POINT OF VIEW

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OPPORTUNITIES TO IMPROVE THE TREATMENT EFFECTIVENESS IN CHRONIC ENDOMETRITIS AMONG WOMEN WITH EXTRAGENITAL DISEASES

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The immune status features were assessed in a sample of women with chronic endometritis (CE) after pregnancy termination against the background of extragenital diseases (EGD) (anemia and chronic pyelonephritis). In women with CE a high frequency of bacterial vaginosis, microbial contamination of the cervical canal (Escherichia coli - in one third), moreover, than endometrium (Enterococcus faecalis and Bacteroides fragilis - in hypoplastic type) was found.

The study of the activity of cellular and humoral immunity links at CE, taking into account the type of inflammation and the presence of infection of the uterine mucosa, allows for rehabilitation of women after pregnancy termination against the background of EGH, which is adequate to the identified disorders.

Keywords: termination of pregnancy, chronic endometritis, extragenital diseases.

Introduction. Research interest in the problem of managing women with chronic endometritis (CE) is explained by the lack of clear ideas about the nature of changes in humoral and cellular immunity in response to persistence in the mucosa of low-virulent or aggressive microorganisms.

Features of the organism immunoreactivity including activation of local anti-infective processes in the uterine mucosa or systemic changes, often - with the induction of autoantibody synthesis are discussed in scientific works with a fair share of doubt [1]. It is believed that the disadaptation of the body on the CE background is realized in various immune responses to antigenic irritation due to the failure of mechanisms protective against bacterial and viral infections. There is an opinion that chronic inflammatory diseases of the pelvic organs should be considered as an autoimmune process, in the outcome of the preserved abnormal immunoreactivity after elimination of infections [4].

The largest number of studies is devoted to the analysis of immune reactions in the sample with miscarriage, due to the conclusion that CE presence among more than 70.0% of women determines the violation of the embryonic development processes, starting with implantation processes [5, 9]. The nature of structural and functional changes resulting from the release of inflammatory mediators in endometrial tissue corresponds to the general indications of any inflammatory process [7].

Obviously, ischemia on the background of sclerotic and microcirculatory changes in the inflamed uterine mucosa, which determines unfavorable conditions for the progression of pregnancy, is associated with abnormal immunoreactivity [6]. However, the nuances of the immune response in CE remain poorly understood, as well as the possibilities of medical correction of autoimmune mechanisms that damage the uterine mucosa among women with pregnancy termination on the CEGD background (chronic extragenital diseases). Ideas about the role of the microbial agent in the genesis of inflammation remain controversial: some authors refer to the low frequency of bacterial contamination of the mucosa, others – to difficulties in identifying anaerobic strains or viruses, or insist on the microbial elimination with the maintenance of the autoimmune process [3].

Objective of the research: To evaluate the nature of immune disorders in a sample of women with chronic endometritis after pregnancy termination on the EGD background.

Materials and methods of the research: A group of 360 women with anemia (n = 216) and chronic pyelonephritis (CP) (n = 144) was prospectively examined. Their pregnancy termination in the first trimester took place on the histologically confirmed CE background. The control group (n = 71) was represented by women whose pathological examination of mucosal biopsy specimens revealed no chronic inflammation. Written informed consent for participation in the study was obtained from all patients.

Criteria for inclusion in the research: the presence of reproductive losses (up to two months after abortion) on the CP and anemia background, histologically verified CE.

Exclusion criteria: multiple pregnancy and the one being the result of assisted reproductive technologies; heavy somatic diseases in the stage of decompensation, precancerous and oncological diseases; stillbirths; chromosomal abnormalities and congenital malformations of the fetus.

Methods of the research: clinical and statistical analysis, sonography, hysteroscopy, pathological and microbiological (assessment of biocenosis, PCR diagnostics, bacteriological examination of discharge from the cervical canal, endometrium) examination of the endometrium. Diagnosis of placental polyp among three women within two months after the intervention excluded them from the study.

Addressing the problem of abortion on the EGD background was associated with difficulties in diagnosing morphofunctional endometrium changes, the choice of timely pathogenetically reasonable rehabilitation. By stratification of endometrial inflammation according to the results of hysteroscopy and morphology, two CE types were distinguished: hyperplastic (n = 203) and hypoplastic (n = 154).

The basis of the hysteroscopic CE types' differentiation – hypoplastic and

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hyperplastic – was the peculiarities of thickness, colour and structure of the mucosa and the vascular pattern.

Determination of immunocompetent cells subpopulations was performed by immunotherapies of peripheral blood lymphocytes, flow cytofluorometry on the machine EPICS-XT company COULTER (USA) and multiparameter two-tone cytofluorimetry. To determine the content of immunoglobulins IgA, IgM, IgG (g/l) by radial immunodiffusion in gel (Manchini et al., 1965) monospecific serum sets were used (Vaccine and Serum Research Institute named after I.I. Mechnikov, N-Novgorod). The number of circulating immune complexes (CIC, opt. units) was evaluated by the method of Haskova V. et al (1978) modified by Yu.A. Grinevich and I.A. Alferova (1981) by the method of selective precipitation in 4.16% PEG 6000 (Serva, Germany).

Mathematical processing of the data was performed using standard software packages for Windows version 20 (SPSS Inc., Chicago, IL). Statistical processing of the studied material included descriptive statistics.

To assess the significance of differences in qualitative features in unrelated groups, χ2 criteria were used. Differences between the indices in different groups were considered significant at p <0.05.

Results of the research and their discussion. According to the results of a morphological study of the material after uterine emptying, all women with abortion on the CP and anemia background were diagnosed with CE (chronic endometritis) (n = 360). Age categories of women with abortion on the CP (chronic pyelonephritis) and anemia background were as follows: 31.3% were in the 18-24 years group, 40.3 – 25-30 years, 22.9% –31-35 years. The number of 36-40 years old women in the CP group turned out to be 9 times more than with anemia (48.6% versus 5.5% accordingly, p = 0.0005). The average age in the CP group was 27.1 ± 5.7 years, anemia – 26.5 ± 5.2 years.

The number of births was higher in the group with anemia – one and a half times, than in group with CP (58.3% versus 36.1%, p = 0.0006), a history of abortion was indicated by 41.0% of women, spontaneous miscarriages – 30.1%, tubal pregnancy – 9.8%, non-developing pregnancy – 11.1%.

The average term for pregnancy termination in the CP group was 8.4 ± 2.2 weeks, anemia – 7.6 ± 2.5 weeks.

The analysis of the microbial contamination of the urogenital tract loci, comparing the simultaneous occurrence of various strains, was carried out along with the assessment of the organism's immunological reactivity to the persistence of infections in the endometrium.

The conclusion about normocenosis appeared in 6.6% of vaginal discharge samples among women with chronic inflammation of the uterus and 58.1% - without CE (p <0.05). Subnormal cenosis was characteristic of 12.0% of all women with pregnancy termination on the EGD background. Bacterial vaginosis (BV) was diagnosed in 73.4% of women with the hypoplastic CE type, 51.9% - hyperplastic, in 25.7% - was found in the absence of an inflammatory process (p <0.05). Vaginitis was detected in a third of women with the hyperplastic CE variant (32.5%), three times less frequently (10.3%) – among patients with a hypoplastic one.

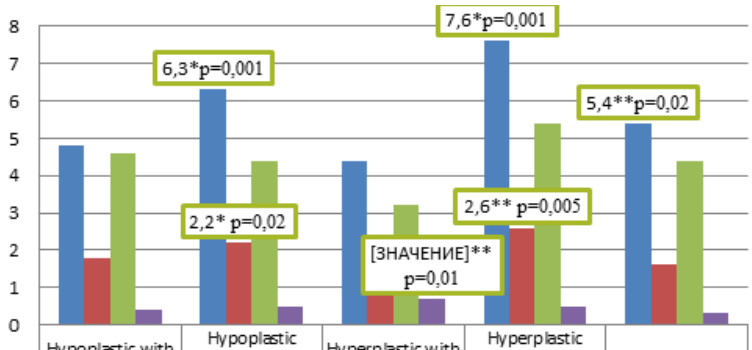
The frequency of infections in the cervical canal in the sample with CE exceeded those in the endometrium – regardless of the type of inflammation. Cultural diagnostics contributed to the identification of a significant contamination of the cervical canal with enteroflora – to confirm the high frequency of vaginal dysbiosis.

The persistence of enterococcus in the endometrium was found in every tenth woman with CE, regardless of the type, in 9.9% on average; the infection in the cervical canal was detected more frequently: in a third of cases with the hypoplastic type of inflammation (p <0.05), a quarter – with the hyperplastic one (p <0.05). The cervical canal contamination with Escherichia coli distinguished a third of all women with CE (32.4% on average), with a greater frequency of its persistence – twice – with the hypoplastic type (18.8% vs. 7.9%) (p<0.05). The frequency of bacteroids detection in the endometrium among patients with the hypoplastic CE type exceeded that in the cervical canal by four times (11.0%) (p<0.05). The intensity of β-streptococcus colonization (> 104 CFU / ml) in the cervical canal was higher (14.0% vs. 4.9% on average) than in the uterine mucosa of all women with CE.

The analysis of the immunological profile of women with EGD whose pregnancy termination was due to chronic endometrial inflammation included assessment of leukogram (Fig. 1), cellular and humoral reactivity factors, depending on the presence of the uterine mucosa contamination (Fig. 2).

Microbial contamination of the endometrium with infections was accompanied by a lower leukocyte reaction — as compared with the intact uterus mucosa — in both CE types (p <0.05). A similar tendency was revealed in relation to lymphocytes and neutrophils, whose indices, when the mucous membrane of the uterus is contaminated on the background of CE hyperplastic type, were lower (p <0.05). The monocytic reaction was more significant in the group of women with endometrial infection in hyperplastic inflammation (p <0.05).

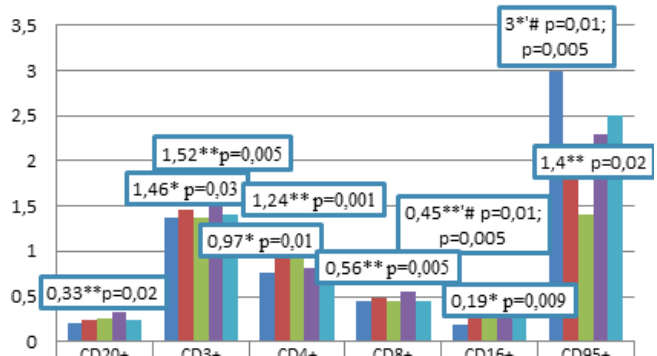
Contamination by uterine mucosal infections in the hypoplastic CE type was accompanied by some inhibition of



	Hypoplastic with contamination	Hypoplastic without contamination	Hyperplastic with contamination	Hyperplastic without contamination	Healthy
Leukocytes	4,8	6,3	4,4	7,6	5,4
Lymphocytes	1,8	2,2	1,5	2,6	1,6
Neutrophils	4,6	4,4	3,2	5,4	4,4
Monocytes	0,4	0,5	0,7	0,5	0,3

Note: * Fig. 1-3 (p <0.05) - the differences in the indices are statistically significant between the groups with / without contamination – for the hypoplastic type, ** - the hyperplastic type, # - for another CE histotype with contamination

Fig. 1. Leukocyte profile depending on the presence of the uterine mucosa contamination



	CD20+	CD3+	CD4+	CD8+	CD16+	CD95+
Hypoplastic with contamination	0,21	1,38	0,76	0,45	0,19	3
Hypoplastic without contamination	0,24	1,46	0,97	0,49	0,32	1,8
Hyperplastic with contamination	0,25	1,37	1,24	0,45	0,45	1,4
Hyperplastic without contamination	0,33	1,52	0,82	0,56	0,25	2,3
Healthy	0,24	1,4	0,83	0,44	0,26	2,5

Fig. 2. Markers of cellular immunity of lymphocytes in various CE types, depending on the uterine mucosa contamination

the production of CD3 +, CD4 +, CD20 +, CD16 + cellular immunity clusters on the background of high proapoptotic directionality (p <0.05). The vectorial character of immune-mediated reactions in hyperplastic CE type with endometrial persistence of infections consisted in the greater activity of individual clusters (CD4 +) (p <0.05) induced by NK CD16 + (p <0.05).

The assessment of humoral immunity depending on the presence of the uterine mucosa contamination is presented in the.

The level of IgA was elevated in the group with the uterine mucosa contamination (Fig. 3), to an irrespective of the CE type (p<0.05). The IgM content in the group with the hyperplastic type of inflammatory process in the endometrium exceeded that of women with intact mucosa (p <0.05), as well as with the hypoplastic type (p<0.05).

The distinctive features of the humoral immunity of women with the hyperplastic CE type were the presence of high immunoglobulin indices in case of the uterine mucosa contamination – more than in the intact (p<0.05). In the group with the hypoplastic CE variant the IgA content (p<0.05) dominated at the microbial contamination of the uterine mucosa. IgM (p<0.05) and IgG (p<0.05) were low compared to the "sterile" uterine mucosa.

The content of immunoglobulins among women with CE and the intact uterine mucosa differed from healthy women by in slightly increased values, IgM and IgA – in the hyperplastic one.

The revealed violations of the mucosal architectonics (macro- and microstigmas) in the groups with reproductive losses on the EGD background (CP and anemia) indicated high CE frequency – as a predictor of the mucosal instability in both CE variants.

The role of CE being one of the leading causes of early pregnancy termination is confirmed by studies of both domestic and foreign authors [2,4,10].

The nature of local damage and the functional activity of the reserve of immunocompetent cells (humoral immunity factors, population and subpopulation structure of blood lymphocytes) in the presence of high microbial contamination of the urogenital tract loci justifies the reasonability of a comprehensive rehabilitation therapy [7, 8].

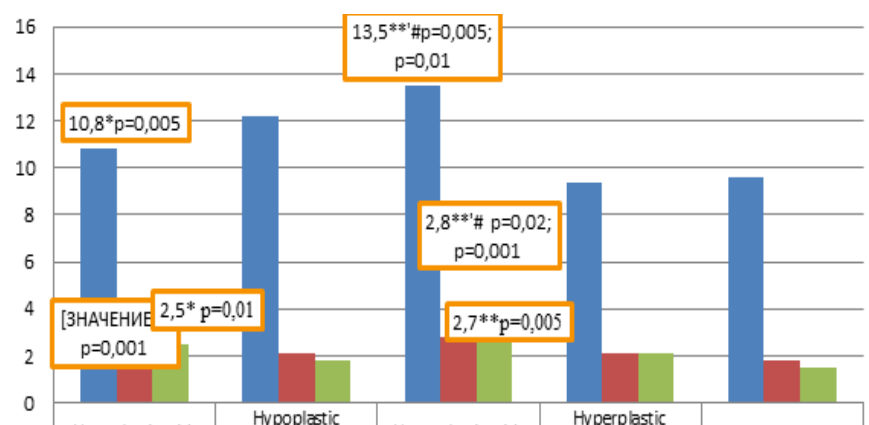
It is obvious that the traditional CE treatment among women with chronic EGD (CP and anemia), being an additional factor of immune imbalance, does not contribute to the restoration of cell-humoral systems to the state of "resource", especially for the elimination of the existing infection. The presence of endometrial contamination indicated productive inflammation and the need for elimination antibiotic therapy, taking into account the sensitivity of infections detected in various urogenital loci in diagnostically significant titers – vaginally and in the cervix, often in combination with urinary tract infection (in CP presence) and subsequent delayed treatment of chronic inflammatory diseases of the pelvic organs.

Analysis of the state of cell and humoral markers in various CE types – hypo- and hyperplastic – depending on the presence of mucosal infection will allow adequate rehabilitation of women after pregnancy termination on the EGD background, which is adequate to the identified changes in immunoreactivity.

Conclusion. The role of CE in the genesis of reproductive losses confirms the formation of abnormal embryo-placental relationship on the background of defective cytotrophoblast invasion into an inflammatory modified endometrium.

We consider it appropriate to organize a step-by-step examination of women with anemia and CP in order to identify causes of abortion, verification of chronic inflammation of the mucosa, with determining a certain CE type – hypoplastic or hyperplastic – during hysteroscopy.

Significant microbial contamination



	Hypoplastic with contamination	Hypoplastic without contamination	Hyperplastic with contamination	Hyperplastic without contamination	Healthy
Ig G	10,8	12,2	13,5	9,4	9,6
Ig M	1,4	2,1	2,8	2,1	1,8
Ig A	2,5	1,8	2,7	2,1	1,5

Fig. 3. Reactivity of humoral factors depending on the presence of the uterine mucosa contamination

of the lower parts of the genital tract among women with abortion on the anemia and CP background was revealed: normocenosis only in 6.6%, BV (bacterial vaginosis) predominance with a hypoplastic CE type – 73.4%, one and a half times, vaginitis –with a hyperplastic type – 32.5%, three times.

The frequency of the cervical canal infection among women with CE was higher than the endometrium infection, regardless of the type of inflammation. E. coli was detected among a third of all women with CE, enterococcus – among a third with a hypoplastic CE type. Endometrial persistence was found with a hypoplastic CE type, E. coli – twice as often as in the cervical canal, bacteroids – four times as often (11.0%).

The presence of productive inflammation in the uterine mucosa was accompanied by a decrease in the level of leukocytes, lymphocytes and neutrophils with the formation of an immunodeficiency state. Features of the immune phenotype of women with a hyperplastic CE type in the persistence of infections in the endometrium are the following: monocytic blood reaction, NK CD16+, CD4+ activity; with a hypoplastic type – the decrease of cellular factors production CD3+, CD4+, CD20+, CD16+ on the background of redundant – CD 95+.

Microbial contamination of the endometrium with a hypoplastic CE type is accompanied by a decrease in the level of immunoglobulins IgG, IgM with an increased rate of IgA in comparison with intact mucosa, with a hyperplastic type –all values of immunoglobulins are reduced.

An integrated approach for women with abortion on the anemia and CP background based on the results of microbiological and immunological studies contributes to the choice of pathogenetic therapy for various CE types. Unreasonable antibiotic therapy for autoimmune endometritis was proved (in the absence of microbial contamination of genital loci, immunodeficiency state).

Correction of identified immune changes seems to be a significant rehabilitation strategy tool for women with reproductive losses due to CP and anemia. The reduction of anti-infection protection of the organism predetermines the lack of pregravid recovery.

References

1. Трунов А.Н., Маринкин И.О., Кулешов В.М. [и др.] Активность местного иммуновоспалительного процесса у пациенток с бесплодием на фоне хронических инфекционно-воспалительных заболеваний урогенитальной сферы в стадии клинической ремиссии. *Медицина и образование в Сибири.* 2012; 6: 55–55. [Trunov A.N., Marinkin I.O., Kuleshov V.M. [et al]. The activity of the local immuno-inflammatory process among patients with infertility on the background of chronic infectious and inflammatory diseases of the urogenital sphere in the stage of clinical remission. *Meditsina i obrazovaniye v Sibiri.* 2012; 6: 55–55. (In Russ.)]

2. Илизарова Н.А., Кулешов В.М., Маринкин И.О. [и др.] Анализ морфоструктурных изменений эндометрия на фоне различных схем предгравидарной подготовки у пациенток с привычным невынашиванием беременности. *Вестн. Нац. медико-хирургического центра им. Н. И. Пирогова.* 2009; 4(1): 100–104. [Ilizarova N.A., Kuleshov V.M., Marinkin I.O. [et al]. The analysis the morfostrukturnykh of changes of endometrium against the background of various schemes of predgravidarny preparation at patients with habitual not incubation of pregnancy. *Vestn. Nats. mediko-khirurgicheskogo tsentra im. N. I. Pirogova.* 2009; 4(1): 100–104. (In Russ.)]

3. Зайнетдинова Л.Ф. Восстановление репродуктивной функции у женщин с трубно-перитонеальным бесплодием, ассоциированным с актуальными генитальными инфекциями. *Вестн. ЮУрГУ.* 2010; 6: 33–36. [Zaynetdinova L.F. Restoration of reproductive function at women with the pipe and peritoneal infertility associated with relevant genital infections. *Vestn. YuUrGU.* 2010; 6: 33-36. (In Russ.)]

4. Радзинский В.Е., Запертова Е.Ю., Полина М.Л. Иммунная партитура беременности. Привычное невынашивание и хронический эндометрит. *Status Praesens.* 2011; 5 (2): 30–35. [Radzinskiy V.E., Zapertova E.Yu., Polina M.L. Immune score of pregnancy. Habitual not incubation and chronic endometritis. *Status Praesens.* 2011; 5 (2): 30-35. (In Russ.)]

5. Сидельникова В.М., Сухих Г.Т. Невынашивание беременности: рук. для практ. врачей. М.: Мед. информ. агентство. 2010. 536 с. [Sidelnikova V.M., Sukhikh G.T. Pregnancy miscarriage: guide for pract doctors. M.: Med. inform. agentstvo. 2010; 536. (In Russ.)]

6. Сухих Г.Т., Ванько Л.В. Иммунология беременности. М.: РАМН, 2003; 400. [Sukhikh G.T., Vanko L.V. Immunology of pregnancy. M.: RAMN, 2003; 400.

7. Сухих Г.Т., Шуршалина А.В. Хронический эндометрит: рук-во. М.: ГЭОТАР–Медиа, 2010: 64. [Sukhikh G.T., Shurshalina A.V. Chronic endometritis: guide. M.: GEOTAR Media, 2010: 64. (In Russ.)]

8. Хаитов Р.М., Пинегин Б.В., Ярилин А.А. Руководство по клинической иммунологии. Диагностика заболеваний иммунной системы: рук. для врачей. М.: ГЭОТАР – Медиа, 2009; 352. [Khaitov R.M., Pinegin B.V., Yarilin A.A. Guide to clinical immunology. Diagnostics of diseases of the immune system: hands. for doctors. M.: GEOTAR Media, 2009; 352. (In Russ.)]

9. McQueen DB, Perfetto CO, Hazard FK, Lathi RB. Pregnancy outcomes in women with chronic endometritis and recurrent pregnancy loss. *Fertil Steril.* 2015; 104: 927–931.doi: 10.1016/j.fertnstert.2015.06.044.

10. Johnston-MacAnanny EB, Hartnett J, Engmann LL et al. Chronic endometritis is a frequent finding in women with recurrent implantation failure after in vitro fertilization. *Fertil Steril.* 2010; 93 (2): 437-41. doi: 10.1016/j.fertnstert.2008.12.131.