ONCOLOGY

Vascular Endothelial Growth Factor in the Serum of Breast Cancer Patients

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Here we present the results of comparative immunoenzyme assay of the initial serum levels of VEGF in breast cancer patients (stages $T_1N_0M_0$ and $T_2N_0M_0$) and apparently healthy women (controls). It was found that VEGF concentrations in the serum of patients with breast cancer stages $T_1N_0M_0$ and $T_2N_0M_0$ significantly surpassed the control levels. Increased levels of VEGF surpassing the threshold values were more often observed in patients with $T_2N_0M_0$ breast cancer compared to patients with $T_1N_0M_0$ tumor. At the same time, this marker cannot be used in the diagnostics of this disease because in only 21.4% patients serum level of VEGF surpassed the upper boundary for this growth factor observed in the serum of control women. Serum concentration of VEGF in patients with stages $T_1N_0M_0$ and $T_2N_0M_0$ breast cancer did not depend on patient's age and reproductive function and receptor status of the primary tumor (estrogen and progesterone receptors), but was closely associated with tumor histogenesis and differentiation degree. Significantly higher levels of VEGF were observed in patients with lobular infiltrative breast carcinoma compared to patients with ductal tumors and in patients with low-differentiated tumors compared to highly and moderately differentiated tumors. High initial concentrations of VEGF (>300 pg/ml) were more often detected in patients with $T_2N_0M_0$ breast cancer developing relapses within the first 3 years of follow-up compared to patients without relapses during the corresponding period (p=0.001). These findings suggest that serum level of VEGF in patients with $T_2N_0M_0$ breast cancer before treatment can be used as an additional marker in parallel with standard clinical and morphological signs of the disease for more precise prognosis of early relapse (during the first 3 years of follow-up).

Key Words: *breast cancer; VEGF; prognosis*

Antiangiogenic approach to the therapy of tumors, including breast cancer (BC), attracts now more and more attention [9,11,12,16]. Some studies of neoangiogenesis in BC demonstrated a correlation between high content of angiogenic factors in the serum, high density of blood vessels in the tumor tissue, and unfavorable prognosis of the disease [2,3-5]. These experiments were aimed at identification of angiogenic factors and possible use of these factors for limit-

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ing neoangiogenesis activity and inhibition of tumor growth and metastasizing [1,6,10,13]. It should be also noted that antiangiogenic therapy deserves special attention because the genome of endothelial cells is stable in contrast to tumor cells, which are genetically unstable and rapidly acquire resistance to many therapeutic agents [10]. However, despite great attractiveness of this approach and certain advances in antiangiogenic therapy in clinical oncology, the results suggest that this treatment is not universally effective. Some authors believe, that the results obtained in practical investigations contradict to theoretical concept on the mechanisms of action of target preparations [15]. This can be explained by activation of alternative (probably, non-antiogenic) mitogenic pathways and mechanisms of tumor progression [14]. In this case, the patients should be stratified on the basis of the results of biomolecular marker analysis, including analysis of angiogenic status of the serum and tumor, not only for evaluation of disease prognosis, but also for selection of patients for target therapy of the tumor [2,4,7,8,13].

Here we compared the concentrations of vascular endothelial growth factor (VEGF) in the serum of patients with $T_1N_0M_0$ and $T_2N_0M_0$ breast cancer with consideration for clinical and morphological signs of the disease, receptor status of the tumor, and parameters of relapse-free survival depending on the initial VEGF concentration.

MATERIALS AND METHODS

The study included 248 patients with $T_1N_0M_0$ and $T_2N_0M_0$ BC observed and treated at Moscow regional oncologic dispensary (Balashikha) from September 2002 to December 2005. The mean age of patients was 57.9±0.7 years (median 57.5 years). Stage $T_1N_0M_0$ was diagnosed in 76 (31%) and stage $T_2N_0M_0$ in 172 (69%) patients. The mean age of patients with stages $T_1N_0M_0$ и $T_2N_0M_0$ BC was similar (58.1±0.2 and 57.8±0.8 years, respectively). Seventy-one patients were in reproductive age and 177 were in the postmenopause.

For evaluation of the stage of the disease in the preoperation period, mammography of both mammary glands in two projections, X-ray examination of the lungs, pelvic ultrasonography, ultrasonic examination of the liver, axillary, supraclavicular, and subclavicular lymph nodes, and bone scanning were performed in all BC patients. In none participants of the study regional and distant metastases were revealed.

In all examinees, BC was diagnosed for the first time by clinical and X-ray examination and confirmed by histological analysis of the primary tumor after surgery according to International Classification of Tumors (WHO, 2002). Lobular infiltrative carcinoma was diagnosed in 27 (10.9%) patients, ductal infiltrative tumor in 106 (42.8%) patients, mixed type tumor in 30 (12.1%) patients, mucinous carcinoma in 9 (3.6%), medullary cancer in 1 (0.4%) patient, and invasive carcinoma in 75 (30.2%) patients.

The control group comprised 55 healthy women of the corresponding age and reproductive status.

Radical mastectomy was performed in 87.5% BC patients and radical resection in 12.5%. After surgery, 39 (15.7%) patients received no specific therapy, radiation therapy was performed in 142 (57.2%) patients, 71 (28.6%) patients received chemotherapy with various drugs (CMF protocol, anthracyclines), hormones were administered to 123 (49.6%) patients.

Of 248 patients with $T_1N_0M_0$ и $T_2N_0M_0$ BC, 53 (21.4%) had relapse of the disease after 5-58.5 month (mean time to relapse 27.0±2.0 months, median 26.3 months). Bone metastases were detected in 15 patients, metastases in the lungs and pleura were detected in 6 and 1 patients, respectively, 2 patients had metastases in the liver 1 in supraclavicular lymph nodes, and 1 in pelvic organs, 2 patients had metastases in the ovaries and 5 in the contralateral mammary gland, 14 patients had metastases in several organs. Relapse in the postoperation scar was revealed in 6 patients, in 5 of them the relapse was associated with metastases in other organs.

The concentration of VEGF was determined by immunoenzyme assay using Quantikine human VEGF kits (R&D Systems Inc.) and ELX800 automatic universal reader for microtitration plates (Bio-Tek Instruments, Inc.).

The expression of protein marker Her-2/neu was measured by the streptavidin-biotin immunoperoxidase method. Paraffin sections of the tumor tissue from 248 BC patients were stained using monoclonal antibodies to Her-2/neu protein (Dako). The patients were divided into two groups by the intensity of Her-2/neu protein expression in membranes of tumor cells: without (0, +) and with protein expression (++, +++).

Expression of receptors for estrogen (ER) and progesterone (PR) in the tumor tissue was evaluated using routine immunohistochemical methods by staining paraffin sections of primary tumor tissue from BC patients with monoclonal antibodies (Dako). The patients were divided into two groups by the intensity of ER and PR expression in the cytoplasm and nuclei of tumor cells: in group 1 expression was absent (ER^-, PR^-) and in group 2 repectors were present (ER^+, PR^+).

The results were processed statistically using Statistica and SPSS software. The statistical procedures were chosen using international methodological recommendations.

RESULTS

The concentration of VEGF in the serum of healthy women was significantly lower than in the total group of BC patients (Table 1). The initial serum level of VEGF in apparently healthy women varied from 48 to 300 pg/ml, therefore the concentration of 300 pg/ml was used as the threshold value. In only 53 (21.4%) BC patients the concentration of VEGF in the serum surpassed the threshold value.

In patients with $T_2N_0M_0$ BC, the suprathreshold concentrations of VEGF were 2-fold more incident than in patients with $T_1N_0M_0$ BC (25.0 and 13.2%, respectively).

The level of VEGF in the serum of patients with $T_1N_0M_0$ and $T_2N_0M_0$ BC did not depend on the age and reproductive status.

Further analysis included evaluation of the initial levels of VEGF in the serum of BC patients with consideration for histological structure of the tumor and stage of the disease (Table 2).

In patients with lobular infiltrative BC, stages $T_1N_0M_0$ and $T_2N_0M_0$, the content of VEGF in the serum was significantly higher than in patients with ductal infiltrative BC (p=0.002). These differences appeared as a trend in patients with $T_1N_0M_0$ BC (p=0.052). Serum concentration of VEGF in patients with mucinous BC were significantly lower that in ductal infiltrative BC (p=0.03).

High serum concentrations of VEGF (≥300 pg/ml) were detected in 37.0% of 27 patients with lobular infiltrative BC, in 11.3% of 106 patients with ductal infiltrative BC, in 36.7% of 30 patients with mixed type BC, in 26.7% of 75 patients with invasive carcinoma, and 0% of 9 patients with mucinous BC (p=0.002).

TABLE 1. Serum Concentrations of VEGF in Patients with BC

Group	Number of patients	Incidence of VEGF ≥300 pg/ml		VEGF, pg/ml		
		n	%	*M*±*m*	median	interval
Control	55	3	5.5	104.8±13.8	103	45-377
$T_1N_0M_0$ and $T_2N_0M_0$ BC (total group)	248	53	21.4	217.3±8.0	185	3.8-620
$T_1N_0M_0$ BC	76	10	13.2	193.4±11.6	180	3.8-560
$T_2N_0M_0$ BC	172	43	25	227.8±10.3	190	28-620

Note. p<0.05 for $T_1N_0M_0$ and $T_2N_0M_0$ compared to the control; p<0.001 $T_1N_0M_0$ compared to the control; p<0.0001 $T_2N_0M_0$ compared to the control. Here and in Tables 2-4: *n*: number of observations

TABLE 2. Concentrations of VEGF in Patients with BC with Consideration for Histological Variant of the Tumor and Stage of the Disease

Stage	Histological variant of the tumor									
	lobular infiltrative carcinoma		ductal infiltrative carcinoma		mixed-type tumor		invasive carcinoma		mucinous carcinoma	
	M±*m*	median	*M*±*m*	median	*M*±*m*	median	*M*±*m*	median	*M*±*m*	median
$T_1N_0M_0$	266.3±44.8* (*n*=8)	225	170.6±12.4** (*n*=34)	182.5	213.7±51.3 (*n*=9)	140	197.4±60.0 (*n*=23)	160	152.5±37.5 (*n*=2)	152.5
$T_2N_0M_0$	279.6±41.4* (*n*=19)	240	201.0±10.8** (*n*=72)	182.5	263.5±40.6 (*n*=21)	180	239.3±20.1 (*n*=52)	195	175.7±24.4 (*n*=7)	190
Total	275.6±31.5 (*n*=27)	240	191.3±8.4 (*n*=106)	182.5	248.5±32.1 (*n*=30)	165	226.5±15.4 (*n*=75)	180	170.6±20.0 (*n*=9)	190

Note. *,**p=0.052 T_1N_0M; *,**p=0.015 $T_2N_0M_0$.

TABLE 3. Serum Concentration of VEGF (pg/ml) in Patients with $T_1N_0M_0$ and $T_2N_0M_0$ BC with Consideration for Tumor Differentiation Degree and Stage of the Disease

BC stage	BC differentiation degree					
	high		moderate		low	
	M±*m*	median	*M*±*m*	median	*M*±*m*	median
$T_1N_0M_0$	150.1±30.7 (*n*=7)	160	198.4±12.3 (*n*=44)	182.5	302.5±45.0* (*n*=8)	295.0
$T_2N_0M_0$	172.9±36.0 (*n*=12)	115	210.5±11.6 (*n*=88)	190	329.7±23.6** (*n*=26)	352.5
Total	164.5±25.0 (*n*=19)	120	206.5±8.7 (*n*=132)	187.5	323.3±20.7 (*n*=34)	330

Note. *p=0.02 $T_1N_0M_0$; **p=0.0002 $T_2N_0M_0$.

The degree of tumor differentiation was evaluated in 185 (74.5%) BC patients. The serum level of VEGF tended to increase with increasing the degree of malignancy of the primary tumor, but significant differences by this parameter were noted only for patients with low-differentiated BC (Table 3).

High serum concentrations of VEGF (≥300 pg/ml) were detected in 10.5% of 19 patients with highly differentiated BC, in 15.2% of 132 patients with moderately differentiated BC, and sharply increased in the group of 34 patients with low-differentiated BC (58.8%; p=0.0001). The incidence of detection of serum VEGF ≥300 pg/ml in patients with low-differentiated $T_1N_0M_0$ and $T_2N_0M_0$ BC was 50.0 and 61.5%. Thus, serum concentration of VEGF in more than one-half of patients with low-differentiated $T_1N_0M_0$ и $T_2N_0M_0$ BC surpassed the threshold value for this marker in the control group.

At the same time, we detected no significant relationships between VEGF concentrations in BC patients and receptor status of the tumor. However, the maximum initial concentrations of VEGF in the serum were detected in patients with $T_1N_0M_0$ BC with unfavorable negative receptor status of the tumor (ER^-PR^-).

We carried out a retrospective analysis of the data for evaluation of prognostic value of VEGF concentration in the serum of patients with $T_1N_0M_0$ and $T_2N_0M_0$ BC. The study included 111 BC patients observed for 3 years and longer. Group 1 comprised patients with tumor relapse during the first 3 years of treatment (*n*=53), group 2 included patients with 3-year relapse-free period (*n*=58; Table 4).

It was found that patients with $T_2N_0M_0$ BC and relapse of the disease within 3 years had significantly higher serum levels of VEGF compared to patients with relapse-free course of the disease.

Blood VEGF concentrations surpassing the threshold values were detected in 2 of 20 patients with $T_1N_0M_0$ BC without relapses (10%) and in 4 of 14 patients with $T_1N_0M_0$ BC with relapse (28.6%; p=0.2). In the group of patients with $T_2N_0M_0$ BC, the corresponding values were 3 of 38 (7.9%) in the group of patients without

TABLE 4. Concentrations of VEGF (pg/ml) in Patients with BC with Consideration for the Stage of the Disease and the Presence of Relapses during the First 3 Years of Follow-Up

Stage	First 3 years of follow-up								*p*
	with relapse				without relapse				
	n	*M*±*m*	median	interval	*n*	*M*±*m*	median	interval	
$T_1N_0M_0$	14	226.9±42.1	207.5	75-560	20	161.4±15.0	150	60-310	0.1
$T_2N_0M_0$	39	361.5±29.2	400	65-620	38	164.9±12.7	150	28-375	0.0001
Total	53	335.4±24.8	360		58	163.7±9.7	150		0.0001

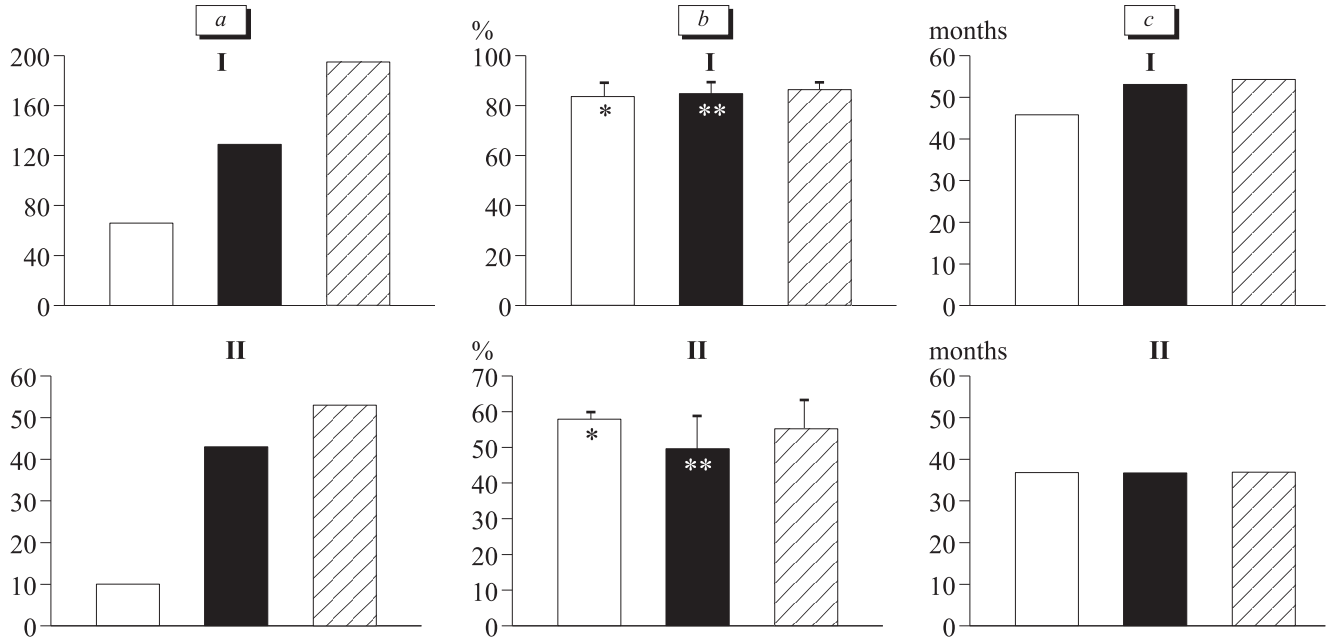

Fig. 1. Delayed results of treatment of BC patients with different initial concentration of VEGF in the serum and stage of the disease. *I*: VEGF<300 pg/ml, *II*: VEGF≥300 pg/ml. *a*) number of patients, *b*) survival, *c*) median. Light bars: $T_1N_0M_0$ BC; dark bars: $T_2N_0M_0$ BC; shaded bars: total. **p*=0.3; ***p*=0.001.

relapses and 25 of 39 (64.1%) in the group of patients with relapse (p=0.0001). Thus, the prognostic value of the threshold concentration of VEGF in blood serum of patients with BC for prediction of early relapse became appreciable only for $T_2N_0M_0$ BC.

Comparison of the parameters of 3-year relapse-free survival in patients with BC with consideration for the threshold value of serum VEGF concentration and stage of the disease in patients with $T_1N_0M_0$ BC revealed only a tendency to worsening of the prognosis for patients with concentration of VEGF≥300 pg/ml. For patients with $T_2N_0M_0$ BC, the differences in delayed outcomes of treatment significantly correlated with the threshold concentration of VEGF (p=0.001; Fig. 1). In patients with $T_1N_0M_0$ BC, the differences in relapse-free survival became appreciable only 3 years after treatment, while in patients with $T_2N_0M_0$ BC the differences were significant throughout the observation period. Thus, the initial serum content of VEGF above 300 ng/ml in patients with $T_2N_0M_0$ BC significantly correlated with reduced (by 30%) 3-year relapse-free survival.

Parameters of 3-year relapse-free survival in groups of patients with $T_1N_0M_0$ and $T_2N_0M_0$ BC and serum level of VEGF <300 pg/ml were similar (83.6±5.8 and 84.8±4.5%, respectively).

Thus, in 21.4% patients with $T_1N_0M_0$ and $T_2N_0M_0$ BC, the initial serum levels of VEGF were above the threshold concentration of this growth factor in the group of healthy women. The concentration of VEGF did not depend on patient's age and, receptor status of the primary tumor (ER, PR), but was higher in lobular infiltrative BC compared to ductal carcinoma and in low-differentiated tumors compared to highly and medium-differentiated tumors. High initial concentrations of VEGF (>300 pg/ml) were more often detected in patients with $T_2N_0M_0$ breast cancer developing relapses within the first 3 years of follow-up compared to patients without relapses during the corresponding period (p=0.001). The initial levels of VEGF can be used as an additional factor for prediction of early relapse in patients with $T_1N_0M_0$ and $T_2N_0M_0$ along with standard clinical and morphological characteristics of the disease in these patients.

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