

## CLINICAL STUDY

# Significance of serum c-erbB-2 oncoprotein, insulin-like growth factor-1 and vascular endothelial growth factor levels in ovarian cancer

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**ABSTRACT**

**OBJECTIVES:** Our aim was to determine the predictive values of serum levels of several growth factors in ovarian cancer, including soluble c-erbB-2 oncoprotein, insulin-like growth factor-1 (IGF-1) and vascular endothelial growth factor (VEGF).

**BACKGROUND:** Previous studies have shown that growth factors play an important role in carcinogenesis.

**METHODS:** Two groups were established. One of them was the malignant group which included 41 patients with ovarian carcinoma and the other was the control group that was made up of 28 healthy volunteers. Preoperative serum samples were obtained from the patients, and c-erbB-2, IGF-1 and VEGF levels were measured in these samples using ELISA. Serum CA-125 levels were also determined, by chemiluminescent microparticle immunoassay. **RESULTS:** VEGF levels of the malignant group were significantly higher than those of the control group ( $p < 0.01$ ). CA-125 levels were also significantly higher than the in control group ( $p < 0.001$ ). Area under the ROC curve (AUC) was 0.982 for CA-125, 0.780 for VEGF, 0.603 for c-erbB-2, and 0.467 for IGF-1 in differentiating cancers from controls.

**CONCLUSION:** Serum VEGF levels might be a predictor for diagnosis in ovarian cancer patients, while serum c-erbB-2 and IGF-1 levels do not have a clinical significance in terms of ovarian cancer (Tab. 1, Fig. 1, Ref. 46).

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**KEY WORDS:** c-erbB-2, insulin-like growth factor-1, vascular endothelial growth factor, CA-125, ovarian cancer.

**Introduction**

Cell proliferation has a major role in carcinogenesis. While many factors have an effect on this process, growth factors play the main role. Accumulation of permanent changes in the genes that code growth factors causes the cell to proliferate in an uncontrolled manner and consequently transform into tumor cells. Growth factors bind with the receptors on the surface of tumor cells from which they originate and thus trigger auto-stimulation, causing increased proliferation of tumor cells (1).

c-erbB-2 oncoprotein (also known as HER2/neu) is an analogue of epidermal growth factor (EGF) receptor. In bladder tumors, amplification and increased expression of the c-erbB-2 gene has been demonstrated through immunohistochemical technique, and it has also been determined that c-erbB-2 positivity correlates with histological grade (2–4).

Insulin-like growth factor 1 (IGF-1) is a mitogenic, anti-apoptotic growth factor which causes the proliferation and differentiation of various cell types. High plasma IGF-1 levels have been shown to be associated with increased risk for prostate, lung, and colon cancer (5–7). In two studies investigating the serum levels of IGF 1 in bladder cancer patients, no significant difference was observed between the patient and control groups (8, 9).

Vascular endothelial growth factor (VEGF) is a mitogenic and angiogenic growth factor that increases vascular permeability. It is responsible for the growth and metastases of tumors. VEGF also increases the proliferation and migration of endothelial cells, and causes the degradation of extracellular matrix by activating urokinase, plasminogen activator and serin proteases (10).

The aim of this study was to determine the preoperative serum levels of c-erbB-2 oncoprotein, insulin-like growth factor 1 and vascular endothelial growth factor, compare these values to those of the control group, and discuss the clinical significance of these parameters.

**Materials and methods**

41 patients who were admitted to the Gynecology and Obstetrics Department at Cerrahpasa Faculty of Medicine and were diagnosed with ovarian cancer but did not receive any treatment

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prior to recruitment were included in the study. The exclusion criteria comprised pathologies related to growth hormone such as acromegaly, gigantism and dwarfism, thyroid function disorders, type I and II diabetes mellitus, and obesity. The control group was made up of 28 healthy volunteers with ages similar to those of the study group. The ethical committee of Istanbul University Cerrahpaşa Faculty of Medicine approved this study, and informed consent was obtained from each participant.

For staging of ovarian cancer, the FIGO (International Federation of Gynecology and Obstetrics) system of classification was used. The postoperative histological analyses were carried out at the Department of Histopathology.

10 mL of blood was drawn from each healthy volunteer and patient prior to surgery, then centrifuged and immediately stored at  $-80^{\circ}\text{C}$  until analysis. Serum levels of c-erbB-2, IGF-1 and VEGF were determined using commercially available ELISA kits (Bender Med Systems Kit cat no: BMS 207 for c-erbB-2, Enzo Life Sciences Assay kit cat no: 900-150 for IGF-1, and Life Technologies cat no: KHG0111 for VEGF). CA-125 levels in serum were analyzed by chemiluminescent microparticle immunoassay (Roche Diagnostics cobas 6000 analyzer).

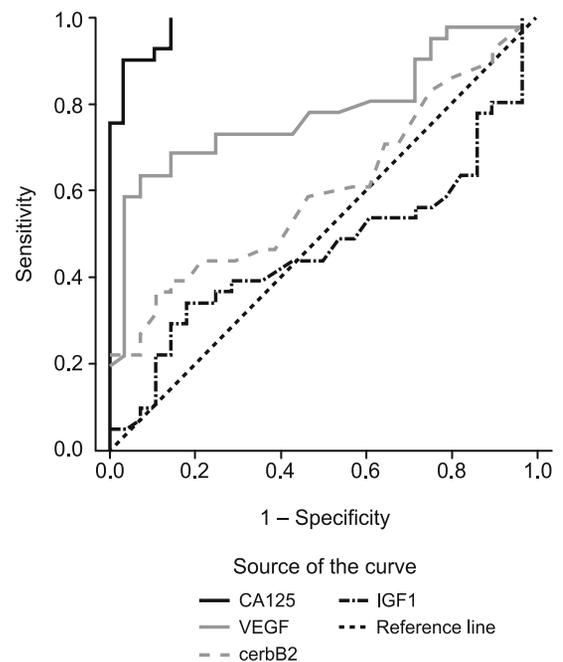
For interpretation of data, SPSS Statistics version 20 was utilized. Groups were first assessed by Kruskal–Wallis method, then pairwise comparisons were carried out by post-hoc Mann–Whitney U test with Bonferroni correction. For correlations, Spearman's rho was used.

## Results

Histopathological examination of the patients' tumors identified their types as follows: 29 serous, 2 mucinous, 3 clear cell, 2 mixed, and 2 undifferentiated. Serum levels of c-erbB-2 oncoprotein, IGF-1, VEGF and CA-125 of the control group and the study group, sorted by the pathology data, are given in Table 1.

Serum VEGF levels were significantly higher in the malignant group, compared to the control group ( $p < 0.01$ ). However, VEGF levels did not differ between early (stage I and II) and advanced (stage III and IV) stages. Both early and advanced stage groups demonstrated significantly higher VEGF levels compared to the control group ( $p < 0.05$  and  $p < 0.01$ , respectively).

CA-125 levels of the malignant group were significantly higher than those of the control group ( $p < 0.001$ ). In addition, they were



**Fig. 1.** Receiver operating characteristic curve for CA-125, vascular endothelial growth factor (VEGF), c-erbB-2, and insulin-like growth factor (IGF-1).

significantly higher in the advanced stage group, compared to the early stage group ( $p < 0.05$ ). Both early and advanced stage groups had significantly higher CA-125 levels compared to the control group (both  $p < 0.001$ ).

As shown in Figure 1, receiver operating characteristic (ROC) curve demonstrates that CA-125 performed best, followed by VEGF, c-erbB-2 and finally IGF-1. The area under the curve (AUC) was 0.982 for CA-125, 0.780 for VEGF, 0.603 for c-erbB-2, and 0.467 for IGF-1 in differentiating ovarian cancer patients from healthy controls. The cut-off points were determined to be 112 U/mL for CA-125; 239 pg/mL for VEGF; 4.7 ng/mL for c-erbB-2; and 111 ng/mL for IGF-1. Using these values, the sensitivity vs. specificity were calculated for CA-125 as 90 % vs 96 %; for VEGF as 73 % vs 75 %; for c-erbB-2 as 59 % vs 54 %; and for IGF-1 as 44 % vs 57 %.

**Tab. 1.** Serum levels of CA-125, vascular endothelial growth factor (VEGF), c-erbB-2 and insulin-like growth factor-1 (IGF-1) in controls and patients in different stages of disease.

	CA-125 (U/mL)			VEGF (pg/mL)			c-erbB-2 (ng/mL)			IGF-1 (ng/mL)		
	mean	median	range	mean	median	range	mean	median	range	mean	median	range
Control (n=28)	31.21	26.3	97.6	226.86	213.5	563	4.88	4.6	5.8	112.28	108.5	283.96
Early Stage (I & II) (n=21)	328.71 <sup>a</sup>	200	953	411.48 <sup>c</sup>	383	690	9.74	5.4	78.2	91.93	72	299.96
Advanced Stage (III & IV) (n=20)	1353.15 <sup>a, b</sup>	386.5	8818	466.85 <sup>d</sup>	570	705	5.33	4.8	11.8	132.06	124	323.6
All Stages (I - IV) (n=41)	828.44 <sup>a</sup>	314	8845	438.49 <sup>d</sup>	412	750	7.59	5	79.6	111.51	104	327.96

<sup>a</sup> $p < 0.001$  – compared to the control group, <sup>b</sup> $p < 0.05$  – compared to early stage group, <sup>c</sup> $p < 0.05$  – compared to the control group, <sup>d</sup> $p < 0.01$  – compared to the control group

VEGF and CA-125 levels were each found to be correlated with disease stage ( $\rho = 0.461$ ,  $p < 0.01$ ; and  $\rho = 0.846$ ,  $p < 0.01$ , respectively). In addition, VEGF levels correlated with CA-125 levels ( $\rho = 0.378$ ,  $p < 0.01$ ).

## Discussion

Having the highest mortality rates among all gynecological cancers, ovarian cancer is more frequently seen in patients with family history or during postmenopause. Treatment options are usually limited because diagnosis often tends to be late (11). The identification of any pelvic mass as benign or malignant achieves certainty mostly after surgical intervention and pathological examination. This necessitates the development of a reliable method to provide early diagnosis for ovarian cancer. A variety of tumor markers are employed for the early diagnosis, guiding the treatment and spotting tumor recurrences of epithelial ovarian cancer.

CA-125, a glycoprotein with a high molecular weight, is secreted in various epithelial cancers by cells of coelomic epithelium (12). Serum levels of this protein have been found to be elevated in 80 % of all epithelial ovarian tumors (13). However, elevated CA-125 levels were also found not only in advanced endometrial, fallopian tube and endocervical cancers (14), but also in non-gynecological cancers, such as breast, colon, pancreas and lung cancer (14, 15). In addition, non-malignant conditions may also cause elevated serum CA-125 levels as in endometriosis, pelvic inflammatory disease, gestation and menstruation (16–18). Despite these limitations, CA-125 is an important tool in monitoring the follow-up of patients diagnosed with ovarian cancer (19, 20). Currently available tumor markers mostly possess low sensitivity and specificity. Their tendency to raise during non-malignant conditions further limits their diagnostic usefulness. Therefore, additional tumor markers should be investigated in serum.

Being one of the best predictive markers, c-erbB-2 determines those patients who are likely to respond to anti-c-erbB-2 (trastuzumab) therapy. Thus, in year 2000, the American Society of Clinical Oncology (ASCO) recommended the measurement of c-erbB-2 levels in every breast cancer patient for its predictive value despite the lack of sufficient data to use it as a prognostic factor. Furthermore, there have been many studies investigating the expression of c-erbB-2 in tissues of lung, gastric, breast and colorectal cancer, although the results are contradictory (21–27). The serum levels and tissue overexpression of c-erbB-2 were found to be correlated in ovarian cancer (28). While there are studies arguing that c-erbB-2 should not be used as a prognostic factor despite having determined that its tissue expression levels correlate with disease stage (29), there are also studies which suggest its use as a diagnostic serum marker even though they haven't found any significant difference in its levels between stage, grade or histological types (30). It has also been reported that serum c-erbB-2 levels may not be related to CA-125 levels, but can still reflect its expression levels in tissue (31). In one of their studies, Meden H et al (32)

first reported an increase of c-erbB-2 in ovarian cancer; however, they argued in a following study that it possessed limited clinical significance (33). Our findings support the latter study (33) on this controversial issue.

IGF-1 has strong mitogenic and anti-apoptotic effects on cells (34, 35). It has been demonstrated that all IGF-peptide binding proteins are present in both normal and malignant ovarian epithelial cells (36), and that overexpression of IGF-1 receptors plays a role in the malignant transformation of these cells (37). While it has been suggested in a study with 47 patients with ovarian cancer (24 benign and 23 malignant) that low IGF-1 levels may be used as a reliable marker to separate benign tumors from malignant ones (38), it has also been recently reported that serum levels of IGF-1 neither were significantly different between the malignant group or healthy controls nor correlated with clinical findings, histological grade or response to chemotherapy although the majority (90 %) of patients were in advanced stage in another study which included 50 patients (39). Similarly, we found no significant difference between the study group and the controls.

VEGF is a potent mitogenic factor through its effects on mitogenesis, angiogenesis, endothelial lifespan and hematopoiesis (40). The first studies investigating the relationship between the prognosis of ovarian cancer and VEGF levels (40–42) found that those patients with elevated levels had worse prognosis. Another study argued that serum VEGF levels are insufficient for distinguishing benign and malignant ovarian lesions (43), while another reported that increased levels were related to reduced lifespan among invasive carcinoma group and that VEGF levels were significantly higher in invasive carcinoma compared to low-malignant and benign groups (44). Also, one study found elevated VEGF levels in ovarian cancer patients compared to healthy women and that these levels were significantly higher in advanced than in early stage ovarian cancer, suggesting VEGF as a new potential tumor marker for ovarian cancer (45). On the other hand, there are studies which indicate that preoperative serum VEGF levels are not correlated with CA-125 levels, but can be used as an independent risk factor for prognosis (46). In our study, VEGF levels were elevated both in early and advanced stage patients and these levels correlated with CA-125 levels, therefore our findings support the study by Cooper BC et al (44).

In conclusion, we suggest that VEGF could be a useful biomarker in combination with CA-125 for the diagnosis of ovarian cancer, however serum IGF-1 and c-erbB2 levels seem limited for this purpose.

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