dependence of expression of steroid receptors between each other, rank correlation between ER and PR (p < 0.0001), ER and TR (p < 0.001), PR and TR (p < 0.001) was reported, it was especially significant between ER and PR with r = 0.928.

Thus, positive HRS was found not only in serous, but also in mucinous OC and SCST, the highest percentage of receptor-positive tumors at that was determined in patients with mucinous OC, which is characterized by unfavorable clinical course of tumor process, and in patients with SCST, which are known to have high hormonal activity [2, 31]. It was confirmed by the determined correlation between morphological structure of tumors and expression of steroid hormone receptors. Represented results have showed that ovary with malignant tumor of different histogenesis is a target tissue for systemic-local classic and non-classic steroids independently of grade of differentiation of tumor tissue.

Most numerous group in our study consisted of patients with serous OC (n = 232), therefore, the further analysis has been carried out for these category of patients. Mean period of observation of patients with serous OC was 39.5 ± 1.7 months, mean relapse-free period — 33.7 ± 1.8 months, median of relapse-free survival — 30.0 ± 2.1 months. Out of total number of patients, 28.4% were in reproductive period, 31.0% — in perimenopausal and 40.5% — in postmenopausal period (in early postmenopausal — 15.1%, in late menopausal — 25.4%). In 149 (64.2%) patients, the relapse of disease has been determined. Patients with relapse of disease depending on age period have been divided as follows: 30 (20.1%) patients of reproductive period, 55 (36.9%) — perimenopause, number of patients of postmenopausal period was

the highest — 64 (42.0%), among them — 22 (14.8%) patients in early menopause, and 42 (28.2%) — late postmenopause. It should be mentioned that the highest number of relapses in patients with OC has been observed in perimenopause and in postmenopause that indicates most aggressive course of disease and unfavorable prognosis.

When analyzing expression of steroid hormone receptors in serous OC depending on age of patients (Table 5), we have noticed that mostly estrogen-, progesterone-, testosterone-receptor-positive tumors have been observed in late postmenopause — 79.7%, 79.7 and 59.3%, respectively. Significant rank correlation (see Table 3) between frequency of expression of ER, PR and age of patients has been established ($r = -0.253$ at $p < 0.001$ and $r = -0.173$ at $p = 0.021$, respectively). Correlation coefficient for TR composed −0.076 at $p = 0.297$ (see Table 3) that is the evidence of absence of correlation of this factor with age of patients. Second place by frequency of receptor-positive tumors belongs to the patients of perimenopausal age. As it has been determined earlier, patients with serous OC in the exactly perimenopausal and postmenopausal periods have most aggressive course and unfavorable prognosis of disease [4, 5, 9]. In postmenopausal period taken as a whole, ER at serous OC has been detected in 70.2% of patients, PR — in 73.4%, and TR — in 54.3%. The lowest percentage of receptor-positive tumors has been observed in patients of reproductive age.

**Table 5.** Frequency of ER, PR and TR in patients with serous OC depending on age period (n = 232)

| Age period | Number of patients with receptor-positive tumor | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ER + | | PR + | | TR + | | Total | |
| | n | % | n | % | n | % | n | % |
| Reproductive period | 42 | 63.6 | 35 | 53.0 | 32 | 48.5 | 66 | 100.0 |
| Perimenopause | 46 | 63.9 | 43 | 59.7 | 41 | 56.9 | 72 | 100.0 |
| Postmenopause | 66 | 70.2 | 69 | 73.4 | 51 | 54.3 | 94 | 100.0 |
| Early postmenopause | 19 | 54.3 | 22 | 62.9 | 16 | 45.7 | 35 | 100.0 |
| Late postmenopause | 47 | 79.7 | 47 | 79.7 | 35 | 59.3 | 59 | 100.0 |
| Total | 154 | | 147 | | 124 | | 232 | |

As seen in Table 3, there is no rank correlation between frequency of expression of steroid hormone receptors and grade of differentiation of tumors. However, it has been determined that neoadjuvant chemotherapy influences only PR expression ($p = 0.0005$), but, at the same time, has no influence on the concentration of ER and TR ($p = 0.1056$ and $p = 0.6528$, respectively). In serous OC, the same as in all MOT, we have registered significant correlation between expression of ER and PR ($r = 0.913$ at $p < 0.0001$), ER and TR ($r = 0.362$ at $p < 0.0001$), PR and TR ($r = 0.374$ at $p < 0.0001$).

As seen in Table 6, the highest number of patients with serous OC had tumor phenotype, which was positive by all steroid hormone receptors (82/35.3%). Second place by frequency was taken by group of patients with tumor phenotype, which was positive only by ER and PR (49/21.1%). Third and fourth places belong to the patients with negative tumor phenotype by all receptors (37/15.9%) and phenotype, which was positive only by TR (25/10.8%). The fact was of interest that the highest number of patients with tumor phenotype ER+PR+TR+ has been observed in late postmenopause — 39.0%, and in postmenopausal period taken as a whole, number of patients with such tumor phenotype has been constituted 43 (52.4%). The lowest percentage of patients with mentioned phenotype has been registered in reproductive age — 20.7%. Tumor phenotype ER+PR+TR− was in equal number of patients in postmenopausal and in reproductive period by 17 (34.7%) and only in perimenopause such tumor phenotype has been detected in 15 (30.6%) patients. It should be mentioned that the highest number of patients of reproductive age had tumor phenotype ER-PR-TR− (13/35.1%), and in late postmenopause this phenotype has been observed only in 16.2% of patients. When comparing patients of reproductive and late postmenopausal periods, significant differences for the phenotype of serous OC ($p = 0.004$) have been determined, such significant differences have been detected between patients in perimenopause and late postmenopause ($p = 0.009$). When comparing tumor phenotype of patients of reproductive, perimenopausal and early postmenopausal periods, no significant differences were found ($p = 0.850$ and $p = 0.873$, respectively). Also, there are no significant differences by tumor phenotype between patients of perimenopause and early postmenopause ($p = 0.739$).

**Table 6.** Distribution of patients with serous OC by tumor phenotype and age period (n = 232)

| Tumor phenotype | Number of patients | | Age periods | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Reproductive | | Perimenopause | | Early postmenopause | | Late postmenopause | |
| | n (%) | % | n | % | n | % | n | % | n | % |
| ER+PR+TR+ | 82 (35.3) | 100.0 | 17 | 20.7 | 22 | 26.9 | 11 | 13.4 | 32 | 39.0 |
| ER+PR+TR− | 49 (21.1) | 100.0 | 17 | 34.7 | 15 | 30.6 | 6 | 12.2 | 11 | 22.5 |
| ER+PR−TR+ | 10 (4.3) | 100.0 | 5 | 50.0 | 4 | 40.0 | 0 | 0.0 | 1 | 10.0 |
| ER+PR−TR− | 13 (5.6) | 100.0 | 3 | 23.1 | 5 | 38.5 | 2 | 15.4 | 3 | 23.1 |
| ER−PR+TR+ | 7 (3.0) | 100.0 | 0 | 0.0 | 5 | 71.4 | 2 | 28.6 | 0 | 0.0 |
| ER−PR+TR− | 9 (3.9) | 100.0 | 1 | 11.1 | 1 | 11.1 | 3 | 33.3 | 4 | 44.4 |
| ER−PR−TR+ | 25 (10.8) | 100.0 | 10 | 40.0 | 10 | 40.0 | 3 | 12.0 | 2 | 8.0 |
| ER−PR−TR− | 37 (15.9) | 100.0 | 13 | 35.1 | 10 | 27.0 | 8 | 21.6 | 6 | 16.2 |
| Total | 232 (100.0) | | n = 66 | | n = 72 | | n = 35 | | n = 59 | |

We have carried out univariate analysis of overall and relapse-free survivals of patients with serous OC depending on expression of each steroid hormone receptor (Fig. 4–9). Rates of overall and relapse-free survivals of patients are represented in Table 7. Data in Table 7 demonstrate that 3-year survival rate in patients with serous receptor-negative tumor to estrogens and progesterone was higher, than in patients with receptor-positive tumor, but differences between them were insignificant ($p = 0.594$ and $p = 0.452$, respectively), since 5-year survival rate in two groups of patients turned out to be the same and equaled 39.3%. Results of 3- and 5-year survivals in patients with receptor-negative tumor to testosterone (see Fig. 6, Table 7) were interesting. They turned out to be significantly higher, than in patients with receptor-positive tumors ($p = 0.0345$). It should be mentioned that median of relapse-free survival in patients with receptor-negative tumor concerning all steroid hormones was higher, than in patients with receptor-positive tumors, but significant rate has been determined only for TR ($p < 0.0500$).

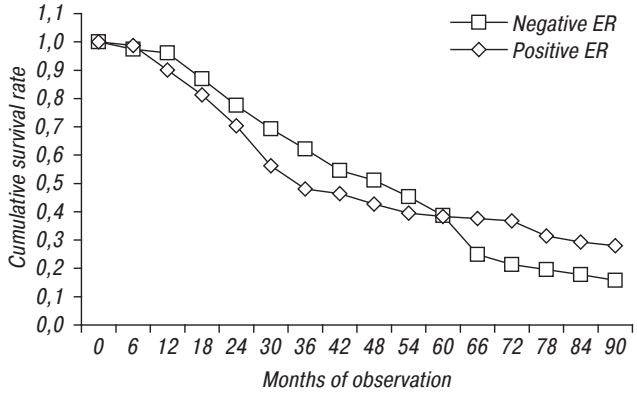


**Fig. 4.** Overall survival of patients with serous OC with expression of estrogen receptors Kaplan— Meier survival curves, *log-rank* criterion; p = 0.594

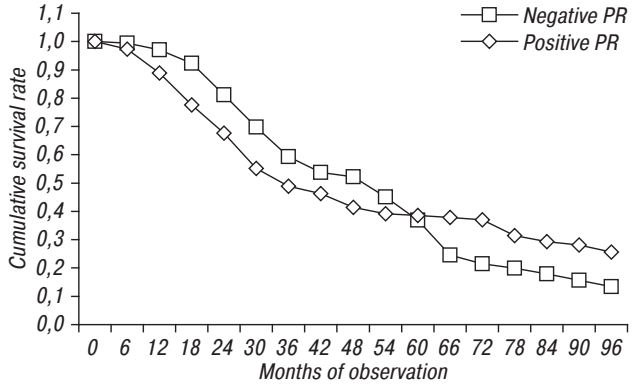


**Fig. 5.** Overall survival of patients with serous OC with expression of progesterone receptor. Kaplan — Meier survival curves, *log-rank* criterion; p = 0.452

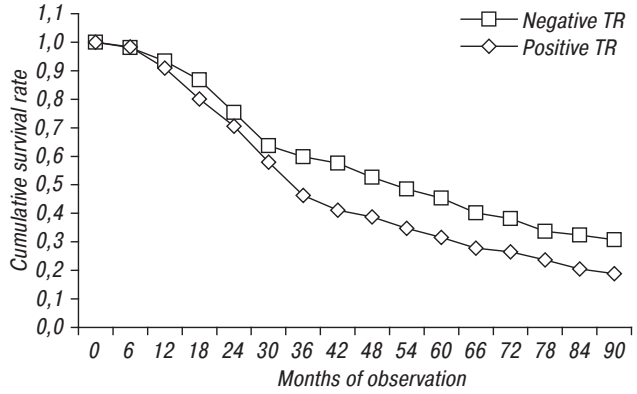


**Fig. 6.** Overall survival of patients with serous OC with expression of testosterone receptor. Kaplan — Meier survival curves, *log-rank* criterion; p = 0.0345

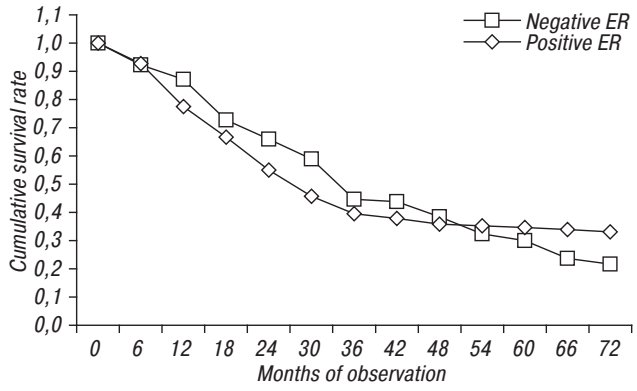


**Fig. 7.** Relapse-free survival of patients with serous OC with expression of estrogens receptor. Kaplan — Meier survival curves, *log-rank* criterion; p = 0.6876

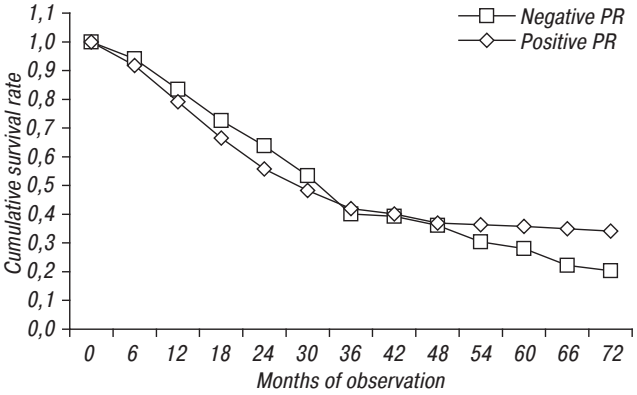


**Fig. 8.** Relapse-free survival of patients with serous OC with expression of progesterone receptor. Kaplan — Meier survival curves, *log-rank* criterion; p = 0,9534

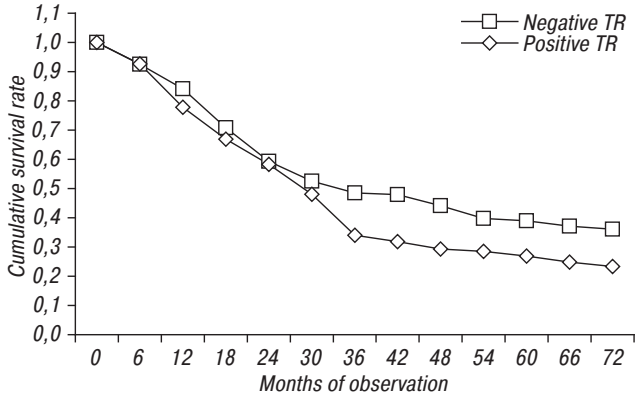


**Fig. 9.** Relapse-free survival of patients with serous OC with expression of testosterone receptor. Kaplan — Meier survival curves, *log-rank* criterion; p < 0.0500

**Table 7.** Overall and relapse-free survival in patients with serous OC depending on expression of steroid hormone receptors

| Steroid hormone receptors | Overall survival, % | | | | Median of relapse-free survival, months | |
|---|---|---|---|---|---|---|
| | Serous OC R+ | | Serous OC R– | | Serous | Serous |
| | 3-year | 5-year | 3-year | 5-year | OC R+ | OC R– |
| ER | 48.2±4.2 | 39.3±4.4 | 63.4±5.7 | 39.3±6.4 | 26.9±2.3 | 34.8±2.7 |
| PR | 49.7±4.3 | 39.3±4.4 | 60.0±5.5 | 39.3±6.1 | 27.8±2.4 | 32.0±2.5 |
| TR | 45.7±4.7 | 31.4±4.8 | 62.2±4.8 | 47.9±5.3 | 27.7±2.2 | 35.7±2.8 |

*Notes*: Serous OC R+ – receptor-positive serous OC; Serous OC R– – receptor-negative serous OC

Hereafter, differences in 3- and 5-year overall survival of patients with serous OC depending on tumor phenotype have been analyzed (Fig. 10). Statistically significant intergroup differences in survival of patients with the following phenotypes have been determined: ER+PR+TR+ and ER–PR–TR– (p = 0.006), between ER+PR+TR+ and ER+PR+TR– (p = 0.0150), between ER+PR+TR+ and ER–PR–TR+ (p < 0.050). For this reason, we have evaluated survival of patients, who had above-listed phenotypes of serous OC only. Patients with tumor phenotype, which was negative by all steroid hormone receptors, had higher 3- and 5-year survival rates, than patients, who had positive HRS of tumor: 67.8/44.5% and 39.9/29.5%, respectively. In patients with tumor phenotype ER+PR+TR–, rates of 3- and 5-year survival have constituted 60.5/49.2% compared to 39.9/29.5% — rates of overall survival of patients with positive HRS OC. However, in group of patients with phenotype tumor, which was positive only by TR, high survival rates have also been recorded (60.5/49.5%) compared to the same in OC patients,

which was a positive tumor by all steroid hormone receptors (39.9/29.5%). Thus, the lowest survival rates have been observed in patients, who had positive HRS tumors.

When evaluating relapse-free survival of patients depending on serous OC phenotype (Fig. 11), significant intergroup differences only for tumors with phenotypes ER+PR+TR+ and ER+PR+TR– have been obtained ($p < 0.050$). The highest rate of relapse-free survival has been determined in patients with ER+PR+TR– OC phenotype (50.8/43.3%) if compared with survival of the patients, who had positive HRS (33.5/25.5%), the absence of TR expression play the key role.

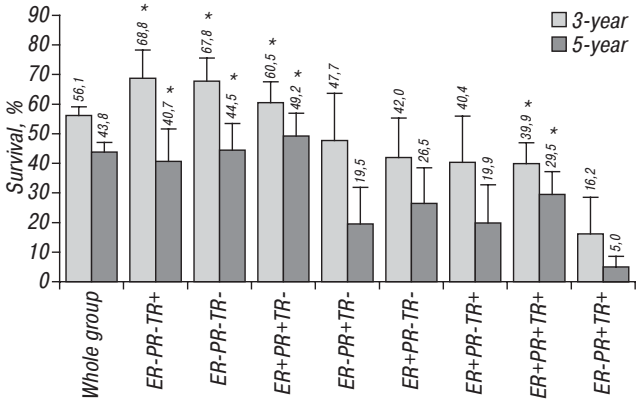


**Fig. 10.** Overall survival of patients with serous OC depending on tumor phenotype. *$p < 0.05$ if 3-year and 5-year groups are compared

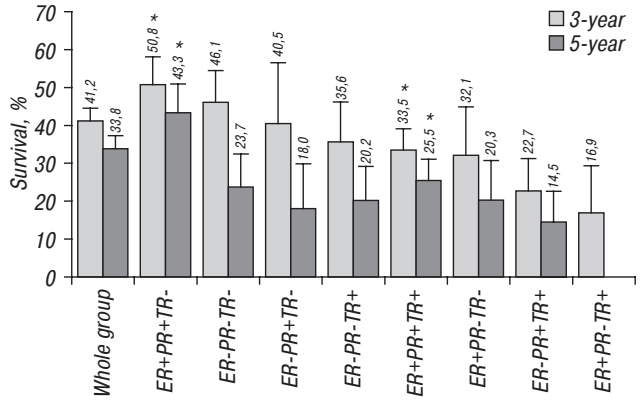


**Fig. 11.** Relapse-free survival of patients with serous OC depending on tumor phenotype. *$p < 0.05$ if 3-year and 5-year groups are compared

Represented results have demonstrated significant correlation between ER, PR, and TR in MOT that corresponds to data of some studies carried out before [31]. It is known that steroid hormones are able to regulate the level of not only own receptors, but also to modulate content of receptors of the other hormones. For instance, estradiol stimulates the formation of PR, while progesterone decreases expression of ER in reproductive organs. Moreover, estradiol is able to increase level of ovarian androgenic receptors several times [32].

The highest frequency of receptor-positive tumors has been observed in patients with serous OC of postmenopausal period, which, by own observations and data of literature, is characterized by aggressive course and unfavorable prognosis of disease [2]. Such similarity by clinical course and tumor HRS is being detected also in patients with BC of postmenopausal period, but, at luminal A type of tumor in BC patients, the favorable prognosis of disease is being predicted [33]. Thus, the highest number of patients with serous OC with positive HRS has been observed in late postmenopausal period. It is the evidence of possible high sensitivity of tumor cells of patients of this age category to the impact of steroid hormones, most of which are formed due to extragonadal aromatization of androgens [6].

Univariate analysis has showed that overall and relapse-free survivals are higher in patients with receptor-negative tumor by ER, PR and TR, than with receptor-positive tumors, but significant differences have been registered only for TR.

Represented results have demonstrated low overall and relapse-free survivals in patients with OC phenotype, which was receptor-positive for all steroid hormones. The highest rates of overall 5-year survival have been determined in patients with not only tumor phenotype ER–PR–TR–, but also with ER+PR+TR–. Relapse-free survival also was the highest in patients with serous OC phenotype ER+PR+TR– that indicates certain role of TR in clinical course of tumor process.

Obtained results, on the one hand, corresponds to the data of some studies, which have demonstrated that strong expression of androgen receptors is being observed in case of invasive OC, and hyperandrogenia can be a risk factor of occurrence and progression of this disease [34]. On the other hand, results of our study contradict the data of other studies, which have determined that high expression of androgen receptors is a favorable prognostic factor in patients with serous OC [35].

Today it is known that androgens, when interacting with receptors, cause strong proliferative processes in ovarian tissue (hyperplasia of stroma, thecal tissue and cells of hilum of ovary) at endocrine syndromes of reproductive system, in particular, polycystic ovary syndrome. Moreover, level of androgens and expression of receptors at these syndromes are able to increase synchronously with a lapse of time [36]. Postmenopausal ovary is androgenic ovary, which stroma is the only source of gonadal hormone reproduction in this age period. It has been proved that concentration of testosterone in ovarian vein is 15 times higher, than level of the last one in blood serum from peripheral vein [37]. As it has been stated above, peripheral synthesis of non-classic estrogens (phenol steroids) becomes more intensive in postmenopause. These estrogens possess weaker inhibiting effect on hypothalamus-hypophysis system, than classic estrogens [6], and increase of gonadotropins production stipulates hyperplasia of ovarian thecal tissue that, in turn, can intensify synthesis of androgens [30, 36]. Non-classic phenol steroids and estrone are main hormones in postmenopause. They are formed as the result of aromatization from androstenedione, secretion of which in menopause is provided mostly by adrenal glands and less — by ovaries [30, 36]. Phenol steroids are strong agonists of estradiol, activity of these

metabolites increases activity of the latter in several times (in particular, 16-α-hydroxyestrone 1 in 8 times), they form stable connections with specific receptors of cells of different tissues [38, 39].

It has been proved that transformation of androstenedione in estrone in percentage correlates with weight of women. Entering of estrogens through aromatization of androgens is not limited only by fat tissue — almost all tissues of organism have this property. ER (α and β) are found in brain, blood vessels, heart, bones, mammary glands, uterus, ovaries. ER-β were detected only in lungs, kidneys, urinary bladder, intestine. Extragonadal estrogen- and androgen-formation serves as source of additional hormonal stimulation [6, 36, 38, 39].

Among age endocrine-metabolic changes in women, which are manifested by obesity and insulin-resistance, compensatory hyperinsulinemia arises [7]. Insulin indirectly, via receptors of insulin-like growth factor I, the same as luteinizing hormone, intensifies enzyme activity of biosynthesis of androgens in ovaries that causes certain increase of their functions [36]. It has been proved for today that androgen-regulating genes stimulate expression of epidermal growth factor, vascular endothelial growth factor and cyclin-dependent kinases 2 and 4. At the same time, they have repressing effect on the expression of TGF-β and Bcl-2 [32].

In postmenopause, along with switching off of ovulatory function of ovaries, takes place strong switching on of series of compensatory mechanisms providing sufficient level and wide range of biologically active metabolites, and at certain factors there are all necessary conditions for stimulation of target organs by them [6].

Thus, obtained results have demonstrated that endocrine factors and hormonal-receptor changes in ovaries is a considerable element of not only pathogenesis, but also important prognostic criterion in postmenopausal patients with MOT. It represents integral part of general mechanism of complicated molecular-genetic interactions.

In conclusion, positive HRS has been determined in serous, mucinous OC and in SCST. High percentage of tumors with the expression of all steroid hormone receptors has been observed in them. However, the highest percentage of receptor-positive tumors has been detected in patients with mucinous OC and SCST that is confirmed by established correlation between morphological structure of tumors and expression of steroid hormone receptors.

Weak correlation between frequency of expression of ER, PR and lack of such expression between expressions of TR in tumors of different genesis and stage of disease has been established. Expression of steroid hormone receptors does not depend on grade of differentiation of tumor tissue.

In patients with serous OC of late menopausal period, the highest frequency of tumors with positive HRS is registered that allows to assume that there is a high sensitivity of these tumors both to endogenous steroids and hormonal therapy.

In patients with serous OC with receptor-positive tumor phenotype, rates of overall and relapse-free survivals are significantly lower compared to the patients with receptor-negative OC phenotype.

Positive HRS, the same as strong expression of TR in patients with serous OC, is a predictive factor of unfavorable course of tumor process.

HRS of MOT can be regarded as the additional criterion for solution of a question concerning application of hormonal therapy as a component of complex treatment for the patients that needs further large multi-center studies in this direction.

## REFERENCES

**1.** Heintz APM, Odicino F, Maisonneuve P, ***et al***. Carcinoma of the ovary. FIGO 6th Annual Report on the Results of Treatment in Gynecological Cancer. Int J Gynaecol Obstet 2006; **95**: 161–9.

**2.** Disaia PJ, Creasman WT. Clinical Oncologic Gynecology (3) (transl from Eng edit by EG Novikova) M.: Rid Elsiver, 2012: 346 (in Russian).

**3.** Vorobyova LI. Practical oncogynecology. K., 2012: 228 (in Ukrainian).

**4.** Urmancheyeva AF, Kutusheva GF, Ulrich EA. Ovarian tumors (clinics, diagnostics and treatment). St. Petersburg: N-L, 2012: 68 (in Russian).

**5.** Svintsitsky VS. Ovarian Cancer: dynamics of certain endocrinological parameters under the complex therapy effect. Clinical Endocrinology and Endocrine Surgery, 2004; **3**: 25–30 (in Ukrainian).

**6.** Vorobyova LI, Svintsitsky VS, Tkalia IG. Hormonal carcinogenesis and substantiation of application of hormonal therapy in treatment of ovarian cancer. Clin Oncol 2013; **1**: 56–64 (in Ukrainian).

**7.** Landen JCN, Birrer MJ, Sood AK. Early stages of the pathogenesis of ovarian cancer. J Clin Oncol 2008; **26**: 149–60.

**8.** Vorobyova LI, Tkalia IG. Clinical significance of concomitant hyperplastic processes of endometrium in patients with malignant ovarian tumors. Onkologiya 2013; **4**: 286–93 (in Ukrainian).

**9.** Bondar GV, Lisovska NYu, Kajriak OV, ***et al***. Chemohormonotherapy in the combined treatment of advanced ovarian cancers. Probl Modern Medic Sci Educat 2009; **2**: 33–35 (in Ukrainian).

**10.** Gorbunova VA. Diagnostics and treatment of ovarian cancer. M: MIA, 2011: 248 (in Russian).

**11.** Bookman MA, Brady MF, McGuire WP, ***et al***. Evaluation of new platinum-based treatment regimens in advanced-stage ovarian cancer: a phase III trial of the Gynecologic Cancer InterGroup. J Clin Oncol 2009; **27**: 1419–25.

**12.** Bristow RE, Puri I, Chi DS. Cytoreductive surgery for recurrent ovarian cancer: a meta-analysis. Gynecol Oncol 2009; **112**: 265–74.

**13.** Perevodchikova NI. Guidelines for chemotherapy of tumors. M.: Pract Med, 2011: 512 (in Russian).

**14.** Maksimov SY, Huseynov KD. Targeted therapy in ovarian cancer. Pract Oncol 2010; **11**: 54–64 (in Russian).

**15.** Svintsitsky VS, Vorobyova LI. Malignant ovarian tumors: optimization of complex treatment. Onkologiya 2010; **12**: 52–6 (in Ukrainian).

16. Cancer in Ukraine, 2010–2011, Morbidity, mortality, oncology service statistic data. Bull Ukrain Nat Cancer Reg 2013; (14): 52–3 (in Ukrainian).

**17.** Urmancheyeva AF, Tyulyandin SA, Moiseyenko VM. Practical oncogynecology: Selected lectures. St. Petersburg: Center TOMM, 2008: 400 (in Russian).

**18.** Schepotin IB, Zotov AS, Anikusko NF, ***et al.*** Neoadjuvant hormonal therapy of locally disseminated breast cancer. Clin Oncol 2011; **3**: 36–9 (in Ukrainian).

**19.** Burges A, Brüning A, Dannenmann C, ***et al.*** Prognostic significance of estrogen receptor alpha and beta expression in human serous carcinomas of the ovary. Arch Gynecol Obstet 2010; **281**: 511–7.

**20.** Aust S, Horak P, Pils D, ***et al.*** The prognostic value of estrogen receptor beta and proline-, glutamic acid- and leucine-rich protein 1 (PELP1) expression in ovarian cancer. BMC Cancer 2013; **13**: 115.

**21.** Halon A, Materna V, Drag-Zalesinska M, ***et al.*** Estrogen receptor alpha expression in ovarian cancer predicts longer overall survival. Pathol Oncol Res 2011; **17**: 511–8.

**22.** Alonso L, Gallego E, Jesús González F, ***et al.*** Gonadotropin and steroid receptors as prognostic factors in advanced ovarian cancer: aretrospective study. Clin Transl Oncol 2009; **11**: 748–52.

**23.** Ayadia L, Chaabounia S, Khabira A, ***et al.*** Correlation between immunohistochemical biomarkers expression and prognosis of ovarian carcinomas in Tunisian patients. World J Oncol 2010; **1**: 118–28.

**24.** Chakraborty A, Chatterjee S, Roy P. Progesterone receptor agonists and antagonists as anticancer agents. Mini Rev Med Chem 2010; **10**: 506–17.

**25.** García-Velasco A, Mendiola C, Sánchez-Muñoz A, ***et al.*** (2008) Prognostic value of hormonal receptors, p53, ki67 and HER2/neuexpression in epithelial ovarian carcinoma. Clin Transl Oncol 2008; **10**: 367–71.

**26.** Nourieh Sharifi, Zohreh Yousefi, Shohreh Saeed, ***et al***. Prognostic Values of Estrogen and Progesterone Expression Receptors in Ovarian Papillary Serous Carcinoma. Ir J Pathology 2009; **4**: 9–12.

**27.** Dudnichenko AS, Yakimova TP, Kartashov SM. Receptor status of ovary cells depending on the morphological features and chemotherapeutic effects. Onkologiya 2001; **3**: 271–4 (in Ukrainian).

**28.** Puzin SN, Payanidi YG, Ogay DS, ***et al.*** Medical expertise in oncogynecology. Oncogynecology 2012; **2**: 60–7 (in Russian).

**29.** Lenhard M, Tereza L, Heublein S, ***et al***. Steroid hormone receptor expression in ovarian cancer: progesterone receptor B as prognostic marker for patient survival. BMC Cancer 2012; **12**: 553.

**30.** Manukhin IB, Tumilovich LG, Gevorkyan MA. Clinical lectures on gynecological endocrinology. Moskow: MIA, 2003: 247 (in Russian).

**31.** Bokhman YV. Guidelines in Oncogynecology. St. Petersburg: Foliant, 2002: 542 (in Russian).

**32.** Korman DB. Endocrine therapy of malignant tumors. Moskow: Practical Medicine, 2010: 400 p. (in Russian).

**33.** Schepotin IB, Zotov AS, Lyubota RV, ***et al.*** Molecular subtypes of breast cancer determined on the basis of immunohistochemical markers: clinical and biological features and prognosis of course. Klin Onkol 2012; **8**: 1–4 (in Ukrainian).

**34.** Wang PH, Chang C. Androgens and ovarian cancers. Eur J Gynaec Oncol 2004; **25**: 157–63.

**35.** Nodin B, Zendehrokh N, Brandstedt J, ***et al***. Increased androgen receptor expression in serous carcinoma of the ovary is associated with an improved survival. J Ovarian Res 2010; **3**: 14.

**36.** Vikhlyayeva EM. Guide to the endocrine gynecology. Moskow: MIA, 2002: 768 (in Russian).

**37.** Risch HA. Hormonal etiology of epithelial ovarian cancer, with a hypothesis concerning the role of androgens and progesterone (review). J Natl Cancer Inst 1998; **90**: 1774–86.

**38.** Dilman VM. Four models of medicine. L.: Medicine, 1987: 288 (in Russian).

**39.** Berstein LM. Extragonadal production of estrogens. St. Petersburg: Nauka, 1998; 13–9 (in Russian).