

# Pathogenetic treatment strategies in nulliparous women with ovarian endometriomas

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The modern approach to the treatment options and assessment of its effectiveness in nulliparous women with endometrial cysts is build on optimal pathogenetically substantiated therapy based on features of the disease not only on the system, organ and tissue levels, and taking to consideration the cellular and molecular features of the functioning of the body. *The objective:* to determine the effectiveness of various treatment regimens for nulliparous women with ovary tumor-like formations based on features of the immune status.

*Materials and methods.* 80 women aged 17 to 40 years were examined: the first group consisted of 50 healthy nonpregnant women, the second group included 30 women, with endometriomas receiving the traditional (subgroup 2a) and complex (subgroup 2b) treatment.

*Results.* In the study of immune status in nulliparous women with endometrioid tumor-like formations of ovaries (n=30) was the inconsistency of the phagocytic system, the imbalance of T- and B-cell immunity, dysimmunoglobulinemy. Based on the identification of changes, the patients were assigned with pathogenetically substantiated combination therapy that included, in addition to conventional treatment regimens, the appointment of immunomodulators and systemic enzyme therapy.

*Conclusions.* Treatment results show the full normalization of phagocytic, T- and B-cells and partially humoral systems.

*Key words:* immunomodulating therapy, nulliparous women, tumor-like endometrioid ovarian formation.

Nowadays an increase of incidence of hyperplastic syndrome of female genital organs is observed. [1]. Despite of numerous scientific reports about endometriosis and its treatment the frequency of this pathology increased significantly [2].

A surgical removal of heterotopic lesions in the ovaries is considered to be the most common treatment regimen of external genital endometriosis. But such strategy is not always possible to recover the specific functions of the female body [3, 4]. The thing is that surgical treatment of ovarian endometriomas may negatively effect ovarian reserve [5–9] and cause relapse process [10].

Most clinicians prefer the combined therapy: the first stage is the surgical removal of foci of heterotopic endometrium, and the second step is the hormonal therapy aimed the suppression of the menstrual function [11, 12, 13]. However, these recent studies indicate that hormone replacement therapy is highly effective against pain, but not for women of the reproductive function.

*The objective:* was to determine the effectiveness of various treatment regimens for nulliparous women with ovary tumor-like formations considering features of the immune status.

## MATERIALS AND METHODS

We examined 80 women aged 17–40 years.

The first group (control group) consisted of 50 healthy non-pregnant women of reproductive age, who at the time of the examination had no signs of gynecological and chronic somatic pathology, administered to the facility for the choosing of contraception.

The second group (comparison group) formed of 30 women with established ovary endometriosis stage I–II, who did not give birth for various reasons (social, religious, unsettled personal life, etc.). Surgical and conservative treatment in this group have not been conducted.

Group 2 women were divided into two subgroups based on their ongoing therapy. Group 2A – consisted of 15 women who did not give birth to a variety of reasons, with a confirmed diagnosis of endometrioid ovary cyst treated with conventional hormone therapy with Dienogest. Group 2B – consisted of 15 women who did not give birth to a variety of reasons, with a confirmed diagnosis of endometrioid ovary cyst treated with comprehensive treatment. Comprehensive treatment consisted of supplemental drugs with immunomodulating and metabolic action: Likopid and Vobenzim.

All patients were conducted physical examination, pelvic examination, transvaginal ultrasound of the pelvic organs and the immune status of the study.

For nonspecific immunological reactivity studies were conducted determination of phagocytic activity of blood monocytes and neutrophils, based on the method of determining the absorbent capacity and digesting them with respect to the microbial culture test after preincubation joint (according to N. Frimel) [14].

Determination of oxygen-dependent metabolism of neutrophils (NBT-test) and functional reserve of cells (stimulated NBT-test) was carried out by M.E. Viksman and A.N. Mayansky [15].

Determination of the activity of myeloperoxidase (MP) of neutrophils was performed by the modified method cytochemically by R.P. Nartsissova [16].

Determination of cationic proteins (CP) in neutrophils was performed by a bromophenol blue by M.G. Shubych [17].

Determination of subpopulations of lymphocytes was carried out by using monoclonal antibodies against antigens CD3<sup>+</sup> (total number of T-lymphocytes), CD4<sup>+</sup> (T-helper), CD8<sup>+</sup> (T-suppressors), CD16<sup>+</sup> (NK-cells), CD19<sup>+</sup> (B cells) produced by NPO «Granum» (Kharkiv).

Determination of humoral immunity IgA, IgM, IgG was performed by using monospecific serums against these immunoglobulins by the method of Manchini G. [18]

Statistical data processing performed using computer software package STATISTICA (StatSoftStatistica v.6.0).

## RESULTS OF THE STUDY AND THEIR DISCUSSION

Study conducted by a number of authors point to the direct involvement of immune mechanisms in the pathogenesis of the ovaries, which coincides with our results [19, 20]. Thus, in the study of the immune status in nulliparous women with ovarian tumor-like formations, we have identified changes in the immune status, which were characterized by incomplete phagocytosis due to lower functional-metabolic reserve, reduced microbicidal capacity imbalance of cellular and humoral immunity (reduction in the number of T-lymphocytes and a sharp increase in the number of B-lymphocytes) disimmunoglobulinemy. In this regard, many authors point to the need for the complex treatment of immunomodulators, however, these drugs for various reasons are not always used, preferring the more traditional methods of treatment. One common cause of this approach is to apply the previously inadequate immunotherapy, leads to deeper disturbances.

Based on the above, we carried out a comparative analysis of the immune status of women treated with traditional and complex treatment, the main component of which is an immunocorrective therapy.

**Condition of functional-metabolic status of neutrophils in nulliparous women with endometriosis, depending on (considering) the applied treatment, Me (75%Q – 25%Q = RQ)**

Indicator, values	Group 1, n=50	Group 2, n=30	Group 2A, n=15	Group 2B, n=15
FIN on 30 min, %	67,5 (74,2–49,1=25,1)	60,1 (82,6–37,9=44,7)	62,7* (71,0–42,4=28,6)	64,5 (83,2–47,3=35,9)
FNN on 30 min, U	3,1 (6,5–1,3=5,2)	3,9* (5,8–1,4=4,4)	3,5 (4,9–1,8=3,1)	3,2 (4,7–1,6=3,1)
FIN on 120 min, %	58,4 (68,3–39,8=28,5)	50,4 (75,1–30,8=44,3)	52,7 (62,1–29,8=32,3)	56,3** (67,2–31,6=35,6)
FNN on 120 min, U	2,7 (4,8–1,9=2,9)	6,2*** (7,2–0,9=6,3)	5,0*** (5,9–2,1=3,8)	3,7** (5,1–2,4=2,7)
NBTsp, y. e.	1,2 (1,3–1,0=0,3)	1,2 (1,5–0,9=0,6)	1,2 (1,7–0,6=1,1)	1,2 (2,2–0,5=1,7)
NBTst, U	1,3 (2,2–0,8=1,4)	1,0 (1,9–0,5=1,4)	1,1 (1,8–0,4=1,4)	1,3 (2,1–0,6=1,5)
CP, U	2,2 (3,0–1,4=1,6)	2,0 (2,8–0,7=2,1)	2,0 (2,6–0,6=2,0)	2,1 (2,9–0,7=2,2)
MP, U	2,3 (3,4–0,7=2,7)	1,6* (2,0–0,3=0,7)	1,7* (2,3–1,0=1,3)	2,1 (2,8–0,6=2,2)

Notes: \* – statistically significant differences (p<0,05) relative to the control group; \*\* – Statistically significant differences (p<0,05) relative to Group 2.

In assessing of functional parameters characterizing metabolic status of neutrophils in group 2A – showed an increase in the functional activity (the fagocyte neutrophile index – FIN) comparing with the control group up to 12% (30') and 85% (120'), whereas with respect to the comparison group – it was reduced by 10% (30') and 19% (120) accordingly.

Indicators characterizing absorptive (30') and digestive (120') ability – the fagocyte neutrophile number (FNN) were reduced relative to the control group at 7% and 10%, and increased by 4% and 4% relative to the comparison group (which is statistically insufficient, but clinically significant). Indicators of NBTsp-test, characterize functional and metabolic reserve of neutrophils, did not change, whereas indicators of NBTst-tests were reduced by 15% relative to the values of the control group and increased by 24% in relation to the values of the comparison group.

Indicators of microbicidal activity: cationic proteins (CP) and myeloperoxidase (MP) were reduced with respect to the values of the control group by 9% and 26%, CP indicators matched the values in comparison group, MP – were increased by 6%, which is not statistically significant, but clinically significant.

When assessing cellular immunity in Group 2A women was shown a reduction of T-cell level: CD3<sup>+</sup>, CD4<sup>+</sup>, CD8<sup>+</sup>, and NK-cells – CD16<sup>+</sup> – 5%, 11%, 8%, 9%, relative values of the control group, and increased by 8%, 6%, 12% and 4% – relative to the

values of the comparison group, in both cases, that is not statistically significant but clinically significant.

Assessing humoral immunity in Group 2A women shown an increase in the number of B-lymphocytes – CD19<sup>+</sup> 16% relative to a control group and a 6% decrease relative to the comparison group.

The concentration of IgA was reduced by 11%, while the IgM, IgG – corresponded to the values of the control group. There was an increase of IgA concentration by 33%; reducing the concentration of IgM by 15%, while the concentration of IgG corresponded to the value of the comparison group.

Thus, the presence of immune disorders, remaining after conventional therapy by Dienogest, proved the necessity of using immunomodulators, which is pathogenetically substantiated in this case.

Considering the features of the immune status of the study group of women, we found it necessary to extend the standard treatment, the purpose of immunoregulatory drugs, the main point of application which would serve as phagocytes, and then their action switched to the T-cell system.

In this case, the drug of choice was Licopid – a synthetic drug, which is the main structural component of the cell wall of almost all known bacterias, which met all the requirements set by us. The drug was administered at a dose of 10 mg 2 times a day sublingual for 21 days.

Table 2

**Indicators of T-cell immunity in nulliparous women with endometriosis, depending on (considering) the applied treatment, Me (75%Q – 25%Q = RQ)**

Indicator, values	Group 1, n=50	Group 2, n=30	Group 2A, n=15	Group 2B, n=15
CD3 <sup>+</sup> , %	63,7 (67,1–59,8=7,3)	56,2 (60,0–49,3=10,7)	60,7 (71,4–56,2=15,2)	65,3* (72,7–57,1=15,6)
CD4 <sup>+</sup> , %	38,7 (44,2–31,8=12,4)	32,4 (42,8–23,6=19,2)	34,4 (45,6–19,9=25,7)	37,2 (48,0–23,4=24,6)
CD8 <sup>+</sup> , %	25,6 (39,0–21,3=17,7)	20,9 (39,3–17,5=21,8)	23,5* (38,8–15,7=23,1)	24,9 (39,6–19,2=20,4)
CD16 <sup>+</sup> , %	16,5 (24,6–11,9=12,7)	14,3* (21,5–9,4=12,1)	15,0** (20,4–10,1=10,3)	16,0 (23,1–12,8=10,3)

Notes: \* – statistically significant differences (p<0,05) relative to the control group; \*\* – Statistically significant differences (p<0,05) relative to Group 2.

Indicators of humoral immune system in nulliparous women with endometriosis, depending on the applied treatment, Me (75%Q – 25%Q = RQ)

Indicator, values	Group 1, n=50	Group 2, n=30	Group 2A, n=15	Group 2B, n=15
CD19 <sup>+</sup> , %	17,2 (23,1–15,6=7,5)	21,3 <sup>*</sup> (32,9–17,1=15,8)	20,0 <sup>*</sup> (30,1–16,8=13,3)	18,1 <sup>**</sup> (26,4–14,7=11,7)
IgA, g/l	1,8 (2,3–0,9=1,4)	1,2 (1,5–0,6=0,9)	1,6 <sup>**</sup> (2,3–0,9=1,4)	1,7 (2,5–1,0=1,5)
IgM, g/l	1,1 (2,5–0,4=2,1)	1,3 (2,2–0,8 = 1,4)	1,1 (2,0–0,5=1,5)	1,0 (2,1–0,4=1,7)
IgG, g/l	10,2 (13,1–6,7=6,4)	10,7 (14,0–5,9=8,1)	10,5 (13,4–6,8=6,6)	10,3 <sup>**</sup> (13,5–6,1=7,4)

Notes: \* – statistically significant differences (p<0,05) relative to the control group; \*\* – Statistically significant differences (p<0,05) relative to Group 2.

Appointment of Vobenzym (a combination of high-protease) enhances the effectiveness of therapies and reduces the risk of adverse effects associated with hormonal drugs. The drug was administered 5 tablets three times a day orally for two months.

In assessing the indicators characterizing the functional and metabolic status of neutrophils in Group 2B, – changes in the functional activity of neutrophils at 30' have been identified, while the 120' was an increase by 37% on the value of the control group. With respect to the comparison group scores were reduced by 18% and 40%, respectively.

Showed a decrease absorptive (30') and digestive (120') ability of neutrophils relatively to the control group by 7% and 5% (which was not statistically significant, but clinically significant) and an increase by 7% and 12% relative to the comparison group.

The indicators characterizing the functional-metabolic reserve of neutrophils – NBTsp-test – to respect the values of the control group and the comparison group, the indicators NSTst test – corresponded the values of the control group, but higher than the value of the comparison group at 30%, respectively.

Indicators of microbicidal system – CP and MR – were reduced relative to the control group by 4% and 8% (which was not statistically significant, but clinically significant) and increased relatively to the comparison group by 5% and 31%, respectively.

Number of T-lymphocytes with a phenotype: CD3<sup>+</sup>, CD4<sup>+</sup>, CD8<sup>+</sup>, and NK-cells – CD16<sup>+</sup> values substantially matched control, but values higher than the comparison group of 16%, 15%, 19% and 21% respectively.

The number of B-lymphocytes with CD19<sup>+</sup> phenotype exceeds control values at 5%, but was reduced by 15% relatively to the values of the comparison group.

The concentration of IgM and IgA was reduced relatively to the control group and by 5% to 9% (which is not statistically significant but clinically significant), Ig concentration was not

different from control values. In relation to the values of the comparison group showed an increase in the concentration of IgA by 42% and reducing of the concentration of IgM and G by 13% and 4%, respectively.

Using the therapy proposed by us with Licopid and Vobenzym number of T-lymphocytes with a phenotype CD3<sup>+</sup>, CD4<sup>+</sup>, CD8<sup>+</sup> recovered to physiological values, decreased the number of B-cells with the phenotype CD19<sup>+</sup>, moreover was observed activation of neutrophils, increasing of phagocytic index, completeness of phagocytosis, a gradual increase in the concentration of IgA.

Thus, the effect of the proposed method of treatment achieved by exposure to one of the major factors in the pathogenesis of tumor-like endometrioid ovarian formations and by appointment of Licopid combined with Vobenzym, that led to the normalization of immune status and allowed to recommend the scheme for the treatment of nulliparous women with endometrioid tumor-like ovary formations.

## CONCLUSIONS

1. On the pre-treatment stage in nulliparous women with endometrioid tumor-like ovary formations were identified changes in the immune system, manifested by failure of phagocytic system (incomplete phagocytosis and functionally deficient metabolic reserve), an imbalance of T- and B-cell units, disimmunoglobulinemia, that indicated a direct participation of immune mechanisms in the pathogenesis of the disease.

2. Normalization of cellular and humoral parameters of innate and adaptive immunity, occurring after a comprehensive therapy, confirms the validity of pathogenic inclusion in the traditional regimen of drugs with immunomodulative and antioxidant effects, which will significantly improve the quality of life of patients.

## Патогенетические лечебные стратегии ведения нерожавших пациенток с овариальными эндометриозами

О.С. Шаповал, Л.Л. Воронцова

Современный подход выбора варианта лечения и оценки его эффективности у нерожавших женщин с эндометриозными кистами состоит в формировании оптимальной патогенетически обоснованной терапии с учетом особенностей заболевания не только на системном, органном и тканевом уровнях, а и с учетом клеточно-молекулярных особенностей функционирования организма.

**Цель исследования:** определение эффективности применения различных схем лечения нерожавших женщин с опухолеподобными образованиями яичников с учетом особенностей иммунного статуса.

**Материалы и методы.** Обследованы 80 женщин в возрасте 17–40 лет: первая группа – 50 здоровых небеременных женщин, вторая

группа – 30 женщин с овариальными эндометриозами, получающих традиционное (подгруппа 2а) и комплексное (подгруппа 2б) лечение.

**Результаты.** При исследовании иммунного статуса у нерожавших женщин с опухолеподобными эндометриозными образованиями яичников (n=30) была выявлена несостоятельность фагоцитарной системы, дисбаланс Т- и В-клеточного иммунитета, дисиммуноглобулинемия. Пациенткам была назначена патогенетически обоснованная комбинированная терапия, включающая, помимо традиционной схемы лечения, назначение иммуномодуляторов и системной энзимотерапии.

**Заключение.** Результаты лечения свидетельствуют о полной нормализации показателей фагоцитарной, Т- и В-клеточной и, частично, гуморальной систем.

**Ключевые слова:** иммуномодулирующая терапия, нерожавшие женщины, овариальные эндометриозы.

**Патогенетичні лікувальні стратегії ведення пацієнток, які не народжували, з оваріальними ендометріомами**

**О.С. Шаповал, Л.Л. Воронцова**

Сучасний підхід вибору варіанту лікування та оцінювання його ефективності у жінок, які не народжували, з ендометріодними кістами полягає у формуванні оптимальної патогенетично обгрунтованої терапії з урахуванням особливостей захворювання не тільки на системному, органному і тканинному рівнях, а й з урахуванням клітинно-молекулярних особливостей функціонування організму.

**Мета дослідження:** визначення ефективності застосування різних схем лікування жінок, які не народжували, з пухлиноподібними утвореннями яєчників з урахуванням особливостей імунного статусу.

**Матеріали та методи.** Обстежено 80 жінок у віці 17–40 років: перша група – 50 здорових невагітних жінок, друга група – 30 жінок з оваріальними ендометріомами, які отримували традиційне (підгрупа 2а) і комплексне (підгрупа 2б) лікування.

**Результати.** Під час дослідження імунного статусу у жінок, які не народжували, з пухлиноподібними ендометріодними утвореннями яєчників (n=30) була виявлена неспроможність фагоцитарної системи, дисбаланс Т- і В-клітинного імунітету, дисімуноглобулінемія. Пацієнткам була призначена патогенетично обгрунтована комбінована терапія, що включає, окрім традиційної схеми лікування, призначення імуномодуляторів і системної ензимотерапії.

**Заключення.** Результати лікування свідчать про повну нормалізацію показників фагоцитарної, Т- і В-клітинної і, частково, гуморальної систем.

**Ключові слова:** імуномодуляторна терапія, жінки, які не народжували, оваріальні ендометріоми.

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