Prognostic Value of Serum Vascular Endothelial Growth Factor (VEGF) in Women with Ovarian Cancer

Yehia M., Abu-Seeda M., Kamal K., El-Lamie I., Hassan M., Shafik A. * and Kassim G

Department of Obstetrics and Gynecology, Ain Shams University
shafikadel@hotmail.com

Abstract: Objective: The aim of the current study was to evaluate the association between serum vascular endothelial growth factor (VEGF) and both prognostic variables and disease recurrence in women with ovarian cancer. **Methods:** The current study was conducted at Ain Shams University Maternity Hospital over the period between October 2006 and July 2010. The study included women admitted to the Gynecologic Oncology Unit for having an adnexal mass and planned for exploration laparotomy and abdominal cytoreductive surgery. All included women were subjected to the unit protocol for management of ovarian masses suspected to be of a malignant nature, including preoperative serum samples for tumor markers (including CA125 and vascular endothelial growth factor [VEGF]). The included women underwent the appropriate surgical management. Women were followed up every 3 months for the first year. Serum VEGF was rechecked at each visit. **Results:** A total of 45 women were included. The mean serum VEGF was significantly higher in women who had FIGO stages III/IV, tumor grades 2/3, ascites, positive omental metastases, bilateral tumors and in those where optimal cytoreduction was feasible, as well as in those who had disease recurrence within 12 months. Both women who had disease recurrence within 12 months and those who were disease-free after 12 months had significant reduction of serum VEGF level 3 and 6 months postoperatively. In those who had recurrence, the serum VEGF level significantly re-rose after 12 months, whereas in those who were disease-free, the serum VEGF level remained low. **Conclusion:** High preoperative serum VEGF was significantly associated with advanced FIGO stage and high tumor grade, and was a significant predictor of suboptimal cytoreduction and disease recurrence within 12 months. Serum VEGF seems to be a promising novel biomarker in prognosis of women with ovarian cancer.
[Yehia M., Abu-Seeda M., Kamal K., El-Lamie I., Hassan M., Shafik A. and Kassim G. **Prognostic Value of Serum Vascular Endothelial Growth Factor (VEGF) in Women with Ovarian Cancer.** *J Am Sci* 2014;10(1):173-181]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 27

Key word: ovarian cancer – ovarian malignancy – vascular endothelial growth factor – prognosis of ovarian cancer

1.Introduction

Ovarian malignancy is the fifth most common gynecological cancer, yet the leading cause of death from malignancy in women [1]. The main reason for this high potential mortality is the late diagnosis, owing to early extensive tumor invasion and peritoneal metastases [2]. The growth and spread of solid tumors, in general, and ovarian cancer, in particular, depend partly on formation of adequate vascular supply (angiogenesis). Angiogenesis has been proven to be an essential component of malignant tumor growth and spread [3]. Various factors have been implicated in angiogenesis. Vascular endothelial growth factor (VEGF), a potent cytokine, has been shown to be a key regulator in both physiological and pathological angiogenesis and a major contributor to many pathological processes, including tumor growth and spread [4]. Ovarian cancer, being a highly vascularized tumor with early and extensive local and peritoneal spread, was shown to be dependent on VEGF-mediated angiogenesis [5]. Peritoneal spread is the most common pathway for spread of ovarian cancer [6]. Indeed, more than 66% of women who present with ovarian cancer present when they have already developed peritoneal metastases; with more than 30% having malignant ascites. Both peritoneal metastases and ascites are one of the important prognostic factors in ovarian cancer [7]. VEGF has been strongly implicated in the peritoneal spread of ovarian malignant tumors and subsequent development of malignant ascites [4]. This widely-proven significant association between VEGF and both growth and spread of ovarian malignant tumors pushed efforts into introducing agents that target VEGF signaling pathways as adjuvant treatment options in ovarian cancer [8-9]. Although there is abundant evidence that VEGF plays a central role in the development and growth of ovarian cancer, information regarding the clinical utility of serum VEGF levels in ovarian carcinoma is limited [10]. The aim of the current work was to evaluate the association between serum VEGF (as a promising tumor biomarker) and prognostic variables in women with ovarian cancer managed at a large tertiary center: the Gynecological Oncology Unit, Ain Shams University Maternity Hospital.

2.Methods

The current study was conducted at Ain Shams University Maternity Hospital during the period between October 2006 and July 2010. The study protocol was in agreement to the World Medical

Association Declaration of Helsinki for Ethical Medical Research, and was approved by the Ethical Research Committee at Obstetrics and Gynecology Department, Faculty of Medicine, Ain Shams University. All included women signed an informed consent after explanation of the purpose and procedures of the study. The study included women admitted to the Gynecologic Oncology Unit for having an adnexal mass and planned for exploration laparotomy and abdominal cytoreductive surgery. Women with secondary or recurrent ovarian tumors or those who had dual malignancy (i.e. coexisting endometrial, breast or colonic malignancy) were not included in the current study. All included women were subjected to the unit protocol for management of ovarian masses suspected to be of a malignant nature, including thorough history revision, preoperative serum samples for tumor markers (including CA125 and the vascular endothelial growth factor [VEGF]) as well as hemoglobin concentration, coagulation profile and blood chemistry, pelvi-abdominal imaging (ultrasonography ± CT scan) and plain chest film. All included women who no longer seek for fertility preservation underwent abdominal cytoreductive surgery by a senior gyne-oncologic surgeon. Cytoreduction was considered optimal when the residual lesion was less than 2 cm in average dimension. All excised specimens were sent for histopathological assessment at the Early Cancer Detection Unit, Ain Shams University Maternity Hospital. Malignant tumors were staged according to the FIGO Staging for Malignant Ovarian Tumors and graded into grade 1 (well-differentiated), grade 2 (moderately differentiated) or grade 3 (poorly differentiated). Postoperatively, women who had primary malignant lesions were followed up every 3 months for the first year, when they were subjected to pelvi-abdominal examination, pelvi-abdominal imaging and/or serum tumor markers reassessment, when needed. Serum VEGF was rechecked at each postoperative visit. Women who received postoperative adjuvant chemotherapy had their first postoperative serum VEGF recheck between the 4th and 5th course of the chemotherapy.

Serum VEGF Assay

Two mL of peripheral venous blood was collected from each patient and immediately centrifuged at 3400 rpm for 10 minutes. The serum was collected and stored at –70°C until analysis. All serum VEGF analyses were performed at the same time and they were not sawed before analysis. Serum samples were stored and analyzed at the Oncology Diagnostic Unit, Biochemistry Department, Faculty of Medicine, Ain Shams University. For Measurement of serum VEGF level, a commercially available enzyme-linked immunosorbent assay (ELISA) kit was used (AviBion Human VEGF ELISA kit®, Ani Biotech Oy, Orgenium® Laboratories Business Unit, Finland).

Statistical analysis

Statistical analysis was performed using SPSS® for Windows® version 16.0 and Microsoft® Excel® version 2010. Difference between metric variables of two independent groups was estimated using the independent student's t-test. Difference between metric variables of two related groups was estimated using the paired student's t-test. Receiver operator characteristics (ROC) curves were constructed for serum VEGF and CA125 levels as predictors of suboptimal cytoreduction and disease recurrence. Validity of predictability was expressed in terms of sensitivity, specificity, positive and negative predictive values. Significance level was set at 0.05.

Sample size justification

Sample size was estimated using the Power Analysis and Sample Size Software (PASS®, NCSS, LLC, Kaysville, Utah, US). Based on retrospective local institution data, the rate of one-year recurrence in women with ovarian cancer was 25%. It was estimated that a sample size of 40 women with ovarian cancer (in whom 10 would have recurrence within one year) would achieve a power of 80% to detect a difference of 0.3 between area under the ROC curve of 0.5 under the null hypothesis and 0.8 under the alternative hypothesis, setting the two-sided confidence level at 95%.

3.Results

A total of 45 women with primary malignant ovarian tumor were included in the current study. The mean age of included women was 46.5 ± 13.7 years (range: 12 – 73 years). Table-1 shows the histopathological type, FIGO staging and tumor grading of ovarian malignant tumors in included women. Tumor features are shown in table-2. Of the included 45 women, 38 (84.4%) received postoperative adjuvant chemotherapy. All of the included 45 women were disease-free after 6 months postoperatively. After 12 months postoperatively, 35 (77.8%) were disease-free, while 10 (22.2%) had recurrent disease (Table-3).

The mean serum VEGF was slightly higher in women who had sex cord – stromal tumor, when compared to those with epithelial or germ cell tumors; the differences were, however, statistically insignificant (Table-4).

The mean serum VEGF was significantly higher in women who had FIGO stages III/IV, tumor grades 2/3, ascites, positive omental metastases, bilateral tumors and in those where optimal cytoreduction was feasible, as well as in those who had disease recurrence within 12 months. The mean serum VEGF was higher in women who had positive lymph node

metastases; the latter difference was statistically insignificant (Table-5).

Table-1 Histopathological Type, FIGO Staging and Grading of Ovarian Tumors in Included Women

Histopathological Type	
Epithelial Tumors	36/45 (80%)
Serous Cystadenocarcinoma	17/45 (37.8%)
Mucinous Cystadenocarcinoma	4/45 (8.9%)
Endometrioid Adenocarcinoma	14/45 (31.1%)
Clear cell Adenocarcinoma	1/45 (2.2%)
Sex-Cord Stromal Tumors	5/45 (11.1%)
Granulosa Cell Tumor	2/45 (4.4%)
Sertoli-Leydig Cell Tumor	3/45 (6.7%)
Germ Cell Tumors	4/45 (8.9%)
Immature Teratoma	1/45 (2.2%)
Endodermal Sinus Tumor	2/45 (4.4%)
Mixed Germ Cell Tumor	1/45 (2.2%)
FIGO Staging	
Epithelial Tumors	
Stage I	7/36 (19.4%)
Stage II	4/36 (11.1%)
Stage III	23/36 (63.9%)
Stage IV	2/36 (5.6%)
Other Types	
Stage I	9/9 (100%)
Tumor Grading	
Grade 1	8/45 (17.8%)
Grade 2	23/45 (51.1%)
Grade 3	10/45 (22.2%)
Not graded*	4/45 (8.9%)

Data presented as number (percentage)

* Tumors in 4 cases were not graded having the following histopathological types: mixed germ cell tumor (1 case), endodermal sinus tumor (2 cases) and clear cell adenocarcinoma (1 case)

Table-2 Tumor Features in Included Women

Ascites	28/45 (62.2%)
Minimal (< 500 ml)	7/45 (15.6%)
Massive (≥ 500 ml)	21/45 (46.6%)
Omental Metastases	23/45 (51.1%)
Iliac Lymph Node Metastases	6/45 (13.3%)
Laterality	
Unilateral	20/45 (44.4%)
Bilateral	25/45 (56.6%)
State of Cytoreduction	
Optimal	42/45 (93.3%)
Suboptimal	3/45 (6.7%)

Data presented as number (percentage)

Table-3 Follow-up Status in Included Women

Within 3 months Postoperatively	
Received Adjuvant Chemotherapy	38/45 (84.4%)
Did not receive Chemotherapy*	7/45 (15.6%)
After 6 months	
Disease-free	45/45 (100%)
Recurrent Disease	0/45 (0%)
After 12 months	
Disease-free	35/45 (77.8%)
Recurrent Disease**	10/45 (22.2%)

Data presented as number (percentage)

* Seven cases did not receive chemotherapy: epithelial tumors stage Ia, grade 1 (3 cases), Sertoli-Leydig cell tumor (2 cases) and granulosa cell tumor (2 cases)

** Ten cases had recurrent disease within 12 months postoperatively: epithelial tumor (8 cases) and germ cell tumor (2 cases)

Table-4 Comparison between Histopathological Types regarding Serum VEGF

	Epithelial Tumors	Sex Cord – Stromal Tumors	Germ Cell Tumors	*P*
Serum VEGF (pg/ml)	842.8 ± 300.4	1008.2 ± 305	642 ± 496.9	0.257* 0.225** 0.160***

Data presented as mean ± SD Analysis using Independent Student's t-Test
* Difference between Epithelial and Sex Cord – Stromal Tumors
** Difference between Epithelial and Germ Cell Tumors
*** Difference between Sex Cord – Stromal and Germ Cell Tumors

Table-5 Association between Serum VEGF and Various Prognostic Factors

FIGO Stage (I/II vs. III/IV)	548.5 ± 119.8 vs. 1079.2 ± 104.3	<0.001
Tumor Grade (1 vs. 2/3)	514.2 ± 288.3 vs. 954.92 ± 241.8	<0.001
Ascites (no vs. present)	549.7 ± 268.8 vs. 1026.7 ± 147.2	<0.001
Omental Metastases (no vs. present)	602.4 ± 264 vs. 1073.8 ± 123.8	<0.001
Lymph Node Metastases (no vs. present)	814.2 ± 312 vs. 1032.7 ± 265.2	0.112
Laterality (unilateral vs. bilateral)	519.1 ± 190.4 vs. 1059.5 ± 142.7	<0.001
Cytoreduction (optimal vs. suboptimal)	815.4 ± 304.8 vs. 1234.3 ± 6.7	<0.001
Disease State after 12 months (free vs. recurrent)	778.7 ± 301.2 vs. 1069.5 ± 248.8	0.008

Data presented as mean ± SD of serum VEGF (pg/ml) Analysis using Independent Student's t-Test
* Difference between Epithelial and Sex Cord – Stromal Tumors
** Difference between Epithelial and Germ Cell Tumors
*** Difference between Sex Cord – Stromal and Germ Cell Tumors

Both women who had disease recurrence within 12 months and those who were disease-free after 12 months had significant reduction of serum VEGF level 3 and 6 months postoperatively. In those who had recurrence, the serum VEGF level significantly re-rose after 12 months, whereas in those who were disease-free, the serum VEGF level remained low (Table-6, Figure-1).

Table-6 Serum VEGF in Women who had Disease Recurrence and Those who were Disease-Free

Serum VEGF (pg/ml)	Preoperative	After 3months	After 6 months	After 12 months	*P*
Cases who had Disease Recurrence within 12 months	1069.5 ± 248.8	502.7 ± 295.9	247.8 ± 80.4	678.9 ± 140.5	0.001* <0.001** <0.001***
Cases who were Disease-Free after 12 months	778.7 ± 301.2	345.8 ± 290.5	167.5 ± 42.6	152.8 ± 33.4	<0.001* 0.002** 0.058***
P	0.008†	0.141††	0.012†††	<0.001††††	

Data presented as range, mean ± SD
* Difference between serum VEGF after 3 months and postoperatively - Analysis using Paired Student's t-Test
** Difference between serum VEGF after 6 and 3 months postoperatively - Analysis using Paired Student's t-Test
*** Difference between serum VEGF after 12 and 6 months and postoperatively - Analysis using Paired Student's t-Test
† Difference between those who had disease recurrence and those who were disease-free regarding serum VEGF preoperatively - Analysis using Independent Student's t-Test
†† Difference between those who had disease recurrence and those who were disease-free regarding serum VEGF after 3 months - Analysis using Independent Student's t-Test
††† Difference between those who had disease recurrence and those who were disease-free regarding serum VEGF after 6 months - Analysis using Independent Student's t-Test
†††† Difference between those who had disease recurrence and those who were disease-free regarding serum VEGF after 12 months - Analysis using Independent Student's t-Test

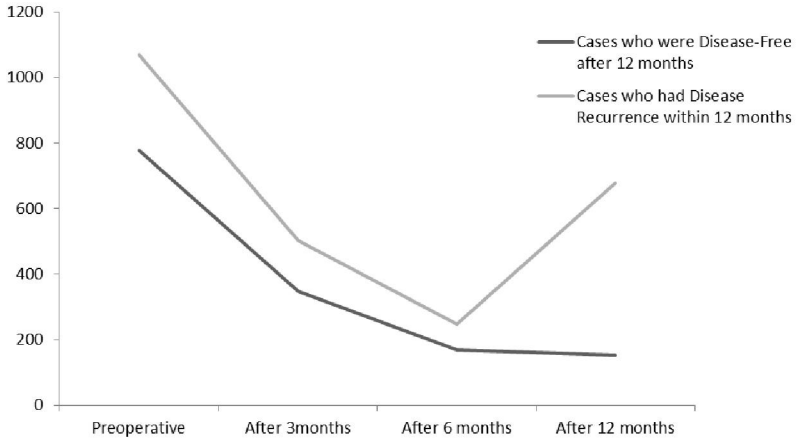
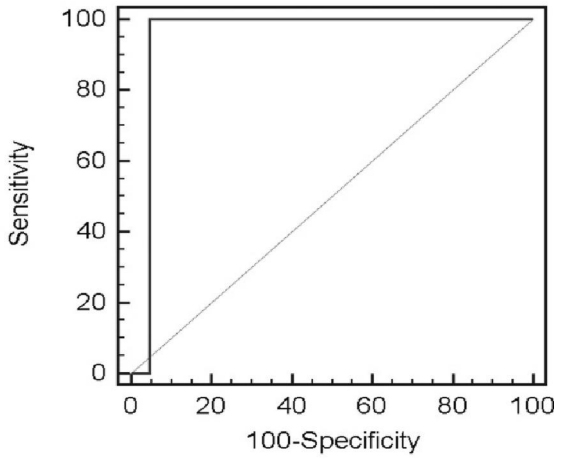
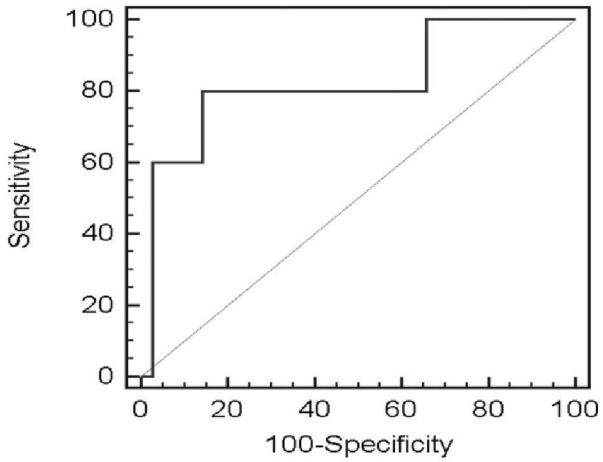


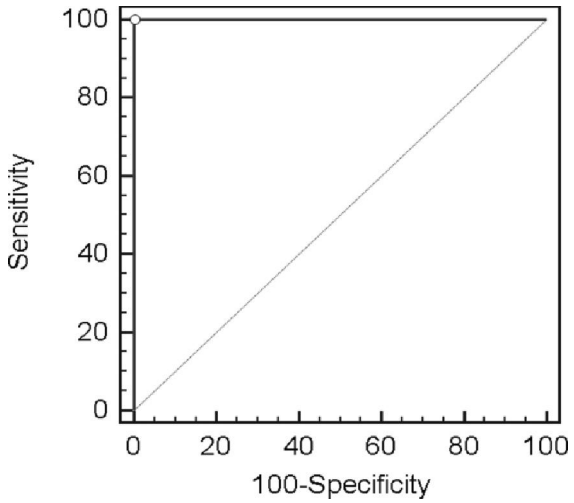
Figure-2 ROC Curves for Serum VEGF as Predictor of State of Cytoreduction and Disease Recurrence within 12 months



a: ROC Curve for Preoperative VEGF as Predictor of Suboptimal Cytoreduction
AUC = 0.952, 95% CI (0.822 to 1.003), p<0.001



b: ROC Curve for Preoperative VEGF as Predictor of Recurrence within 12 months
AUC = 0.823, 95% CI (0.672 to 0.982), p<0.001



c: ROC Curve for VEGF at 12 months as Predictor of Recurrence
AUC = 1.0, 95% CI (1.0 to 1.0), p<0.001

Table-7: Validity of Serum VEGF and CA125 as Predictors of State of Cytoreduction and Disease Recurrence within 12 months

		Sensitivity	Specificity	PPV	NPV
Suboptimal Cytoreduction	**Preoperative VEGF ≥ 1181 pg/ml**	100%	95.2%	60%	100%
	Preoperative CA125 ≥ 115.2 IU/ml	100%	94.9%	63.3%	100%
Disease recurrence within 12 months	**Preoperative VEGF ≥ 1015 pg/ml**	80%	85.7%	61.5%	93.7%
	Preoperative CA125 ≥ 87.3 IU/ml	82%	84.4%	62.3%	94.4%
	VEGF at 6 months ≥ 182 pg/ml	87.5%	71.4%	46.7%	95.2%
	CA125 at 6 months ≥ 9.2 IU/ml	87.5%	78.6%	53.8%	95.7%
	VEGF at 12 months ≥ 250 pg/ml	100%	100%	100%	100%
	CA125 at 12 months ≥ 101 IU/ml	100%	100%	100%	100%

PPV positive predictive value NPV negative predictive value

4.Discussion

Limited reports have addressed the value of serum VEGF in prediction of the prognosis in women with ovarian cancer, despite the evident role of VEGF expression in the growth and spread of solid tumors, in general, and ovarian malignant neoplasms, in particular [11]. The current study showed no association between preoperative serum VEGF and histopathological type of the tumor (whether epithelial, germ cell or sex cord – stromal type). Similar findings were previously reported [12-16]. Specifically, Yamamoto *et al.,* found that VEGF expression was significantly higher in clear cell carcinoma than in other types of epithelial carcinomas [17]. The significant association between higher preoperative serum VEGF and advanced stages (FIGO stages III/IV) found in the current study, was previously found in several relevant studies [13,16,18-21]. Kraft *et al.* stated that, frequently elevated VEGF levels in patients with advanced disease, and significant decrease in VEGF levels in sera after surgery, suggest that large tumor masses release high amounts of VEGF which might contribute to elevated VEGF levels in serum [19]. Yamamoto *et al.* stated that VEGF expression strongly correlated with prognosis and that the prognostic significance of VEGF was related to its correlation with FIGO stage and, that VEGF expression was not an independent prognostic indicator on its own [17]. On the contrary, some studies showed no significant correlation between preoperative serum VEGF and FIGO stage [12,15,22-24]. Cooper *et al.* proposed some explanations for this latter finding, including different assay techniques, and small samples in some studies [24]. Tempfer *et al.* concluded that the lack of association between serum levels of VEGF and tumor stage in their series could be due to that VEGF production is described as an early event in ovarian cancer and indicates that VEGF-promoted angiogenesis is continuous during all stages of ovarian cancer growth [23]. Yamamoto *et al.* stated that serum VEGF levels in ovarian carcinoma patients were not linearly correlated with either tumor fluid levels or ascitic levels, and that the elevation of serum VEGF levels may be influenced not only by the expression level of VEGF but also by other factors, such as tumor vasculature and/or expression of other cytokines regulating vascular permeability [17]. Significantly higher preoperative serum VEGF was found in women who had high tumor grades (grades 2/3). Similar findings were reported in previous relevant studies [14,16,23]. Chen *et al.* suggested that proliferation and differentiation of tumor cells could reflect angiogenic activity in ovarian cancer [14]. Dirix *et al.* showed that high grade malignancies characterized by fast progression were found to have about five times higher VEGF serum levels than those of patients with low grade tumors [25]. Tempfer et al. assumed that serum levels of VEGF are not indicative of tumor bulk, but strong tumor proliferation [23]. Significantly higher preoperative serum VEGF was found in women who had ascites. This was in agreement with the results of other studies [13,16,24]. In fact, the precise mechanism of peritoneal and pleural fluid accumulation in women with malignant (and some benign) ovarian neoplasms remains unresolved. Few reports have demonstrated an association between high concentrations of VEGF and refractory fluid retention [26-27]. Besides stimulating endothelial cell proliferation, VEGF increases vessel permeability to circulating macro-molecules, thus enhancing extravasations of plasma-rich exudates into the peritoneal cavity and leading to ascites generation. There was also clinical evidence that besides increasing capillary permeability, VEGF facilitates the entry and implantation of tumor cells into the peritoneum, which in turn stimulated surface peritoneal vessel development and further induced ascites production [19]. This also explains the significant association between higher serum VEGF and positive omental metastases found in the current and other studies [16,19], and explains the poor association between serum VEGF and lymph node metastases encountered in the current and previous studies [23,28].

Most importantly, preoperative serum VEGF was found to be a significant predictor of the state of cytoreduction, which was shown to be the single most significant prognostic factor in recurrence and survival. Similar findings were reported by Hefler *et al.* [28] and Li *et al.* [16]. This may indicate that high preoperative serum VEGF may reflect a high metastatic potential and therefore, a higher likelihood of suboptimal cytoreduction and recurrence within 12 months. Numerous studies have demonstrated the association between angiogenesis (which VEGF was shown to be one of the major factors responsible for) and tumor aggressiveness and metastatic potential [29-33]. Among the included women who underwent cytoreduction, postoperative follow-up showed a significant reduction of serum VEGF levels. Li *et al.* found that the postoperative serum levels of VEGF correlate with the residual tumor size [16]. Oehler and Caffier found that postoperative serum VEGF returned to normal or declined to a level below 320 pg/ml more often in women who had optimal rather than suboptimal cytoreduction. The authors concluded that postoperative serum level of VEGF may reflect the optimality of surgical treatment [15]. The serum VEGF levels remained low in women who remained disease-free, and re-rose in women who had recurrence, thus introducing serum VEGF as a novel marker for detection of disease recurrence during postoperative follow-up. These findings were consistent with those reported by previous studies [17,24]. Moreover, high preoperative serum VEGF level was shown to be a significant predictor of disease recurrence within 12 months. These findings were consistent with the results of similar studies [12,28,34]. Results of the current study showed that a preoperative serum VEGF ≥ 1181 pg/ml or ≥ 1015 pg/ml was significant predictor of suboptimal cytoreduction and disease recurrence within 12 months, respectively. Hefler *et al.* showed close results, regarding prediction of suboptimal cytoreduction, and concluded that preoperative serum VEGF may be a useful biomarker for detection of women who may benefit from neoadjuvant chemotherapy before undergoing cytoreduction to optimize the surgical benefit [28]. Interestingly, Hefler *et al.* [28] and Cooper *et al.* [24] have showed that preoperative serum VEGF was an independent prognostic factor for disease recurrence within 12 months, when other variables (stage, grade and residual tumor size) were adjusted. The results of the current study showed that a serum CA125 ≥ 9.2 IU/ml at 6 months postoperatively (2-2.5 months following completion of adjuvant chemotherapy) was a significant predictor of recurrence. This finding was comparable to reported by van Altena *et al.* and Varughese *et al.* [36] who found that postoperative nadir CA125 >10 IU/ml was an independent factor to predict ovarian cancer recurrence.

In conclusion, serum VEGF seems to be a promising biomarker in ovarian cancer. High preoperative levels seem to be associated with poor prognostic features (advanced FIGO stages, high tumor grading, ascites, omental metastases, suboptimal cytoreduction). Postoperative re-rise of serum VEGF was shown to be a significant predictor of disease recurrence.

References

1. **Jacobs IJ and Menon U.** Progress and challenges in screening for early detection of ovarian cancer. Mol Cell Proteom 2004; 3 (4): 355–366.
2. **Wang J, Sun T, Zhao X, Zhang S, Zhang D, Qiang G, Wang X, Zhao N, Qie S and Sun B.** Functional significance of VEGF-a in human ovarian carcinoma. Cancer Biol Therapy 2008; 7:5, 760-768.
3. **Mesiano S and Ferrara N.** Role of Vascular Endothelial Growth Factor in Ovarian Cancer - Inhibition of Ascites Formation by Immunoneutralization. Am J Path 1998; 153 (4): 1249-1256.
4. **Moghaddam SM. Amini A, Morris DL and Pourgholami MH.** Significance of vascular endothelial growth factor in growth and peritoneal dissemination of ovarian cancer. Cancer Metastasis Rev 2012; 31:143–162.
5. **Ramakrishnan S, Subramanian IV, Yokoyama Y and Geller M.** Angiogenesis in normal and neoplastic ovaries. Angiogenesis 2005; 8(2):169-182.
6. **Pannu HK Bristow RE, Montz, FJand Fishman, EK** Multidetector CT of peritoneal carcinomatosis from ovarian cancer1. RadioGraphics 2003, 23(3), 687–701.
7. **Meunier L, Puiffe ML, Le Page C, Filali-Mouhim A, Chevrette M, Tonin, PN, Provencher DM and Mes-Masson AM.** Effect of ovarian cancer ascites on cell migration and gene expression in an epithelial ovarian cancer in vitro model. Transl Oncol 2010, 3(4), 230–238.
8. **Wulff C, Wilson H, Rudge JS, Wiegand SJ, Lunn SF and Fraser HM.** Luteal angiogenesis: prevention and intervention by treatment with vascular endothelial growth factor trap (A40). J Clin Endocrinol Metab 2001; 86(7):3377-3386.
9. **Mabuchi S, Kawase C, Altomare DA, Morishige K, Hayashi M, Sawada K, Ito K, Terai Y, Nishio Y, Klein-Szanto AJ, Burger RA, Ohmichi M, Testa JR and Kimura T.** Vascular endothelial growth factor is a promising therapeutic target for the treatment of clear cell

carcinoma of the ovary. Mol Cancer Ther 2010; 9(8):2411-2422

10. **Mahner S, Woelber L, Eulenburg C, Schwarz J, Carney W, Jaenicke F, Milde-Langosch K and Mueller V.** TIMP-1 and VEGF-165 serum concentration during first-line therapy of ovarian cancer patients. BMC Cancer 2010; 10:139.
11. **Masoumi MS, Amini A, Morris DL and Pourgholami MH.** Significance of VEGF in growth and peritoneal dissemination of ovarian cancer. Cancer Metastasis Rev., 2011; 20: 136-145.
12. **Secord AA, Sayer R, Snyder SA, Broadwater G, Rodriguez GC, Berchuck A and Blackwell K.** The relationship between serum vascular endothelial growth factor, persistent disease, and survival at second-look laparotomy in ovarian cancer. Gynecol Oncol 2004, 94: 74-79.
13. **Gadducci A, Ferdeghini M, Fanucchi A, Annicchiarico C, Ciampi B, Prontera C and Genazzani AR.** Serum preoperative vascular endothelial growth factor (VEGF) in epithelial ovarian cancer: relationship with prognostic variables and clinical outcome. Anticancer Res 1999, 19: 1401-1405.
14. **Chen CA, Cheng WF, Lee CN, Chen TM, Kung CCS, Hsieh FJ and Hsieh CY**. Serum vascular endothelial growth factor in epithelial ovarian neoplasms: correlation with patient survival. Gynecol Oncol 1999, 74 (2): 235-240.
15. **Oehler MK and Caffier H.** Prognostic relevance of serum vascular endothelial growth factor in ovarian cancer. Anticancer Research 2000, 20: 5109-5112.
16. **Li L, Wang L, Zhang W, Tang B, Zhang J, Song H, Yao D, Tang Y, Chen X, Yang Z, Wang G, Li X, Zhao J, Ding H, Reed E and Li QQ.** correlation of serum VEGF level with clinical stage, therapy efficacy, tumor metastasis and patient survival in ovarian cancer. Anticancer Research 2004, 24: 1973-1980.
17. **Yamamoto S, Konishi I, Mandai M, Kuroda H, Komatsu T, Nabu K, Sakahara H and Mori T.** Expression of vascular endothelial growth factor (VEGF) in epithelial ovarian neoplasms: correlation with clinico-pathology and patient survival, and analysis of serum VEGF levels. Br J Cancer 1997; 76 (9): 1221-1227.
18. **Kassim SK, El-Salahy EM, Fayed ST, Helal SA, Helal T, Azzam ED and Khalifa A**. Vascular endothelial growth factor and interleukin-8 are associated with poor prognosis in epithelial ovarian cancer patients. Clin Biochem 2004; 37 (5): 363-369.
19. **Kraft A, Weindel K, Ochs A, Marth C, Zmija J, Schumacher P, Unger C, Marm D and Gastl G.** Vascular endothelial growth factor in the sera and effusions of patients with malignant and nonmalignant disease. Cancer 1999; 85 (1): 178-187.
20. **Shen GH, Ghazizadel M, Kawanami O, Shimizu H, Jin E, Araki T and Sugisaki Y**. Prognostic significance of vascular endothelial growth factor expression in human ovarian carcinoma. Br J Cancer 2000; 83: 196-203.
21. **Brustmann H and Naude S**. Vascular endothelial growth factor expression in serous ovarian carcinoma: relationship with high mitotic activity and high FIGO stage. Gynecol Oncol 2002; 84: 47-52.
22. **Obermair A, Tempfer C, Hefler L, Preyer O, Kaider A, Zeillinger R, Leodolter S and Kainz C.** Concentration of vascular endothelial growth factor (VEGF) in the serum of patients with suspected ovarian cancer. Br J Cancer 1998; 77 (11): 1870-1874.
23. **Tempfer C, Obermair A, Hefler L, Haeusler G, Gitsch G and Kainz C.** Vascular endothelial growth factor serum concentrations in ovarian cancer. Obstet Gynecol 1998; 92 (3): 360-363.
24. **Cooper BC, Ritchie JM, Broghammer CLW, Coffin J, Sorosky JI, Buller RE, Hendrix MJC and Sood AK.** Preoperative serum vascular endothelial growth factor levels: significance in ovarian cancer. Clin Cancer Res 2002; 8 (10): 3193-3197.
25. **Dirix LY, Vermeulen PB, Hubens G, Benoy I, Martin M, DePooter C and Van Oesterom AT.** Serum basic fibroblast growth factor and vascular endothelial growth factor and tumour growth kinetics in advanced colorectal cancer. Ann Oncol 1996; 7 (5): 843-848.
26. **Ishiko O, Yoshida H, Sumi T, Hirai K and Ogita S.** Vascular endothelial growth factor levels in pleural and peritoneal fluid in Meigs' syndrome. Eur J Obstet Gynecol Reprod Biol 2001; 98:129–30.
27. **Abramov Y, Anteby SO, Fasouliotis SJ and Barak V.** Markedly elevated levels of vascular endothelial growth factor, fibroblast growth factor, and interleukin 6 in Meigs syndrome. Am J Obstet Gynecol 2001; 184:354–5.
28. **Hefler LA, Zeillinger R, Grimm C, Sood AK, Cheng WF, Gadducci A, Tempfer CB and Reinthaller A**. Preoperative serum vascular endothelial growth factor as a prognostic parameter in ovarian cancer. Gynecol Oncol 2006; 103: 512-517.
29. **Weidner N, Semple JP, Welch WR and Folkman J.** Tumor angiogenesis and metastasis--correlation in invasive breast cancer. N Engl J Med 1991; 324 (1): 1-8.

30. **Maeda K, Chung YS, Ogawa Y, Takatsuka S, Kang SM, Ogawa M, Sawada T and Sowa M.** Prognostic value of vascular endothelial growth factor expression in gastric carcinoma. Cancer 1996; 77 (5): 858-863.
31. **Toi M, Hoshina S, Takayanagi T and Tominaga T.** Association of vascular endothelial growth factor expression with tumor angiogenesis and with early relapse in primary breast cancer. Jpn J Cancer Res 1994; 85 (10): 1045-1049.
32. **Takahashi Y, Kitadai Y, Bucana CD, Cleary KR and Ellis LM**. Expression of vascular endothelial growth factor and its receptor KDR correlates with vascularity, metastasis, and proliferation of human colon cancer. Cancer Res 1995; 55 (18): 3964-3968.
33. **Takahashi Y, Cleary KR, Mai M, Kitadai Y, Bucana CD and Ellis LM.** Significance of vessel count and vascular endothelial growth factor and its receptor (KDR) in intestinal-type gastric cancer. Clin Cancer Res 1996; 2 (10): 1679-1684.
34. **Paley PJ, Staskus KA, Gebhard K, Mohanraj D, Twiggs LB, Carson LF and Ramakrishnan S.** Vascular endothelial growth factor expression in early stage ovarian carcinoma. Cancer 1997; 80 (1): 98-106.
35. **van Altena AM, Kolwijck E, Spanjer MJ, Hendriks JC, Massuger LF and de Hullu JA.** CA125 nadir concentration is an independent predictor of tumor recurrence in patients with ovarian cancer: a population-based study. Gynecol Oncol 2010; 119 (2): 265-269.
36. **Varughese E, Kondalsamy-Chennakesavan S and Obermair A.** The value of serum CA125 for the development of virtual follow-up strategies for patients with epithelial ovarian cancer: a retrospective study. J Ovarian Res 2012; 22: 5-11.

12/25/2013