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## ADVANTAGES OF IMMUNOHISTOCHEMISTRY IN THE DIAGNOSIS OF CHRONIC ENDOMETRITIS

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**Abstract.** In Russia, there is currently a high incidence of infertile marriages and spontaneous abortions. One of the main causes of infertility in women is endometrial pathology. Structural and functional deficiencies of the endometrium caused by pathological inflammatory processes lead to the disruption of implantation, clinical infertility, and prenatal losses. The identification of new treatment solutions for the endometrial pathology is extremely relevant for the reproductive health of society. The main causes of endometrial pathology include inadequate hormonal regulation and acute and chronic inflammation (endometritis). Chronic endometritis is often characterized by a long asymptomatic period and challenging initial diagnosis, which underwent significant changes during the past decade. Currently, the diagnosis is based on a comprehensive assessment of clinical, morphological, and instrumental data. Particular attention is paid to the verification of chronic endometritis via immunohistochemical examination of the endometrium. This method allows not only for the identification of infection but also for the establishment of the specific phase of the disease, which has a significant role in the decision making. It is particularly important to immunohistochemically identify the viral etiology of chronic endometritis because this method can reliably detect the presence of antigens from different viral agents.

**Key words:** chronic endometritis; immunohistochemical research; simple herpes virus; cytomegalovirus; Epstein–Barr virus.

## ПРЕИМУЩЕСТВА ИММУНОГИСТОХИМИЧЕСКОГО МЕТОДА В ДИАГНОСТИКЕ ХРОНИЧЕСКОГО ЭНДОМЕТРИТА

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**Резюме.** В настоящее время в России отмечается высокая частота бесплодных браков и спонтанных абортов. Одной из основных причин нарушения fertильности женщин является патология эндометрия. Структурная и функциональная неполноценность эндометрия, вызванная патологическими воспалительными процессами, закономерно приводит к нарушению процесса имплантации и клинически проявляется бесплодием и пренатальными потерями. Поиски решений лечения патологии эндометрия исключительно актуальны для профилактики репродуктивного здоровья общества. Основными причинами патологии эндометрия являются неадекватная гормональная регуляция, острый и хронический воспалительные процессы (эндометрит). Для хронического эндометрита часто характерны длительное бессимптомное течение и сложность первичной диагностики. Диагностические подходы в верификации хронического эндометрита непрерывно видоизменялись на протяжении последних десятилетий. Сегодня диагноз базируется на комплексной оценке целого ряда клинических, морфологических, инструментальных данных. Особое внимание при верификации хронического эндометрита уделяется иммуногистохимическому (ИГХ) исследованию эндометрия. Метод позволяет не только идентифицировать тип инфекционного агента, но и установить фазу процесса, что имеет существенную роль для определения тактики лечения. Особая роль отводится иммуногистохимическому методу при выявлении вирусной этиологии хронического эндометрита, так как этот метод позволяет достоверно выявить наличие антигенов к различным вирусным агентам.

**Ключевые слова:** хронический эндометрит; иммуногистохимический анализ; вирус простого герпеса; цитомегаловирус; вирус Эпштейн–Барр.

Recently, a high frequency of infertile marriages (up to 15%) and spontaneous abortions (up to 10%–15%) in Russia was reported [4], which has significantly influenced the social and economic policy of the country in order to support the reproductive health of the nation.

Endometrium pathology is one of the main causes of impaired female fertility. The endometrium is a unique tissue that undergoes cyclic modification induced by the sex steroids produced in the ovaries. Tissue remodeling for the embryonic implantation involves sequential processes, including cell proliferation, differentiation, and apoptosis [8]. Structural and functional endometrium deficiencies caused by pathological inflammatory processes lead to implantation failure, which is clinically manifested as infertility or prenatal loss. The development of a treatment for endometrium pathology is crucial for the preventive care of reproductive health [7].

Inadequate hormonal regulation and acute and chronic inflammatory processes (endometritis) are the main causes of endometrium pathology. Chronic endometritis (CE) is frequently characterized by long-term asymptomatic development; and challenging initial diagnostics.

CE is a clinical and histopathologic syndrome that causes long-term damage of the endometrium by an infectious agent, resulting in the morphofunctional changes that impairs the cyclic transformation and receptors of the endometrium [3].

CE was first described as a specific nosological entity in the International Classification of Diseases (ICD-9) in 1975. The event was preceded by a semicentennial discussion on the possibility of chronic inflammation of the endometrium taking into account cyclic transformation and potential for functional layer desquamation on monthly basis.

CE according to its causes can be divided into non-specific and specific forms (Table 1).

According to Smetnik (2007), CE is both clinical and anatomic concept. The following morphologic forms of the disease are stated:

- Atrophic form, in which gland atrophy, stromal fibrosis and infiltration with lymphoid elements are observed;
- Cystic form, in which fibrous tissue constricts the gland ducts resulting in thickening of their contents and cysts' formation;
- Hypertrophic form, in which the mucosal membrane undergoes hyperplasia as a result of chronic inflammation.

Regardless of multiple scientific studies and data on its high prevalence (60%–65%) chronic endometritis continues to be poorly studies in modern gynecology [9]. The significance of this pathology is determined by the considerable difficulty in its diagnostics. The physicians continue to underestimate the role of timely prophylaxis and treatment CE as way to increase fertility. A constantly high frequency of CE suggests that it is a common problem, which may be caused by multiple mechanisms, thus moving away from a one-sided understanding of the disease as an exclusively classic pyoinflammatory process that, according to the statistics, occurs as a complication of every 4<sup>th</sup> to 5<sup>th</sup> surgical abortion [5].

According to current knowledge, CE has several characteristic features [1, 3]:

- Change of the etiologic structure with increased significance of viral and opportunistic flora;
- Increase of flora resistance to drug therapy;
- Transformation of clinical symptoms towards sub-clinical and atypical forms;
- Mismatch of clinical manifestations and structural changes in the endometrium.

Table 1

Classification of chronic endometritis according to the etiologic factor (C. Buckley, 2002)

Endometritis	Characteristic
Nonspecific	Specific flora in the endometrium is not detected. Inflammation develops secondary to the intrauterine devices, radiation therapy for pelvic organs, and bacterial vaginosis in HIV-infected patients when using oral contraceptives
Specific	1. Chlamydial: <i>Chlamydia trachomatis</i> 2. Viral: HSV, CMV, and HIV 3. Bacterial: <i>Mycobacterium tuberculosis</i> , <i>Neisseria gonorrhoeae</i> , <i>Neisseria meningitidis</i> , <i>Actinomyces israelii</i> , and <i>Treponema pallidum</i> 4. Mycoplasmal: <i>Mycoplasma hominis</i> 5. Fungal: <i>Candida</i> , <i>Blastomyces dermatitidis</i> , <i>Coccidioides immitis</i> , and <i>Cryptococcus glabratus</i> 6. Protozoal: <i>Toxoplasma gondii</i> and <i>Schistosoma haematobium</i> 7. Parasitic: <i>Enterobius vermicularis</i> 8. Sarcoidosis

For example, an inflammatory process in the endometrium presents an association of obligate anaerobes and also the persistence of opportunistic flora and viruses. According to many studies, the most specific feature of CE in women is the associations of 2 or 3 species of obligate anaerobes and viruses [1, 2]. The most important persistent viruses include herpes simplex virus (HSV) and herpes zoster, cytomegalovirus (CMV), enteroviruses (Coxsackieviruses A and B), and adenoviruses [1, 2]. In the literature, a range of clinical cases of HSV or CMV monoinfection has been described.

Herpes virus infection plays a major role in the natural history of CE. Its prevalence among the young population is relatively high. Herpes virus infection can damage most organs and systems of the host's body. Environmental degradation, emotional stress, and improper drug therapy can increase immunodeficiency, allowing the presence of an active and persistent herpes virus infection. Herpetic endometritis generally occurs in patients with atypical or asymptomatic forms of genital herpes and is caused by a long-term HSV persistence in the endometrium [2, 6].

Diagnostic approaches for CE verification have been continuously changed over several decades. Currently, its diagnosis is based on the integral assessment of all clinical, histological, and instrumental data. Compared with histologic CE confirmation after endometrial curettage, the integral method of CE diagnosis has improved the verification of this pathology by 64.6%.

Because of its high specificity and sensitivity, it is crucial to perform immunohistochemical (IGC) studies of the endometrium as a part of diagnostic work-up. Furthermore, it makes possible not only to identify the type of infectious agent but also to determine the stage of the process, which plays an important role in the decision making process. IGC study allows the accurate detection of the antigens of various viral agents to determine the viral etiology of CE.

We describe two clinical cases showing the significance of the IGC method in the verification of infectious agent.

### CLINICAL CASE 1

A 29-years-old female patient, K., visited a women's health clinic after uterine cavity curettage for missed abortion.

The menstrual cycle was regular and had remained unchanged from the date of menarche, with unchanged hormonal state. The patient had three pregnancies and one delivery (2007). No complications after the pregnancies, delivery, and in the postnatal period were observed. In 2009 and 2013, uterine cav-

ity curettages were performed because of the missed abortion at the early stages of pregnancy. During the postoperative period, the patient received antibacterial therapy. No postoperative complications were observed.

During the ultrasound examination no signs of pelvic organ pathology was found; the results of real-time PCR for significant infections of the reproductive organs (examination material: secretions of the cervical canal) were negative. On the 10th day of the menstrual cycle, an aspirate from the uterine cavity using a Pipelle curette was obtained. The histopathologic and IGC studies and mass spectrometry were performed to verify the presence of bacterial agents. The histopathologic examination of the endometrium showed glands at the early and medium proliferation stages, with moderate lymphoid stromal infiltration.

According to the IGC study, HSV and CMV antigens were detected (intensity of response to HSV and CMV was 2+). The mass spectroscopy data indicated the presence of *Actinomyces viscosus* and *Clostridium coccoides*.

### CLINICAL CASE 2

A 32-years-old female patient, N., visited a women's health clinic with complaints of inability to get pregnant for 3 years.

The menstrual cycle was regular and had remained unchanged from the date of menarche, with an unchanged hormonal state. The patient had pregnancies; the first was terminated with a therapeutic abortion in 2003 during 8–9 weeks of pregnancy (according to the patient the postoperative period was without complications), and the second pregnancy ended by a term birth with threatened miscarriage at the early stages. No complications after the delivery and in the postnatal period were observed. The patient's husband, 35-years-old, was examined by an urologist, and a spermogram was performed. No pathology signs of pathology were found.

During the ultrasound examination no signs of pelvic organ pathology was found. The results of real-time PCR examination for significant infections of the reproductive organs (examination material: secretions of the cervical canal) were negative. On the 10th day of the menstrual cycle an aspirate from the uterine cavity using a Pipelle curette was obtained. The histopathologic and IGC studies and mass spectrometry were performed to verify the presence of bacterial agents. The histopathologic examination of the endometrium showed glands at early and medium proliferation stages, with moderate lymphoid stromal infiltration.

According to the IGC study, antigens of HSV and Epstein-Barr virus (EBV) were detected (reaction in-

tensities to HSV and EBV were 3+ and 2+, respectively; Fig. 1).

Mass spectrometry results showed the presence of *Propionibacterium freudenreichii* and *C. coccoides*.

In both cases, CE of mixed bacterial and viral etiology was diagnosed based on clinico-laboratory data. Both clinical cases clearly demonstrate the role of IGC study in determining viral infection in the diagnosed CE for recovering the reproductive function of patients.

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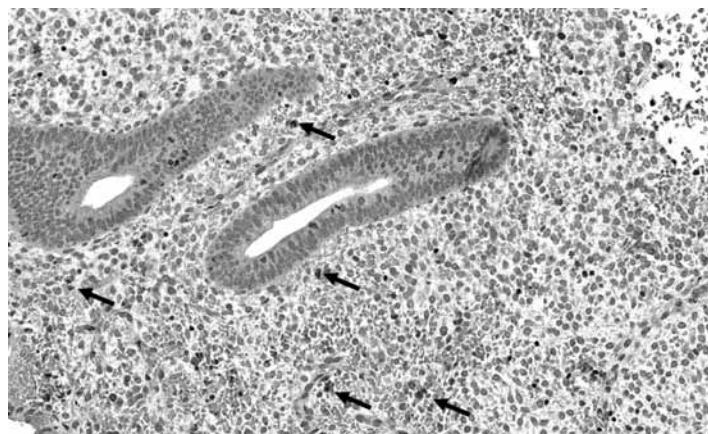


Fig. 1. Antigens to herpes simplex virus in stromal endometrium cells (x 400)

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