MORPHOLOGICAL CHARACTERIZATION OF ENDOMETRIUM IN WOMAN WITH VIRAL-BACTERIAL ENDOMETRITIS.

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ABSTRACT
This paper presents data of 120 examined women diagnosed with chronic endometritis, 80 of them underwent needle-biopsy of the endometrium and 40 - hysteroscopy with diagnostic curettage of the uterine cavity. The diagnosis in all women is confirmed histologically. An analysis of endometrial microflora was performed in these women with an assessment of the morphofunctional state of the endometrium. It was found that in women with the viral component of chronic endometritis, the Epstein-Barr virus was the predominant infectious agents, HPV infection of high and medium oncogenic risk (with prevalence of types 16 and 18), and herpes simplex virus types 1 and 2, leading to chronic inflammatory process not independently, but most often in conjunction with the bacterial flora. Thus, viruses in the endometrium were found in 49% of women; in most cases, virus-bacterial associations were observed. It was also determined that certain differences in the chronic viral endometritis can be detected using immunohistochemistry and morphometric analysis. The data obtained make it possible to state that the immune component of the inflammatory reaction is more pronounced in the endometrium of the viral etiology, the viral infection enhances sclerotic processes and reduces the number of receptors for steroid hormones, especially for progesterone.

Keywords: chronic endometritis, endometrium, bacterial-viral flora, immunohistochemical study, PCR diagnostics

INTRODUCTION
Chronic endometritis (CE) still remains an actual problem today due to its widespread prevalence - from 14 to 25% [1,2], as well as its social significance associated with the violation of reproductive function in women. According to some data, the frequency of CE with spontaneous abortion reaches 90%, undeveloped pregnancies - 91.7%, unsuccessful attempts of ECO - 57%-83.3%, infertility - 50-65% [3,4,5].

CE is considered as a clinical and morphological syndrome, where due to persistent damage of the endometrium by an infectious agent multiple secondary morphological and functional changes occur, which disrupt the cyclic biotransformation and receptivity of the mucous membrane of the uterine body (Shurshalina A.V.) [1]. However, to date, there has been a clear shift in the paradigm of the concepts of the uterus as a sterile organ, and endometritis is regarded as a violation of peaceful coexistence between microorganisms and the host's immune system in the endometrium, which leads to a fertile pathology [6]. The paradigm of the sterile uterus, invented by French pediatrician G. Tisser at the turn of the twentieth century, is a persistent dogma stating that human babies develop in a sterile environment [7]. There has already been convincing evidence that the endometrium is full of a number of microorganisms, making up the uterine microbiome, and their balance with the immune system of a woman determines the state of her health. The same conclusions are made in veterinary science regarding the physiology of the uterus of healthy nulliparous heifers [8]. Thus, the infectious component plays the main role in the development of chronic endometritis, when bacteria, viruses, as well as bacterial-viral associations can be detected in the...
endometrium, while the inflammatory reaction is morphologically observed in cases when the persistent microorganisms invade the endometrium. According to K.M. Mitchell et al. (2014), the uterine cavity is not sterile in most women who underwent a hysterectomy, and the persistence of bacterial flora in a small amount does not entail significant inflammation. The most common microbes in the uterus were \textit{lactobacillus iners} (45\%), \textit{Frevotella} spp. (33\%), \textit{L. crispatus} (33\%) [9]. The American study of 2013 [10] in 27\% of cases detected intracellular bacteria in the basal plate of the placenta, and these pathogens were fixed in the placenta without any signs of chorioamnionitis, suggesting the potential role of natural intrauterine colonization in placental infection.

Detection of a viral infection in the endometrium is not uncommon too and its role in the morphological changes of the endometrium continues to be studied. In our earlier study (2015), a high incidence of herpesvirus and HPV infection in the endometrium associated with the microbial flora was established [11]. According to the data presented by V.I. Krasnopolskii et al. (2004), genital herpes was found in the endometrium in 33.6\% of cases, CMV in 18.9\% [20].

Objective of this research was to study the morphological features of the endometrium in women with chronic endometritis in bacterial and viral infection.

MATERIALS AND METHODS
The study involved 120 women aged 22-40 years diagnosed with chronic endometritis, confirmed histologically by endometrial pipelle biopsy in 80 women and hysteroscopy with separate diagnostic curettage in 40 patients. Most of the patients were followed-up for reproductive abnormalities: sterility - in 84, with 50 women with primary sterility; miscarriage - in 30; 6 women had chronic endometritis not associated with reproductive dysfunction. All women underwent general clinical examination and a culture assay for examining the endometrial biopsy. 40 women underwent immunohistochemical examination of the endometrial biopsy; these women were divided into 2 groups: group 1 - women with chronic endometritis without viral flora, group 2 - women with chronic endometritis with viral flora. To diagnose HPV infection, the real-time polymerase chain reaction (PCR) was used, and an immunohistochemical determination of the antigen of the herpes virus type 1-2, CMV, Epstein-Barr virus, enteroviruses and adenoviruses in the endometrium was carried out using test systems Diagnostic Biosystems, Cell Marque, Thermo Fisher, and Abcam, respectively. The PCR method was used for detecting in the endometrium \textit{Chlamydia trachomatis}, \textit{Mycoplasma genitalium}, \textit{Mycoplasma hominis}, \textit{Ureaplasma parvum} and \textit{Urealyticum}, T960, \textit{Gardnerella vaginalis}, \textit{Trichomonas vaginalis}. It was mandatory to conduct a morphological and immunohistochemical study of the endometrial biopsy. For the immunomorphological study, an immunoperoxidase method was used with a set of monoclonal antibodies to CD45, myeloperoxidase (MPO), CD3, CD20, CD38 and CD56, CD68, CD31, vimentin, type IV collagen, pancytokeratin (CKR), produced by Thermo, Dako, Diagnostic Biosystems, BioGenex, and Lab Vision, respectively, and estrogen and progesterone receptors by Thermo. Morphometric assay of cellular elements was carried out using G.G. Avtandilov’s morphometric ocular mesh [14, 15] with a quantitative evaluation of the signs in 20-40 fields of view of each histological preparation, with 500-1000 cells taken into account.

The detected changes were assessed in accordance with the recommendations by O.K. Khmelnitsky [12], developed for the pathomorphological diagnosis of inflammatory endometrium diseases. Statistical processing of the obtained data was carried out with the help of the Statistica program (StatSoft Inc - USA).

RESULTS AND DISCUSSION, SUMMARY
The nature of extragenital diseases, representing chronic inflammatory foci of different localization, was as follows in the examined women: frequent ARVI occurred in 21.3\% (25) patients, chronic tonsillitis - in 10.2\% (12), chronic gastritis - in 4\% (2). The analysis of clinical data revealed complaints of pain in the lower abdomen in 19.7\% (14), perimenstrumary spotting - in 58.1\% (41), dyspareunia - in 17.4\% (12). A
frequent event was chronic cervicitis, detected in 38 (32%) of the observed women. The nature of reproductive disorders is presented in Table 1.

Table 1. The nature of reproductive disorders in women with chronic endometritis (%).

<table>
<thead>
<tr>
<th>Nature of abnormalities</th>
<th>Examined women, n=120</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>N</td>
</tr>
<tr>
<td>Miscarriage</td>
<td>30</td>
</tr>
<tr>
<td>Sterility I and II</td>
<td>84</td>
</tr>
<tr>
<td>Premature delivery</td>
<td>2</td>
</tr>
<tr>
<td>Ectopic pregnancy</td>
<td>2</td>
</tr>
</tbody>
</table>

Bacteriological and immunohistochemical studies of endometrial biopsies, in addition to PCR diagnostics, revealed a wide spectrum of the microflora of the uterus cavity in 80 women. Viral infection (Table 2) was detected in 61% (49) patients, Epstein-Barr virus in 25% (20), HPV of high and medium oncogenic risk in 20% (16), HSV type 1, 2 - 16.3% (8), CMV - 3.75% (3), enteroviruses - 6.25% (5), adenoviruses - 6.25% (5). Thus, the prevalence of Epstein-Barr virus and HPV infection of high and medium oncogenic risk in the endometrium is consistent with our data on HPV infection in the study as of 2015 [33]. It is important to note that similar viruses in the cervical canal have been found in only a third of patients and it is not possible to assume a contamination of the endometrium from the cervix. This applies equally to bacterial infection, which in overwhelming majority did not coincide with the microflora of the cervical canal. Chlamydial infection in the endometrium among all examined women was detected in 2.5% of cases (2), Mycoplasma genitalium - in 5.0% (4), Mycoplasma hominis - in 3.75% (3), Ureaplasma parvum - in 10% (8). Bacterial viral associations are found in 32.5% of women (39). Among the bacterial flora, *Staphylococcus aureus* prevailed in 14.3% (10), *Enterococcus faecalis* - in 6.25% (5), *Klebsiella pneumonia* - in 6.25% (5), *Escherichia coli* - 5.0% (4). In 18.5% (22) of the examined women microflora was not isolated.

Routine histological studies revealed a stereotyped morphological pattern of the endometrium in all the women examined. Thus, all observations detected focal and diffuse lymphoid-cellular infiltration with the presence of plasma cells, histiocytic elements and a small amount of neutrophils. The immunohistochemical analysis allowed us to reveal the detailed nature of changes in the endometrium, and showed the swelling and desquamation of the endothelium of blood vessels up to the obliteration of their lumen, which was determined by the pronounced expression of MKAT to CD31 in all patients and suggests in this case sclerosis of the endometrial vessels with the corresponding violation of endometriosis function. However, in the first study group, these cell elements were 1.5 times more (t=24.6, p=0.00). The basal membranes were split, fragmented or thickened, which was confirmed by the MKAT reaction to collagen IV. Endometrial glands got stained, often unevenly, the immunohistochemical picture was confirmed by the unequal expression of MKAT to pancitokeratins, which is confirmed by the proliferative changes in endometrial epitheliocytes in the examined women. It turned out that these changes in women with chronic endometritis without a viral infection (group 1) were observed more frequently than in patients with a monoviral and mixed infection (group 2), with the differences being significant (t=12.7,
The total number of CD45(+) leukocyte cells in the examined women did not differ significantly and was
25.7% and 24.4% in women with CE without a viral infection and women with CE with viral infection in the endometrium, respectively (t=-1.5, p=0.17). But the distribution of cell elements in the general structure of CD45(+) cells was somewhat different. Thus, in women with chronic endometritis and viral flora the number of CD3(+) T-lymphocytes was 1.3 times greater than in women without viral chronic endometritis (t=6.1, p=0.00), CD20(+) B-lymphocytes - 1.4 times greater (t=7.3, p=0.00), CD56(+) NK-cells - 1.6 times greater (t=7.7, p=0.00). The content of CD38(+) plasma cells and CD68(+) macrophages in the second study group was also significantly higher (t=9.5, p=0.00 and t=12, p=0.00, respectively). At the same time, the group 1 had 1.6 times more MPO(+) neutrophilic leukocytes detected - 3.6% (t=4.2, p=0.00). The greatest quantitative difference was noted for fibroblasts expressing MKAT to vimentin - 21.6% in patients without viral infection and 27.7% in patients with viral infection, respectively, which indicates fibrosis of the endometrial stroma in women with a predominant viral component of chronic endometritis (t=19.3, p=0.00).

Analysis of the receptor status (Table 4) in women with CE without viral infection showed a high content of estrogen- and progesterone-positive cellular elements both in glands and in stroma of the endometrium. In women with viral CE, the expression of MKAT to these receptors was reduced. The number of progesterone (+) cells decreased significantly up to 75.3% in the glands and up to 80.9% in the stroma of the endometrium, the differences were also significant (t=111.1, p=0.00 for glands and t=38.8, p=0.00 for the stroma of the endometrium). In this case, a decrease in the number of progesterone-positive cells was more noticeable in the endometrial glands, which probably indicates a decrease in the receptivity of the endometrium in women with viral endometritis, and also suggests the possibility of the formation of hyperplastic processes in the endometrium.

**Table 3.** The content of estrogen- and progesterone-positive cell elements in the glands and stroma of the endometrium in chronic inflammation (%).

<table>
<thead>
<tr>
<th>cell elements</th>
<th>Women with CE without viral infection (n=20)</th>
<th>Women with CE with viral infection (n=20)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Glands</td>
<td>Stroma</td>
</tr>
<tr>
<td>estrogen(+)</td>
<td>89.4±4.49*</td>
<td>90.4±5.00*</td>
</tr>
<tr>
<td>progesteron(+)</td>
<td>91.4±5.22*</td>
<td>87.4±3.80*</td>
</tr>
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Note:* - significance of differences between the groups - p<0.01

**CONCLUSION**

It was established that a viral infection plays a significant role in the etiology of chronic endometritis, with a significant proportion of infectious agents belonging to the Epstein-Barr virus, HPV infection of high and medium oncogenic risk (with prevalence of type 16 and 18), and type 1 and 2 herpes simplex virus that lead to the chronic inflammatory process most often in conjunction with the bacterial flora. The study also found that certain differences in the chronic endometritis of the viral etiology can be detected using immunohistochemistry and morphometric analysis. The obtained data, with a certain degree of certainty, allow us to state that viral endometritis is characterized by more pronounced immune component of the inflammatory reaction (according to the higher percentage of immunocompetent cells), the tendency towards the development of fibroplastic processes (as indicated by a higher number of fibroblasts), as well
as by changes in the receptor apparatus of cells (as evidenced by a decrease in the amount of estrogen- and, especially, progesterone-positive elements). The data of our study are confirmed in a recent study by Gatagazheva Z.M. (2017), which determined that herpes and HPV viruses in the endometrial biopsy are found in 40.7% of women with chronic endometritis, and microbial and viral associations - in 35%. Morphologically, focal endometrial hyperplasia is observed on the background of chronic endometritis [21], which is also confirmed by other researchers [22].

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REFERENCES