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Clinical usefulness of assessing VEGF and soluble receptors sVEGFR-1 and sVEGFR-2 in women with breast cancer

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Abstract

Introduction. The biological activity of VEGF depends on the presence of its specific receptors on the endothelial surface: VEGFR-1, VEGFR-2, and on their soluble forms sVEGFR-1 and sVEGFR-2. The binding of the membrane-bound receptors with VEGF affects the permeability, proliferation and migration of vascular endothelial cells. This creates the necessary conditions for the vascularisation of solid tumours and for the spread of remote metastases. The sVEGFR-1 and sVEGFR-2 receptors are believed to be natural inhibitors of VEGF.

Objective. To determine the clinical usefulness of VEGF and the sVEGFR-1 and sVEGFR-2 receptors level assay in women with primary breast cancer. The assessment also took into account: patient's age, stage of the disease, histological grade, status of the axillary lymph nodes and size of the primary tumour.

Material and methods. The concentrations of VEGF, sVEGFR-1 and sVEGFR-2 were ascertained in 103 women with primary breast cancer. The concentrations of VEGF in the plasma, and those of the soluble receptors sVEGFR-1 and sVEGFR-2 in the serum, were assessed by ELISA, R&D Systems.

Results. The study found significantly raised concentrations of VEGF, sVEGFR-1 and sVEGFR-2 in the serum of women with breast cancer, relative to the values obtained from the control group. It was found that with increasing clinical stages of the disease, the levels of VEGF and concentrations of sVEGFR-1 and sVEGFR-2 also increased. Similar findings were noted when assessing the degree of the histological grade of the tumours. Significantly higher values of VEGF protein and the assessed receptors were obtained from women with metastases to the axillary lymph nodes. A positive relationship, though without statistical significance, was noted between the concentration of sVEGFR-2 and the size of the tumour.

Conclusions. The high concentrations of the VEGF cytokine and the sVEGFR-1 and sVEGFR-2 receptors in women with breast cancer are responsible for giving rise to the processes of tumour angiogenesis. The concentrations of the VEGF protein and the soluble forms of the receptors sVEGFR-1 and sVEGFR-2 in the serum of breast cancer patients showed positive correlations with the clinical stage of the disease. These results point to the usefulness of VEGF assessment and its soluble receptors in the clinical evaluation of patients with breast cancer.

Key words

breast cancer, VEGF, tumour metastasis

INTRODUCTION

The formation of vessels in solid tumours is associated with the presence of factors stimulating the growth of the tumour and, in particular, with the vascular endothelial growth factor (VEGF) [1, 2]. This factor activates the processes of migration and proliferation in endothelial cells and increases the permeability of vessel walls, resulting in the escape of blood plasma proteins, including fibrinogen, to the tissues surrounding the vessels [3, 4]. Studies on the mechanism of its biological function have shown VEGF to be a cytokine affecting endothelial cells after binding with specific surface receptors, such as VEGFR-1 (Flt-1) and VEGFR-2 (KDR/Flk-1) [5]. VEGFR-1 receptors (Flt-1 – fms-like tyrosine kinase-1) are mainly found on the surface of endothelial cells in vessels and on the surface of monocytes and macrophages [6, 7, 8]. They also play a role in the early stages of organogenesis, in neovascularisation and in wound healing. Further, VEGFR-1 receptors are important in the processes of growth and differentiation of endothelial cells and in vessel repair [9]. VEGFR-2 receptors (Flk-1/KDR – foetal liver kinase-1/ kinase domain receptor) are found on the surface of the endothelial cells of the vessels, blood platelets, haemopoietic cells, osteoblasts, and on the stem cells of the retina. These receptors are thought to be the main regulators of vasculogenesis during embryonic development and during angiogenesis in adult individuals [10]. Receptors for the vascular endothelial growth factor, apart from those bound to cellular membranes, also occur in soluble form [11, 12]. It is possible that sVEGFR-1 receptors, binding with each isoform of VEGF, act as negative regulators in the process of angiogenesis by reducing the availability of the cytokine to the endothelial cells of the vessels. Similarly, soluble sVEGFR-2 receptors bind with VEGF before it reaches

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membrane-bound receptors on endothelial cells, thus slowing their migration and proliferation, and consequently, the formation of new blood vessels in solid malignant tumours. The sVEGFR-1 and sVEGFR-2 receptors are regarded as physiological inhibitors of the processes of angiogenesis and neoangiogenesis [13].

The aim of this study was to determine the usefulness of assessing the concentration of VEGF and its soluble receptors: sVEGFR-1 and sVEGFR-2 in women with breast cancer. Prognostic factors, such as patient age, clinical stage of the disease, histological grade of the tumour, state of the axillary lymph nodes and size of the primary tumour were also taken into consideration.

MATERIALS AND METHODS

Clinical characteristics of the studied group. VEGF protein and the soluble receptors VEGFR-1 and sVEGFR-2 were demonstrated prior to surgery in the plasma and serum of 103 women with breast cancer. The women were aged 29–89 years (average age 56 years). The patients were treated in the Department of Oncological Surgery in the Oncology Division of the University of Medical Sciences in Poznan, Poland. The control material comprised of plasma and serum drawn from 40 healthy women aged 24–75 years (average age 47 years). The Bioethics Committee of the Poznan University of Medical Sciences gave consent prior to undertaking the study. Patients' characteristics are shown in Table 1.

Table 1. Patients' characteristics.

CHARACTERISTIC	No. OF PATIENTS	PERCENTAGE OF WOMEN TESTED [%]
MENOPAUSAL STATUS		
premenopausal	31	30.1
postmenopausal	72	69.9
TNM CLASSIFICATION		
I	47	45.6
II	38	36.9
III	18	17.5
HISTOLOGICAL GRADE		
G1	11	10.7
G2	50	48.5
G3	42	40.8
AXILLARY LYMPH NODES STATUS		
pN0	51	49.5
pN1	52	50.5
TUMOUR SIZE		
<20 mm	54	52.4
≥20<50 mm	40	38.9
≥50 mm	9	8.7

The concentration of VEGF and the soluble receptors VEGFR-1 and sVEGFR-2 were determined using an immuno-enzymatic method (ELISA), (Quantikine tests, R&D Systems).

Statistical analysis. A statistical analysis was carried out using the non-parametric Mann-Whitney U test and the Kruskal-Wallis test. Results with a value of $p<0.05$ were deemed to be statistically significant. Calculations were carried out using the programme Statistica for Windows (StatSoft, Inc., 2001).

RESULTS

The median concentration of VEGF in the group of patients with malignant tumours of the breast was more than 6 times greater than that found in the plasma of the control group, a difference that was found to be statistically significant (Tab. 2). The concentrations of sVEGFR-1 and sVEGFR-2 in the serum of the breast cancer patients were significantly higher than in control group (Tab. 3). The median concentrations of VEGF and the sVEGFR-1 receptor rose in line with the increasing grade of histological malignancy (Tab. 2) VEGF, sVEGFR-1 and sVEGFR-2 concentrations also rose together with the advancement of the disease process (Tab. 2). The median concentration of VEGF in women in clinical stage III of disease was twice that of women in stage II, and as much as three times greater than that of women in stage I of disease (Tab. 2). Analysis of the data also showed significantly raised levels of VEGF and the receptors sVEGFR-1 and sVEGFR-2 among women with metastases to the axillary lymph nodes, relative to those without metastases (Tab. 2,3).

The results of the presented study show that there is a positive correlation between the average concentration of VEGF and sVEGFR-2 and size of the tumour. However,

Table 2. Values of VEGF to clinicopathologic parameters.

Clinicopathologic parameter	Average VEGF concentration	Median VEGF concentration	Range of values
		[pg/ ml]	
Control n = 40	22.7±18.3	17.2	(1.2–68.3)
Cancer patients n = 103	154.7±124.7*	120.0*	(3.5–490.2)
Histological grade			
G1 n= 11	61.2± 90.3	17.5	(3.5–267.2)
G2 n= 50	119.1± 82.6* ♦	103.6* ♦	(17.3–331.6)
G3 n= 42	221.5±141.6* ■	199.6* ■	(12.1–490.2)
Stage of clinical according to TNM			
I n = 47	95.4± 96.4*	71.8*	(3.5–490.2)
II n = 38	152.8± 91.1* ◙	160.8* ◙	(12.1–438.0)
III n =18	313.3±117.5* ●	315.2* ●	(58.8–468.7)
Axillary lymph nodes status			
N0 n= 51	81.4± 74.6*	67.4*	(3.5–298.8)
N1 n= 52	226.0±122.6* ✔	199.6* ✔	(12.2–490.2)
Tumour size			
T_1< 20 mm n= 54	100.7± 92.4*	76.7*	(3.5–438.0)
T_2 ≥ 20 < 50 mm n= 40	196.6± 123.5*□	182.4* □	(12.1–490.2)
T_3 ≥ 50 mm n= 9	292.4±133.1* ⊠	289.8* ⊠	(58.8–468.7)
Menopausal status			
Premenopausal n =31	130.4±105.5*	96.6*	(12.1–468.7)
Postmenopausal n= 72	165.2±150.9*	150.9*	(3.5–490.2)

* statistically significant difference compared to control group; $p < 0.05$
■ statistically significant difference compared to grade G2 and G1 tumours; $p<0.05$
♦ statistically significant difference compared to group of women with grade G1tumours; $p<0.05$
● statistically significant difference compared to group of women in stages I and II; $p< 0.05$
◙ statistically significant difference compared to group of women in stage I; $p< 0.05$
✔ statistically significant difference compared to women in the group without axillary lymph nodes metastases; $p< 0.05$
⊠ statistically significant difference compared to the group of women where T_1 and T_2 ; $p<0.05$
□ statistically significant difference as compared to the group of women where T_1; $p<0.05$

Table 3. Values of sVEGFR-1 and sVEGFR-2 to clinicopathologic parameters.

Characteristic	sVEGFR-1			sVEGFR-2		
	Average sVEGFR-1 concentration	Median sVEGFR-1 concentration	Range of values	Average sVEGFR-2 concentration	Median sVEGFR-2 concentration	Range of values
	[pg/ml]			[pg/ml]		
Control; n=40	41.0±8,7	43.5	20.1-49.7	5086±1162	5142	2455–6759
Cancer patients; n=103	102.8* ±54.9	79.3*	43.2- 259.8	9452*± 4600	8758*	1006–21770
Stage according to TNM						
I n=47	74.1±25.6*	67.7*	43.2-159.6	7093±2748*	6444*	1006–18820
II n=38	115.0±63.5*&	87.6 *&	48.8-256.9	9584±3636 *&	9380*&	1311–16721
III n=18	132.6±60.1*&	136.0*&	48.5-259.8	15334±5070*&#	15902*&#	2037–21770
Status of axillary lymph nodes						
pN0; n=51	76.9±35.3*	67.4*	43.2-226.8	**7**893±3375*	6755*	4003–19277
pN1; n=52	128.2±59.0*▼	114.6*▼	49.8-259.8	10983±51320*▼	10174*▼	1006–21770
Tumour size						
pT < 20 mm; n=54	88.7±49.8*	70.8*	43.5-257.4	7735 ±3431*	7276*	1006–19277
pT ≥20< 50 mm; n=40	121.5±56.9*♦	97.4*♦	50.8-259.8	10705 ± 4823*♦	10731*♦	1311–21770
pT ≥ 50 mm; n=9	99.2±54.4*	72.8*	48.8-195.0	15180 ± 4583*♦●	14673*♦●	9137–20754
Histological grade						
G1 n=11	59.7±8.5*	61.8*	46.8-77.6	7936±2802*	6755*	5274–13255
G2 n=50	98.9±44.5*	83.0*	43.2-226.8	9007±4045*	8749*	1349–20315
G3 n=42	118.8±65.9*■	85.0*■	48.5-259.8	10379±5426*	9422*	1006–21770
Menopausal status						
Premenopausal; n=31	80.8±32.5*	71.9*	43.2-162.1	8910±3674*	8758*	4003–20754
Postmenopausal; n=72	112.3±59.8*	84.4*	46.8-259.8	9686 ± 4951*	8898*	1006–21770

* – statistically significant difference in sVEGFR-1 and sVEGFR-2 concentration in comparison to control group, where p< 0.05
& – statistically significant difference in sVEGFR-1 and sVEGFR-2 concentration in comparison to women in stage I of disease, where p< 0.05
# – statistically significant difference in sVEGFR-2 concentration in comparison to women in stage II of disease, where p< 0.05
▼ – statistically significant difference in sVEGFR-1 and sVEGFR-2 concentration in comparison to women without axillary lymph nodes, where p< 0.05
♦ – statistically significant difference in sVEGFR-1 and sVEGFR-2 concentration in comparison to women with tumours sized <20 mm, where p<0.05
● – statistically significant difference in sVEGFR-2 concentration in comparison to women with tumours sized 20-50 mm, where p< 0.05
■ – statistically significant difference in sVEGFR-1concentration in comparison to women with grade G1, where p<0.05

no relationship was found between the concentration of sVEGFR-1 and size of the tumour (Tab. 3). The presented study also shows no significant differences between the concentrations of VEGF, sVEGFR-1 or sVEGFR-2 in cancer patients before menopause and those after menopause (Tab. 2,3).

DISCUSSION

After lung cancer, cancer of the breast is the next most common cause of death (due to malignancies) among Polish women. Breast cancer is characterised by high invasiveness and an ability to metastasise to distant organs [3]. The process which enables cancer cells to grow and migrate to neighbouring tissue is known as angiogenesis [14]. Clinical observations have shown that the process of neovascularisation is under the strict control of angiogenic substances, working locally, to produce new vessels for the tumour. Vascular endothelial growth factor (VEGF) belongs to this group of substances [15, 16]. This cytokine is a glycoprotein, with strong mitogenic properties which, by increasing the permeability of blood vessels, allows cancer cells to pass through to extra-vascular spaces and form distant metastases [17, 18].

From the results of the presented study, it appears that the concentration of VEGF in the serum of breast cancer patients is significantly higher than that in the control group. If it is accepted that VEGF is the most important angiogenic factor, then its high concentration in the serum of patients must induce the process of neovascularisation in tumours. The protein has its biological effects only after binding with its specific membrane receptors: VEGFR-1 (Flt-1) and VEGFR-2 (KDR/Flk-1), present on endothelial surfaces [19].

In recent years, the presence of soluble VEGF receptors-sVEGFR-1 and sVEGFR-2 in the supernatant of endothelial cells has also been discovered. Currently, many oncological investigation are attempting to explain the role of these soluble receptors in the angiogenic process and in the formation of metastases. It may be noted that, through their high affinity for VEGF, these receptors slow the biological functions of VEGF and are its natural antagonists. The receptors sVEGFR-1 and sVEGFR-2 bind VEGF even before it contacts the endothelial surface and, in so doing, reduces VEGF availability for the membrane-bound receptors [20]. The effect of this may be the blocking of the signalling pathway for the receptors VEGFR-1 and VEGFR-2, as well as the slowing of proliferation and migration of the endothelial cells. As a result, this limits the formation of new blood vessels in malignant tumours [21].

The results of our earlier study have shown a high correlation between the concentration of the sVEGFR-2 receptor and the level of VEGF (r=0.67854, where p<0.05), which may indicate a high affinity of this receptor for VEGF [22]. A weaker correlation was discovered between concentration values of sVEGFR-1 and VEGF (r=0.29122, where p<0.05), which may suggest the influence of yet other not fully recognised factors on their mutual interactions. The results of the study showed significantly higher concentrations of the receptors sVEGFR-1 and sVEGFR-2 in the plasma of women with breast cancer than in the control group. Kumar et al [23], in their study determined that sVEGFR-1 receptors were present in the plasma of breast cancer patients, but they did not confirm

the presence of these forms in the plasma of healthy women. Similarly, Toi et al. [24] and Belgore et al. [25] observed raised concentrations of these receptors in the plasma of patients with breast cancer, in comparison with control group levels, but did not show any statistical significance. Gershtein et al. [26] showed that in about 85% of breast cancer patients there is a raised level of sVEGFR-2 in tumour homogenates. Taking into consideration the principle that soluble VEGF receptors are natural inhibitors of angiogenesis, it may be supposed that the high levels found in this study may be associated with a natural defence mechanism against the spread of malignant cells. A lack of new blood vessels effectively blocks cancer cells from increasing tumour mass, and from migration to remote organs and the formation of metastases within them.

The presented study shows that in cancer patients, as the disease becomes more clinically advanced, the level of VEGF increases significantly as does the concentration of the receptors sVEGFR-1 and sVEGFR-2. Benoy and Fuhrmann also confirmed a positive relationship between the concentration of VEGF and the advancement of the disease process [27, 28]. Similar observations, although in patients with tumours of the large bowel, were made by Rmali et al. in 2006 [29].

The present study also confirms the significant role of the studied markers in the development of cancer and the formation of metastases. The results showed VEGF levels to be three times higher in cases of breast cancer with metastases to lymph nodes than in cases with no metastases, and levels of sVEGFR-1 in cases with metastases to be twice as high as in cases with no metastases. These results are in agreement with those of Wu et al. [16], who observed that, among patients with breast cancer, a high sVEGFR-1/VEGF ratio was a better prognostic factor than VEGF concentration alone. Toi had a similar opinion, and found that in patients where the level of sVEGFR-1 in cancer tissues exceeded 10 times the level of VEGF, this ratio was a useful prognostic marker [24]. Toi's study, however, did not show any significant relationship between the concentration of sVEGFR-1 and metastases to the axillary lymph nodes. The results of the presented study showed that there was a significantly higher concentration of sVEGFR-2 in the plasma of women with metastases that in patients with no metastases. Meunier-Carpentier et al., however, showed no significant correlation between the levels of VEGFR-2 receptors and the occurrence of metastases [30].

In the presented study it was observed that the concentration of VEGF in women with breast cancer rose in line with the increase in tumour size and mass. This may suggest that the main source of VEGF in these patients are the cells of the tumour itself. A statistically significant relationship was observed between the median concentrations of sVEGFR-1 in women with tumours measuring less than 20mm, and with tumours between 20–50mm. Similar observations were made by Wu et al. [16]. A significant correlation between the level of sVEGFR-1 and size of the tumour occurring in patients with pancreatic tumours, was also shown by Chang [31]. It is currently difficult to evaluate the significance of VEGF and its soluble receptors, sVEGFR-1 and sVEGFR-2 in the early diagnosis of breast cancer.

CONCLUSIONS

The results of the presented study indicate that the assessment of the vascular endothelial growth factor concentration in the serum and the concentration of the soluble receptors, sVEGFR-1 and sVEGFR-2 in the plasma, may represent a valuable additional clinical test for patients with breast cancers. This is confirmed by the statistical dependencies found between the concentrations of the tested parameters in patients with cancer and the stage of disease, the state of the axillary lymph nodes and size of the tumour.

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