CHARACTERISTICS OF SYSTEMIC INFLAMMATION IN PATIENTS WITH ENDOMETRITIS-ASSOCIATED INFERTILITY

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Chronic endometritis (CE) is the most significant endometrial disease in terms of its impact on reproductive potential. Thus far, pathogenetically significant inflammatory markers — cytokines, inflammatory proteins, and hematological indices — have not been widely used in medical practice for the diagnosis of CE. This study aimed to evaluate indicators of systemic inflammation, including hematological parameters and inflammatory indices, in patients with infertility, and to determine their diagnostic significance for CE. We analyzed the data of examinations of 50 patients with infertility. The standard hematological method was used to determine the leukocyte count. Based on the blood cell composition, we calculated the inflammatory indices NLR, MLR, PLR, and SII. The concentration of CRP in the blood serum was determined by enzyme immunoassay. In patients with CE-associated infertility, the absolute and relative numbers of lymphocytes were lower ($p = 0.0451$ and $p = 0.0089$, respectively), whereas the relative numbers of monocytes and basophils were higher ($p = 0.0469$ and $p = 0.0005$, respectively) than in patients without CE. In the study group, the concentration of CRP in the blood was almost four times higher than in the control group ($p = 0.0191$), but all indicators remained within the normal range. A comparative analysis of the NLR, MLR, PLR, and SII indices revealed their significant growth in CE-associated infertility cases ($p = 0.0387$, $p = 0.0058$, $p = 0.0335$, and $p = 0.0333$, respectively). ROC analysis established the predictive significance of NLR (0.871 (95% CI: 0.767–0.974); $p < 0.0001$) and MLR (0.848 (95% CI: 0.737–0.958); $p < 0.0001$) indices for detecting CE in infertility patients.

Keywords: chronic endometritis, infertility, inflammatory status, hematological inflammatory indexes

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Compliance with ethical standards: the study was approved by the Ethics Committee of St. Petersburg State Medical University (Minutes No. 18/07 of October 27, 2022), conducted in accordance with the federal laws of the Russian Federation (No. 152, 323, etc.) and Declaration of Helsinki 1964, with all subsequent additions and amendments regulating scientific research on biomaterials obtained from humans. All participants signed an informed consent form before participating in the study.

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ОСОБЕННОСТИ СИСТЕМНОГО ВОСПАЛИТЕЛЬНОГО СТАТУСА ПАЦИЕНТОК ПРИ ЭНДОМЕТРИТ-АССОЦИИРОВАННОМ БЕСПЛОДИИ

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Среди заболеваний эндометрия, снижающих его репродуктивный потенциал, ведущую роль играет хронический эндометрит (ХЭ). Патогенетически значимые воспалительные маркеры, такие как цитокины, воспалительные белки и гематологические индексы, пока не нашли широкого применения в медицинской практике для диагностики ХЭ. Цель исследования — оценить у пациенток с бесплодием показатели системного воспалительного статуса, включая гематологические лабораторные параметры и воспалительные индексы, и определить их диагностическую значимость при ХЭ. Представлены данные исследования 50 пациенток с бесплодием. Подсчет количества лейкоцитов крови проводили стандартным гематологическим методом. На основе клеточного состава крови рассчитывали воспалительные индексы NLR, MLR, PLR и SII. Концентрацию СРБ в сыворотке крови определяли методом иммуноферментного анализа. У пациенток с бесплодием, ассоциированным с ХЭ, абсолютное и относительное количество лимфоцитов было ниже ($p = 0{,}0451$ и $p = 0{,}0089$ соответственно), а относительное количество моноцитов и базофилов было выше ($p = 0{,}0469$ и $p = 0{,}0005$ соответственно), чем у пациенток без ХЭ. У пациенток из исследуемой группы концентрация СРБ в крови была почти в 4 раза выше, чем у пациенток из группы сравнения ($p = 0{,}0191$). При этом все показатели оставались в пределах нормальных значений. Сравнительный анализ индексов NLR, MLR, PLR и SII показал их статистически значимое повышение при бесплодии, ассоциированном с ХЭ ($p = 0{,}0387$, $p = 0{,}0058$, $p = 0{,}0335$ и $p = 0{,}0333$ соответственно). ROC-анализ позволил установить предиктивную значимость у индексов NLR (0,871 (95% ДИ: 0,767–0,974); $p < 0{,}0001$) и MLR (0,848 (95% ДИ: 0,737–0,958); $p < 0{,}0001$) для выявления ХЭ у пациенток с бесплодием.

Ключевые слова: хронический эндометрит, бесплодие, воспалительный статус, гематологические воспалительные индексы

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Currently, there is a clear upward trend in cases of pelvic inflammatory disease becoming chronic among women, with the immune and endocrine systems involved in the pathological process [1]. This adversely affects the reproductive function of patients and can lead to infertility.

Chronic endometritis (CE) is the most significant endometrial disease in terms of its impact on reproductive potential. According to various sources, from 0.2 to 46.3% of women of reproductive age suffer from CE [2]. The considerable breadth of this range arises from variations in diagnostic approaches among researchers. Chronic endometritis is a persistent low-intensity inflammation of the endometrium with infiltration of the stroma by immunocompetent cells, leading to disruption of its cyclic biotransformation and receptivity [2, 3].

Currently, the diagnosis, prevention, and restoration of reproductive function in patients with CE are considerably complex matters. This pathology is often asymptomatic, or presents with nonspecific symptoms such as chronic pelvic pain, mucous or mucopurulent vaginal discharge, dyspareunia, abnormal uterine bleeding, or recurrent cystitis [1]. An objective assessment of the prevalence of CE is hampered by the said lack of symptoms (in most cases) and the need for a biopsy to verify the diagnosis, as a definitive conclusion requires pathomorphological examination [3].

In recent years, the number of patients with CE-associated infertility has been steadily increasing. It has been established that from 2.8 to 56.8% of women with confirmed CE are diagnosed infertile [4]. In cases of infertility associated with a chronic inflammatory process, laboratory test results may appear normal, clinical symptoms may be absent, yet immune cell function is often disrupted at both local and systemic levels. The resulting destructive immune-inflammatory process requires appropriate anti-inflammatory and immunomodulatory therapy [5–6]. In addition, CE is one of the causes of spontaneous pathological termination of pregnancy and repeated implantation failures in the context of using assisted reproductive technology to treat infertility [7]. Nevertheless, this disease is still not on the list of conditions checked for before in vitro fertilization (IVF).

The foregoing necessitates the development of new methods for diagnosing and treating CE-associated endometrial pathology and disorders of endometrial receptivity. One of the promising areas of research is determination of the inflammatory status of infertile women. Hematological inflammatory indices can be used for this purpose in clinical practice [8]. For example, the NLR inflammatory index, defined as the ratio of neutrophils to lymphocytes, serves as an indicator of systemic inflammation and reflects the overall state of the immune system under various stress conditions [9]. The PLR index is the ratio of platelets to lymphocytes; platelets are a source of TGFb and VEGF, which contribute to the chronicity of the inflammatory process [10]. The use of these indices as markers of chronic inflammation has become increasingly common [8]. The MLR index, which is the ratio of monocytes to lymphocytes, is not as popular. This index is associated with systemic inflammation [11]. Another inflammatory marker that clinicians have begun to use more frequently in recent years is the SII. When its value is high, the patient's immune function is suppressed and inflammatory processes are activated [12].

Thus, hematological inflammatory indices may prove to be highly informative systemic markers of chronic subclinical inflammation associated with CE, which could facilitate the development of new strategies for anti-inflammatory and immunomodulatory therapy and methods of restoration of reproductive function.

This study aimed to evaluate indicators of systemic inflammation, including hematological parameters and inflammatory indices, in patients with infertility, and to determine their diagnostic significance for CE.

METHODS

Patients

We analyzed the examination results of 50 patients aged 24–43 years (median age 37 [32–40] years). The study group included 27 patients diagnosed with CE-associated infertility, while the control group comprised 23 patients without signs of CE, whose infertility was attributed to the male factor. The participants were women preparing for IVF in the Department of Assisted Reproductive Technologies. The patients underwent ultrasound examination with Doppler assessment of the uterine arteries on the 12th day or later of the menstrual cycle, followed by a Pipelle biopsy of the endometrium, and histological and immunohistochemical examination. Furthermore, all patients had urethral, cervical canal, and vaginal secretions examined by bacterioscopy and PCR (for bacterial and viral infections), as well as by light microscopy (for HPV, CMV, and HSV antigens) using an Axio Lab.A1 microscope (Carl Zeiss, Germany). This step was intended to exclude the influence of infectious factors on the overall results of the study. The patients in both groups were comparable in age, weight, and medical history, including menstrual and reproductive function and the presence of chronic somatic diseases. Inclusion criteria: CE-associated female infertility; infertility associated with the male factor (no signs of CE). Exclusion criteria: bacterial and viral infections of the genitourinary tract; other infectious, allergic, autoimmune, inflammatory, oncological, mental diseases; hormonal, anti-inflammatory therapy; malformations of the genitals. The clinical and diagnostic characteristics of the patients are given in Table 1.

Assessment of parameters of systemic inflammatory status, including inflammatory indices

All patients underwent quantitative determination of blood leukocyte subsets by a standard hematological method with a hematology analyzer (Abbott Laboratories, USA).

Based on the cellular composition of peripheral blood, we calculated the following inflammatory indices: NLR (neutrophil-lymphocyte ratio); PLR (platelet-lymphocyte ratio); MLR (monocyte-lymphocyte ratio); SII (systemic inflammatory index) [10–12].

The indices were calculated using these formulas:

NLR = absolute neutrophil count / absolute lymphocyte count;
PLR = absolute platelet count / absolute lymphocyte count;
MLR = absolute monocyte count / absolute lymphocyte count;
SII = absolute platelet count — absolute neutrophil count / absolute lymphocyte count.

The concentration of CRP in blood serum was measured by enzyme immunoassay using the CRP-IFA kit (KHEMA, Russia) in accordance with the manufacturer's instructions and expressed in mg/ml.

Statistical analysis of the data

For statistical data processing, we used IBM SPSS Statistics 22 (Armonk; USA) and GraphPad Prism 8.3.1 (GraphPad Software; USA). The normality of distribution of all the data

Table 1. Characteristics of patients with infertility

Parameter	Parameter value	Patients with CE (n = 27)	Patients without CE (n = 23)
Median age, years		40,0 (34,0–42,0)	34,0 (30,0–38,0)
Median duration of infertility, years		4,0 (2,0–6,0)	3,0 (2,0–5,0)
Ultrasound signs of CE, frequency of occurrence, % (abs.)	yes	59.3% (16)	0% (0)
	no	40.7% (11)	100% (23)
IHC signs of CE, frequency of occurrence, % (abs.)	yes	100% (27)	–
	no	0% (0)	–
History of infectious diseases of the genitourinary tract	yes	33.3% (9)	4.3% (1)
	no	33.7% (18)	95.7% (22)

considered was checked with the Shapiro–Wilk test. The parameters were described using the median (Me) and the interquartile range (Q_1–Q_3). To assess the differences in the parameters, we used the Mann-Whitney test. The differences were considered significant at $p < 0.05$ (5%).

We used ROC to establish diagnostic significance of the systemic inflammatory status parameters in predicting the presence of CE. The cut-off value for the quantitative marker was the one that maximized Youden's index. The differences were considered significant at $p < 0.05$ (5%).

RESULTS

A comparative analysis of systemic inflammatory parameters — including ESR, total platelet count, leukocyte count, and the absolute and relative numbers of leukocyte subsets — revealed significant differences between patients with and without CE (Table 2). In patients with CE-associated infertility, the absolute and relative lymphocyte counts were significantly lower than those in patients without CE (p = 0.0451 and p = 0.0089, respectively). At the same time, the relative number of monocytes and basophils in the study group significantly exceeded that in the control group (p = 0.0469 and p = 0.0005, respectively). In addition, blood CRP levels in the study group were about four times those in the control group (p = 0.0191) (Table 2). All indicators in both groups remained within the reference ranges.

A comparative analysis of hematological inflammatory indices in patients with CE-associated infertility and patients without CE also revealed significant intergroup differences. In the study group, the NLR (p = 0.0387), MLR (p = 0.0058), PLR (p = 0.0335) and SII (p = 0.0333) indices were significantly higher than those of the control group (Table 3).

We performed ROC analysis and determined the optimal threshold values of the evaluated parameters to assess the diagnostic significance of hematological inflammatory indices in detecting subclinical endometrial inflammation.

The analysis demonstrated a significant association of NLR and MLR indices with chronic endometrial inflammation among infertile patients. NLR values above the threshold of 1.56 absolute units were associated with the probability of CE (Table 4; Fig. 1). The area under curve was 0.871 (95% CI: 0.767–0.974), sensitivity — 87.0%, specificity — 73.7%. The resulting model was statistically significant ($p < 0.0001$).

A similar relationship was found for the MLR index. ROC analysis showed that MLR values exceeding 0.24 absolute units are an independent sign of a high risk of CE in patients with infertility (Table 4; Fig. 2). The area under curve was 0.848 (95% CI: 0.737–0.958), sensitivity — 80.0%, specificity — 75.0%. The resulting model was statistically significant ($p < 0.0001$).

Our assessment of the diagnostic applicability of the PLR and SII indices revealed no significant association between their values and the presence of chronic endometrial inflammation ($p > 0.05$) (Table 4). In addition, we found no diagnostic significance for such cellular parameters of systemic inflammation as the total platelet and leukocyte counts, the absolute and relative numbers of different leukocyte subsets, or ESR and CRP ($p > 0.05$).

Table 2. Hematological parameters of systemic inflammation in patients with infertility (Me (Q_1–Q_3))

Indicator	Patients with CE (n = 27)	Patients without CE (n = 23)	p
ESR, mm/h	6.0 (3.0–15.0)	10.0 (2.0–15.0)	0.8879
CRP, mg/l	2.25 (1.03–3.80)	0.60 (0.06–0.82)	0.0191
Absolute cell count, 10^9/l			
Platelets	255.0 (226.0–287.0)	262.0 (235.0–297.0)	0.2633
Leukocytes	5.7 (4.8–6.8)	6.0 (5.3–6.7)	0.7392
Neutrophils	3.4 (2.6–3.9)	3.1 (2.7–3.8)	0.7028
Lymphocytes	1.6 (1.4–2.2)	2.0 (1.7–2.4)	0.0451
Monocytes	0.50 (0.40–0.50)	0.40 (0.33–0.49)	0.0963
Eosinophils	0.10 (0.10–0.20)	0.11 (0.07–0.17)	0.9635
Basophils	0.00 (0.00–0.10)	0.02 (0.00–0.03)	0.8979
Relative cell count, %			
Neutrophils	58.1 (54.1–61.9)	56.1 (49.5–59.3)	0.1012
Lymphocytes	29.5 (26.8–34.1)	36.0 (31.0–40.0)	0.0089
Monocytes	8.3 (7.1–9.7)	7.4 (5.5–8.4)	0.0469
Eosinophils	2.1 (1.7–2.7)	2.1 (1.5–3.1)	0.7098
Basophils	0.7 (0.5–1.0)	0.4 (0.1–0.5)	0.0005

Table 3. Parameters of systemic inflammatory status in patients with infertility (Me (Q_1–Q_3))

Indicator	Patients with CE (n = 27)	Patients without CE (n = 23)	p
Inflammatory indices, abs. u.			
NLR	1.93 (1.50–2.38)	1.55 (1.30–1.93)	0.0387
MLR	0.27 (0.23–0.30)	0.20 (0.14–0.27)	0.0058
PLR	148.7 (122.6–183.0)	126.8 (111.1–138.7)	0.0335
SII	469.9 (385.3–628.0)	396.4 (329.8–511.7)	0.0333

DISCUSSION

Inflammation plays a key role in the pathogenesis of endometritis-associated infertility and contributes to the risk of adverse reproductive outcomes. It has been shown to suppress the expression of estrogen and progesterone receptors in the endometrium, thereby delaying its maturation and hindering embryo implantation [13].

The diagnosis of CE is a complicated process: the disease is often asymptomatic, ultrasound examination is insufficiently effective, and there are no uniform diagnostic criteria. The absence of signs of CE on ultrasonography cannot be interpreted as the absence of endometrial inflammation; this limitation necessitates an invasive and costly procedure, aspiration biopsy, to obtain pathomorphological confirmation of the diagnosis [14].

Recently, hematological inflammatory indices have been proposed as new biomarkers for various socially significant diseases accompanied by a subclinical inflammation. There is evidence of the usefulness of these indices in the prognosis of cardiovascular [15], oncological [16], and mental [17] diseases, as well as systemic inflammatory diseases of connective tissue [18]. Hematological inflammatory indices are non-invasive, inexpensive, easy to calculate, and require no specialized equipment.

In the context of non-malignant gynecological pathologies, the diagnostic and predictive significance of hematological inflammatory indices has been largely unstudied. Recent studies in the field of obstetrics and gynecology have demonstrated their benefits in the cases of endometriosis, ovarian hyperstimulation syndrome, as well as the adverse course and complications of pregnancy (in particular, preeclampsia) [8]. However, neither Russian nor foreign sources contain any data on the applicability of the said indices in CE cases. Thus, it is reasonable to continue investigating their prognostic significance in patients with chronic inflammatory diseases of the endometrium, particularly in the context of infertility treatment using assisted reproductive technologies.

When the inflammation is subclinical and chronic, the clinical significance of hematological cellular and molecular parameters is low. Earlier, we found significant differences in the concentrations of CRP and the counts of lymphocytes, monocytes, and basophils between the study and control groups; however, these indicators were ultimately deemed uninformative, as their values remained within the reference range.

At the same time, the values of the inflammatory hematological indices NLR, PLR, MLR and SII in these groups were significantly different (Table 3), and NLR and MLR (Table 4, Figures 1 and 2) were deemed diagnostically significant. NLR reflects the balance of the innate and adaptive immune response to various stressors. Its value characterizes the severity of the systemic inflammatory reaction [19]. The increase of the said value in women with CE-associated infertility may be caused by the dropping number of lymphocytes in the blood as they are constantly recruited to the locus of chronic inflammation. A declining NLR may reflect attenuation of the inflammatory response and can be associated with a more favorable prognosis. MLR is the ratio of monocytes to lymphocytes in the blood. Monocytes do not circulate in the blood for long; they migrate into tissues, replenishing the pool of tissue macrophages. In addition, they can differentiate into dendritic cells and tissue-specific phagocytes [20]. Monocytes/macrophages are responsible for phagocytosis and removal of damaged cells, processing of antigens, stimulation of T- and B-cell immune responses, and also secrete regulatory and effector cytokines that ensure the development of a systemic inflammatory response [21]. In patients with CE-associated infertility, a high MLR may have two causes: first, an insufficient rate of monocyte migration from the blood to the site of chronic inflammation; and second, as mentioned above, redistribution of lymphocytes between the blood and the inflamed endometrium. This theory is supported by the decreased lymphocyte counts and increased monocyte counts observed in the blood of patients with CE (Table 2).

Introduction of the inflammatory indices to the laboratory testing and clinical practice requires further validation of the discriminatory levels of these indicators in an independent sample of patients suffering from various forms of infertility. However, it can already be argued that the NLR and MLR indices are applicable in the treatment of infertile women to identify those with asymptomatic CE even if they have normal hematological parameters and no signs of the inflammatory process on the ultrasonography. For such patients, it is feasible to perform additional immunohistochemical examination of the endometrium to confirm the diagnosis and prescribe anti-inflammatory therapy before IVF.

CONCLUSIONS

Patients with infertility associated with chronic endometrial inflammation had a higher relative number of monocytes and basophils, and a lower absolute and relative number of lymphocytes than patients without signs of chronic endometritis, but the values of these indicators remained within the normal

Table 4. Characteristics of the predictive significance of inflammatory hematological indices for detecting the presence of chronic endometrial inflammation in patients with infertility

Indicator	cut-off	AUC	95% CI	p	Sensitivity, %	Specificity, %
NLR	1.56	0.871	0.767–0.974	< 0.0001	87	73.7
MLR	0.24	0.848	0.737–0.958	< 0.0001	80	75
PLR	130.4	0.679	0.528–0.831	0.0339	61.5	54.6
SII	469	0.683	0.531–0.837	0.0333	52	61.9

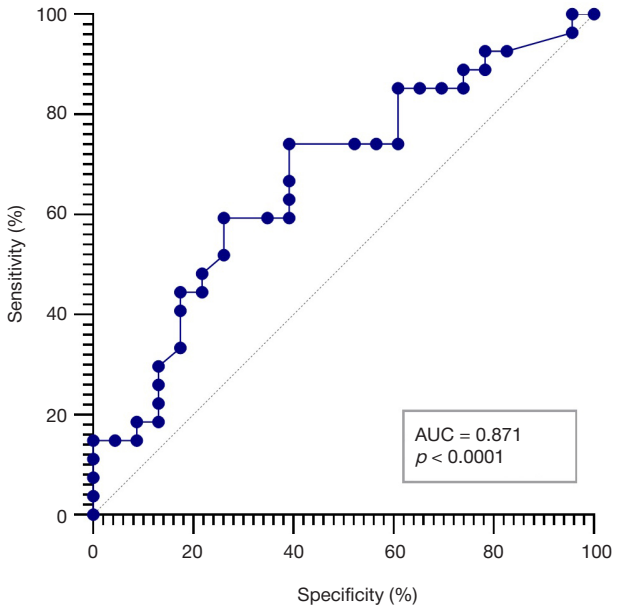

Fig. 1. ROC curve of predictive significance of the NLR index for detecting the presence of chronic endometritis in patients with infertility

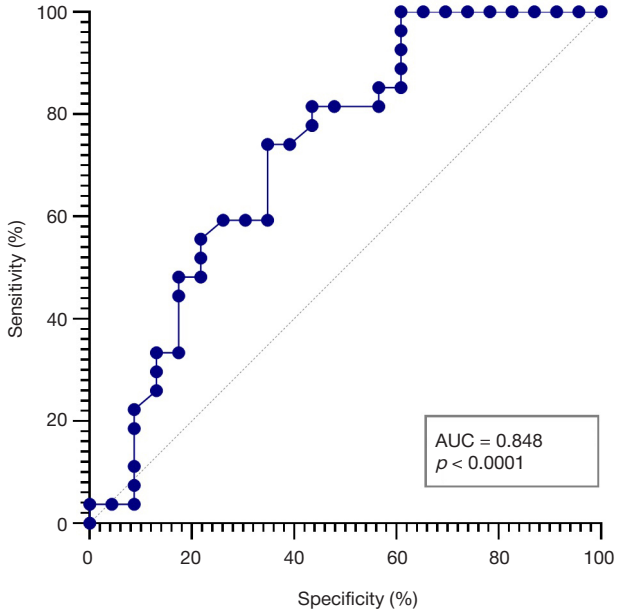

Fig. 2. ROC curve of predictive significance of the MLR index for detecting the presence of chronic endometritis in patients with infertility

range. A comparative analysis of hematological inflammatory indices showed a significant increase in their value in cases of CE-associated infertility. However, ROC analysis allowed establishing that only NLR and MLR indices can be used as predictors of CE in infertile patients, as other inflammatory markers remain within normal values. Ultimately, it should be noted that even when hematological cellular parameters are normal and ultrasound signs of endometritis are absent, infertile patients may still have chronic subclinical endometrial inflammation — a condition that can be ruled out by assessing the NLR and MLR indices. These markers may be useful in clinical practice for screening IVF patients. The assessment of these indices will make it possible to identify individuals at high risk of asymptomatic chronic endometritis, refer them for further immunohistochemical examination, and, if necessary, provide appropriate treatment before IVF. This approach may help reduce the risk of IVF failure.

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