

# Determination of serum CRP, VEGF, Leptin, CK-MB, CA-15-3 and IL-6 levels for malignancy prediction in adnexal masses

## *Adneksiyel kitlelerde malignite prediksyonunda tümör belirteci olarak serumda CRP, VEGF, Leptin, CK-MB, CA-15-3, IL-6 bakılması*

Serhat Şen<sup>1</sup>, Oğuzhan Kuru<sup>1</sup>, Özgür Akbayır<sup>2</sup>, Hilal Oğuz<sup>3</sup>, Vildan Yasasever<sup>3</sup>, Sinan Berkman<sup>1</sup>

<sup>1</sup>Department of Obstetrics and Gynecology, İstanbul Faculty of Medicine, İstanbul University, İstanbul, Turkey

<sup>2</sup>Department of Obstetrics and Gynecology, Kanuni Sultan Süleyman Research and Training Hospital, İstanbul, Turkey

<sup>3</sup>Department of Basic Oncology, Institute of Oncology, İstanbul University, İstanbul, Turkey

### Abstract

**Objective:** Investigation of serum markers which could be used in the malignancy prediction of adnexal masses.

**Material and Methods:** Vascular endothelial growth factor (VEGF), interleukin 6 (IL-6), leptin, C-reactive protein (CRP), creatine-kinase-MB (CK-MB) and cancer antigen 15-3 (CA 15-3) levels were determined prospectively in serum samples that were obtained from patients who underwent surgery for an adnexal mass and who were referred to İstanbul University, Faculty of Medicine, Department of Obstetrics and Gynecology, between 2009 and 2011, and then were compared with the serum samples of completely healthy outpatient patients as a control group. Based onto the ovarian cancer status, cases were divided into four groups: 13 patients were included in the early-stage malignant group, 12 patients were included in the advanced-stage malignant group, 25 in the benign group and 19 in the healthy control group. Patients with only epithelial ovarian cancer were included into the cancer group. Ethics Committee approval was obtained for this study. The budget was supported by the İstanbul University Scientific Research Projects Unit.

**Results:** Results related with sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and odds ratio (OR), respectively, and the following values were calculated: 48%, 95%, 92%, 59% and +OR 9.6 -OR 0.5 for CA; 15-3; 52%, 75%, 72%, 55%, +OR 2.08 -OR 0.64 for leptin; 72%, 70%, 75%, 66% 2.4-0.5 for IL-6; 24%, 80%, 60%, 45%, 1.2-0.92 for VEGF; 68%, 30%, 55%, 43%, 0.97-1.06 for CRP; and 8%, 70%, 25%, 38%, 0.26-1.31 for CK-MB.

**Conclusion:** CA 15-3, IL-6, Leptin, VEGF and CRP were effective in the prediction of benign and malignant masses; however they may be more suitable in selected cases as they have a limited use because of their inadequate potential regarding sensitivity and specificity.

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**Key words:** Adnexal masses, malignancy prediction, epithelial ovarian cancer, serum biomarkers

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### Özet

**Amaç:** Adneksiyel kitlelerde malignite ayırımında kullanılabilecek serum markerlarının araştırılması.

**Gereç ve Yöntemler:** İstanbul Üniversitesi İstanbul Tıp Fakültesi Kadın Hastalıkları ve Doğum Anabilim Dalı'nda 2009-2011 yılları arasında prospektif olarak, adneksiyel kitle endikasyonu ile opere olan hastalardan alınan serum örneklerinde vascular endotelial growth factor (VEGF), interlökin 6 (IL-6), leptin, C-reaktif protein (CRP), kreatin kinaz-MB (CK-MB) ile kanser antijeni 15-3 (CA 15-3) düzeyleri belirlendi ve kontrol grubu olan tamamen sağlıklı poliklinik hastalarının serum örnekleriyle karşılaştırıldı. Olgular over kanseri durumuna göre 4 grupta toplandı: 13 olgu erken evre malign, 12 olgu ileri evre malign, 25 olgu benign, 19 olgu sağlıklı kontrol grubunda yer aldı. Over kanseri grubunda sadece epitelyal over kanseri olan olgular çalışmaya dahil edildi. Çalışma için etik kurul onayı alındı ve bütçe ise İstanbul Üniversitesi Bilimsel Araştırma Projeleri Birimi tarafından desteklendi.

**Bulgular:** Belirteçlerin duyarlılık, özgüllük, pozitif prediktif değer (PPD), negatif prediktif değer (NPD) ve odds ratio (OR) sonuçlarına bakıldığında CA 15-3 için sırasıyla %48, %95, %92, %59 ve +OR 9.6 -OR 0.5; leptin için sırasıyla %52, %75, %72, %55, +OR 2.08 -OR 0.64; IL-6 için %72, %70, %75, %66 2.4-0.5; VEGF için %24, %80, %60, %45, 1.2-0.92; CRP için %68, %30, %55, %43, 0.97-1.06; CK-MB için %8, %70, %25, %38, 0.26-1.31 değerleri hesaplandı.

**Sonuç:** CA 15-3, IL-6, Leptin, VEGF ve CRP benign-malign over tümörü ayırımında etkin bulundu ancak duyarlılık ve özgüllükleri yeterli kadar kuvvetli olmadığı için rutinde kullanımı kısıtlı olup seçilmiş vakalarda kullanımı uygun olabilir.

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**Anahtar kelimeler:** Adneksiyel kitleler, malignite prediksyonu, epitelyal over kanseri, serum biomarkerler

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## Introduction

Ovarian cancer is the second most common and lethal gynecological cancer seen in women (1). The condition ranks in 5<sup>th</sup> place in cancers among women. Ovarian cancers are listed in 4<sup>th</sup> place for death due to cancer in women. In the U.S.A, more than 15.000 women die every year because of ovarian cancer (2). At the early stage, life expectancy can be as high as 90%, but unfortunately only 19% of the patients can be diagnosed during the early stage of the disease (3). Most of the patients are diagnosed at the advanced stage when consequently their life expectancy falls below 30%. Early ovarian cancer may not cause obvious symptoms. Ovarian cancer is generally diagnosed when considerable abdominal fluid is observed (at this stage the tumor can be at an advanced stage) or when a mass in the pelvic region is found by the imaging methods (ultrasonography, magnetic resonance imaging, computerized tomography). However, these imaging methods may not be capable of having a high specificity to differentiate benign and malignant tumors. Furthermore, additional surgical interventions may be required when an abnormal image is determined. Consequently, imaging methods may not be reliable in detecting ovarian cancers at the early stage and can be variable (4).

However, there are no biomarkers that are known to diagnose ovarian cancer. Cancer antigen 125 (CA-125) was frequently employed to help the diagnosis of ovarian cancer, but it was basically approved to determine the recurrent disease and monitor response to the therapy (5-7). The sensitivity of CA-125 in detecting an early stage ovarian cancer varies between 29% - 75%. Additionally, CA-125 level may also increase during certain circumstances such as endometriosis, pregnancy, menstruation, cardiac failure and cirrhosis, which are considered as normal or benign conditions. In a recent study, it was observed that an abnormal level of CA-125 failed to indicate the presence of a cancer in 86% of the cases, whereas the mentioned level returned to normal within 3-6 months (8). Today, we still require good biomarkers which can be used in the diagnosis of ovarian cancer.

## Materials and Methods

Blood samples were collected from patients who were operated on due to an adnexal mass and from outpatient patients who were completely healthy as a control group, prospectively between 2009-2011 at the Istanbul University, Istanbul Medical Faculty Department of Obstetrics and Gynecology. Blood samples were kept 30 minutes in room temperature and then were centrifuged for 10 minutes at 4000 rpm. Separated serums were stored at -80°C until the number of tests were completed.

Serum C-reactive protein (CRP) levels of patients and control groups were determined by a Toshiba Accute auto-analyzer and immuno-turbidimetric method, while creatine-kinase-MB (CK-MB) and cancer antigen 15-3 (CA 15-3) levels were determined with an Abbott AxSYM device and micro-particle enzyme immune-assay (MEIA) method. Vascular endothelial growth factor (VEGF), interleukin 6 (IL-6) and leptin levels were determined by a dual antibody sandwich enzyme immune-assay (ELISA) method.

All pathological samples were examined and diagnosed by 3 specialists from Istanbul Faculty of Medicine, Department of Gynecopathology. The FIGO surgical staging system was used in the staging of ovarian cancer. Histopathological classification was carried out according to the World Health Organization. Serum samples obtained from patients were examined at the Istanbul University, Institute of Oncology, Department of Cancer Biochemistry.

Patients were classified in four groups: Early Stage (Stage 1 and 2), Advanced Stage (Stage 3 and 4), benign ovarian tumors and healthy control groups. Only patients with epithelial ovarian cancer were included into the cancer groups. Patients were distributed into appropriate groups according to their postoperative diagnosis and surgical stage. Accordingly, 13 patients were included in the early stage malignant group, 12 patients in the advanced stage malignant group, 25 patients in the benign group and 19 patients in the healthy (control) group. The control group was used to determine the cut-off values of markers which did not have a cut-off value. The upper limit value was estimated by an average +2 standard deviation formula at the 95% confidence interval. Demographic similarities of patients' data were compared by the student's t-test. Statistical power analysis was also made. Because of time limitation and inadequate cases, only 70% statistical power was reached with 25 ovarian cancer cases.

A comparison was made by the student's t-test and Mann-Whitney U-test to determine if there was a difference between the mean value of each tumor marker in the groups. The Wilcoxon method was used to calculate the value of markers regarding the difference between benign and malignant tumors. However, the number of patients with a malignant tumor in the study group was low and therefore ANOVA testing was not applied. Instead, the Kruskal-Wallis test was used to determine the relationship of the stage of the tumor of each parameter. The correlation between markers in malignant tumors was determined by a Pearson correlation analysis. A linear regression study was carried out on markers which showed significant correlation. Cut-off values were obtained as the following:

CA 15-3: 0-30 U/mL

CRP: 5 mg/dl

Cut-off values were obtained from the healthy control group for CK-MB, IL-6, VEGF and Leptin. The following values were estimated: 9.65 pg/mL for IL-6, 201 pg/mL for VEGF, 1.225 pg/mL for Leptin and 0.9 ng/mL for CK-MB. Ethics Committee approval was obtained for this study. The budget was supported by the Istanbul University Scientific Research Projects Unit.

## Results

Clinical characteristics of patients showed that the most frequent histological diagnosis was serous carcinoma; 20% of the patients were at the early stage malignant, 18.75% at the advanced stage malignant, 31% at the benign ovarian tumor and 29.6% were the healthy control group. The distribution of these ratios are displayed in Table 1. Average age and parity were found statistically significant at higher levels in the

advanced stage cancer group, respectively 49.6 and 3.4 when compared to other groups ( $p < 0.05$ ) (Table 2).

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and odds ratio (OR) for CA 15-3, respectively, were 48%, 95%, 92%, 59% and +OR 9.6 -OR 0.5.

Sensitivity, specificity, PPV, NPV and OR for IL-6, respectively, were 72%, 70%, 75%, 66%, 2.4-0.5.

**Table 1. Clinical characteristics of patients**

	Number of Patients (%)
Ovarian Cancer Groups	
Early (I and II)	13 (20.3%)
Advanced (III and IV)	12 (18.75%)
Benign Ovarian Tumor	25 (31.25%)
Healthy (control) group	19 (29.6%)
Histology	
Serous carcinoma	12 (17.39%)
Endometrioid carcinoma	5 (7.24%)
Mucinous carcinoma	6 (8.69%)
Mixed carcinoma	2 (2.89%)
Endometrioma	9 (13.04%)
Serous cystadenoma	7 (10.14%)
Mucinous cystadenoma	4 (5.79%)
Teratoma	3 (4.3%)
Fibrotectoma	2 (2.8%)

**Table 2. Demographic data of the groups**

Group		Year	Gravida	Parity
Early stage	N	13	10	10
	Mean	44.6	3.8	2.1
	Standard deviation	4.45	1.1	0.6
	Minimum	25	0	0
	Maximum	62	6	4
Advanced Stage	N	12	10	10
	Mean	49.6	4.2	3.4
	Standard deviation	6.4	1.4	0.8
	Minimum	36	0	0
	Maximum	66	10	6
Benign	N	25	19	19
	Mean	41.6	3.5	2.1
	Standard deviation	10.3	1.3	1.0
	Minimum	23	0	0
	Maximum	68	10	7
Control	N	19	17	16
	Mean	40.1	3.4	2.3
	Standard deviation	8.9	1.4	1.1
	Minimum	27	0	0
	Maximum	57	13	12

Sensitivity, specificity, PPV, NPV and OR for leptin, respectively, were 52%, 75%, 72%, 55%, +OR 2.08 -OR 0.64.

Sensitivity, specificity, PPV, NPV and OR for VEGF, respectively, were 24%, 80%, 60%, 45%, 1.2-0.92.

Sensitivity, specificity, PPV, NPV and OR for CRP, respectively, were 68%, 30%, 55%, 43%, 0.97-1.06.

Sensitivity, specificity, PPV, NPV and OR for CK-MB, respectively, were 8%, 70%, 25%, 38%, 0.26-1.31.

Accordingly, it was significant that CA 15-3 was the most specific and IL-6 the most sensitive markers (Table 3). Pearson correlation analysis displayed a correlation coefficient value of 0.193 among these two values, while p value was 0.013 which was considered statistically significant. On the other hand, leptin with relatively high positive and negative predictive ratios, showed a significant correlation with CA 15-3 (correlation coefficient: 0.185,  $p = 0.020$ ) (Table 4).

The logistic regression analysis of CA 15-3 and IL-6 was found more sensitive in the malignancy prediction of ovarian tumors when used concomitantly, but failed to reach a level which can be considered as significant.

Among tumor markers, CA 15-3, IL-6 and CRP showed a significant increase in the malignant tumor group, while VEGF and Leptin displayed a significant increase both in the malignant and benign tumor groups when compared with the control group (Table 5). Also, a statistical difference was found in CA 15-3, IL-6 and CRP levels between the early stage and the advanced stage groups. However, these markers were not capable of making a prediction related with stage differentiation (Table 6).

We have analysed the success of these markers by the Wilcoxon rank test to understand what parameter they were successful in detecting an early stage ovarian tumor, and after separate measurements, it was concluded that CA 15-3, IL-6, Leptin and VEGF were suitable to be used in the early stage differentiation of ovarian tumors ( $p < 0.05$ ) (Table 7). Also, a ROC curve (Figure 1) was determined. It was also concluded that CA 15-3, IL-6 and CRP were useful in differentiating ovarian cancers from benign tumors (Table 8). The sensitivity, specificity, PPV and NPV of these markers must be considered.

## Conclusion

There is no ideal model for scanning of an ovarian cancer nor is there an approved clinical test for diagnosis at the early stage. Currently, early stage sensitivity of the many biomarkers which were used to clinically diagnose ovarian cancer, is low (9). At present CA 125 is the most reliable serum marker in ovarian cancer but its role in scanning the disease is challengeable. High CA 125 levels can be encountered in 30% during Stage I and 90% during the advanced stage (10). Additionally, mucinous ovarian tumors may secrete a lesser degree of CA 125 when compared to non-mucinous type ovarian cancers (11). Many researchers agree that a minimum 10% positive predictive value must be achieved in the scanning process of an ovarian cancer. To attain this value, it may be necessary to reach a minimum ratio of 99.6% specificity. Nevertheless, specificity of CA 125 is considerably lower than this value (12-14). Therefore,

**Table 3. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) of 6 biomarkers**

Marker	Cut-off value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
CA 15-3	30 U/mL	48	95	92	59
IL-6	9.65 pg/mL	72	70	75	66
Leptin	1225 pg/mL	52	75	72	55
VEGF	201 pg/mL	24	80	60	45
CRP	5 mg/dl	68	30	55	43
CK-MB	0.9 ng/mL	8	70	25	38

**Table 4. Pearson’s correlation coefficients of 6 biomarkers**

	CA 15-3	IL-6	Leptin	VEGF	CRP	CK-MB
<b>CA 15-3</b>						
CC	1	0.193*	-0.185 <sup>¥</sup>	0.131	0.095	0.024
p value		0.013	0.020	0.087	0.120	0.95
<b>IL-6</b>						
CC	0.193	1	-0.108	0.101	0.126	0.031
p value	0.013		0.091	0.130	0.130	0.87
<b>Leptin</b>						
CC	-0.185	-0.108	1	-0.113	-0.098	0.28
p value	0.020	0.091		0.081	0.24	0.98
<b>VEGF</b>						
CC	0.131	0.101	-0.113	1	0.361	0.020
p value	0.087	0.112	0.081		0.361	0.91
<b>CRP</b>						
CC	0.095	0.126	-0.098	0.071	1	0.076
p value	0.120	0.130	0.24	0.361		0.210
<b>CK-MB</b>						
CC	0.024	0.031	0.28	0.020	0.076	1
p value	0.85	0.87	0.88	0.91	0.210	

CC: correlation coefficient, \*: significant positive correlation p<0.05, <sup>¥</sup>: significant negative correlation p<0.05

to achieve this purpose; various biomarkers must be used synchronically (15, 16).

Because of the heterogenic structure of the ovarian cancer, a single biomarker may not be capable of covering the entire types and stages. Use of multiple biomarkers may be necessary to determine ovarian cancer at an early stage (17). Gorelik et al. were successful in differentiating 44 early stage ovarian cancers in the serum samples of 45 healthy women by using a combination of IL-6, IL-8, VEGF, EGF and CA 125 biomarkers, where a ratio of 84% for sensitivity and 95% for specificity were obtained (18). In a study performed by Zhang et al. early stage ovarian cancer was diagnosed with 72% sensitivity after CA 125, CA 72-4, CA 15-3 and M-CSF biomarkers were used in combination according to an artificial estimation system (19). Similarly, Skates et al. determined early stage ovarian cancer with a 98% specificity and 75% sensitivity by using a CA 125, CA 15-3, CA 72-4 and M-CSF combination (20). Additionally, different biochemical and ultrasonographic parameters can be used to carry out a differentiated diagnosis of adnexal masses based on logistic regression models (21). In a study performed by Woolas et al.

**Table 5. Comparison of levels of tumor markers among the groups (mean ± standard deviation)**

Tumor marker	Ovarian Cancer n=25	Benign ovarian tumor n=25	Control n=19
CA 15-3	61.3±14*	13.86±7.1	15.2±5.1
IL-6	70±11.0*	26±7.7	26.5±8.4
Leptin	745.5±39*	862.11±41.9 <sup>¥</sup>	941±28.4
VEGF	162.2±10.7*	142.2±12 <sup>¥</sup>	96.4±9.2
CRP	16±1.5*	11.8±1.3	11.6±1.1
CK-MB	0.63±0.01	0.74±0.04	0.58±0.02

\* Statistical difference was determined in the cancer group compared with the control group, p<0.05. (Negative correlation was also observed in the leptin levels), <sup>¥</sup> Statistical difference was determined in the benign ovarian tumor group compared with the control group, p<0.05

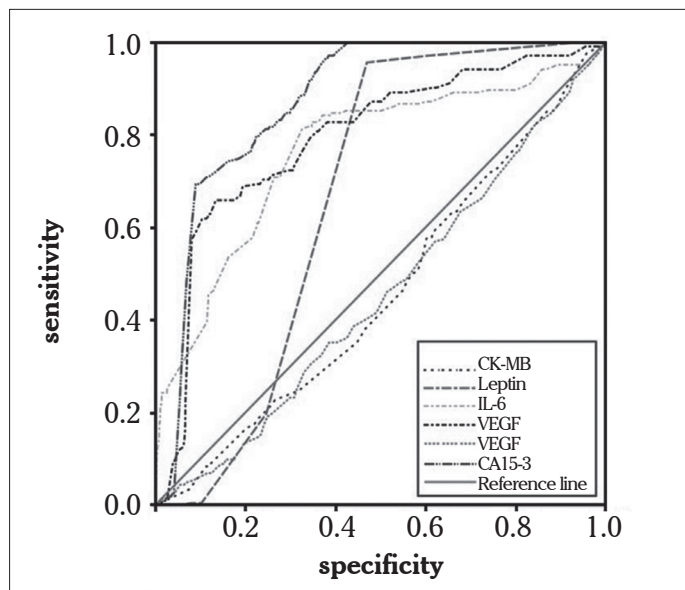
effectiveness was enhanced by combining 8 different tumor markers (MCSF, OVX1, LASA, CA 15-3, CA 72-4, CA 19-9, CA 54 and CA 61) in 492 patients, where 192 of these patients had ovarian cancer. The single sensitivity and specificity of CA 125

was found as 78.1% and 76.8% respectively. Different combinations were used in logistic regression analysis where sensitivity achieved a ratio of 90.6% and a specificity of 93.2% where this improvement can be considered as satisfactory (22).

**Table 6. Deviation of significant tumor markers among the stages (mean±standard deviation)**

Tumor Marker	Stage I and II n=13	Stage III and IV n=12
CA 15-3	23.21±2.6	102.7±19.7*
IL-6	31.1±4.4	113.3±14.3*
Leptin	731±38.9	761.2±39.3
VEGF	178.9±13.6	144.9±6.6
CRP	7.5±0.6	25.5±1.7*

\*statistical difference was encountered between the early stage and the advanced stage groups p<0.05



**Figure 1. ROC Curve**

**Table 7. p values of the markers for the detection of early-stage ovarian cancer**

	CA 15-3	IL-6	Leptin	VEGF	CRP	CK-MB
p value	0.044	0.038	0.001	0.0003	0.213	0.762

**Table 8. Analysis of the ROC Curve**

Marker	Area Under the Curve (AUC)	Standard Deviation	p value	95% confidence
CA 15-3	0.861*	0.009	0.000	0.810- 0.823
IL-6	0.785*	0.016	0.000	0.751- 0.814
Leptin	0.063	0.012	0.000	0.638- 0.681
VEGF	0.458	0.022	0.040	0.404- 0.462
CRP	0.761*	0.021	0.000	0.718- 0.796
CK-MB	0.451	0.023	0.051	0.398- 0.458

\*CA 15-3, IL-6 and CRP were useful in differentiating ovarian cancers from benign tumors

In our study, CA 15-3 was found to be the most specific and IL-6 the most sensitive marker. Pearson correlation analysis demonstrated that concomitant use of CA 15-3 and IL-6 in logistic regression analysis, as these markers display a positive correlation, could be more sensitive in the differentiation of benign-malignant ovarian tumors, but these findings were unable to reach a significant level. In a similar study, CA 15-3 showed a lower sensitivity ratio, while higher specificity in the malignancy prediction of adnexal masses was found (23).

Gil Mor et al. found that 4 biomarkers (leptin, prolactin, osteopontin, insulin-like growth factor-II) may not be useful alone in the prediction of malignancy, but the combination of these four markers may be effective in the prediction of an early stage ovarian cancer as they possess a 95% ratio of sensitivity, 95% ratio of specificity, 95% ratio of PPV and 94% ratio of NPV (15). In our study, leptin alone was also found effective in the prediction of early stage ovarian cancer. However its 52% ratio of sensitivity and 75% ratio of specificity may cause a handicap for clinical significance.

The most comprehensive study in the literature was carried out by Bertenshaw et al. where a total number of 204 molecules were investigated. Among these molecules, 104 antibodies, 44 autoimmune markers, and 56 infection molecules were significant. Also, levels of 77 biomarkers were found different and the most significant molecules were CA 125, CRP, EGFR, IL-10, IL-8, CTGF, haptoglobin, and TIMP-1. Excluding CA 125, no specificity higher than 80% was determined in any of the mentioned markers. The 8 markers herein mostly consist of inflammation markers and acute phase reactants. CRP level was found significantly increased and was accepted as the second most determinative biomarker. Increased IL-6 level was stated to be responsible for the high correlation of CRP level. The inflammatory response and the active state of the tumor, may prepare a suitable environment for angiogenesis and metastasis, as there is also some information that it may contribute to the progression of the cancer (24, 25). In our study CRP and IL-6 levels were also found significant in the scanning of ovarian cancer (Table 8). Angiogenesis plays a significant role in the tumorigenesis. Bertenshaw et al. (25) showed a significant increase in the VEGF level, which is a

potent angiogenetic factor. In our study, a similar increase was also detected in the VEGF levels of the ovarian cancer group. We found higher ratios of mean age and higher parity values in patients included in the advanced stage ovarian cancer group when compared with other groups ( $p < 0.05$ ). Even though this finding is compatible with the information in the literature related with the increased risk of ovarian cancer due to higher age, it is contrary to the information that ovarian cancer risk may decrease when parity is increased (26-28).

Although a ROC curve was created in this study, the number of early stage ovarian cancer cases in the sample group was fewer than expected and could cause a bias for our study. Information was obtained from a limited number of patients and therefore clinical significance is readily decreased. However, according to the current data, CA 15-3, IL-6, leptin, VEGF and CRP were found effective in the differentiation process of benign-malignant ovarian tumors. Parameters such as sensitivity and specificity alone were not as effective as estimated, and may not be convenient for routine applications, but differential diagnosis can be used in solving problematic cases.

There is a strong need for large-scale serial studies which may confirm and strengthen the findings of this study. Additionally, this study could be an important indicator that it may not be suitable to use CK-MB as a biomarker in future studies.

#### Conflict of interest

The budget was supported by the Istanbul University, Scientific Research Projects Unit.

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