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Serum Vascular Endothelial Growth Factors A, C and D in Human Breast Tumors

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Abstract Available evidence suggests that vascular endothelial growth factor (VEGF) a potent regulator of vasculogenesis and tumor angiogenesis may be a predictor of recurrence in breast cancer patients. We sought to determine whether VEGF serum levels (VEGF-A, VEGF-C and VEGF-D) in 377 patients with malignant and benign breast tumors differ and whether there is association between vascular growth factors, clinicopathologic features and prognosis. There was no significant difference in investigated circulating angiogenic markers between patients with malignant and non malignant lesions. We found strong correlation between VEGF-A and VEGF-D and between VEGF- C and VEGF-D. Besides serum VEGF-D levels and estrogen receptor (ER) expressions no other correlations between VEGF and clinicopathologic variables were observed. However, elevated VEGF-A and VEGF-C concentrations were associated with increased number of erythrocytes, leukocytes and platelets. In Cox model values of angiogenic serum markers and recognized prognostic markers in breast cancer, VEGF-C turned out as independent prognostic factor. Our study is the first analysis showing correlation between serum concentrations of three angiogenic factors: VEGF-A, VEGF-C, VEGF-D. Associations between angiogenic cytokines and number of blood cells may be due to release of VEGF from platelets and leucocytes. Prognostic role of VEGF is still uncertain, though VEGF-C has a potential to serve as a prognostic marker.

Keywords Vascular endothelial growth factor-A · Vascular endothelial growth factor-C · Vascular endothelial growth factor-D · Serum assay · Breast cancer

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Introduction

Solid tumors require new blood vessels and remodeling of existing ones to grow larger than 1–2 mm. Angiogenesis is a process of new blood vessel formation. Blood supply is essential to obtain nutrients for growth and to metastasize to other organs. Over the last two decades, data from number of laboratory, non-clinical and clinical studies support the crucial role of angiogenesis in breast cancer progression. Moreover, antiangiogenic therapies have recently emerged as a new option in the treatment of several malignancies such as: breast, colorectal, lung, renal and hepatocellular

cancer. Since the response to antiangiogenic agents is still limited and relatively short, the important issue is defining molecular markers to predict response and select patients' population which is likely to achieve benefits from such therapy. Modulation of biological targets may be also helpful in monitoring *in vivo* treatment efficacy. Currently, extensive research is focusing on establishing the value of prognostic and predictive angiogenic markers [1].

Invasive human breast cancer commonly express variety of angiogenic factors [2]. Vascular endothelial growth factor (VEGF), also called VEGF-A plays a key role in the neovascularisation of human tumors. VEGF–C and VEGF-D influence the growth of lymphatic vessels (lymphangiogenesis) [3]. The effect of VEGF-A is mediated through binding to two homologous VEGF receptors, VEGF receptor-1 (Ftl-1) and VEGF receptor-2 (KDR), which are expressed on vascular endothelial cells. A third receptor, VEGF receptor-3 (Ftl-4) has been shown to be involved in VEGF-C and VEGF-D mediated lymphangiogenesis. VEGF C, VEGF D and two of four isoforms of VEGF-A that are expressed, $VEGF_{121}$ and $VEGF_{165}$, are soluble and hence detectable in serum and plasma [4]. Several additional members of the VEGF family (i.e. VEGF B, E) have been also identified.

The serum VEGF level has been correlated with outcome in a variety of solid tumors including cervical [3], ovarian [5], colorectal [6], colon [7], gastric [8], and lung cancer [9]. Although there is growing evidence indicating prognostic value of angiogenesis markers in breast cancer, methods of assessment varies across published studies [10–12]. Besides intratumoral microvessel density (MVD), in most studies tumor cytosolic VEGF was measured. VEGF expressions have been reported to have independent prognostic significance and it is associated with inferior survival in breast cancer. What is more, pattern of relapse may be also associated with level of VEGF in primary tumor. It has been observed that patients with visceral metastases have higher level of VEGF, compared to the group with relapse limited to bone and soft tissue [11]. VEGF may be also a predictive marker in patients treated with hormonal therapy and chemotherapy. Unfortunately, clinical utility of these findings is limited by different methods of assessment, different patients' populations and their retrospective nature. However, due to variety of angiogenic factors and their complex interactions it is likely that assessment of multiple angiogenic markers may be more useful. Since blood biomarkers are minimally invasive, relatively easy to assess in almost all patients, may provide a less subjective analysis than other measurement such as microvessel density (MVD) or immunohistochemical analysis of VEGF expression. There are several reports suggesting that soluble VEGF might be potential markers of angiogenesis. We examined the serum VEGF-A, VEGF-C and VEGF-D levels in patients with malignant and benign breast tumours to determine their association with clinicopathologic parameters and potential prognostic value.

Materials and Methods

Patients

Our analysis is based on a group of 377 consecutive patients with operable breast tumors treated in Department of Surgical Oncology of Lower Silesian Oncology Centre (Poland) between May 2003 and May 2005. All women with breast cancer underwent primary surgery: in 255 patients radical mastectomy and in 94 breast conserving treatment (lumpectomy with axillaries node dissection). Women with pathologically proven benign tumors had lumpectomy. Surgical treatment in breast cancer patients was followed by standard adjuvant therapy.

The median patients age was 57 years (range 28–83). Patients with benign tumors were significantly younger (median 50, mean 52 years) compared to the group diagnosed with malignant tumors (median 58, mean 58.31 years, $p=0.0018$). Pathology revealed non malignant lesion in 28 patients and breast cancer in 349 women (228 cases of invasive ductal carcinoma, 65—invasive lobular carcinoma, 44—other types of invasive carcinoma and 12—ductal or lobular carcinoma in situ).

According to AJCC staging we classified 227 patients as having pT1 tumors, 93-pT2 tumors and 12 as having pT3 tumors. Tumor size was not determined in 5 cases. Palpable axillary lymph nodes (cN+) were found in 134 patients. Pathology reports confirmed lymph node metastases in 129 women (pN1-pN3). All patients were followed up for the median of 42 months (range 30–54 months).

Study was approved by the local Ethical Committee and all patients provided written informed consent before the initiation of the analysis.

Serum Assay

All serum samples were collected before starting the treatment. Peripheral venous blood samples (5 ml) from all subjects were collected and allowed to coagulate at +4°C, centrifuged at 2,000 g for 10 min, and stored in aliquots at −80°C. Serum VEGF concentration was determined using the quantitative sandwich enzyme immunoassay technique (Quantikine human VEGF, R&D Systems Minneapolis, USA). Briefly, serum samples were incubated in a micro-well plate pre-coated with anti-human VEGF-A_{165}, VEGF-C and VEGF-D monoclonal antibodies. The amount of all studied vascular endothelial growth factors

were detected by complex peroxidase-antibody and the amount of peroxidase was determined by addition of tetramethylbenzidine (TMB) substrate. Reactions were stopped by adding a sulphuric acid solution and the optical densities were read at 450 nm in a micro-titer plate spectrophotometer. All measurements in serum samples were done in duplicates, undiluted or appropriately diluted and the mean value was chosen to represent the VEGF-A_{165}, VEGF-C and VEGF-D value of each patient.

Statistical Analysis

Association between VEGF-A, VEGF-C, VEGF-D, established prognostic and predictive factors were tested by Pearsons' χ^2 test. Survival was estimated using the Kaplan-Meier method, and comparison between study groups was performed with log-rank test. Mean and median levels of vascular endothelial growth factors were reported. Overall survival was assessed from the date of diagnosis to date of death or date of last follow up. Multivariate analysis using Cox's proportional hazard regression model was carried out to assess, the independent correlations between VEGF A, VEGF C and other variables. All tests were two-sided and the significance level was set at 0.05. Statistical analysis was performed using R Development Core Team, Vienna, Austria [13].

Results

We observed several extremely high serum levels of vascular endothelial growth factors which were excluded from statistical analysis. Table 1 shows detailed data regarding VEGF serum levels (after excluding the outlying scores) and its correlations with clinicopathological factors.

Benign and Malignant Breast Lesions

Mean serum concentrations of VEGF-A, VEGF-C and VEGF-D in the group of cancer patients and women with benign breast diseases were presented in Table 1. There were no significant differences between markers serum levels in patients with malignant and non malignant lesions (VEGF-A-p=0.97, VEGF-C-p=0.78 and VEGF-D-p=0.18).

VEGF

Correlations between serum concentrations of those three vascular endothelial growth factors were also tested. We found strong correlation between VEGF-A and VEGF-D serum levels (p=0.0000000000395) and between VEGF-C and VEGF-D levels (p=0.000333). There was no significant correlation between VEGF-A and VEGF-C serum levels (p=0.0908).

The analysis did not revealed any statistically significant correlations between serum levels of VEGF A, C, D and tumor histology, its size, grade, stage of disease, patients age and menopausal status. Although the analysis did not found any statistically significant association between serum levels of VEGF A, C and hormone receptor status, in hormone receptor positive tumors, the higher expressions of estrogen receptor (+ vs. +++ and ++ vs. +++) correlated with higher serum levels of VEGF-D (p=0.044 and p=0.017). The analysis revealed that when two groups were constructed: first with 0 and 1 expression according to ImmunoReactive Score (IRS) of HER 2; second with 2 to 12 (IRS) HER2 expression—the statistical correlations between VEGF D levels and HER were observed (p=0,0186): VEGF D levels were higher in the second patients group.

In the group of 30 premenopausal patients the correlation between VEGF serum levels and phase of menstrual cycle were analysed, but did not show any differences in luteal and follicular phase (details in Table 1).

Statistically significant correlations were found between erythrocytes count and VEGF-C levels (p=0.0368), leukocytes count and both VEGF-A and VEGF-C levels (p=0.00072 and p=0.043). There were also correlations between platelets count and VEGF-A levels (p=0.0134) and VEGF-C levels (p=0.000027). There was no dependency between VEGF-D levels and blood cells count.

Patients with a serum VEGF C > 1784.699 pg/ml and patients with a serum levels <1784.699 pg/ml had a 4 year survival rate 0.93 and 0.82 respectively (p=0.00628). Similar correlation had been found assessing cut off limit for VEGF A—711.1 pg/ml and for VEGF D—95.599 pg/ml but statistical significance had not been achieved (p = 0.255 and p=0.0771 respectively) Figs. 1, 2, 3.

In first iteration we considered a set of Cox model with one explanatory variable only (details in Table 2). We assessed significant correlations between disease free survival (DSF) and VEGF C levels—p=0.011, age—p=0.00016, pT stage—p=0.003 and pN stage—p=0.00091. In second iteration we considered one model with all variables selected as significant in first iteration and the effect of variable VEGF C was still considered as significant (p=0.0058).

Discussion

The recognition that tumor growth is strongly dependent on angiogenesis prompted investigations assessing the prognostic and predictive value of angiogenic factors in breast cancer. Angiogenesis can be quantified by several different

Table 1 Correlations between pre-treatment serum VEGF-A, VEGF-C and VEGF-D levels and clinical parameters of patients with breast tumors

Tumor characteristics	Number of patients (%)	Serum VEGF-A levels (pg/ml)		Serum VEGF-C levels (pg/ml)		Serum VEGF-D levels (pg/ml)	
		Mean + − SD	p	Mean	p	Mean	p
All tumors	377 (100%)	797.8 +/− 727.57		3035 +/−949.27		170.5 +/− 157.79	
Benign tumors	28 (7.43%)	808 +/− 709.12	0.8	2989 +/−867.32	0.5	179.4 +/− 144.55	0.26
Malignant tumors	349 (92.57%)	797.1 +/− 731.08		3040 +/−957.42		168.7 +/− 158.94	
pTis	12 (3.44%)	591	0.95 / 0.9 / 1	2393	0.4 / 0.7 / 0.29	359.1	0.25 / 0.2 / 0.67
pT1	227 (65.04%)	1277	0.94 / 0.63	3187	0.27 / 0.49	336.7	0.39 / 0.27
pT2	98 (28.08%)	1706	0.67	3043	0.39	399.6	0.2
pT3	12 (3.44%)	537.8	0.91	3608	0.29	245.3	0.11
pN0	208 / 337 (61.7)	1489	0.51	3194	0.16	360.7	0.74
pN1-3	129 / 337 (38.28%)	1165		3027		321.8	
p I	153 (45.4%)	1469	0.33 / 0.69 / 0.97 / 0.31 / 0.49	3234	0.42 / 0.47 / 0.1 / 0.57 / 0.45	310.8	0.28 / 0.4 / 0.58 / 0.14 / 0.23
p IIA	80 (23.74%)	1357	0.36 / 0.51 / 0.42 / 0.9	3118	0.84 / 0.33 / 0.8 / 0.81	407.3	0.11 / 0.83 / 0.29 / 0.68
p IIB	21 (6.23%)	1485	0.78 / 0.24 / 0.32	3072	0.57 / 0.9 / 0.97	231.1	0.29 / 0.075 / 0.11
p IIIA	47 (13.95%)	1159	0.42 / 0.45	2927	0.8 / 0.61	523	0.36 / 0.65
p IIIB	8 (2.37%)	888.2	0.59	3026	0.93	106	0.58
p IIIC	28 (8.31%)	1239		3061		225.2	
Ductal histology	228 (67.65%)	1490	0.36 / 0.71	3105	0.56 / 0.61	375.4	0.2 / 0.39
Lobular histology	65 (19.29%)	891.2	0.28	3199	0.98	211.3	0.88
Other carcinomas	44 (13.06%)	1429		3204		399.7	
Grade 1	27 (7.74%)	1044	0.93 / 0.46	3242	0.39 / 0.94	247.4	0.96 / 0.84
Grade 2	144 (41.26%)	1576	0.086	3018	0.16	471.7	0.78
Grade 3	63 (18.05%)	1348		3220		310.7	
ER (−)	108 (30.95%)	1749	0.7 / 0.95 / 0.97	3148	0.27 / 0.8 / 0.6	538.5	0.5 / 0.41 / 0.097
ER(+)	55 (15.76%)	1291	0.63 / 0.93	3341	0.21 / 0.19	234.7	1 / 0.044
ER(++)	97 (27.79%)	1191	0.9	3107	0.75	278.4	0.017
ER(+++)	40 (11.46%)	987.3		3041		291.7	
PgR(−)	114 (32.66%)	1576	1 / 0.93 / 0.93	3092	0.74 / 0.68 / 0.63	326.8	0.7 / 0.057 / 0.74
PgR(+)	42 (12.03%)	2007	1 / 0.88	3158	0.98 / 0.9	558.6	0.059 / 0.94
PgR(++)	84 (24.07%)	1111	0.81	3154	0.86	350.1	0.07
PgR(+++)	49 (14.05%)	1044		3191		357.1	
Follicular phase	12	1022	0.46	3542	0.92	494.3	0.44
Luteal phase	18	1594		3492		1643.9	

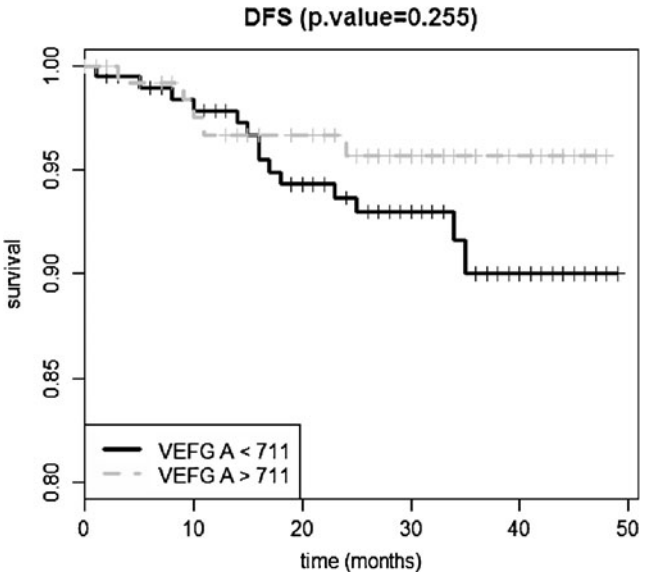

Fig. 1 Kaplan–Meier curves for disease free survival and serum VEGF-A levels

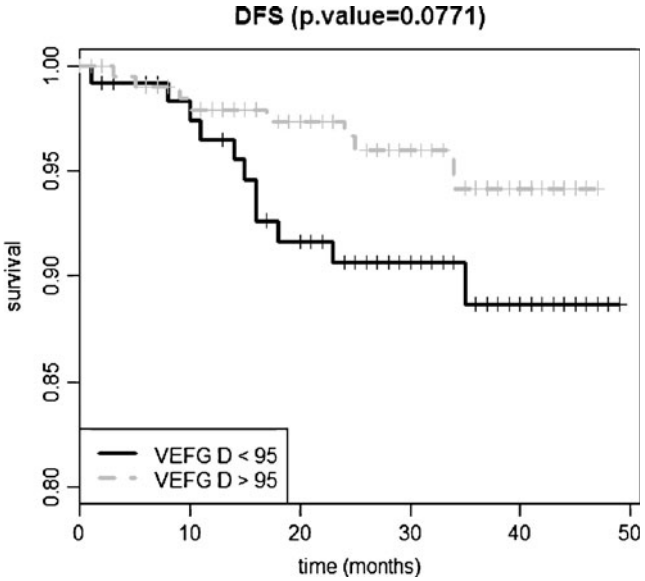

Fig. 3 Kaplan–Meier curves for disease free survival and serum VEGF-D levels

methods, such as counting new blood vessels or measuring angiogenic factors by immunochemistry, in tumor cytosol or in the blood. Investigated angiogenic factors include: VEGF, hypoxia inducible factor (HIF1A), fibroblast growth factor (FGF) and receptors (e.g. VEGFR2, sVEGFR1 and sVEGFR2). As tissue VEGF may not represent sensitive marker, in some studies activated receptor VEGFR2 (phosphorylated VEGFR2 [p-VEGFR2]) was also examined [10]. These factors were studied as potential prognostic and predictive markers in breast cancer patients in adjuvant and metastatic settings. Due to the complex nature

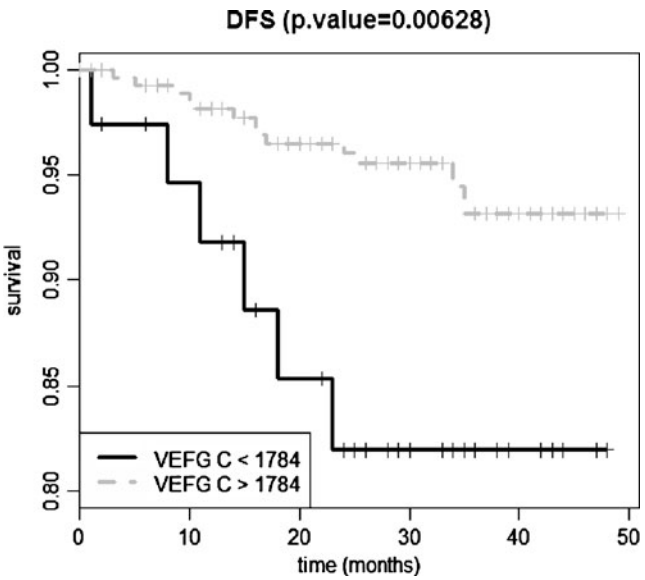

Fig. 2 Kaplan–Meier curves for disease free survival and serum VEGF-C levels

of angiogenesis and multiple factors involved in this process it seems to be worth to study different angiogenic factors. This study investigated for the first time serum levels of three important angiogenic factors: VEGF-A, VEGF-C and VEGF-D in patients with breast tumors.

Serum markers seem not to be necessarily associated with malignancy, and data from published studies are conflicting. We found no significant difference in VEGF-A, VEGF-C and VEGF-D concentrations between patients with invasive breast cancer and benign breast lesions. Unfortunately most studies assessing serum angiogenic growth factors did not compare results of measurements between malignant and benign tumors [1,2,12]. Konukoglu et al. [14] in their small study reported significantly higher serum concentrations of VEGF-A in 20 patients with operable breast cancer compared with 11 women with benign breast diseases ($p<0.01$). Design of very interesting study performed by Adams et al. [15] allowed the comparison between different groups of healthy individuals and women with breast cancer in different stages of disease. They examined both plasma ($VEGF_P$) and serum ($VEGF_S$) VEGF concentrations in 201 blood samples taken from: pre- and postmenopausal healthy controls, patients with benign breast lesions, localized and metastatic breast cancer and patients with breast cancer in remission. There were seen substantial differences between all groups. $VEGF_P$ but not $VEGF_S$ concentrations of patients with localized disease were significantly elevated compared with normal controls ($p=0.016$). Patients with metastatic disease had higher $VEGF_P$ and $VEGF_S$ levels than normal controls ($p<0.001$, $p=0.044$ respectively), and higher $VEGF_P$, but not $VEGF_S$, than patients with benign disease ($p=0.009$) and

Table 2 Multivariate Cox regression model of prognostic covariates in patients with breast cancer

Clinical Parameters	Coefficient	p-value
VEGF -A	−0.58	0.26
VEGF -C	−1.26	0.011
VEGF -D	−0.80	0.085
Age	0.092	0.00016
pT	1.47	0.003
pN	2.09	0.00091
ER	−1.34	0.2
PgR	−0.50	0.19
Her2/neu	−0.50	0.61
Treatment-chemotherapy	0.24	0.65
Treatment-hormonotherapy	−15.22	0.34
Erytrocyte count	−0.82	0.19
Leucocyte count	−0.15	0.3
Platelets count	0.0071	0.053

patients with localized disease (p=0.004). The highest $VEGF_P$ and $VEGF_S$ concentrations were seen in patients in remission compared with normal controls (p<0.001 and p=0.008) and $VEGF_P$ concentrations in this group were also higher than in patients with benign disease (p=0.01) or patients with localized disease (p=0.005). In above study significantly higher VEGF concentrations were found in the serum samples ($VEGF_S$) compared with the corresponding citrated plasma samples ($VEGF_P$; p<0.0001). This observation indicates that serum and plasma VEGF are different markers and may explain the lack of differences seen in our series between patient with malignant and benign lesions. There are several other reports in the literature showing results consistent with our observation. In the work of Mathur et al. [16] although elevated serum VEGF-C levels was seen in squamous cervical cancer, premalignant lesions and cervical intraepithelial neoplasia (CIN) compared to healthy controls, no increase in serum VEGF-C was found in patients with ovarian and endometrial adenocarcinomas.

In study of Mitsuhashi et al. [3] exploring serum VEGF-A and VEGF-C in 78 patients with cervical carcinoma and 30 healthy controls, both markers were significantly elevated in patients with squamous cell carcinoma patients, which correlated with more advanced FIGO stages. But again, there was no significant difference in serum VEGF-A and VEGF-C between patients with adenocarcinoma and healthy controls.

The association between serum VEGF and clinicopathologic parameters in cancer is uncertain. We found no significant correlations between the pretherapeutic serum levels of VEGF-A, VEGF-C, VEGF-D and clinicopathologic variables. Similar results were observed in other studies assessing VEGF-A serum concentration in breast cancer [2] and other tumors such as head and neck [4], colon [7] and ovarian cancer [16]. In contrast, Adams et al. [15] in the study that was described above, found that VEGF expression was inversely correlated with tumor grade. There are also several reports from studies performed in non-small cell lung cancer [9,17] and gastric cancer [8] which indicate that patients diagnosed with more advanced disease tend to exhibit higher level of VEGF.

Interaction between ER and VEGF is an interesting area of investigation. There are experimental studies showing evidence of an inhibitory relationships between ER-α expression and production of angiogenic factors. Some studies assessing tissue expression of VEGF A and D [18] showed inverse correlation between those markers and ER expression. Heer at al. [2] in his analysis performed in 200 breast cancer patients, found a significantly higher levels of VEGF-A in women with ER positive comparing to ER negative tumors. Also Adams et al. [15] reported significant correlation between VEGF expression and estrogen receptor status. Our results did not confirm it. Only in hormone receptor positive tumors, we observed that higher expression of estrogen receptor (+ vs. +++ and ++ vs. +++) was associated with higher levels of VEGF-D serum level (p= 0.044 and p=0.017). But this correlation can not be considered specific and clinically relevant since there was no difference in VEGF concentration between ER positive and ER negative group. Furthermore we did not find any association between serum VEGF-A, VEGF-C levels and hormone receptors. Experimental data indicates that both estrogen and tamoxifen can stimulate VEGF secretion, which may result in counteracting the tamoxifen effect. Interesting observation comes from studies investigating predictive value of VEGF in patients treated with adjuvant tamoxifen. They suggest, that there is a strong link between VEGF pathway and tamoxifen response. High level of tumor-specific VEGFR2 expression seems to indicate tumors resistant to tamoxifen in premenopausal patients [19]. Reduced disease free survival has been reported in postmenopausal women with elevated VEGF levels [20]. Since there is an association between extracellular regulated kinase 1/2 (ERK1/2) and VEGFR2 in breast cancer and phosphorylated ERK1/2 is linked to resistance to tamoxifen, it is likely, that VEGF pathway can be important in predicting benefits from hormonal therapy [21].

Our findings regarding association between soluble VEGF and blood cells, may be explained by the fact that circulating neutrophils and platelets contain considerable amounts of VEGF, which may contribute to high VEGF levels in serum and lysed whole blood [22]. Thus liberation of VEGF from these compartments could well be of importance for tumor angiogenesis. Elevated serum VEGF may indicate increased tumor activity but on the other hand it may be the consequence of increased platelet numbers in

cancer patients caused by intratumoral platelet activation and subsequent release of thrombopoetin. The importance of platelet-derived VEGF-A in cancer may be due to thrombin activation by platelets resulting in VEGF releasing. VEGF-A inducing vascular permeability further promotes coagulation [23]. A similar mechanism has been postulated for the leukocyte—VEGF-A interaction [24]. Some studies suggest that leukocytes are more important sources of VEGF in cancer patients. Surprisingly, Kut et al. [23] showed that even in cancer hosts the largest source of VEGF in the body is not malignant tumor, but other tissues, in particular skeletal muscle which appear to contain a large reservoir of VEGF. Unpublished results of our study investigating serum levels of VEGF A, C, D in young healthy sportsmen showed that elevated levels of those angiogenic markers may be the result of forced physical effort and repeated micro-injuries. The mechanism of interactions between platelets, leukocytes, other members of vascular endothelial growth factors family, erythrocytes and VEGF-C levels is unclear.

VEGF expression has been reported to be associated with reduced RFS and OS in breast cancer patients. In one study prognostic value of soluble VEGF receptors was examined together with tumor cytosolic VEGF. The relative ratio of tumor cytosolic VEGFR1 to total VEGFR has been shown to be a prognostic indicator of RFS (p=0,0008) and OS (p=0,0002) [10]. Reduced DFS and OS was seen in patients with ovarian cancer and high serum levels VEGF-A [5]. Similar results in patients with colon adenocarcinoma were presented by De Vita et al. [7]; they proved that preoperative high VEGF-A serum levels are associated significantly with worse DFS and OS. Our results did not suggest the correlation between serum VEGF-A levels and survival. In our study in Cox model we showed that serum VEGF C levels was independent prognostic factor for DFS as well as OS. In breast cancer the studies on prognostic significance of angiogenic factors were conducted only on tumor tissue samples. Yang et al. [25] observed that overexpression of VEGF-A in breast cancer tissue was associated with shorter DFS. In their study patients with tumor expressing high levels of VEGF-C or VEGF-D showed a notable trend for worse DFS, however, it was not statistically significant.

Conclusions

In summary, to our knowledge, this is the first study which examined serum VEGF-A, VEGF-C and VEGF-D concentrations in patients with malignant breast tumors and benign lesions and proved significant correlation between these angiogenic markers. We have demonstrated that VEGF-C serum levels in breast cancer patients was independent prognostic factor. Although correlation between levels of VEGF- A and C and D were found, the combination of all the three angiogenic factors does not seem to be helpful in predicting outcome. The correlation between serum levels of VEGFs and clinicopathologic parameters in malignant tumors remains uncertain. Observed association between different serum VEGF and number of blood cells is likely to be a consequence of VEGF release from blood cells such as platelets and leucocytes and further investigations, preferably measuring both plasma and serum VEGF are warranted. The confirmation of prognostic and predictive role of angiogenic markers in breast cancer patients requires assessment in prospective studies.

Angiogenesis has become an attractive target for cancer therapy. Bevacizumab, a humanized monoclonal antibody directed against VEGF-A ligand was approved for the treatment of metastatic breast cancer in combination with paclitaxel and variety of antiangiogenic agents are being studied in this population. Results of clinical studies are encouraging. But despite recent widespread use of these agents, in the absence of validated predictive factors, these therapies are given on the population basis rather than targeted therapy. Thus, there is an urgent need to better understand complex interaction between angiogenic factors and their possible prognostic and predictive values.

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