CLINICAL SCIENCE

THE ROLE OF SOME INFLAMMATORY MARKERS, CYTOKINS AND TUMOR MARKERS IN DIAGNOSIS OF ENDOMETRIOSIS

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Citation: Georgievska J, Tofoski G, Dimitrov G, Daneva-Markova A, Jovanovska V, Dabeski D, Jovcevski S, Dzikova E, Atanasova A. The role of some inflammatory markers, cytokins and tumor markers in diagnosis of endometriosis Arch Pub Health 2022; 14 (2) 58-71.

doi.org/10.3889/aph.2022.6060

Key words: endometriosis, inflammation, cytokines, interleukin, serum tumor markers

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Received: 3-Mar-2022; **Revised:** 10-Jul-2022; **Accepted:** 30-Jul-2022; **Published:** 30-Dec-2022

Competing Interests: The author have declared that no competing interests

Abstract

Endometriosis is a multifactorial disease which etiopathogenesis has not been elucidated. One of the theories of etiopathogenesis is the inflammatory theory. Aims of the study: To develop a practical non-invasive test for the diagnosis of endometriosis by examining some inflammatory markers and cytokines; to compare the highly sensitive C-reactive protein (hsCRP), cytokines (interleukin-6-IL-6 and tumor necrotizing factor alpha) and the tumor marker cancer antigen 125 (CA-125) among healthy patients and patients with endometriosis; to determine the sensitivity and specificity of each biomarker separately in the diagnosis of endometriosis and to determine their role in the diagnosis of endometriosis. Materials and methods: In a prospective study conducted at the University Clinic for Gynecology and Obstetrics, Ss. Cyril and Methodius University in Skopje, North Macedonia 138 patients were included of a reproductive age between 18-50 years (83 with diagnosis endometriosis operated laparoscopically or with laparotomy) and a control group of 55 healthy women, in a period between 01.09.2018 to 01.05.2021. Serum levels of IL-6, TNF-α, hs-CRP and tumor marker CA-125 were evaluated in both groups. Results: Serum levels of CA-125, IL-6 and TNF-α and hs-CRP were significantly higher in patients with endometriosis compared to the control group. The surface under the ROC curve (AUC) for IL-6, CA-125, hs-CRP, and TNF-α has shown that as individual markers they all have a discriminatory capacity to diagnose patients with endometriosis. Conclusions: Results obtained in our study showed statistically significantly higher serum concentrations of CA-125, IL-6 and TNF-α and hs-CRP in patients with endometriosis compared to the control group of patients. However, none of these biomarkers showed a high sensitivity for diagnosis of endometriosis. It is necessary to find a panel combination of biomarkers with a high sensitivity of about 100% that will enable early diagnosis of endometriosis.

КЛИНИЧКИ ИСТРАЖУВАЊА

УЛОГАТА НА НЕКОИ ИНФЛАМАТОРНИ МАРКЕРИ, ЦИТОКИНИ И ТУМОР МАРКЕРИ ВО ДИЈАГНОЗА НА ЕНДОМЕТРИОЗАТА

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Цитирање: Георгиевска Ј, Тофоски Г , Димитров Г, Данева-Маркова А, Јовановска В, Дабески Д, Јовчевски С, Џикова Е, Атанасова А. Улогата на некои инфламаторни маркери, цитокини и тумор маркери во дијагноза на ендометриозата. Арх Ј Здравје 2022;14(2) 58-71.

doi.org/10.3889/aph.2022.6060

Клучни зборови: ендометриоза, воспаление, цитокини, интерлеукин, серумски тумор маркери

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Примено: 3-мар-2022 **Ревидирано:** 10-јул-2022; **Прифатено:** 30-јул-2022; **Објавено:** 30-дек-2022

Конкурентски интереси: Авторот изјавува дека нема конкурентски интереси.

Извадок

Ендометриозата е мултифакторно заболување, чија етиопатогенеза не е разјаснета. Една од теориите за етиопатогенезата е инфламаторната теорија. Цели на истражувањето: Да се развие практичен неинвазивен тест за дијагноза на ендометриозата со иследување нанекои инфламаторни маркери и цитокини; да се направи споредба на високосензитивниот Ц-реактивен протеин (hsCRP), цитокините (интерлеукин 6 –IL-6 и тумор-некротизирачки фактор алфа - TNF-α) и туморскиот маркер cancer antigen 125 (CA-125) кај здрави пациентки и пациентки со ендометриоза; да се утврди сензитивноста и специфичноста на секој биомаркер посебно во дијагнозата на ендометриозата и да се утврди нивната улога во дијагноза на ендометриозата. Материјал и методи: Во проспективна студија спроведена на Универзитетската клиника за гинекологија и акушерство, Универзитет „Св. Кирил и Методиј“ во Скопје, Северна Македонија беа вклучени 138 испитанички на репродуктивна возраст помеѓу 18-50 години (83 со дијагноза ендометриоза, оперирани со лапароскопија или лапаротомија) и контролна група од 55 здрави жени, во период од 01.09.2018 година до 01.05.2021. Серумските вредности на интерлеукин 6 (IL-6), тумор-некротизирачки фактор алфа (TNF-α), високоспецифичен Ц-реактивен протеин (hsCRP) и туморскиот маркер CA-125 беа евалуирани во двете групи. Резултати: Серумските вредности на CA-125, IL-6 и TNF-α и hsCRP беа сигнификантно повисоки кај пациентките со ендометриоза во споредба со оние во контролната група. Површината под ROC кривата (AUC) за IL-6, CA-125, hs-CRP и TNF-α покажа дека како поединечни маркери сите имаат дискриминаторен капацитет за дијагноза на пациентки со ендометриоза. Заклучоци: Иследувањата во нашата студија покажаа статистички сигнификантно повисоки концентрации на CA-125, IL-6 и TNF-α и hs-CRP кај пациентките со ендометриоза во однос на контролната група пациентки. Меѓутоа, ниту еден од овие биомаркери не покажа висока сензитивност за дијагноза на ендометриозата. Потребно е да се најде панел комбинација на биомаркери со висока сензитивност од околу 100% кои ќе овозможат рана дијагноза на ендометриозата.

Introduction

Endometriosis is a disease characterized by the presence of endometrial glands and endometrial stroma outside the uterine cavity. This tissue responds to reproductive hormones and this results in the formation of endometrial cysts in the ovaries and endometrial foci along the peritoneum, intestines, and other sites in the abdominal cavity.

The clinical picture of these patients includes: chronic pelvic pain, dysmenorrhea, dyspareunia, menstrual disorders, infertility, but the disease can be asymptomatic and can be detected as a random finding by laparoscopy or laparotomy. It is found in 6-10% of women in the reproductive period and in 30-50% of patients with infertility[1]. Ultrasound and magnetic resonance imaging[2] can help diagnose this disease. Today the gold standard for the diagnosis of endometriosis is laparoscopy, which allows visualization of endometrial foci as well as their biopsy for histopathological confirmation of the diagnosis[3].

At the same time, laparoscopy as well as laparotomy make it possible to determine the stage of the disease according to the revised classification of endometriosis by the American Society for Reproductive Medicine - rASRM [4]. Because laparoscopy as well as laparotomy are risky and expensive methods, several blood markers are being investigated for non-invasive diagnosis of endometriosis that would allow early diagnosis of this disease.

Despite a long history of clinical experience and experimental research, the pathogenesis of endometriosis has not yet been accurately established.

There are several theories, such as: implantation theory as a consequence of retrograde menstruation, theory of complete metaplasia, genetic theory, immune theory, inflammation theory and others[5, 6].

According to inflammatory theory, the peritoneal environment in patients with endometriosis may be involved in the pathogenesis of the disease[7]. Peritoneal fluid in patients with endometriosis is thought to be filled with activated macrophages that secrete a range of local products such as growth factors and cytokines, and therefore endometriosis is considered a chronic inflammatory disease. Chronic inflammation is accompanied by fibrous tissue formation and local peritoneal adhesions, angiogenesis, and proliferation. The inflammatory process in endometriosis causes pelvic pain and infertility [8].

There are many studies suggesting elevated levels of activated macrophages and several cytokines in the peritoneal fluid in patients with endometriosis, such as: interleukin-6 (IL-6), interleukin 1ß, interleukin-8 (IL-8), tumor necrotizing factor α (TNF-α), vascular endothelial growth factor (VEGF), macrophage migration inhibitory factor (MIF), and others [9, 10, 11].

Elevated values of several inflammatory biomarkers have also been found in the serum of patients with endometriosis: C-reactive protein (CRP), interleukins (interleukin-4, interleukin-6, interleukin-8, TNF-α) and others [12].

IL-6 is thought to play a major role

in the growth and survival of ectopic endometrial tissue. Interleukin-6 regulates inflammation and the immune response by modulating the secretion of other cytokines, promoting T-cell activation, B-cell differentiation, and inhibiting the growth of other cell lines.Interleukin-6 (IL-6) is a cytokine that is a mediator of the immune system and has a number of biological actions. It is also known as B-cell stimulating factor.

TNF-α is secreted by activated macrophages and has inflammatory, cytotoxic and angiogenic effects. TNF-α stimulates the expression of matrix metalloproteinase from endometrial tissue involved in endometrial invasion and remodeling.

Serum cancer antigen (CA-125) is a surface cellular antigen with elevated serum values in most patients with endometriosis and therefore this tumor marker is recommended in the screening of this disease [13,14]. Several studies have combined studies of serum concentrations of CA-125, CA 19-9, and CA 15-3, but the diagnostic value of these tumor markers in endometriosis is still inconsistent [15].

C-reactive protein (CRP) is a protein of the acute inflammatory phase and is widely used in clinical practice as a marker of inflammation. Some studies have found higher CRP and hs-CRP values in patients with endometriosis, especially in stages 3 and 4 of the disease, but without a significant difference between patients with endometriosis and patients without endometriosis[16]. On the other hand, in a study by Lermann J. *et al*. hs-CRP values were significantly lower than CRP values in women without endometriosis and therefore hs-CRP could serve as a marker for the absence of endometriosis [17].

Objectives of the study were to determine the role of serum markers of inflammation (hs-CRP), cytokines (IL-6, TNF-α) and tumor marker CA-125 in the diagnosis of endometriosis, to determine the sensitivity and specificity of each biomarker separately in the diagnosis of endometriosis. Based on the results obtained, to determine which biomarkers can be used in the early diagnosis of endometriosis.

Materials and methods

In a prospective study conducted at the University Clinic for Gynecology and Obstetrics, Ss. Cyril and Methodius University in Skopje, North Macedonia 148 women were included of a reproductive age between 18-50 years. Of these, 93 were diagnosed with endometriosis and were hospitalized at the Clinic for surgical treatment (laparoscopy or laparotomy) and 55 patients were in the control group. In 83 patients the diagnosis of endometriosis was confirmed histopathologically postoperatively and they were included in the examination.

Ten patients whose diagnosis of endometriosis was not confirmed postoperatively were excluded from the study. The intraoperative stage of endometriosis was determined according to a revised classification ofthe American Society for Reproductive Medicine (rASRM). The control group consisted of 55 healthy women in the reproductive period in whom the presence of endometri-

osis or some other form of inflammation was excluded.

The study was performed in the period from 01.09.2018 to 01.05.2021 and was approved by the Human Research Ethics Committee of the Faculty of Medicine in Skopje.

Exclusion criteria were: malignancy, menopause, pelvic inflammatory disease, previous anti-inflammatory therapy for a period shorter than 6 months before the start of the study.

After receiving an informed consent forparticipation in the study, a detailed history was taken from each woman who voluntarily participated in the study. A preoperative echosonographic evaluation was then performed. After appropriate preoperative preparation, patients were operated on by laparoscopy or laparotomy in the proliferative phase of the menstrual cycle. Intraoperative stage of the disease was determined according to the rASRM classification. Venous blood (5 ml) was taken from each patient preoperatively in the proliferative phase of the menstrual cycle. Blood samples were left at room temperature for 60 minutes to coagulate. The sample was then centrifuged for 10 min at 3000 rpm. Serum concentrations of interleukin-6 (IL-6), tumor necrotizing factor alpha (TNF-α), highly specific C-reactive protein (hs-CRP) and tumor marker CA-125were determined in the Clinical Biochemical Laboratory of the University Clinic for Gynecology and Obstetrics in Skopje.

Method for determination of interleukin-6 (IL-6) concentration

Serum IL-6 concentration was quantified by the immunometric assay (Immulite 2000 HP, Diagnostic Products Corp). Analytical sensitivity of the test is 2 pg / ml with a measuring range up to 1000 pg / ml. Reference values for IL-6 <5.9 pg / ml.

Method for determination of tumor necrotizing factor α (TNF-α) concentration

TNF-α concentrations were quantified by the immunometric method (Immulite 1000, Diagnostic Products Corp.). TNF-α is a solid phase, chemiluminescence immunometric essay. The test shows an analytical sensitivity of 1.7 pg / ml with a measuring range up to 1000 pg / ml. Reference value for TNF-α <8.1 pg / ml.

Method for determination the concentration of highly sensitive CRP (hs-CRP)

The serum concentration of hs-CRP in patients was determined by the immunoturbidimetric method at 522 nm, on a biochemical analyzer Cobas Integra 400 plus, Roshe Diagnostic, Germany. Human CRP agglutinates with latex particles coated with monoclonal anti-CRP antibodies. The precipitate is determined turbidimetrically 552 nm with a measurement range of 0.1-20mg / L (0.952-190 nmol / L). The lowest level of detection is 0.1mg / L (0.952 nmol / L). Reference values for hs-CRP <5mg / ml.

Method for determination of CA-125 concentration

Serum CA-125 tumor marker concentration was quantified by the immunometric assay (Immulite 2000 HP, Diagnostic Products Corp). It is

a sequential, two-sided, solid-phase, enzymatic chemiluminescence essay. Analytical sensitivity of the test is 2 pg / ml with a measuring range up to 1000 pg / ml. Reference value for CA-125 <35mIU / ml.

Statistical analysis

The statistical analysis of the data was performed with the statistical program SPSS 23.0. The Kolmogorov-Smirnov test was used to test the normality of data distribution. The statistical characteristics of the categorical variables are presented by absolute and relative numbers, while the quantitative variables are presented by average, standard deviation, minimum and maximum values, median value and interquartile range.

The statistical significance of the intergroup differences was tested with the Chi-square test and the Mann-Whitney test.

Logistic regression analysis was used to determine the independent predictors of endometriosis.

Sensitivity, specificity, positive and negative predictive value were calculated for each marker. Because the study was designed as a case-control study, predictive values were calculated by Bayesian approximation, assuming an endometriosis prevalence of 10%.

ROC analysis by constructing a ROC curve was used to determine the discriminant ability of IL-6, hs-CRP, TNF-alfa, and CA-125 as tests for the diagnosis of endometriosis.

A ROC curve was constructed for all of these tests, as a graphical representation of the sensitivity and specificity of each possible test result.

Statistical significance was defined at the level of $p < 0.05$.

Results

A total of 138 patients of a reproductive age from the University Clinic for Gynecology and Obstetrics were included in the study, of which 83 patients with endometriosis (IG) and 55 patients without endometriosis (control group - CG). Both groups were homogeneous in terms of age of patients ($p = 0.56$); the mean age was 33.4 ± 6.4 and 32.8± 5.5 years, respectively in the study and control groups(Table 1).

Table 1. Patients from both age groups

Variable	Variable calculated	groups		p-level
		CG	IG	
Age	mean ±SD	32.8 ±5.5	33.4± 6.4	t=0.58 p=0.56 ns
	min - max	22 – 45	22 – 54	

IG-investigated group with endometriosis
t - (Student's t-test)
CG - control group

Table 2. Comparison of investigated biomarkers (IL-6, CA-125, hs-CRP and TNF-α) between CG and IG

Calculated parameter	groups		p-level
	CG	IG	
IL - 6			
min – max	2.5 – 18.3	2 – 367	Z=2.84
median (IQR)	4.98(3.99 – 5.9)	6.97(3.99 – 13.2)	**p=0.0045 sig
IL - 6			
min – max	1.2 – 29.5	2.53 – 384.4	Z=8.12
median (IQR)	7.4(5.2 – 10.7)	27.6(16.3 – 64.5)	***p=0.000000 sig
IL - 6			
min – max	0.2 – 23.5	0.2 – 243	Z=3.41
median (IQR)	1.1(0.5 – 3.3)	2.7(1.0 – 9.2)	***p=0.00064 sig
IL - 6			
min – max	4 – 25.7	4 – 238	Z=3.89
median (IQR)	5.3(4.6 – 6.25)	6.34(5.2 – 9.64)	**p=0.0001 sig

IG- investigated group **Z** (Mann-Whitney test)
CG- control group *p<0.05 **p<0.01 ***p<0.0001
The results presented in Table 2 show that patients with endometriosis had higher values for all 4 analyzed biomarkers compared to patients from CG.

Significantly higher median serum IL-6 values were confirmed for p = 0.0045 in the endometriosis group compared to CG (6.97 vs. 4.98) (Figure 1).

Median serum concentrations of the tumor marker CA-125 were significantly higher in the endometriosis group compared toCG (27.6 *vs.* 7.4, p <0.0001). These results are shown in Figure 2.

The biomarker of inflammation, hs-CRP, showed significantly higher serum concentrations in patients with endometriosis versus the control group (2.7 *vs.* 1.1, p = 0.00064) (Figure 3). Significantly higher serum concentrations of TNF-α were measured for p = 0.0001 in the group of patients with endometriosis compared to CG patients (6.34 *vs.* 5.3) (Figure 4).

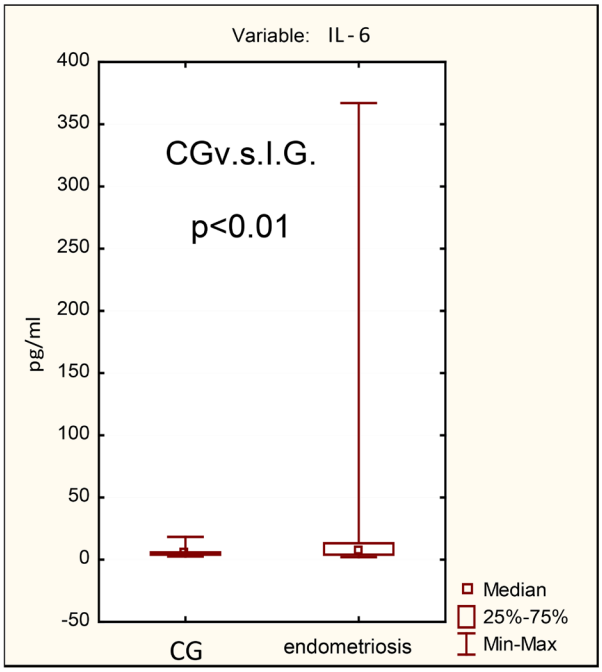

Fig 1. Distribution of IL-6 (CG vs.IG),

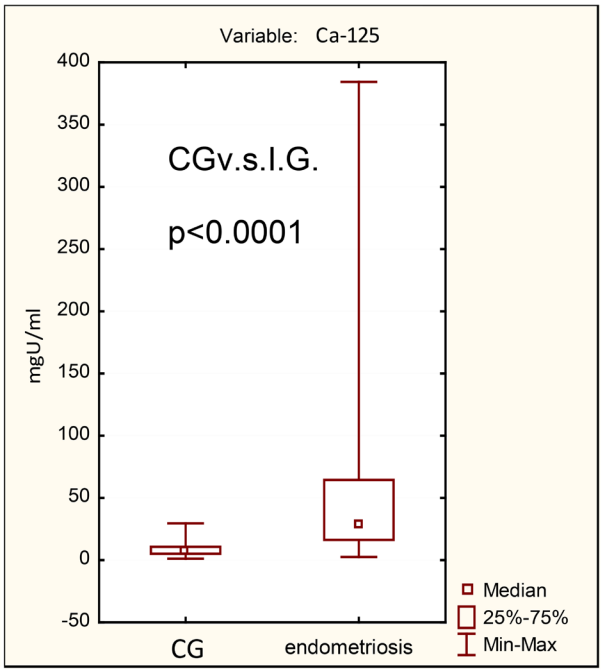

Fig. 2 Distribution of CA-125 (CG vs. IG)

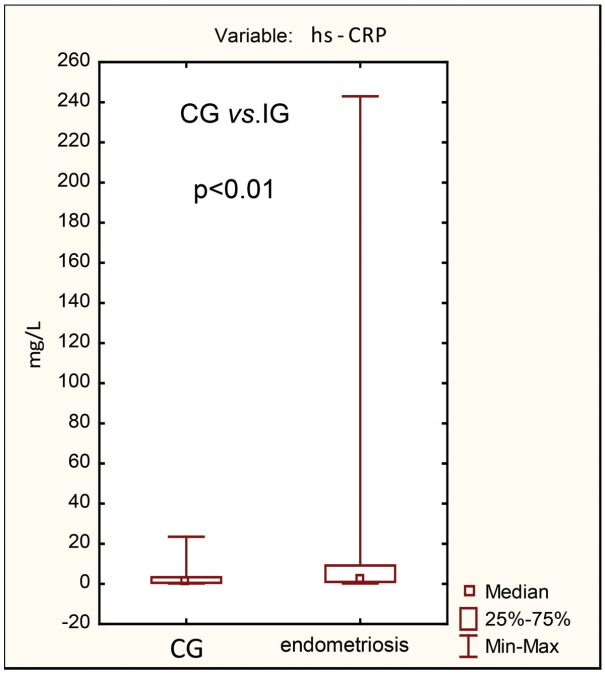

Fig 3. Distribution of hs-CRP (CG vs. IG),

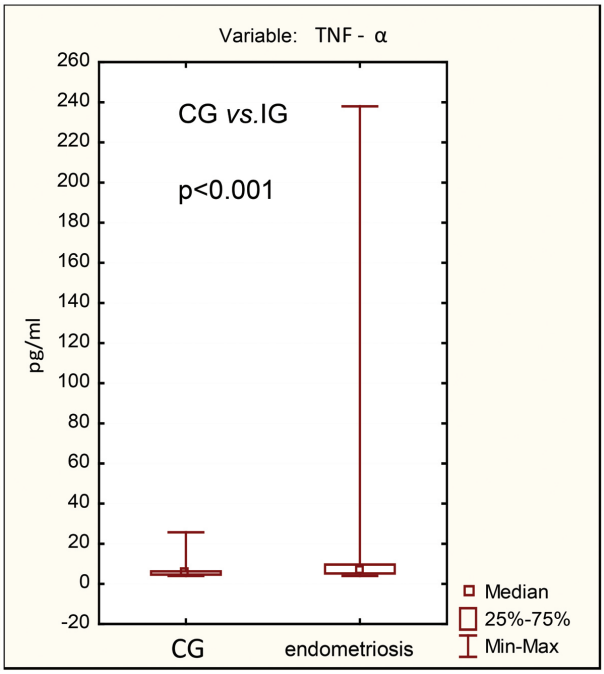

Fig. 4 Distribution of TNF-α (CG vs. IG)

Table 3 presents the results of the univariate and multivariate logistic regression analysis showing the variables associated with endometriosis. The variables that proved to be significant by univariate analysis (IL-6, CA-125, hs-CRP and TNF-α) were included in a multivariate analysis model, which determined serum CA-125 as a marker significantly associated with endometriosis. Increasing serum concentrations of CA-125 by 1mIU / ml increased the chance of endometriosis by 19.7% (OR = 1.197; 95% CI 1.108-1.294).

Table 3. Univariate and multivariate analysis of the examined biomarkers (IL-6, CA-125, hs-CRP and TNF-α)

	Univariate analysis				Multivariate analysis			
			95% CI for Exp (B)				95% CI for Exp (B)	
	p	Exp (B)	Lower	Upper	p	Exp (B)	Lower	Upper
age	0.557	1.017	0.961	1.077				
IL6	0.011	1.117	1.025	1.218	0.714	1.031	0.875	1.215
CA 125	0.000	1.220	1.132	1.314	0.000	1.197	1.108	1.294
CRP	0.019	1.093	1.015	1.177	0.617	1.030	0.917	1.157
TNF alfa	0.023	1.161	1.021	1.320	0.119	1.105	0.975	1.252

Elevated serum IL-6 levels were significantly more frequently reported in patients with endometriosis compared to CG (56.6% vs. 23.6%, p = 0.00013). Table 4 shows the diagnostic performance of IL-6 as a predictor of endometriosis. IL-6 had a sensitivity of 56.63% and a specificity of 76.36% in discrimination between patients with endometriosis and the control group at cut-off values of 5.9 pg / ml.

Table 4. Contingency table of the diagnostic performance of IL-6 as a predictor of endometriosis

IL-6	IG-with endometriosis	CG	Total
>5.9	47 (56.63%)	13 (23.64%)	60
<5.9	36 (43.37%)	42 (76.36%)	78
total	83	55	138
Chi-square = 14.65 ***p=0.00013			
sensitivity	56.63%		
specificity	76.36%		
positive predictive value	21.02%		
negative predictive value	94.06%		

***p<0.0001

There were no patients in the CG with elevated serum CA-125 concentrations, while in the endometriosis group 37.35% of patients had elevated CA-125. The intergroup comparison in the frequency of elevated serum CA-125 was statistically significant (p <0.0001).

Table 5 shows the diagnostic performance of CA-125 as a predictor of endometriosis at a cut-off value of 35 mIU / ml.

Table 5. Diagnostic performance of CA-125 as a predictor of endometriosis

CA – 125	IG	CG	Total
>35	31(37.35%)	0	31
<35	52(62.65%)	55(100%)	107
total	83	55	138
Chi-square = 26.65 ***p<0.0001			
sensitivity	37.35%		
specificity	100%		
positive predictive value	100%		
negative predictive value	93.49%		

Table 5 shows that CA-125 had a sensitivity of 37.35% and a specificity of 100% in the diagnosis of endometriosis.

For p = 0.011, a statistically significant difference in the frequency of increased hs-CRP in the serum between the two groups was confirmed. Values were increased in 38.55% and 18.2% of patients, respectively in the endometriosis and control groups.

Table 6 illustrates the diagnostic performance of hs-CRP as a predictor of endometriosis. Hs-CRP had a sensitivity of 38.55% and a specificity of 81.82% in the diagnosis of endometriosis, at a cut-off value of 5mg / ml.

Table 6. Diagnostic performance of CA-125 as a predictor of endometriosis

hs-CRP	IG	CG	Total
>5	32(38.55%)	10(18.18%)	42
<5	51(61.45%)	45(81.82%)	96
total	83	55	138
Chi-square = 6.48 *p=0.011 sig			
Chi-square = 6.48 *p=0.011 sig	37.35%		
sensitivity	38.55%		
specificity	81.82%		
positive predictive value	19.07%		
negative predictive value	92.30%		

*p<0.05

The TNF-α biomarker was elevated in the serum of 30.1% of patients with endometriosis, and in 9.1% of CG patients, with a significant difference of p = 0.0034.

Table 7 shows the diagnostic performance of TNF-α as a predictor of endometriosis. TNF-α had a sensitivity of 30.12% and a specificity of 90.91% in the diagnosis of endometriosis at a cut-off value of 8.1pg / ml.

Table 7. Contingency table of the diagnostic performance of IL-6 as a predictor of endometriosis

TNF-α	IG	CG	Total
>8.1	25(30.12%)	5(9.09%)	30
<8.1	58(69.88%)	50(90.91%)	108
total	83	55	138
Chi-square = 8.59 **p=0.0034			
sensitivity	30.12%		
specificity	90.91%		
positive predictive value	26.91%		
negative predictive value	92.13%		

**p<0.01

ROC analysis of the area under the curves was performed to determine the discriminant ability of IL-6, Ca-125, hs-CRP, and TNF-alfa as tests for the diagnosis of endometriosis. A ROC curve was constructed for all of these tests.

The results showed that based on the size of the area under the ROC curve (AUC), IL-6, CA-125, hs-CRP and TNF-alpha were tests with a satisfactory ability to distinguish patients with endometriosis from the control group: IL-6 (AUC = 0.665), CA-125 (AUC = 0.687), hs-CRP (AUC = 0.602), TNF-alpha (AUC = 0.605).

Discussion

In patients with severe endometriosis, the clinical picture is dominated by pelvic pain, dysmenorrhea, infertility, and ultrasound evaluation indicating the presence of endometriotic cysts in the ovaries. In these patients, gynecologists will advise laparoscopic extirpation of the endometriomas and endometriotic lesions, so that the diagnosis of endometriosis will be made quickly. However, a large percentage of patients with mild endometriosis can be asymptomatic. Early diagnosis is important for them, which will allow them to start therapy for endometriosis immediately, before severe irreversible damage to the

female genitals occurs. The use of non-invasive biomarkers would allow early suspicion of endometriosis and early therapy and invasive procedures such as laparoscopy would be avoided.

In our study we found elevated values of markers of inflammation (hs-CRP), cytokines (IL-6, TNF-α) and tumor marker CA-125 in the investigated group of patients with endometriosis compared to the control group of healthy patients, which corresponded to the results obtained in the studies of Irungu S. et *al*., and May KE et *al*. [18,19]. None of the patients operated for endometriosis in our study had an infection of the operative wound postoperatively, and the blood for analysis was taken preoperatively, so that the surgical treatment had no effect on the results of the study.

In the group of patients with endometriosis, we found elevated values of IL-6 compared to the levels in the control group of patients (6.97 vs. 4.98, p = 0.0045). Similar results were presented by Kashanian M. et *al*., who in their study found higher CA-125 and IL-6 values in patients with endometriosis than in the control group of patients, but AUC (CA-125) and AUC (IL -6) did not show significant difference and diagnostic value ofthese tests in the diagnosis of endometriosis[20].

Recent studies have shown that there is a correlation between CA-125 values and the stage of endometriosis. In the study of Tian Z. et *al*., the sensitivity of this biomarker was found to be 63.1% in stage 3 and 4 endometriosisversus 24.8% for the first and second stage endometriosis[21]. The CA-125 tumor marker values had diagnostic value in diagnosing advanced stages of endometriosis as well as in monitoring the treatment of these patients in thestudy of Wu MH et *al*.[22] and inthe study of Maiorana A. et *al*.[23].

In the study of Sütcü H. K et *al*. significantly elevated values of the tumor marker CA-125 were found in patients in all stages of endometriosis in comparisonto the control group of patients, as well as in patients with stages 3 and 4 of the disease compared to the control group of patients ($p<0.05$). CA-125 had a sensitivity of 70% and a specificity of 79% in discrimination between patients with stage 3 and 4 endometriosis in comparison to the control group of patients, at a cut-off value of 21.7U / ml [24].

In our study, in CG there were no patients with elevated serum CA-125 concentrations. In the group of patients with endometriosis, 37.35% of patients had elevated CA-125 values. Based on the area under the CA-125 curve (AUC) in the diagnosis of endometriosis, at a cut-off value of 35 mIU / ml, the sensitivity was 37.35% and the specificity 100%. Similar results were obtained in a study by Bilibioet *al*., who found higher sensitivity and specificity of CA-125 in the diagnosis of advanced (third and fourth stage) endometriosis [25].

In our study patients with endometriosis were not divided into groups according to the stage of the disease, so the sensitivity and specificity of CA-125 in the diagnosis of endometriosis were lower.

IL-6 is a pleotropic cytokine produced by a range of cell types such as monocytes, lymphocytes, fibroblasts, endothelial cells, and kerati-

nocytes. IL-6 is also produced in the eutopic and ectopic endometrium. IL-6 is a mediator in the active phase of inflammation and angiogenesis.

Bedaiwy et *al.* in their study found that serum IL-6 levels could be used to discriminate patients with and without endometriosis. They found a sensitivity of 90% and a specificity of 67% for IL-6 in the diagnosis of endometriosis, at a cut-off value of 2 pg / ml 26. In our study we found significantly elevated IL-6 values in patients with endometriosis compared to the levels in the control group (56.6% vs. 23.6%, p = 0.00013).

For IL-6 we found a sensitivity of 56.63% and a specificity of 76.36% in the prediction of endometriosis, at a cut-off value of 5.9 pg / ml. According to these results, IL-6 showed the highest sensitivity and specificity of all other inflammatory markers and cytokines in the diagnosis of endometriosis.

Hs-CRP is a marker of inflammatory response and can be used as a non-invasive biomarker of endometriosis. In our study, the hs-CRP biomarker presented significantly different serum concentrations in patients of both groups. Hs-CRP presented elevated serum values in 38.55% of patients with endometriosis versus 18.2% of patients without endometriosis. At cut-off values of 5 mg / ml, the sensitivity of hs-CRP in the diagnosis of endometriosis was 38.55%, and the specificity 81.82%

In their study, Abrao et *al.* reported elevated hs-CRP levels in patients with severe endometriosis (stage 3 and 4) [27].

Endometriosis patients and CG patients in our study differed significantly in their serum TNF-α values, with significantly higher median serum TNF-α concentrations in the endometriosis group compared to CG patients (6.34 vs. 5.3, p =0.001).

The TNF-α biomarker was elevated in the serum of 30.12% of patients with endometriosis and in 9.09% of patients with CG, with a significant difference between IG versus CG (p = 0.0034). TNF-α as a single marker is useful in distinguishing patients with endometriosis from healthy patients (AUC = 0.605), with a sensitivity of 30.12% and a specificity of 90.91%, at a cut-off value of 8.1 pg / ml.

In the study of Furucu FN. et *al.* from 2020, the authors observed the expression of TNF-α and IL-6 in normal and endometrial tissue using the immunohistochemical method. They found increased immunoreactivity of TNF-α and IL-6 in the endometrial tissue of the examined patients compared to healthy patients and stated that these two biomarkers play an important role in the pathogenesis of endometriosis [28].

Conclusions

In this study, the use of several biomarkers in the diagnosis of endometriosis (CA-125, IL-6, hs-CRP and TNF-α) was evaluated. The results showed statistically significantly higher concentrations of CA-125, IL-6 and TNF-α and hs-CRP in patients with endometriosis compared to the concentrations in the control group of patients. The results of our study showed that none of these biomarkers individually has high sensitivity and specificity for the diagnosis of endometriosis.

The limitation of our study is the

smaller number of patients examined compared to other studies. It is necessary to find a panel combination of biomarkers with a high sensitivity of about 100% that will enable early diagnosis of endometriosis. Also, this combination of biomarkers will allow patients with infertility and pelvic pain to be singled out as patients at high risk for endometriosis who will undergo laparoscopy with excision of the endometrial foci.

Aknowledgment: This paper is part of the project entitled: "Correlation of inflammatory cytokines and tumor markers in patients with endometriosis" supported by the Faculty of Medicine in Skopje.

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