

# The Determination of VEGF and MVD, among Patients with Primary Breast Cancer

Anna Thielemann · Zygmunt Kopczyński ·  
Violetta Filas · Jan Bręborowicz ·  
Sylvia Grodecka-Gazdecka · Aleksandra Baszczuk

Received: 20 March 2008 / Accepted: 20 March 2008 / Published online: 9 April 2008  
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**Abstract** The purpose of the study was to ascertain the value of assessment of vascular endothelial growth factor (VEGF) levels and microvessel density, and to search for correlations between them, in women with breast cancer. The assessment considered factors such as the stage of clinical disease advancement—according to International Union Against Cancer, the grade of histological malignancy, status of axillary lymph nodes and the size of the primary tumour. The concentration of VEGF was assessed in the plasma of 103 women with breast cancer, using an immunoenzymatic method (Quantikine test of R&D Systems). Assessment of microvessel density was performed using histopathological immunoperoxidase methods, using an anti-von Willebrand factor antibody (DAKO A/S). A statistically significant relationship was found between rising VEGF levels and microvessel density in women with breast cancer, when compared to values from a control group. A correlation was found between VEGF concentration and microvessel density (MVD) values. Statistically significant differences were found between VEGF levels of patients in stages I, II and III of clinical disease advancement.

For MVD, differences were found only between stages I and III. A statistical relationship was also found between VEGF and MVD and tumour size. Similar results were found between VEGF concentrations in women with metastases to the axillary lymph nodes and cytokine levels in women with no metastases. The results of the study suggest that the degree of tumour vascularization and the concentration of VEGF may represent valuable indicators for the assessment of the angiogenic process in women with breast cancer.

**Keywords** Angiogenesis · Breast cancer · Microvessel density · Neoplasm · Vascular endothelial growth factor

## Abbreviations

ELISA	enzyme linked immunosorbent assay
MVD	microvessel density
VEGF	vascular endothelial growth factor
vWF	von Willebrand factor
VEGFR-1	vascular endothelial growth factor receptor 1
VEGFR-2	vascular endothelial growth factor receptor 2
UICC	International Union Against Cancer

A. Thielemann (✉) · Z. Kopczyński · A. Baszczuk  
Department of Laboratory Medicine,  
Poznan University of Medical Sciences,  
Łąkowa St. No 1/2,  
61-878 Poznań, Poland  
e-mail: anna.thielemann@oncology.am.poznan.pl

V. Filas · J. Bręborowicz  
Department of Tumour Pathology, Department of Oncology,  
Poznan University of Medical Sciences,  
Poznań, Poland

S. Grodecka-Gazdecka  
Department of Oncological Surgery, Department of Oncology,  
Poznan University of Medical Sciences,  
Poznań, Poland

## Introduction

Scientific studies have recently shown that the growth of malignant tumors, particularly solid tumors, is associated with an increase in vascularization through the process of angiogenesis [1]. Experiences from tests carried out in mouse models have shown that the flow of blood through a malignant tumor depends on the histological structure, the organization of blood vessel spacing and the mitotic index of the cells [2]. Blood vessels in solid malignant tumors arise from two sources [3, 4]. One of these sources is the

existing network of the body, morphologically mature and active blood vessels, while the second source is capillaries formed through the process of vascularization [5, 6].

The basic vascularization of solid tumors is associated with the presence of factors which stimulate tumour growth, and in particular with vascular endothelial growth factor (VEGF) [7].

VEGF increases the permeability of the endothelium of blood vessels, which in turn allows for cancerous cells to penetrate the vessel walls and begin the process of metastasis, even in very distant organs [8–10].

VEGF is also involved in metabolic processes such as increasing the activity of phospholipase C, stimulating the secretion of von Willebrand factor from endothelial cells and has an effect on the synthesis of collagenases and on the activity of plasminogen. Studies in the area of molecular biology have shown that VEGF is a homodimeric glycoprotein of fractional mass 34–64 kDa, with the ability to bind heparin [11, 12].

The human VEGF gene has been mapped to chromosome 6p21.3 and consists of eight exons, divided by seven introns [13]. It is known that this gene codes for six different isoforms of VEGF which contain 121, 145, 165, 183, 189 and 206 amino acids [13]. The most frequently detected isoform, VEGF<sub>165</sub> is a protein has a significant effect on the mitogenic activity of the endothelium and on its permeability. Studies into the mechanism of action of VEGF have shown that the cytokine works on endothelial cells through binding with two specific receptors of the vascular endothelium: vascular endothelial growth factor receptor (VEGFR)-1 and VEGFR-2 [13, 14]. These receptors belong to a family of proteins containing tyrosine kinase domains. The connection of vascular endothelial growth factor with its receptors KDR/flk-1 and flt-1 significantly increases the mitotic activity of endothelial cells and the chemotaxis of cancer cells. These properties confirm the action of VEGF in the process of angiogenesis in tumors [15, 16].

Experimental and clinical studies have shown that high concentrations of VEGF in systemic fluids expedite the formation of metastases. The higher the level of these cytokines being produced by malignant cells, the higher the observed degree of vascularization of the tumor and the greater its invasiveness [17–23]. Many study centres have concluded that, among patients with colorectal carcinoma, the level of vascularization within the limits of the tumor correlate significantly with the presence of metastases to the liver [24, 25]. Data also exists concerning a relationship between the degree of tumor vascularization and the presence of distant metastases among patients with non-small-cell carcinoma of the lung [20]. Experimental studies have also been carried out to determine the relationship

between VEGF concentration and the degree of vascularization in solid, malignant, primary cancers of the breast [26].

The purpose of our study was to determine the correlation between the concentration of vascular endothelial growth factor in the plasma of patients with primary breast cancer and also the microvessel density (MVD) of the tumors in these patients. In the assessment, prognostic factors such as the histological stage of malignancy, the stage of disease advancement, the status of local axillary lymph nodes and the size of the primary tumor were taken into consideration.

## Material and Methods

### Patients' Clinical Characteristics

The pre-surgical plasma concentration of VEGF was determined in the cases of 103 women with primary breast carcinoma. The ages of the women ranged from 29 to 89 years (average age, 58 years) and all were treated in the Department of Oncological Surgery, within the Oncology Department of the Poznan University of Medical Sciences. The control group consisted of 40 healthy women aged between 24 and 65 years (average age, 47 years) employed in the Oncology Department of the University. In all cases of breast cancer, in which a lesion had undergone histological assessment and had been found to show features of malignant disease, we carried out assessment of MVD. Control material comprised 25 tissue sections taken from non-malignantly altered breast tissues, such as *mastopathia fibrosa et cystica*, *adenosis sclerosans* and *fibroadenoma*.

In each case the clinical diagnosis was confirmed by histopathological studies carried out in the Department of Tumor Pathology of the Poznan University of Medical Sciences. The clinical characteristics of the patients are presented in Table 1.

### Collection of Material for Testing

For the assessment of VEGF concentration, 5 ml of venous blood was taken from each patient and preserved in 3.8% sodium citrate with a ratio of 9:1, after which it was centrifuged at 350×g for 10 min. The plasma was transferred to a 0.5 ml tube and frozen at –20°C. The prepared samples were stored in this state until tested, but not longer than 3 months.

For the assessment of MVD in tumors of the breast, material was taken from paraffin wax blocks containing tumor tissue, previously fixed in 10% formalin and embedded according to conventional histological methods.

**Table 1** Characteristics of breast cancer patients

Clinical characteristics of patients	Number of patients	Percentage of women tested [%]
TNM classification		
I	47	45.6
II	38	36.9
III	18	17.5
State of axillary lymph nodes		
pN0	51	49.5
pN1	52	50.5
Size of primary tumor pT		
<20 mm	54	52.4
≥20<50 mm	40	38.9
≥50 mm	9	8.7
Grade of histological malignancy		
G1	11	10.7
G2	50	48.5
G3	42	40.8

#### Measurement of Vascular Endothelial Growth Factor through Use of an Immunoenzymatic Method

The concentration of VEGF was measured in plasma by use of a quantitative 'sandwich' type enzyme linked immunosorbent assay produced by R&D System, Abingdon, UK.

The sensitivity of the test, as defined by the manufacturer is less than 9.0 pg/ml.

In our own tests for reaction values for the concentration of VEGF were less than 22.7 pg/ml.

Determination of microvessel density in tumor tissues of the breast by means of immunohistochemistry

Blood vessels in tested tissues were identified by use of an immunoperoxidase method, comprising EnVision+™ complex, peroxidase, Rabbit antibodies (DAKO Corporation, USA, Code: K4003) and antibodies directed against von Willebrand's factor (DAKO A/S, Denmark, Code: A 082) which is found on the cell surface of endothelial cells.

The intensity exponent for vascularization of tumor tissue was the number of vessels visualised after the staining reaction for the endothelial cell antigen (von Willebrand's factor). Assessment of microvessel density was performed by Weidner's method.<sup>27</sup> Properly stained slides were first assessed under high magnification (×100) in order to find "hot-spots", fields which were subsequently used to count the number of capillaries at a still higher magnification (×200—Carl Zeiss, Germany). The result was given in terms of blood vessels per squared millimetre [vp/mm<sup>2</sup>].

#### Statistical Analysis

Results obtained for the concentration of VEGF in the plasma of women suffering from breast carcinoma and the micro-

vessel density of the tumour tissue were subjected to statistical analysis. To grade the difference between studied groups of results the non-parametric Wilcoxon–Mann–Whitney and Kruskal–Wallis tests were used. For statistical analysis, version 5.5 of the program "Statistica" was used. In order to determine the correlation between VEGF concentration and MVD, the rank Spearman test was applied. All statistical hypotheses were tested to the level of significance of  $p < 0.05$ .

#### Results

In the control group the average concentration of VEGF in the plasma amounted to 22.7 pg/ml, and in the group of 103 patients with cancer of the breast the average VEGF level was 154.7 pg/ml (Table 2). The median concentration in the group of patients with malignant cancers of the breast was more than six times higher than in the plasma of the control group and this difference was statistically significant (Table 2). Analysis of the results showed that increased VEGF concentration was evident in 86 patients (83.4%) and of these, 30 patients showed high levels exceeding a value of 200 pg/ml (Table 3). A statistically significant difference was also found between the level of microvessel density in the tumors of women with breast cancer, relative to the MVD values for control tissues (Table 2). The average microvessel density in healthy tissue amounted to 2 vp/mm<sup>2</sup> while it was 26 vp/mm<sup>2</sup> in cancer tissue (Table 2).

Further results showed that with increasing histological malignancy of cancers, the average concentration of VEGF rose significantly. The concentration in patients with a stage G3 malignancy was almost twice as great as in patients with G2 malignancies and around four times greater than in those with G1 malignancies (Table 2). Such expressive relationships were not seen when analyzing the MVD of groups of women with breast cancer at various grades of histological malignancy (Table 2).

The median concentration of VEGF rose relative to advancement of the disease process in women with breast cancer (Table 2). Women in stage I of clinical disease advancement showed VEGF concentrations almost twice lower than in women in stage II and four times lower than in stage III of the clinical advancement of the disease process (Table 2). This was a statistically significant difference. A similar pattern, though less marked, was observed for MVD. A statistically significant difference was found between MVD values in stages I and III of clinical disease advancement and the correlation coefficient for the concentration of VEGF and MVD values in stage I of clinical disease advancement was calculated as  $R=0.55502$ ,  $p < 0.05$  (Table 2).

Further analysis of the results showed that, among women with metastases to the axillary lymph nodes, the

**Table 2** Values of VEGF and MVD in relation to clinical advancement of disease according to the TNM system, the status of axillary lymph nodes, tumor size and the grade of histological malignancy in women with breast cancer

Clinical characteristic of patients	Vascular endothelial growth factor			Microvessel density		
	Average VEGF concentration	Median VEGF concentration	Range of values	Average MVD	Median MVD	Range of values
	[pg/ml]			[vp/mm <sup>2</sup> ]		
Control ( <i>n</i> =40)	22.7±18.3 <i>n</i> =40	17.2	1.2–68.3	2±1 <i>n</i> =25	2	0–5
Cancer patients ( <i>n</i> =103)	154.7 <sup>a</sup> ±124.7	120.0 <sup>a</sup>	3.5–490.2	26 <sup>b</sup> ±17	25 <sup>b</sup>	3–64
Stage of clinical advancement according to TNM						
I ( <i>n</i> =47)	95.4±96.4	71.8	3.5–490.2	22.4±13.9	22	3–60
II ( <i>n</i> =38)	152.8±91.1 <sup>c</sup>	160.8 <sup>c</sup>	12.1–438.0	27.1±13.3	23	7–64
III ( <i>n</i> =18)	313.3±117.5 <sup>c, d</sup>	315.2 <sup>c, d</sup>	58.8–468.7	34.7±15.7 <sup>c</sup>	37 <sup>c</sup>	9–62
Status of axillary lymph nodes						
pN0 ( <i>n</i> =51)	81.4±74.6	67.4	3.5–298.8	24.2±15.3	24	3–64
pN1 ( <i>n</i> =52)	226.0±122.6 <sup>c</sup>	199.6 <sup>c</sup>	12.2–490.2	28.4±13.6	25	3–60
Tumor size						
pT<20 mm ( <i>n</i> =54)	100.7±92.4	76.7	3.5–438.0	22.2±13.7	21	3–64
pT≥20<50 mm ( <i>n</i> =40)	196.6±123.5 <sup>f</sup>	182.4 <sup>f</sup>	12.1–490.2	28.8±13.0 <sup>f</sup>	25 <sup>f</sup>	8–60
pT≥50 mm ( <i>n</i> =9)	292.4±133.1 <sup>g</sup>	289.8 <sup>g</sup>	58.8–468.7	40.0±16.8 <sup>f</sup>	44 <sup>f</sup>	14–62
Histological grade of malignancy						
G1 ( <i>n</i> =11)	61.2±90.3	47.5	3.5–267.2	23.0±17.5	25	5–56
G2 ( <i>n</i> =50)	119.1±82.6	103.6	17.3–331.6	26.8±15.3	24	3–64
G3 ( <i>n</i> =42)	221.5±141.6 <sup>h</sup>	199.6 <sup>h</sup>	12.1–490.2	26.5±13.0	24	9–60

<sup>a</sup> Statistically significant difference for VEGF in comparison to the control group,  $p<0.05$

<sup>b</sup> Statistically significant difference for MVD in comparison to the control group,  $p<0.05$

<sup>c</sup> Statistically significant difference for VEGF and MVD in comparison to women in stage I of clinical disease advancement,  $p<0.05$

<sup>d</sup> Statistically significant difference for VEGF in comparison to women in stage II of clinical disease advancement,  $p<0.05$

<sup>e</sup> Statistically significant difference for VEGF in comparison to women with no metastases to the axillary lymph nodes,  $p<0.05$

<sup>f</sup> Statistically significant difference for MVD in comparison to women with tumors sized <20 mm,  $p<0.05$

<sup>g</sup> Statistically significant difference for VEGF in comparison to women with tumors sized <20 mm,  $p<0.05$

<sup>h</sup> Statistically significant difference for VEGF in comparison to groups of women in grades G1 and G2,  $p<0.05$

**Table 3** Percentage increase values for vascular endothelial growth factor in the plasma of breast cancer patients

	% percentage increase in VEGF levels concentration range [pg/ml]		
	22.7–200.0	200.1–400.0	>400.0
Breast cancer ( <i>n</i> =103)	54.3 ( <i>n</i> <sub>1</sub> =56)	22.3 ( <i>n</i> <sub>1</sub> =23)	6.8 ( <i>n</i> <sub>1</sub> =7)
TNM classification			
I ( <i>n</i> =47)	63.8 ( <i>n</i> <sub>1</sub> =30)	6.4 ( <i>n</i> <sub>1</sub> =3)	2.1 ( <i>n</i> <sub>1</sub> =1)
II ( <i>n</i> =38)	60.5 ( <i>n</i> <sub>1</sub> =23)	26.3 ( <i>n</i> <sub>1</sub> =10)	2.6 ( <i>n</i> <sub>1</sub> =1)
III ( <i>n</i> =18)	16.7 ( <i>n</i> <sub>1</sub> =3)	55.5 ( <i>n</i> <sub>1</sub> =10)	27.8 ( <i>n</i> <sub>1</sub> =5)
Metastases to lymph nodes			
pN0 ( <i>n</i> =51)	60.7 ( <i>n</i> <sub>1</sub> =31)	7.8 ( <i>n</i> <sub>1</sub> =4)	0 ( <i>n</i> <sub>1</sub> =0)
pN1 ( <i>n</i> =52)	48.1 ( <i>n</i> <sub>1</sub> =25)	34.6 ( <i>n</i> <sub>1</sub> =18)	13.5 ( <i>n</i> <sub>1</sub> =7)
Tumor size			
pT<20 mm ( <i>n</i> =54)	61.1 ( <i>n</i> <sub>1</sub> =33)	9.3 ( <i>n</i> <sub>1</sub> =5)	1.8 ( <i>n</i> <sub>1</sub> =1)
pT≥20<50 mm ( <i>n</i> =40)	50.0 ( <i>n</i> <sub>1</sub> =20)	35.0 ( <i>n</i> <sub>1</sub> =14)	10.0 ( <i>n</i> <sub>1</sub> =4)
pT≥50 mm ( <i>n</i> =9)	33.3 ( <i>n</i> <sub>1</sub> =3)	44.4 ( <i>n</i> <sub>1</sub> =4)	22.2 ( <i>n</i> <sub>1</sub> =2)
Histological grade of malignancy			
G1 ( <i>n</i> =11)	18.2 ( <i>n</i> <sub>1</sub> =2)	18.2 ( <i>n</i> <sub>1</sub> =2)	0 ( <i>n</i> <sub>1</sub> =0)
G2 ( <i>n</i> =50)	40.5 ( <i>n</i> <sub>1</sub> =17)	14.0 ( <i>n</i> <sub>1</sub> =7)	0 ( <i>n</i> <sub>1</sub> =0)
G3 ( <i>n</i> =42)	74.0 ( <i>n</i> <sub>1</sub> =37)	13.2 ( <i>n</i> <sub>1</sub> =14)	16.7 ( <i>n</i> <sub>1</sub> =7)

*n* Number of patients, *n*<sub>1</sub> number of raised results

median VEGF concentration was almost three times greater when compared to patients without metastases. This was found to be a statistically significant difference. However, no statistically significant difference was found in breast cancer patients in whom the microvessel density had been assessed (Table 2).

In the group of 52 women with metastases to the axillary lymph nodes we discovered a high percentage of results for increased VEGF concentration. Among these patients, the average VEGF level was 226.0 pg/ml (Table 2), among these the percentage of results for increased VEGF concentration from, 22.7 to 400 pg/ml and above, amounted to 96.2% (Table 3). Among patients with no lymph node involvement however, the average VEGF level was 81.4 pg/ml and the percentage of increased results was 68.5% (Tables 2 and 3). Analysis of the MVD values in the group of women with metastases and in the group without lymph node involvement showed a high percentage of raised values in the ranges of 2–32 and 33–49 vp/mm<sup>2</sup>, which represents 92.2% and 90.4% and correlation coefficients as follows:  $R=0.45631$  and  $R=0.48646$ ,  $p<0.05$  (Table 4).

Increasing tumor size rose with increasing VEGF concentration, among women with breast cancer (Table 2). Statistically significant differences were found between average VEGF concentrations in tumors sized less than 20 mm, from 20–50 mm and greater than 50 mm (Table 2). The median VEGF concentration among women with tumors measuring less than 20 mm was 2.5 times lower than in patients whose tumors were 20–50 mm in size, and four times lower than in patients with tumors measuring more than 50 mm. Increases in line with rising tumor size

were also seen in the vascularization of cancer tissues in breast cancer patients (Table 2). In women with tumors measuring less than 20 mm, the MVD value was almost twice lower than among women whose tumors measured greater than 50 mm (Table 2). These differences were statistically significant. The highest percentages of increased MVD results, in the range of 33–66 vp/mm<sup>2</sup>, were found among women with tumors sized 20–50 mm and greater than 50 mm and were 35.0% and 66.6% respectively (Table 4).

## Discussion

Breast cancer is the most frequently diagnosed malignant tumor in Polish women. Not only is this a serious clinical problem but it is, furthermore, a shared problem for current oncology.

One of the greatest co-factors leading to the deaths of women suffering from breast cancer is the metastatic character of the disease. Clinical studies have demonstrated the importance of angiogenesis in neoplasia [27]. The effects of vascularization on tumor growth and the formation of metastases have demonstrated the value of assessing the degree of vascularization in cancer tissue and the assessing the concentration of angiogenic substances in systemic fluids [28]. Presently the most important factor in the physiological and pathological regulation of angiogenesis is thought to be vascular endothelial growth factor [28]. This glycoprotein increases the mitogenic activity of vascular endothelial cells and induces the secretion of

**Table 4** Percentage increase in microvessel density in the tumors of women with breast cancer

	% percentage increase in MVD values vessel count range [vp/mm <sup>2</sup> ]		
	2–32	33–49	50–66
Breast cancers ( $n=103$ )	73.8 ( $n_1=76$ )	17.5 ( $n_1=18$ )	8.7 ( $n_1=9$ )
Stage of clinical disease advancement			
I ( $n=47$ )	85.1 ( $n_1=40$ )	8.5 ( $n_1=4$ )	6.4 ( $n_1=3$ )
II ( $n=38$ )	73.7 ( $n_1=28$ )	18.4 ( $n_1=7$ )	7.9 ( $n_1=3$ )
III ( $n=18$ )	44.5 ( $n_1=8$ )	38.8 ( $n_1=7$ )	16.7 ( $n_1=3$ )
Status of lymph axillary			
pN0 ( $n=51$ )	80.4 ( $n_1=41$ )	11.8 ( $n_1=6$ )	7.8 ( $n_1=4$ )
pN1 ( $n=52$ )	67.3 ( $n_1=35$ )	23.1 ( $n_1=12$ )	9.6 ( $n_1=5$ )
Tumor size			
pT<20 mm ( $n=54$ )	87.0 ( $n_1=47$ )	7.4 ( $n_1=4$ )	5.6 ( $n_1=3$ )
pT≥20<50 mm ( $n=40$ )	65.0 ( $n_1=26$ )	27.5 ( $n_1=11$ )	7.5 ( $n_1=3$ )
pT≥50 mm ( $n=9$ )	33.3 ( $n_1=3$ )	33.3 ( $n_1=3$ )	33.3 ( $n_1=3$ )
Histological grade of malignancy			
G1 ( $n=11$ )	81.8 ( $n_1=9$ )	74.0 ( $n_1=37$ )	71.4 ( $n_1=30$ )
G2 ( $n=50$ )	74.0 ( $n_1=37$ )	14.0 ( $n_1=7$ )	12.0 ( $n_1=6$ )
G3 ( $n=42$ )	71.4 ( $n_1=30$ )	23.8 ( $n_1=10$ )	4.8 ( $n_1=2$ )

$n$  Number of patients,  $n_1$  number of raised results

proteolytic enzymes involved in the degradation of the cellular matrix. It is proven, that the action of such cytokines is a necessary condition for the growth of tumors and for the establishment of remote metastases. Increased concentration of VEGF, in both neoplastic tissues and the plasma has been demonstrated in many cancers, such as colorectal, ovarian and urinary bladder cancers [29–31].

The results of this study revealed significantly higher VEGF concentrations in the plasma of women with breast cancer, relative to levels of the same cytokine in a control group. A similar relationship was found by Granato and Salven and their colleagues [32, 33], whose findings associated increased VEGF levels with vascularization of solid, malignant tumors. In the opinion of Fuhrmann and Munn and their colleagues [34, 35] the determination of VEGF concentration and the degree of tumour vascularization in breast cancer may represent a good indicator for the assessment of angiogenesis in solid tumors.

These findings are supported by the results of our study.

They discovered that, in all the cancerous tissue of women suffering from breast cancer, there was an increased proportion of MVD values when compared to the proportion of vessels in control tissues. This suggests that both tested parameters have roles in the promotion of the process of angiogenesis within the limits of malignant tumors. Similar results were obtained by Valkovic, Ludovini, Toi and their co-workers [36–38]. Their results were obtained through the use of an immunoenzymatic reaction, using an antibody directed against vascular endothelium—von Willebrand's factor—and also demonstrated a strong correlation between VEGF and microvessel density in the tumors of women with breast cancer. In turn, Wu et al. [39] reported a significant relationship between the clinical stage of disease advancement and the concentration of VEGF in the serum of women with breast cancer. Herr et al. [40] published similar results. The results of our study are in agreement with these findings. The studies of Lantzsich and Collozza and their colleagues [41, 42] each showed a strong correlation between the stage of disease progression according to the TNM classification and the level of vascularization in the cancerous tissues of diseased breasts. However, Adams et al. [56] did not find correlation between circulating VEGF with any clinicopathological factor.

From the available published results, it can be seen that establishment and spread of secondary metastases is linked with progression of the tumor from the *in situ* state to an invasive state [43]. This process is associated with increases in the level of the angiogenic factor VEGF, which increases the permeability of vessels and migration of endothelial cells. Increased VEGF concentration in the serum has been described in many cancers including, among others, laryngeal and endometrial cancers [44, 45]. The effect of

the action of vascular endothelial growth factor is the increase in the count of blood vessels within the borders of the tumor.

Seo and colleagues [25] discovered that the level of these cytokines in tumor tissues of the pancreas correlates with the count of blood vessels in the tumor and within the limits of metastases to the liver. It is the opinion of Roselli and colleagues [46] that increased VEGF concentration in the plasma of patients with non-small-cell carcinoma of the lung has a significant link with the presence of metastases to the bones.

In our study we discovered an almost three-fold increase in VEGF concentration in the plasma of women with breast cancer, who showed involvement of the axillary lymph nodes, compared to levels in women without metastases, thus demonstrating the prognostic value of assessment of this marker. A similar observations was made by Linderholm [47], in that the assessment of the concentration of the cytokine may represent a good indicator of the process of malignant spread in women with this malignancy. Many investigations have shown a significant relationship between VEGF concentration and MVD values in patients with breast cancer and have proposed the use of both these indicators in the course of assessing prognosis in these patients [47–50]. The results of our study showed increased numbers of blood vessels in women with metastases to the axillary lymph nodes in comparison with MVD levels in health breast tissue. No statistical relationship was found between the tumor vessel densities of women with metastases to the lymph nodes and those without. The lack of such a relationship was also described by Lantzsich and Medri and their colleagues [41, 51]. In turn, Weidner [52] found a strong correlation between MVD and the number of metastases and reported that they consider the vascularization of tumors to significantly worsen the prognosis for women with breast cancer. With clinical experience, such results as the size of a malignant tumor represent classic prognostic indicators in breast cancer patients [53]. This study has shown that, along with the size of the tumor, increased plasma VEGF levels are important for women with this cancer. A positive correlation between tumor size and VEGF levels was found by Sledge [54]. However, studies carried out by Stimpfl and co-workers [30] found no such relationship. There is also evidence of a close correlation between the number of newly formed vessels and the size of malignant tumors [55–58]. The results of our study show a relationship between the number of vessels in the group of patients with primary tumors less than 20 mm in diameter and MVD values in patients with tumors greater than 50 mm in diameter. Medri et al. [51] found no characteristic statistical differences between MVD values in tumor sizes among women with cancer of the breast.

The results of the study we have presented here showed significantly raised VEGF concentration in the plasma of, and significantly greater tumor microvessel density in, women with breast cancer in our reference group. A correlation was found between increases in the concentration of vascular endothelial growth factor and MVD values among patients with this chronic illness. Both these parameters may be useful for completeness, in the clinical assessment of patients with breast cancer. Our study also showed statistical relationships between vascular endothelial growth factor concentration in the plasma and prognostic factors such as: the stage of clinical disease advancement, the histological grade of malignancy, the presence of metastases to the axillary lymph nodes and the size of the primary tumor. Also found was a statistically significant relationship between MVD and the stage of clinical disease advancement and tumor size. On the basis of our study it may be concluded that VEGF and MVD may represent valuable indicators for the assessment of angiogenesis intensity in solid malignant tumors.

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