OBSTETRICS AND GYNAECOLOGY

DIAGNOSTIC SIGNIFICANCE OF INTERLEUKIN LEVELS IN BLOOD SERUM IN PREMENOPAUSAL WOMEN WITH CHRONIC ENDOMETRITIS AND NORMAL WEIGHT OR OVERWEIGHT

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ABSTRACT

Background. *Chronic endometritis (CE) is an inflammatory hysteropathy causing miscarriage and infertility. High invasiveness of the main method of CE diagnosis and vague clinical picture necessitate the need for the development of less invasive approaches to establish the presence of this disease.*

The aim of the study. *To establish a significant association of the concentration of pro- and anti-inflammatory interleukins in the blood serum with the presence of chronic endometritis in premenopausal women without concomitant endocrine diseases.*

Materials and methods. *This re-analysis of the data is based on the results of a cross-sectional study conducted between May 2017 and December 2019 which included 198 premenopausal women. In all participants, body weight and height were measured with the calculation of the body mass index, the concentration of Creactive protein, pro- and anti-inflammatory cytokines in the blood serum was determined, and a pipelle biopsy was performed to determine the CD138 expression in the endometrial stroma. Non-parametric methods, as well as ROC analysis, were used for statistical analysis.*

Results. *Eighty six women were included in the re-analysis of the data, 37 of them had a confirmed diagnosis of chronic endometritis. Statistically significantly higher values of interleukin 1 (IL-1) concentration ($p = 0.0028$) and IL-1/tumor necrosis factor α ratio ($p < 0.001$) were determined in women with CE and normal body weight; threshold values of these parameters were ≥ 1.35 pg/ml (sensitivity 75 %, specificity 83 %; 95% confidence interval (95% CI): 0.88–2.15) and ≥ 1.03 (sensitivity 85 %, specificity 78 %; 95% CI: 0.81–1.27) respectively. Such a relationship was not revealed in women with overweight.*

Conclusions. *The obtained results can be the basis for conducting a larger-scale study with determining the concentration of cytokines not only in the blood serum, but also in the endometrium of women with CE, which will allow the development of a minimally invasive method for determining the risk of the presence of chronic endometritis in premenopausal women.*

Key words: *chronic endometritis, interleukin 1, tumor necrosis factor α, cytokines, chronic inflammation, overweight*

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ДИАГНОСТИЧЕСКАЯ ЗНАЧИМОСТЬ УРОВНЕЙ ИНТЕРЛЕЙКИНОВ В СЫВОРОТКЕ КРОВИ У ЖЕНЩИН РЕПРОДУКТИВНОГО ВОЗРАСТА С ХРОНИЧЕСКИМ ЭНДОМЕТРИТОМ И НОРМАЛЬНОЙ ИЛИ ИЗБЫТОЧНОЙ МАССОЙ ТЕЛА

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РЕЗЮМЕ

Обоснование. *Хронический эндометрит (ХЭ) является воспалительным заболеванием матки, приводящим к невынашиванию беременности и бесплодию. В связи с высокой инвазивностью основного метода диагностики ХЭ и смазанностью клинической картины является актуальной разработка менее инвазивных подходов установления наличия данного заболевания.*

Цель исследования. *Установление значимой ассоциации концентрации про- и противовоспалительных интерлейкинов в сыворотке крови с наличием хронического эндометрита у женщин репродуктивного возраста без сопутствующих эндокринных заболеваний.*

Материалы и методы. *Настоящий ре-анализ данных проведён на основе результатов кросс-секционного исследования, проведённого в период с мая 2017 по декабрь 2019 г. с участием 198 женщин репродуктивного возраста. У всех участниц измеряли массу тела и рост с расчётом индекса массы тела, определяли концентрацию С-реактивного белка, про- и противовоспалительных цитокинов в сыворотке крови и проводили пайпель-биопсию с определением экспрессии CD138 в строме эндометрия. Для статистического анализа использовали непараметрические методы анализа, а также ROC-анализ.*

Результаты. *В ре-анализ данных включили 86 женщин, из которых у 37 подтвердили диагноз ХЭ. У женщин с ХЭ на фоне нормальной массы тела установлены статистически значимо более высокие значения концентрации интерлейкина (ИЛ) 1 ($p = 0,0028$) и отношения ИЛ-1/фактор некроза опухоли α ($p < 0,001$) с пороговыми значениями данных параметров ≥ 1,35 пг/мл (чувствительность 75 %, специфичность 83 %; 95%-й доверительный интервал (95% ДИ): 0,88; 2,15) и ≥ 1,03 (чувствительность 85 %, специфичность 78 %; 95% ДИ: 0,81; 1,27) соответственно. У женщин с избыточной массой тела такой зависимости не выявлено.*

Выводы. *Полученные результаты могут быть основой для проведения более масштабного исследования с установлением концентрации цитокинов не только в сыворотке крови, но и в эндометрии женщин с ХЭ, что позволит разработать малоинвазивный метод определения риска наличия данного заболевания у женщин репродуктивного возраста.*

Ключевые слова: *хронический эндометрит, интерлейкин 1, фактор некроза опухоли α, цитокины, хроническое воспаление, избыточная масса тела*

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BACKGROUND

One of the main consequences of chronic endometritis (CE) is a decrease in endometrial receptivity against the background of inflammation, which can subsequently cause the development of miscarriage and infertility in women of reproductive age. This occurs as a result of increased expression of proinflammatory cytokines in the uterine cavity, which suppress endometrial growth and angiogenesis factors and lead to dysregulation of the decidualization processes of uterine mucosa cells. These processes cause thinning of the endometrium and reduce its ability to implant [1–3].

CE is a disease that causes difficulties for doctors, since along with decreased receptivity and an increased risk of infertility, this condition is very difficult to diagnose due to non-specific symptoms, and in some women it may not manifest itself clinically. Based on this, it is difficult to establish the exact prevalence of this disease in the general population. According to available data, CE occurs on average in 10-11% of women of reproductive age [4].

To date, the most effective and reproducible method for diagnosing CE is a pipelle biopsy to determine the content of plasma cells expressing CD138 in the endometrial stroma [5, 6]. Despite the high sensitivity and specificity of this research method, there are some difficulties in its use. In particular, the procedure for collecting endometrial samples for analysis is a minor gynecological operation and requires indications for appointment [6, 7]. In this regard, the risk of missing the presence of the disease in the patient is quite high.

For many years, researchers have been trying to develop less invasive and more accessible methods for diagnosing CE, including finding a relationship between some blood serum parameters and the presence of this disease [8, 9]. Of particular interest are serum levels of pro- and anti-inflammatory cytokines, since their imbalance is recorded in the uterine mucosa of women with CE [10]. Some researchers have found an increase in the concentration of proinflammatory cytokines in menstrual or venous blood in patients with CE [11]. However, these studies did not take into account additional parameters that may affect the concentration of cytokines in the blood, such as systemic inflammation due to the presence of concomitant diseases, including hormonal disorders and obesity [12, 13].

Thus, **the aim of this study** was to establish a significant association between the concentration of pro- and anti-inflammatory interleukins in the blood serum and the presence of chronic endometritis in women of reproductive age without concomitant endocrine diseases.

MATERIALS AND METHODS

Research design

A cross-sectional study, described in detail in our previously published works [8, 14], was conducted from May 2017 to December 2019. A total of 198 women of reproductive age (33.71 ± 5.93 years) were examined. All participants were recruited during annual preventive examinations at the Scientific Centre for Family Health and Human Reproduction Problems. All women who took part in the study signed informed consent for the examination. In working with patients, the ethical principles set out in the World Medical Association Declaration of Helsinki (1964, 2013 edition), the Federal Law of the Russian Federation of 11-21-2011 No. 323-FZ «On the Fundamentals of Health Protection of Citizens in the Russian Federation» and the «Rules of Clinical Practice in the Russian Federation», approved by Order of the Ministry of Health of Russia dated 06-19-2003 No. 266 were observed. This study was approved by the local Ethics Committee of the Scientific Centre for Family Health and Human Reproduction Problems (protocol No. 2.1 dated February 24, 2016).

The inclusion criteria for this reanalysis were: the presence or absence of CE; the presence of data on body mass index (BMI) and serum interleukin concentration. The exclusion criterion was the presence of a concomitant diagnosis of polycystic ovary syndrome in patients. At the time of the study, all patients had no signs of acute local or systemic inflammation.

Instrumental methods

Instrumental and clinical research methods have been described in detail previously [8, 14]. Based on the examinations conducted, BMI was calculated for all patients, and the diagnosis of CE was established based on the results of morphological and immunohistochemical studies of endometrial samples.

Laboratory methods

Blood serum for research was obtained by centrifuging tubes at 3000 rpm for 10 min. The serum was stored in disposable Eppendorf tubes at a temperature of –80°C. Quantitative determination of the concentration of interleukin (IL) 1, IL-6, IL-8, tumor necrosis factor (TNF) α, interferon (INF), and C-reactive protein (CRP) was performed using test systems of Vector-Best LLC (Russia) on an ELx808 enzyme immunoassay analyzer (BioTek, USA) according to the manufacturer's instructions.

Statistical methods

The statistical analysis included descriptive statistics, testing of statistical hypotheses, ROC analysis and determination of the odds ratio (OR). The Kolmogorov – Smirnov test was used to determine the proximity to the normal distribution law of continuous variables. The description of continuous variables is presented as the median and the lower and upper quartiles. To test the statistical hypothesis of equivalence in the location of two general populations for independent random samples, the Mann – Whitney U-test was used, and for several general populations, the Kruskal – Wallis test was used. To establish a correlation between the values of the BMI and CRP parameters,

the Spearman's rank correlation test was used. To establish the threshold values of the parameters and their 95% confidence intervals (95% CI), the characteristic curve analysis (ROC analysis) was performed. Sensitivity and specificity were calculated; the area under the ROC curve (AUC) was estimated. The OR was also calculated for each threshold value.

RESULTS

Of the 198 participants, data from 86 women were used for the present reanalysis according to the inclusion and exclusion criteria (fig. 1).

The women were then divided into two groups depending on the presence or absence of CE, and the values of serum pro- and anti-inflammatory cytokine concentrations, as well as their relationships between the groups, were compared (table 1).

Based on the comparison results, we found statistically significantly higher values of IL-1 concentration ($p = 0.0027$) and IL-1/TNF-α ratio ($p < 0.0001$) in women with CE compared to the values of these parameters in women in the control group.

In order to assess at what value of IL-1 concentration or IL-1/TNF-α ratio the presence of CE is observed in women, a ROC analysis was conducted, and based on the results we determined the threshold values for the analyzed parameters (fig. 2).

Thus, in women with CE, the IL-1 concentration values were ≥ 1.3 pg/ml (sensitivity 78 %, specificity 59 %; 95% CI: 0.76; 2.15 pg/ml), and the IL-1/TNF-α ratio was ≥ 0.89 (sensitivity 81 %, specificity 63 %; 95% CI: 0.69; 1.37). We also calculated the OR for the established threshold values, which for IL-1 was 3.732 (95% CI: 1.503; 9.267), for the IL-1/TNF-α ratio – 6.243 (95% CI: 2.356; 16.546).

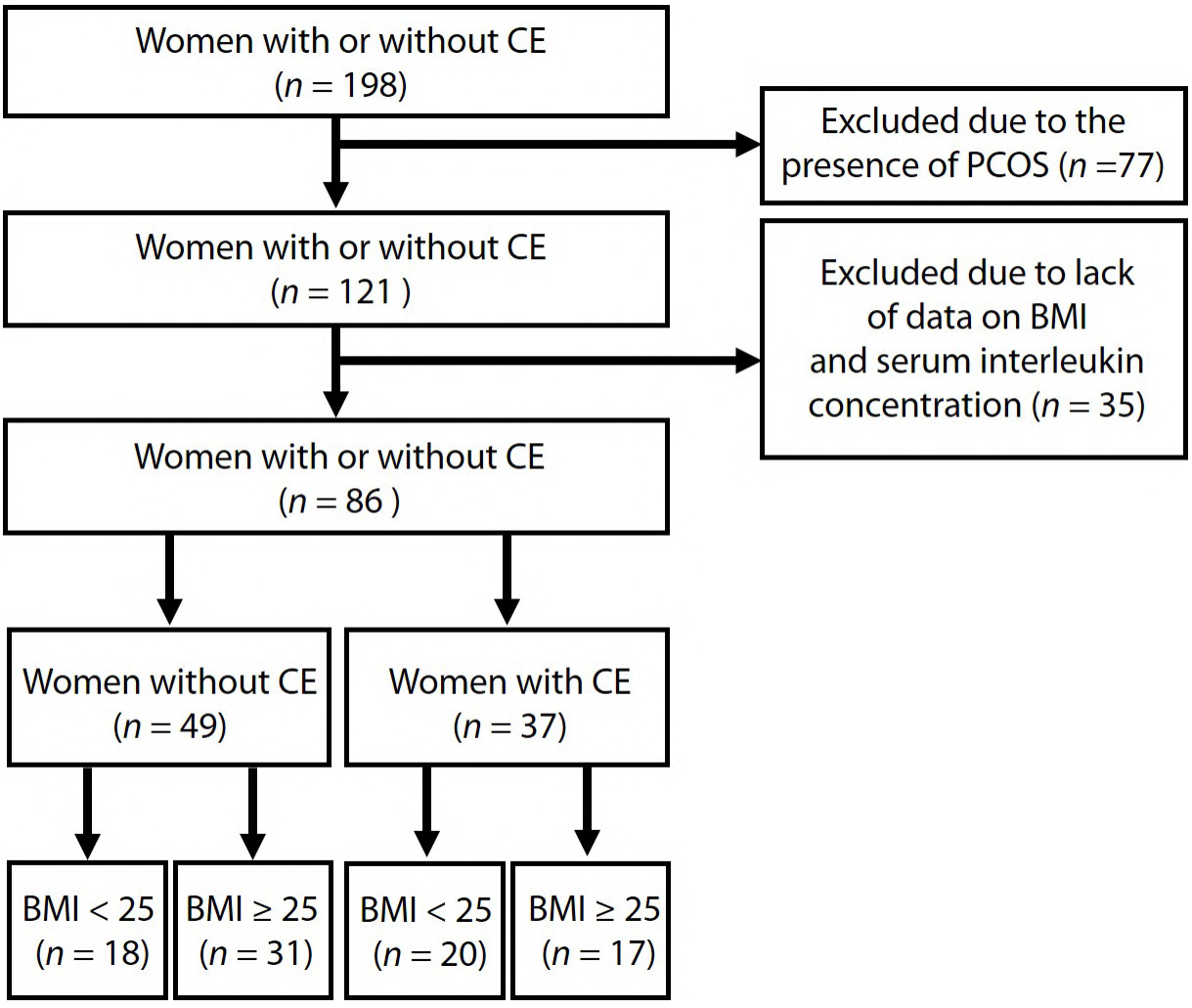

FIG. 1.
Study design: PCOS – polycystic ovarian syndrome

TABLE 1

MAIN CHARACTERISTICS AND CONCENTRATION OF INTERLEUKINS IN PREMENOPAUSAL WOMEN WITH OR WITHOUT CHRONIC ENDOMETRITIS

Parameters	Control (*n* = 49)	CE (*n* = 37)	*p*
Age, years	38 (34; 41)	39 (36; 43)	0.330
BMI, kg/m^2	25.92 (22.51; 30.14)	23.84 (21.05; 28.38)	0.105
	Proinflammatory cytokines		
IL-1, pg/ml	**0.95 (0,70; 1,85)**	**1.7 (1.10; 2.25)**	**0.0027**
IL-6, pg/ml	1.4 (0.75; 2.25)	0.9 (0.50; 2.10)	0.1640
IL-8, pg/ml	14 (6.75; 33.00)	13 (7.30; 28.30)	0.7762
TNF-α, pg/ml	1.7 (1.30; 2.20)	1.3 (0.85; 1.85)	0.0976
INF, pg/ml	0.6 (0.30; 1.05)	0.9 (0.35; 1.40)	0.1271
	Anti-inflammatory cytokines		
IL-10, pg/ml	1.5 (0.27; 2.25)	1.6 (0.70; 3.40)	0.3135
	Relationships between pro- and anti-inflammatory cytokines		
INF/IL-10	0.44 (0.145; 0.790)	0.43 (0.205; 0.89)	0.6712
IL-6/IL-10	1 (0.52; 1.55)	0.46 (0.255; 1.435)	0.0862
IL-1/IL-10	0.68 (0.275; 1.465)	0.88 (0.54; 1.89)	0.1496
IL-8/IL-10	9.6 (5.75; 35.31)	7.5 (2.58; 22.00)	0.1608
IL-6/TNF-α	0.73 (0.510; 1.820)	0.64 (0.395; 1.450)	0.5163
IL-10/TNF-α	0.94 (0.310; 1,680)	1 (0.470; 2.630)	0.1788
IL-1/TNF-α	**0.77 (0.43; 1.1)**	**1.19 (0.890; 2.295)**	**< 0.0001**

Note. Data are presented as medians and lower and upper quartiles; p is the level of statistical significance when comparing parameter values between groups using the Mann – Whitney test.

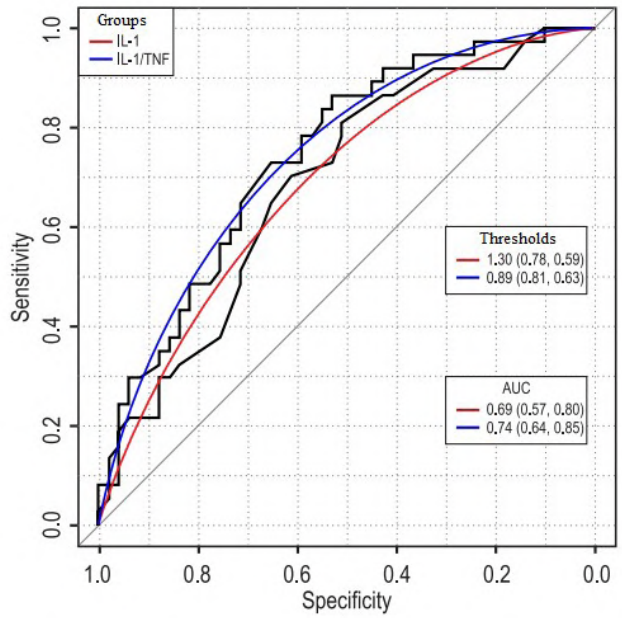

FIG. 2.
ROC curve of IL-1 concentration or IL-1/TNF-α ratio in premenopausal women with or without chronic endometritis against the background of normal weight or overweight: IL1 – ROC curve of IL-1 concentration in patients with or without chronic endometritis; IL1/TNF – ROC curve of IL-1/TNF-α ratio in patients with or without chronic endometritis; the cut-off point for IL-1 concentration is 1.3 (95% CI: 0.76; 2.15); the cut-off point for IL-1/TNF-α ratio is 0.89 (95% CI: 0.69; 1.37)

The low specificity of the obtained cut-off points could be due to the fact that the groups included both women with normal body weight and overweight and obese women, who could have chronic inflammation against the background of elevated BMI values. It should be noted that we did not find statistically significant differences in BMI values in the studied groups: 25.92 (22.51; 30.14) kg/m^2 in healthy women versus 23.84 (21.05; 28.38) kg/m^2 in patients with CE (p = 0.105). Therefore, we further compared the values of interleukin concentrations and their ratios between women with or without CE depending on BMI. We also assessed the concentration of CRP in the blood as a marker of chronic systemic inflammation in overweight and obesity in women of the studied groups (table 2) [15].

TABLE 2

MAIN CHARACTERISTICS AND CONCENTRATION OF INTERLEUKINS IN PREMENOPAUSAL WOMEN WITH OR WITHOUT CHRONIC ENDOMETRITIS DEPENDING ON BODY MASS INDEX

Parameters	Control (n = 49)		CE (n = 37)		
	BMI < 25 kg/m^2 (n = 18) 1	BMI ≥ 25 kg/m^2 (n = 31) 2	BMI < 25 kg/m^2 (n = 20) 3	BMI ≥ 25 kg/m^2 (n = 17) 4	p^a
Age	38 (33.5; 41)	38 (34; 41)	39 (36; 43)	39 (36; 42.75)	p_{all} = 0.4031
BMI, kg/m^2	**21.48 (20.13; 23.29)**	**28.42 (26.27; 31.78)**	**21.18 (20.06; 23.28)**	**28.79 (27.12; 29.77)**	**p_{all} < 0.001**
					p_{1-3} = 0.704^b
p^b	**< 0.001**		**< 0.001**		**p_{1-4} < 0.001^b**
					p_{2-4} = 0.1069^b
	Proinflammatory cytokines				
IL-1, pg/ml	**0.8 (0.5; 1.25)**	**1.2 (0.72; 1.9)**	**1.65 (1.013; 2.975)**	**1.8 (1.1; 2.15)**	**p_{all} = 0.0103**
					p_{1-3} = 0.0059^b
p^b	0.1187		0.982		**p_{1-4} = 0.0024^b**
					p_{2-4} = 0.1069^b
IL-6, pg/ml	0.955 (0.575; 1.825)	1.5 (1.04; 2.4)	0.9 (0.425; 2.5)	0.9 (0.55; 2.1)	0.2159
IL-8, pg/ml	18.5 (6.775; 45.4)	12 (5.4; 33)	12 (7.05; 30)	14 (7.65; 28.3)	0.7762
TNF-α, pg/ml	1.8 (1.325; 2.4)	1.7 (1.3; 2)	1.3 (0.8; 1.775)	1.4 (1; 2)	0.342
INF, pg/ml	0.75 (0.25; 1.35)	0.6 (0.3; 0.9)	0.85 (0.325; 1.575)	0.9 (0.4; 1.3)	0.4036
CRP, IU/l	**1.3 (0.55; 2.20)**	**2.3 (1.15; 6.35)**	**0.7 (0.40; 1.90)**	**2.8 (1.00; 3.05)**	**p_{all} = 0.0018**
					p_{1-3} = 0.2803^b
p^b	**0.0130**		**0.0142**		**p_{1-4} = 0.1036^b**
					p_{2-4} = 0.5538^b
	Anti-inflammatory cytokines				
IL-10, pg/ml	1.55 (0.55; 2.3)	1.5 (0.09; 2.3)	2 (0.75; 3.4)	1.3 (0.6; 3.1)	0.6008
	Relationships between pro- and anti-inflammatory cytokines				
INF/IL-10	0.465 (0.215; 0.805)	0.375 (0.095; 0.553)	0.385 (0.178; 0.618)	0.430 (0.270; 1.620)	0.3679
IL-6/IL-10	0.870 (0.658; 1.485)	0.895 (0.588; 1.615)	0.595 (0.248; 1.160)	0.440 (0.260; 2.000)	0.2020
IL-1/IL-10	0.705 (0.338; 1.833)	0.655 (0.230; 1.225)	0.610 (0.420; 2.833)	1.130 (0.690; 1.600)	0.4176
IL-8/IL-10	18.66 (7.95; 36.82)	8.03 (5.28; 28.06)	7.37 (2.64; 24.84)	7.50 (2.39; 20.00)	0.2687
IL-6/TNF-α	0.725 (0.545; 1.678)	0.880 (0.580; 2.190)	0.655 (0.425; 1.495)	0.640 (0.385; 1.305)	0.6363
IL-10/TNF-α	1.000 (0.410; 1.343)	0.940 (0.050; 1.880)	1.775 (0.405; 3,093)	0.890 (0.540; 2.425)	0.4826
IL-1/TNF-α	**0.675 (0.315; 0.9475)**	**0.79 (0.44; 1.19)**	**1.260 (1.015; 2.683)**	**1.140 (0.815; 2.015)**	**p_{all} = 0.0022**
					p_{1-3} = 0.0011^b
p	**0.7619**		**0.2661**		**p_{1-4} = 0.0157^b**
					p_{2-4} = 0.2661^b

Note. Data are presented as medians and lower and upper quartiles; p is the level of statistical significance when comparing parameter values between groups using the Mann – Whitney test.

We found statistically significantly higher IL-1 concentration and IL-1/TNF-α ratio in women with CE regardless of BMI compared to the results in women without CE with normal body weight. However, we did not find any significant differences in the values of these parameters in women without CE and with CE against the background of overweight and obesity. This can be explained by the presumable presence of chronic systemic inflammation in women with overweight and obesity [15], which is confirmed by a statistically significantly elevated CRP level in such women regardless of the presence of CE. In addition, in women with overweight and obesity, we found a statistically significant positive correlation of CRP with BMI ($r = 0.495$; $p < 0.001$), which was absent in women with normal body weight ($r = 0.050$; $p = 0.763$).

We next performed ROC analysis to establish risk thresholds for the presence of CE for IL-1 or IL-1/TNF-α ratio in women with normal body weight (fig. 3).

We found that in women with CE and normal body weight, the IL-1 concentration was ≥ 1.35 pg/ml (sensitivity 75 %, specificity 83 %; 95% CI: 0.88; 2.15), and the IL-1/TNF-α ratio was ≥ 1.03 (sensitivity 85 %, specificity 78 %; 95% CI: 0.81; 1.27). The OR for the established threshold values was: for IL-1 – 8.167 (95% CI: 1.885; 35.381), for the IL-1/TNF-α ratio – 15 (95% CI: 3.027; 74.32).

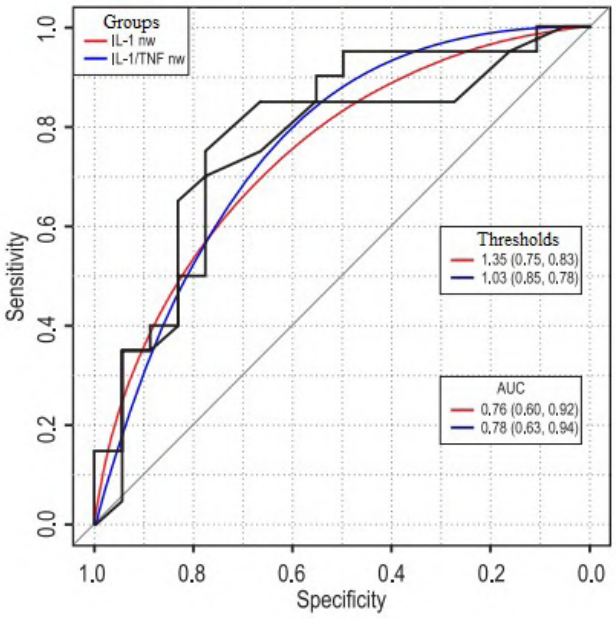

FIG. 3.
ROC curve of IL-1 concentration or IL-1/TNF-α ratio in premenopausal women with or without chronic endometritis against the background of normal weight: IL1 nw – ROC curve of IL-1 concentration in patients with normal weight; IL1/TNF nw – ROC curve of IL-1/TNF-α ratio in patients with normal weight; the cut-off point for IL-1 concentration is 1.35 (95% CI: 0.88; 2.15); the cut-off point for IL-1/TNF-α ratio is 1.03 (95% CI: 0.81; 1.27))

DISCUSSION

In this study, we examined the levels of pro- and anti-inflammatory cytokines in the blood serum of women with and without CE, including their dependence on BMI. Based on the results of the study, we found that women with CE, regardless of BMI, have statistically significantly higher values of serum IL-1 concentrations and IL-1/TNF-α ratios. We also determined the threshold values for these parameters in relation to the presence of CE using ROC analysis. However, the sensitivity of certain threshold values of IL-1 and IL-1/TNF-α concentrations turned out to be relatively low, and therefore we decided to analyze the levels of pro- and anti-inflammatory cytokines in women with and without CE, depending on BMI, since the presence of excess body weight and obesity can provoke chronic systemic inflammation and affect the level of serum cytokines [12], which can clinically manifest itself as an increase in the level of CRP [15]. According to the analysis, we also found a statistically significant association of higher IL-1 and IL-1/TNF-α values with the presence of CE only in women with normal body weight, but not in overweight and obese women. At the same time, in overweight women, regardless of the presence or absence of CE, we found a significantly higher level of CRP than in women with normal body weight, which indirectly confirms the contribution of increased BMI to the development of systemic inflammation. Subsequent ROC analysis with the establishment of threshold values showed more pronounced sensitivity and specificity for certain points compared to the values established for women without taking into account BMI. Thus, we found that for women of reproductive age with normal body weight, CE is accompanied by an increase in the concentration of IL-1 ≥ 1.35 pg/ml and the value of the IL-1/TNF-α ratio ≥ 1.03.

Proinflammatory cytokines in the uterine mucosa are produced by neutrophils, macrophages, and epithelial cells in response to regulatory factors under physiological conditions and bacterial and viral antigens in case of a pathological process. Thus, neutrophils in the uterine mucosa, under the influence of bacterial lipopolysaccharides (LPS), can produce IFN-γ, as well as IL-12 and TNF-α. IFN-γ, in turn, activates macrophages [16], which are located in the subepithelial stroma of the endometrium and are the first to recognize foreign antigens [17]. LPS can also directly stimulate macrophages to produce proinflammatory IL-1β, which causes the secretion of human β-defensin-2 by endometrial epithelial cells to resist bacterial invasion [18]. The development of inflammation in the endometrium can also be promoted by T-helpers 1 (Th1), which produce TNF-α [19]. In addition, proteins that provide direct stimulation of the production of proinflammatory cytokines (TNF-α and IL-1β) are expressed on epithelial cells, monocytes and dendritic cells of the endometrium [20]. Thus, with the development of CE under the influence of bacterial LPS and other proinflammatory factors

in the uterine cavity, the expression of cytokines responsible for the inflammatory response significantly increases.

Proinflammatory cytokines (IL-1, IL-6, IL-8, IL15, TNF-α) along with many hormones (estrogen, progesterone) and factors of decidualization and degradation of the endometrium extracellular matrix (integrin β3, IGFBP1 and metalloproteinases) are factors regulating endometrial receptivity, which determines the success of embryo implantation. In patients with CE, changes in the expression and functioning of these factors are noted, which can cause the development of infertility and habitual miscarriage against the background of this disease [3].

Thus, W.J. Wang et al. (2019) showed that the endometrium of women of reproductive age with CE has increased expression of proinflammatory IL-17 and decreased expression of anti-inflammatory IL-10 [2]. In our previous studies, we found increased secretion of IL-1β, IL-4, IL-6, IL-10, as well as IFN-γ and TNF-α in the endometrium of women with CE compared to the results in healthy women [21, 22]. C. Tortorella et al. (2014) also found an increased concentration of proinflammatory IL-1β, IL-6, and TNF-α in the menstrual blood of women with chronic endometritis. At the same time, with regard to CE screening, they revealed higher sensitivity for such indicators as the IL-6/TNF-α and IL-6/IL-1β ratios [23].

Currently, the most effective and reproducible method for diagnosing CE is the detection of CD138+ cells in the endometrial stroma during a Pipelle biopsy [5]. Despite the high sensitivity and specificity of this diagnostic method, it has a number of limitations. In particular, a Pipelle biopsy is a minor gynecological operation, and its use requires indications. At the same time, it is known that CE can often be asymptomatic. Such difficulties necessitate the search for new, less invasive methods for diagnosing CE [4, 6, 7].

Thus, some researchers have attempted to identify an association between the level of cytokines in the blood serum and the presence of CE. According to the results of the study by L.V. Tkachenko et al. (2020), an increase in the concentration of proinflammatory cytokines was found in uterine cavity aspirates, as well as in the blood serum of women with CE: IL-1β, IL-2, IL-6, TNF-α and IFN-γ [10]. I.P. Koltsov et al. (2011) recorded increased secretion of IL-8 by blood monocytes in women with chronic endometritis [24]. Yu.A. Sorokin et al. (2022) in a study of the effectiveness of CE treatment by introducing cavitated 0.9% sodium chloride solution into the uterine cavity revealed statistically significantly higher serum values of IL-1β (7.65 [6.3–8.98] vs. 1.22 [0.99–1.45]; $p < 0.05$) and TNF-α (2.75 [1.42–4.08] vs. 1.48 [1.29–1.67]; $p < 0.05$) in patients of reproductive age with CE compared to the values of similar parameters in women without CE [25]. These results are partially consistent with the data of our study that in women with CE, the concentration of IL-1 in the blood serum is ≥ 1.3–1.35 mg/ml.

Serum cytokine levels can change under the influence of not only local but also systemic inflammation. One of the common causes of chronic systemic inflammation is obesity. Thus, obese people may have elevated levels of proinflammatory cytokines in the blood serum [12], as well as CRP [15]. Based on this fact, we analyzed the levels of proinflammatory cytokines in women of reproductive age with or without CE depending on the body mass index. As a result, we found that an increase in the serum level of the proinflammatory cytokine IL-1 and the IL-1/TNF-α ratio is statistically significant only in the group of women with CE against the background of normal body weight. At the same time, a significant increase in the CRP level was observed only in overweight and obese women, regardless of the presence of CE. This may indirectly confirm the contribution of excess body weight to the development of chronic systemic inflammation, expressed in a relative increase in the CRP level.

The advantage of this study is that the level of interleukins in women with CE was assessed, among other things, taking into account BMI and CRP levels, since overweight and obesity can provoke chronic systemic inflammation [12, 15]. Also, one of the exclusion criteria was the presence of polycystic ovary syndrome in patients, since this disease is also associated with systemic inflammation and can affect serum cytokine levels [13]. The disadvantages of this study include the small patient sample size, which does not allow extrapolating the research results to the general population. Also, the lack of data on the levels of proinflammatory cytokines directly in the endometrial tissues with the determination of their correlation with the concentration of cytokines in the blood serum does not allow us to fully assert that the established patterns in the form of increased levels of IL-1 and the IL-1/TNF-α ratio are directly related to the presence of CE in patients. In addition, we did not assess the presence of other chronic diseases in patients that may be accompanied by the development of chronic systemic or local inflammation, which could have influenced the analysis results of the level of interleukins and CRP in the examined patients.

CONCLUSION

The conducted reanalysis of the cross-sectional study data of women of reproductive age with or without CE revealed a significantly higher serum concentration of IL-1 and higher values of the IL-1/TNF-α ratio in patients with CE against the background of normal body weight compared to the results in healthy patients with normal body weight with the establishment of threshold values of these parameters. At the same time, we did not find significant changes in the level of serum cytokines in patients with or without CE against the background of overweight and obesity, which can

be explained by the presence of systemic inflammation in them, which also affects the level of pro- and anti-inflammatory cytokines. The absence of a significant change in IL concentrations in patients of this group may be associated with dysregulation of the immune response against the background of overweight and obesity associated with an increased level of CRP.

Thus, it is necessary to conduct additional studies with a larger sample size, as well as assessing the level of cytokines not only in the blood serum, but also in the endometrium of women with or without CE to confirm the obtained results and identify a direct relationship between the studied parameters in the blood serum and endometrium. This will allow developing new a new method of minimally invasive diagnostics or determining the risk of CE in women of reproductive age.

Conflicts of interest

No potential conflict of interest relevant to this article reported.

REFERENCES

1. Danusevich IN. The frequency of occurrence of chronic endometritis in women with different variants of reproductive disorders. *Acta biomedica scientifica*. 2013; 4(92): 14-16. (In Russ). [Данусевич И.Н. Частота встречаемости хронического эндометрита у женщин с различными вариантами репродуктивных нарушений. *Acta Biomedica Scientifica*. 2013; 4(92): 14–16].

2. Wang WJ, Zhang H, Chen ZQ, Zhang W, Liu XM, Fang JY, et al. Endometrial TGF-β, IL-10, IL-17 and autophagy are dysregulated in women with recurrent implantation failure with chronic endometritis. *Reprod Biol Endocrinol*. 2019; 17(1): 1-9. doi: 10.1186/s12958-018-0444-9

3. Wu D, Kimura F, Zheng L, Ishida M, Niwa Y, Hirata K, et al. Chronic endometritis modifies decidualization in human endometrial stromal cells. *Reprod Biol Endocrinol*. 2017; 15(1): 1-10. doi: 10.1186/s12958-017-0233-x

4. Kitaya K, Yasuo T. Immunohistochemistrical and clinicopathological characterization of chronic endometritis. *Am J Reprod Immunol*. 2011; 66(5): 410-415. doi: 10.1111/j.1600-0897.2011.01051.x

5. Asaturova AV, Badlaeva AS, Tregubova AV, Tabeeva GI. Reproducibility of diagnosis of chronic endometritis before and after the use of immunohistochemical examination of plasma cells in the endometrium. *Russian News of Clinical Cytology*. 2023; 27(1): 5-10. (In Russ.). [Асатурова А.В., Бадлаева А.С., Трегубова А.В., Табеева Г.И. Воспроизводимость диагностики хронического эндометрита до и после применения иммуногистохимического исследования плазматических клеток в эндометрии. *Новости клинической цитологии России*. 2023; 27(1): 5-10]. doi: 10.24412/1562-4943- 2023-1-0001

6. Yasuo T, Kitaya K. Challenges in clinical diagnosis and management of chronic endometritis. *Diagnostics (Basel)*. 2022; 12(11): 2711. doi: 10.3390/diagnostics12112711

7. Inflammatory diseases ofthe female pelvic organs. Clinical recommendations. Moscow; 2021. (In Russ.). URL: https://www.infomed39.ru/upload/iblock/007/t7cdo4j9ro5jyc55f0524xi4d2i730iw/Vospalitelnye-bolezni-zhenskikh-tazovykh-organov.pdf [Date Accessed: March 05, 2021].

8. Sharifulin EM, Igumnov IA, Krusko OV, Atalyan AV, Suturina LV. Features of chronic endometritis in women of reproductive age with polycystic ovary syndrome. *Acta biomedica scientifica*. 2020; 5(6): 27-36. (In Russ.). [Шарифулин Э.М., Игумнов И.А., Круско О.В., Аталян А., Сутурина Л.В., Особенности хронического эндометрита у женщин репродуктивного возраста с синдромом поликистозных яичников. *Acta Biomedica Scientifica*. 2020; 5(6): 27–36]. doi: 10.29413/ ABS.2020-5.6.3

9. Ievleva KD, Danusevich IN, Atalyan AV, Sharifulin EM, Lazareva LM, Nadelyaeva IG, et al. Adipokine levels and their association with chronic endometritis in reproductive-aged women. *Gynecology, Obstetrics and Perinatology*. 2023; 22(5): 60-68. (In Russ.). [Иевлева К.Д., Данусевич И.Н., Аталян А.В., Шарифулин Э.М., Лазарева Л.М., Наделяева Я.Г., и др. Уровень адипокинов и их ассоциация с хроническим эндометритом у женщин репродуктивного возраста. *Вопросы гинекологии, акушерства и перинатологии*. 2023; 22(5): 60–68]. doi: 10.20953/1726-1678-2023-5-60-68

10. Tkachenko LV, Sviridova NI, Zharkin NA, Burova NA, Belan EB. Assessing cytokine status of patients with chronic endometritis combined with endometrial hyperplastic processes in reproductive period. *Russian Journal of Infection and Immunity*. 2020; 10(4): 762-768. (In Russ.). [Ткаченко Л.В., Свиридова Н.И., Жаркин Н.А., Бурова Н.А., Белан Э.Б. Оценка цитокинового статуса у пациенток с хроническим эндометритом в сочетании с гиперпластическими процессами эндометрия в репродуктивном периоде. *Инфекция и иммунитет*. 2020; 10(4): 762–768]. doi: 10.15789/2220-7619-AOC-1357

11. Motovilova TM, Kachalina TS, Grechkanev GO, Borovkova LV, Zinov'ev AN, Nikolaeva OA, et al. Determining of biomarkers in menstrual blood as possibility of non-invasive diagnostics of inflammatory process in uterine cavity. *Medical Almanac*. 2016; 45(5): 88-91. (In Russ.). [Мотовилова Т.М., Качалина Т.С., Гречканев Г.О., Боровкова Л.В., Зиновьев А.Н., Николаева О.А., и др. Определение биомаркеров в менструальной крови как возможность неинвазивной диагностики воспалительного процесса в полости матки. *Акушерство и гинекология*. 2016; 5: 88–91].

12. Bays HE, Bindlish S, Clayton TL. Obesity, diabetes mellitus, and cardiometabolic risk: An Obesity Medicine Association (OMA) Clinical Practice Statement (CPS). *Obesity Pillars*. 2023; 2023: 100056. doi: 10.1016/j.obpill.2023.100056

13. Daan NMP, Koster MPH, de Wilde MA, Dalmeijer GW, Evelein AMV, Fauser BCJM, et al. Biomarker profiles in women with PCOS and PCOS offspring; a pilot study.

PLoS One. 2016; 11(11): e0165033. doi: 10.1371/journal.pone.0165033

14. Sharifulin EM, Lazareva LM, Kanya OV, Stefanenkova AA, Belykh DV, Suturina LV. Endometrial morphology in women of reproductive age with PCOS. *Acta biomedica scientifica*. 2018; 3(3): 136-142. (In Russ.). [Шарифулин Э.М., Лазарева Л.М., Каня О.В., Стефаненкова А.А., Белых Д.В., Сутурина Л.В. Состояние эндометрия при синдроме поликистозных яичников в репродуктивном возрасте. *Acta Biomedica Scientifica*. 2018; 3(3): 136-142]. doi: 10.29413/ABS.2018-3.3.21

15. Calabro P, Chang DW, Willerson JT, Yeh ETH. Release of Creactive protein in response to inflammatory cytokines by human adipocytes: Linking obesity to vascular inflammation. *J Am Coll Cardiol*. 2005; 46(6): 1112-1113. doi: 10.1016/j.jacc.2005.06.017

16. Yeaman GR, Collins JE, Currie JK, Guyre PM, Wira CR, Fanger MW. IFN gamma isproduced by polymorphonuclear neutrophils in human uterine endometrium and by cultured peripheral blood polymorphonuclear neutrophils. *J Immunol*. 1998; 160: 5145-5153.

17. Soares MJ, Chakraborty D, Kubota K, Renaud SJ, Rumi MA. Adaptive mechanisms controlling uterine spiral artery remodeling during the establishment of pregnancy. *Int J Dev Biol*. 2014; 58: 247-259. doi: 10.1387/ijdb.140083ms

18. Pioli PA, Weaver LK, Schaefer TM, Wright JA, Wira CR, Guyre PM. Lipopolysaccharide-induced IL-1 beta production by human uterine macrophages up-regulates uterine epithelial cell expression of human beta-defensin 2. *J Immunol*. 2006; 176: 6647-6655. doi: 10.4049/jimmunol.176.11.6647

19. D'Ippolito S, Di Nicuolo F, Pontecorvi A, Gratta M, Scambia G, Di Simone N. Endometrial microbesand microbiome: Recent insights on the inflammatory and immune "players" of the human endometrium. *Am J Reprod Immunol*. 2018; 80: e13065. doi: 10.1111/aji.13065

20. Zhu N, Yang X, Liu Q, Chen Y, Wang X, Li H, et al. "Iron triangle" of regulating the uterine microecology: Endometrial microbiota, immunity and endometrium. *Front Immunol*. 2022; 13: 928475. doi: 10.3389/fimmu.2022.928475

21. Danusevich IN, Sharifulin EM, Nemchenko UM, Kolesnikova LI. Features of the immune system functioning with persistence of infectious agents in women with chronic endometrial inflammation and reproductive disorders. *Int J Biomed*. 2020; 10(4): 362-368. doi: 10.21103/Article10(4)_OA6

22. Danusevich IN, Lazareva LM, Nemchenko UM, Kolesnikova LI. Endometrial cytokines in women with reproductive disorders. *Int J Biomed*. 2021; 11(4): 526-531. doi: 10.21103/Article11(4)_OA20

23. Tortorella C, Piazzolla G, Matteo M, Pinto V, Tinelli R, Sabbà C, et al. Interleukin-6, interleukin-1β, and tumor necrosis factor α in menstrual effluents as biomarkers of chronic endometritis. *Fertil Steril*. 2014; 101(1): 242-247. doi: 10.1016/j.fertnstert.2013.09.041

24. Koltsov IP, Khramova IA. Interrelation between secretory and synthetic processes in monocytes/macrophages and level of interleukine-8 secretion by blood monocytes in case of endometritis. *Pacific Medical Journal*. 2011; (3): 58-60. (In Russ.). [Кольцов И.П., Храмова И.А. Взаимосвязь секреторно-синтетических процессов в моноцитах/макрофагах с уровнем секреции интерлейкина-8 моноцитами крови при эндометрите. *Тихоокеанский медицинский журнал*. 2011; 3: 58–60].

25. Sorokin YuA, Gizinger OA, Radzinsky VE. A clinical and immunological rationale for ultrasonic cavitation use in the complex treatment of infertility in chronic endometritis. Gynecology. 2022; 24(5): 355-361. (In Russ.). [Сорокин Ю.А., Гизингер О.А., Радзинский В.Е. Клинико-иммунологическое обоснование ультразвуковой кавитации в комплексном лечении бесплодия при хроническом эндометрите. *Гинекология*. 2022; 24(5): 355–361]. doi: 10.26442/20795696.2022.5.201866

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