Serum leptin, prolactin and vascular endothelial growth factor (VEGF) levels in patients with breast cancer

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Angiogenesis plays an important role in tumor growth and metastasis in solid tumors. VEGF is an important regulator of tumor angiogenesis. Both leptin and prolactin have also been suggested to have roles in the regulation of angiogenic process. In our study, we measured serum leptin, prolactin and VEGF levels in 30 metastatic, 55 non-metastatic breast cancer patients and 25 control subjects. Serum leptin levels were found to be similar in non-metastatic (38.1 ± 19.5 ng/ml), metastatic patients (39.6 ± 16.3 ng/ml) and control subjects (35.6 ± 13.9 ng/ml) ($p>0.05$). There was no statistically significant difference between patients with visceral metastasis (44.0 ± 16.8 ng/ml) and patients with bone metastasis (35.2 ± 15.0 ng/ml) ($p>0.05$). Serum prolactin levels were found to be similar in non-metastatic (12.2 ± 10.7 ng/ml), metastatic patients (11.6 ± 8.2 ng/ml) and control subjects (12.3 ± 8.1 ng/ml), ($p>0.05$). Moreover, serum prolactin levels were not different in patients with visceral (11.4 ± 8.8 ng/ml) and bone metastasis (11.8 ± 8.0 ng/ml), ($p>0.05$). Metastatic patients had higher serum VEGF levels (249.8 ± 154.9 pg/ml), when compared to the non-metastatic patients (138.7 ± 59.3 pg/ml) and control subjects (108.4 ± 47.7 pg/ml), ($p<0.05$). There was no difference in serum VEGF levels in non-metastatic patients and control subjects ($p>0.05$). Patients with visceral metastasis (337.0 ± 168.0 pg/ml) had higher serum VEGF levels, when compared to patients with bone metastasis (162.6 ± 71.8 pg/ml), ($p<0.05$). Serum VEGF activity may be used to evaluate angiogenic and metastatic activity in breast cancer patients. However, serum leptin and prolactin levels does not seem to be related with angiogenic activity and metastasis in breast cancer patients.

Key words: Breast cancer, VEGF, leptin, prolactin, angiogenesis.

Angiogenesis, the development of new blood vessels, plays an important role in tumor growth and metastasis [22]. Many clinical studies have demonstrated that angiogenesis is a potent prognostic factor for breast cancer patients [32, 33]. Vascular endothelial growth factor (VEGF) is an endothelial cell mitogen that is involved in the multiple process of carcinogenesis [5, 12, 28]. VEGF has been reported to be the major angiogenic factor in breast cancer and appears to play a key role in pathological angiogenesis [15, 32]. Moreover, VEGF expression in tumor tissue has been reported to be an independent prognostic factor for breast cancer patients regardless of nodal status [10, 15, 24]. A number of positive and negative factors besides VEGF may also be involved in the regulation of angiogenesis [14].

Leptin, the adipocyte derived hormone, is a 16 kDa protein that plays a key role in the control of satiety and energy expenditure [9]. Recently, additional biological functions such as antiapoptotic and angiogenic activity have also been reported for leptin [3, 19, 30]. It has been reported that leptin may promote the tumor growth by stimulating angiogenesis in prostate cancer [31]. It has been reported that leptin synergistically stimulates angiogenesis with VEGF [3] and that magnitude of stimulation of angiogenesis by leptin is similar to that induced by VEGF [2, 30].

Prolactin is a major growth and differentiating hormone in the human breast and may play a role in the pathogenesis and progression of breast carcinoma [1, 18]. It has been suggested that an increase in prolactin secretion may be responsible for the elevation of leptin levels [26]. Prolactin is involved in the control of angiogenesis through its cleaved

fragments of 14 kDa and 16 kDa [8, 13]. It has been shown that 16 kDa prolactin inhibits VEGF induced cell proliferation of capillary endothelial cells in humans [6]. Several clinical studies have demonstrated an association between high prolactin levels and poor prognosis in advanced breast cancer patients [1, 18].

In the present study, we measured serum leptin, prolactin and VEGF levels in metastatic and non-metastatic breast cancer patients to determine their roles in the process of angiogenesis in breast cancer.

Material and methods

Eighty-five breast cancer patients, of whom 55 in complete remission (group 1) and 30 with a metastatic disease (group 2) and 25 healthy female controls (group 3) were included in this study. Median follow-up time was 13.4 months for patients in complete remission (range: 7–100 months). Fifteen patients had bone (group 2_b) and 15 patients had visceral (lung or liver) (group 2_v) metastasis. Informed consent was obtained from all patients and control subjects. None of the patients suffered from infectious, allergic, autoimmune or other systemic diseases such as diabetes mellitus, hypertension heart failure, nephropathy or renal failure. Mean age was 51.2 ± 11.1 years in group 1, 48.5 ± 13.5 years in group 2, 46.6 ± 11.6 years in group 2_v, 50.4 ± 15.3 in group 2_b and 44.5 ± 11.2 years in group 3. Body mass index (BMI) was 25.3 ± 2.8 kg/m^2 in group 1, 26.5 ± 3.1 kg/m^2 in group 2, 25.7 ± 4.5 kg/m^2 in group 2_v, 27.3 ± 4.2 kg/m^2 in group 2_b and 25.8 ± 3.3 kg/m^2 in group 3. Blood samples were collected between 9.00 and 11.00 a.m. to minimize possible circadian variations after an overnight fasting. Samples were stored at –30 ˚C until analysis.

Leptin masurement. Serum leptin levels were measured by a commercially available ELISA kit (The DSL-10-23100 Human Leptin Enzyme-Linked Immunosorbent Kit; Diagnostic Systems Laboratories, Webster, Texas). The principle of the test is an enzymatically amplified, two step, sandwich-type immunoassay.

Prolactin measurement. Serum prolactin levels were determined by DPC-Immulate 2000 machine with the chemiluminometric immunoassay method.

VEGF measurement. VEGF was measured in serum by ELISA using commercial reagents [11]. The minimum detectable dose of VEGF is <5 pg/ml. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99 in both cases. The recovery of hVEGF added to human serum averaged 95% (Biosource International 542 Flynn Road. Camarillo, CA 93012, USA).

CEA and CA 15-3 measurement. CEA and CA 15-3 were determined by a commercial enzyme immunoassay adapted to an ES-700 analyzer.

Statistical analysis. The results were presented as mean $\pm$ SD. Mann Whitney U test, Paired-t test and Pearson's correlation analysis were used in statistical analysis. A p value less than 0.05 was considered to be significant.

Results

There was no difference among all breast cancer patient groups and control subjects in terms of age and body mass index (Tab. 1) ($p>0.05$). Serum leptin levels were found to be similar in non-metastatic (group 1: 38.1 ± 19.5 ng/ml), metastatic patients (group 2: 39.6 ± 16.3 ng/ml) and control subjects (group 3: 35.6 ± 13.9 ng/ml) ($p>0.05$). Serum leptin levels were higher in patients with visceral metastasis (group 2_v: 44.0 ± 16.8 ng/ml) than non metastatic patients, patients with bone metastasis (group 2_b: 35.2 ± 15.0 ng/ml) and control subjects, but these were not statistically significant ($p>0.05$) (Tab. 1, Fig. 1). There was a significant correlation with BMI and serum leptin levels in patients and controls ($p>0.05$).

Serum prolactin levels were found to be similar in non-metastatic (group 1: 12.2 ± 10.7 ng/ml), metastatic patients (group 2: 11.6 ± 8.2 ng/ml) and control subjects (group 3: 12.3 ± 8.1 ng/ml) ($p>0.05$). Moreover, serum prolactin levels were not different in patients with visceral (group 2_v: 11.4 ± 8.8 ng/ml) and bone metastasis (group 2_b: 11.8 ± 8.0 ng/ml) ($p>0.05$) (Tab. 1, Fig. 2).

Table 1. Characteristics of patients and control subjects

	n	Age (years)	BMI (kg/m^2)	Leptin (ng/ml)	Prolactin (ng/ml)	VEGF (pg/ml)	CEA (ng/ml)	CA 15-3 (U/ml)
Group 1	55	51.2 ± 11.1	25.3 ± 2.8	38.1 ± 19.5	12.2 ± 10.7	$138.7 \pm 59.3^*$	$2.4 \pm 1.4^{**}$	$27.3 \pm 10.6^{**}$
Group 2	30	48.5 ± 13.5	26.5 ± 3.1	39.6 ± 16.3	11.6 ± 8.2	249.8 ± 154.9	11.1 ± 16.5	135.8 ± 161.9
Group 2_v	15	46.6 ± 11.6	25.7 ± 4.5	44.0 ± 16.8	11.4 ± 8.8	337.0 ± 168.0	8.5 ± 14.7	159.3 ± 204.9
Group 2_b	15	50.4 ± 15.3	27.3 ± 4.2	35.2 ± 15.0	11.8 ± 8.0	162.6 ± 71.8	13.6 ± 18.3	112.2 ± 105.3
Group 3	25	44.5 ± 11.2	25.8 ± 3.3	35.6 ± 13.9	12.3 ± 8.1	$108.4 \pm 47.7^{**}$	–	–

BMI – Body mass index, Group 1 – non-metastatic patients, Group 2 – metastatic patients, Group 2_v – patients with visceral metastasis, Group 2_b – patients with bone metastasis, Group 3 – control subjects. $^*p<0.05$, $^{**}p<0.001$.

Metastatic patients had higher serum VEGF levels (group 2: 249.8 ± 154.9 pg/ml; p>0.05) when compared to the non-metastatic patients (group 1: 138.7 ± 59.3 pg/ml) and control subjects (group 3: 108.4 ± 47.7 pg/ml). There was no difference in serum VEGF levels in non-metastatic patients and control subjects (p>0.05). Patients with visceral metastasis (group 2_v: 337.0 ± 168.0 pg/ml) had higher serum VEGF levels when compared to patients with bone metastasis* (group 2_b: 162.6 ± 71.8 pg/ml), non-metastatic patients* and control subjects** (*p>0.05, **p>0.001). Serum VEGF levels were not different in non-metastatic patients (group 1) and patients with bone metastasis (group 2_b) (p>0.05). Serum VEGF levels were higher in patients with bone metastasis (group 2_b) when compared to the control subjects (group 3) (p>0.05) (Tab. 1, Fig. 3).

There was no significant correlation among serum leptin, prolactin and VEGF levels (p>0.05). Also, these serum levels were not correlated with serum CEA and CA 15-3 levels (p>0.05)

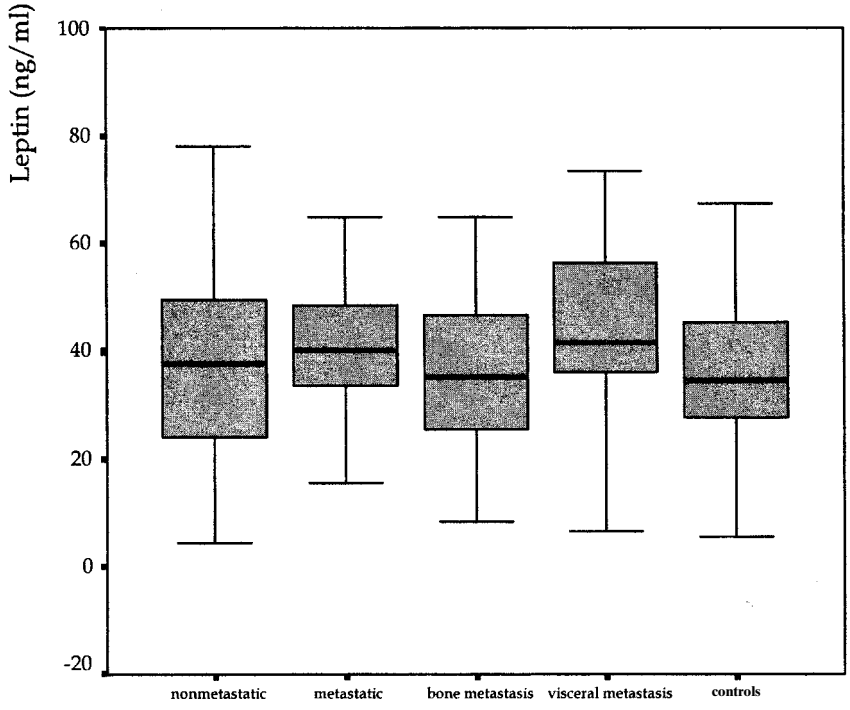

Figure 1. Mean serum leptin levels in patients and controls.

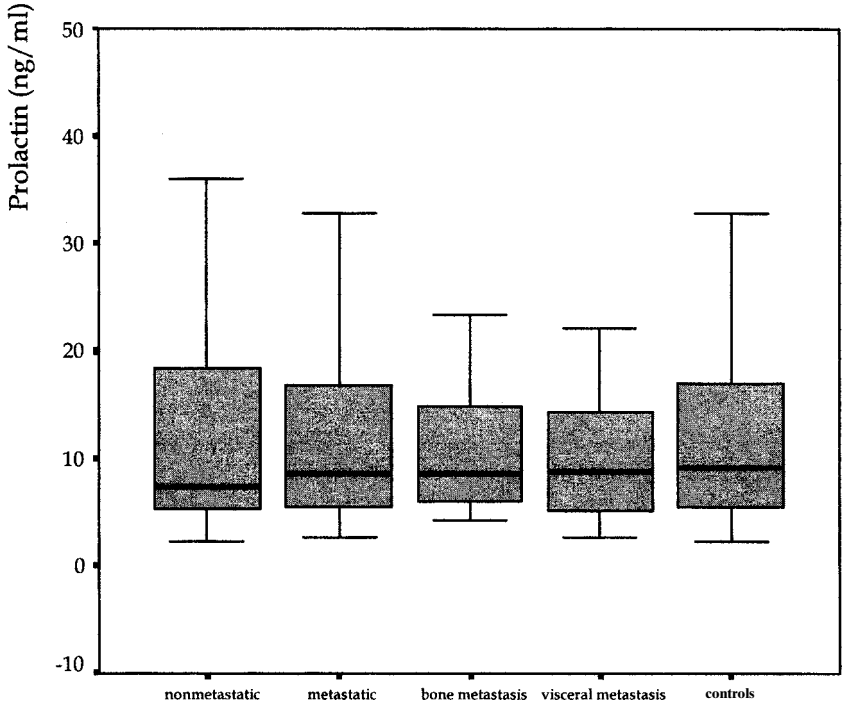

Figure 2. Mean serum prolactin levels in patients and controls.

Discussion

Angiogenesis, the development of new capillaries, is essential for tumor growth and metastasis [22]. The importance of tumor angiogenesis as a prognostic factor has been reported in various solid tumors including breast cancer [4, 32, 33]. VEGF, a potent multifunctional cytokine, has been suggested as being the major angiogenic factor in human tumors [5, 12]. Patients with higher VEGF expression have been reported to have shorter diesease free and overall survival in breast cancer [10, 15, 24]. The expression of VEGF in tumor tissue has been reported to be an independent prognostic indicator for breast cancer patients regardless of the nodal status [10, 15, 24].

It has been reported that increase in serum VEGF levels may be associated with worsened prognosis in patients with various types of cancer [29]. It has been suggested that the extent of disease in breast cancer patients may be accompanied by an elevation of serum VEGF levels [29, 34]. Similar to these findings, serum VEGF levels were found to be higher in metastatic patients when compared to

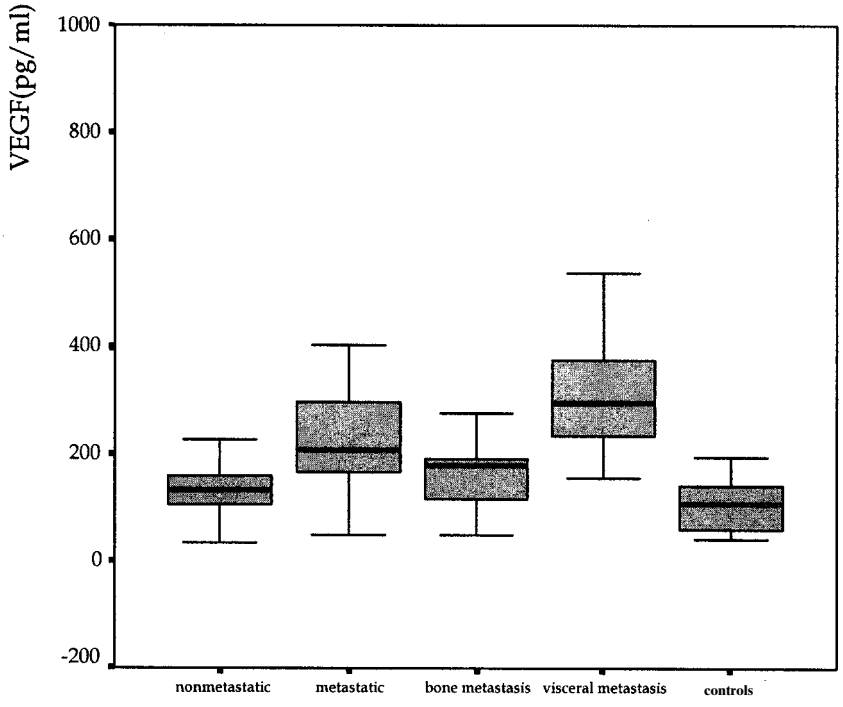

Figure 3. Mean serum VEGF levels in patients and controls.

non-metastatic patients in the present study. However, serum VEGF levels were similar in patients with complete remission and control subjects. Increased serum VEGF levels may reflect tumor growth and metastatic potential in breast cancer patients.

It has been reported that patients with visceral metastasis had higher VEGF expression when compared to patients with bone metastasis [23]. However, in another study, no correlation was found between the degree of angiogenesis and first recurrence site of breast cancer patients [16]. In the present study, serum VEGF levels were found to be higher in patients with visceral metastasis than bone metastatic patients. This can be explained by more aggressive metastatic potential of visceral metastasis than bone metastasis. There is experimental evidence that, besides VEGF, other cytokines and growth factors are also involved in the angiogenic process including leptin [4, 13, 30].

Leptin is a protein product of the ob gene that regulates food intake as well as metabolic and endocrine functions [9]. It has been reported that leptin plays a promoting role in angiogenesis and modulates angiogenic responses induced by VEGF [3, 30]. The different responses of angiogenesis induced by leptin and VEGF imply that different signaling pathways in endothelial cells could be involved in the angiogenic responses stimulated by these factors [3]. Based on its angiogenic potential, a possible association between leptin and cancer has been investigated in several cancer types. It has been reported that elevated serum levels of leptin are associated with later development of prostate cancer and may promote the tumor growth by stimulating angiogenesis [31]. However, both in vulvar [20] and vaginal cancer [21], no correlation was found between serum leptin levels and clinicopathological parameters and prognosis of patients. Only one study [25] investigated the association between breast cancer and serum leptin levels has been found in the literature. The authors suggested that leptin does not appear to affect the risk of ductal carcinoma *in situ*. In our study, we investigated the significance of serum leptin levels in breast cancer patients as a angiogenic marker and also correlated these levels with a well known angiogenic marker, VEGF. However, serum leptin levels were not different in patients and control subjects. Moreover, no correlation was found between serum leptin and VEGF levels. Serum leptin level does not seem to reflect angiogenic activity in breast cancer patients.

In this study, we also measured serum prolactin levels in patients and control subjects. Prolactin, an important hormonal and growth promoting factor, has been suggested to play a role in the pathogenesis and progression of breast cancer [1, 18]. It has been reported that prolactin fragments of 14kDa and 16 kDa bind to endothelial cell receptors and inhibit angiogenesis [8, 13]. It has been reported that 16 kDa prolactin inhibits VEGF induced angiogenesis in humans [6]. In humans, higher prolactin levels have been found in advanced breast cancer patients and suggested to be inversely correlated with survival, estrogen and progesteron receptor status [1, 18]. However, therapies aimed to reduce serum prolactin levels have not found to be successful in the treatment of breast cancer [17, 27]. So, there is still contraversy in this issue. In our study, serum prolactin levels were not found to be higher in metastatic breast cancer patients. Also, serum prolactin levels were not correlated with VEGF and leptin levels.

In conclusion, we found higher serum VEGF levels in patients with metastatic breast cancer. These levels were found to be higher in patients with visceral metastasis than bone metastatic patients. It can be considered that higher serum VEGF levels in visceral metastasis may be a reflection of higher angiogenic and metastatic activity of visceral metastasis. Serum leptin and prolactin levels were not different in patients and control subjects. Serum levels of leptin and prolactin does not seem to be related with angiogenic activity and metastasis in breast cancer patients.

References

[1] Bhatavdekar JM, Shah NG, Balar DB, Patel DD, Bhaduri A, Trivedi SN, Karelia NH, Ghosh N, Shukla MK, Giri DD. Plasma prolactin as an indicator of disease progression in advanced breast cancer. Cancer 1990; 65: 2028–2032.

[2] Bouloumie A, Drexler HC, Lafontan M, Busse R. Leptin, the product of Ob gene, promotes angiogenesis. Circ Res 1998; 83: 1059–1066.

[3] Cao R, Brakenhielm E, Wahlestedt C, Thyberg J, Cao Y. Leptin induces vascular permeability and synergistically stimulates angiogenesis with FGF-2 and VEGF. Proc Natl Acad Sci USA 2001; 98: 6390–6395.

[4] Chung YS, Maeda K, Sowa M. Prognostic value of angiogenesis in gastro-intestinal tumours. Eur J Cancer 1996; 32A: 2501–2505.

[5] Claffey KP, Brown LF, del Aguila LF, Tognazzi K, Yeo KT, Manseau EJ, Dvorak HF. Expression of vascular permeability factor/vascular endothelial growth factor by melanoma cells increases tumor growth, angiogenesis, and experimental metastasis. Cancer Res 1996; 56: 172–181.

[6] Clapp C, Martial JA, Guzman RC, Rentier-Delure F, Weiner RI. The 16-kilodalton N-terminal fragment of human prolactin is a potent inhibitor of angiogenesis. Endocrinology 1993;133: 1292–1299.

[7] Clapp C, Martinez de la Escalera G. Prolactin: novel regulators of angiogenesis. News Physiol Sci 1997; 12: 231–237.

[8] Clapp C, Torner L, Gutierrez-Ospina G, Alcantara E, Lopez-Gomez FJ, Nagano M, Kelly PA, Mejia S, Morales MA, Martinez de la Escalera G. The prolactin gene is expressed in the hypothalamic-neurohypophyseal system and the protein is processed into a 14-kDa fragment with activity like 16-kDa prolactin. Proc Natl Acad Sci U S A 1994; 91: 10384–10388.

[9] Collins S, Kuhn CM, Petro AE, Swick AG, Chrunyk BA, Surwit RS. Role of leptin in fat regulation. Nature 1996; 380: 677.

[10] De Paola F, Granato AM, Scarpi E, Monti F, Medri L, Bianchi S, Amadori D, Volpi A. Vascular endothelial growth factor and prognosis in patients with node-negative breast cancer. Int J Cancer 2002; 98: 228–233.

[11] Dirix LY, Vermeulen PB, Pawinski A, Prove A, Benoy I, De Pooter C, Martin M, VanOosterom AT. Elevated levels of the angiogenic cytokines basic fibroblast growth factor and vascular endothelial growth factor in sera of patients. Br J Cancer 1997; 76: 238–243.

[12] Dvorak HF, Brown LF, Detmar M, Dvorak AM. Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. Am J Pathol 1995; 146: 1029–1039.

[13] Ferrara N, Clapp C, Weiner R. The 16K fragment of prolactin specifically inhibits basal or fibroblast growth factor stimulated growth of capillary endothelial cells. Endocrinology 1991; 129: 896–900.

[14] Folkman J. What is the evidence that tumors are angiogenesis dependent? J Natl Cancer Inst 1990; 82: 4–6.

[15] Gasparini G, Toi M, Gion M, Verderio P, Dittadi R, Hanatani M, Matsubara I, Vinante O, Bonoldi E, Boracchi P, Gatti C, Suzuki H, Tominaga T. Prognostic significance of vascular endothelial growth factor protein in node-negative breast carcinoma. J Natl Cancer Inst 1997; 89: 139–147.

[16] Gasparini G, Weidner N, Bevilacqua P, Maluta S, Dalla Palma P, Caffo O, Barbareschi M, Boracchi P, Marubini E, Pozza F. Tumor microvessel density, p53 expression, tumor size, and peritumoral lymphatic vessel invasion are relevant prognostic markers in node-negative breast carcinoma. J Clin Oncol 1994; 12: 454–466.

[17] Heusen JC, Coune A, Staquet M. Clinical trial of 2-Br- -ergocryptine (CB154) in advanced breast cancer. Eur J Cancer 1972; 8: 155–156.

[18] Holtkamp W, Nagel GA, Wander HE, Rauschecker HF, von Heyden D. Hyperprolactinemia is an indicator of progressive disease and poor prognosis in advanced breast cancer. Int J Cancer 1984; 34: 323–328.

[19] Konopleva M, Mikhail A, Estrov Z, Zhao S, Harris D, Sanchez-Williams G, Kornblau SM, Dong J, Kliche KO, Jiang S, Snodgrass HR, Estey EH, Andreeff M. Expression and function of leptin receptor isoforms in myeloid leukemia and myelodysplastic syndromes: proliferative and anti-apoptotic activities. Blood 1999; 93: 1668–1676.

[20] Lebrecht A, Hefler L, Schneeberger C, Koelbl H. Serum leptin in patients with vulvar cancer. Gynecol Oncol 2001; 83: 164–165.

[21] Lebrecht A, Ludwig E, Huber A, Klein M, Schneeberger C, Tempfer C, Koelbl H, Hefler L. Serum vascular endothelial growth factor and serum leptin in patients with cervical cancer. Gynecol Oncol 2002; 85: 32–35.

[22] Lewis JS, Landers RJ, Underwood JC, Harris AL, Lewis CE. Expression of vascular endothelial growth factor by macrophages is up-regulated in poorly vascularized areas of breast carcinomas. J Pathol 2000; 192: 150–158.

[23] Linderholm B, Grankvist K, Wilking N, Johansson M, Tavelin B, Henriksonn R. Correlation of vascular endothelial growth factor content with recurrences, survival, and first relapse site in primary node-positive breast carcinoma after adjuvant treatment. J Clin Oncol 2000; 18: 1423–1431.

[24] Linderholm B, Tavelin B, Grankvist K, Henriksson R. Vascular endothelial growth factor is of high prognostic value in node negative breast carcinoma. J Clin Oncol 1998; 16: 3121–3128.

[25] Mantzoros CS, Bolhke K, Moschos S, Cramer DW. Leptin in relation to carcinoma in situ of the breast: a study of premenopausal cases and controls. Int J Cancer 1999; 80: 523–536.

[26] Mastronardi CA, Walczewska A, Yu WH, Karanth S, Parlow AF, McCann SM. The possible role of prolactin in the circadian rhythm of leptin secretion in male rats. Proc Soc Exp Biol Med 2000; 224: 152–158.

[27] Peyrat JP, Vennin P, Bonneterre J, Hecquet B, Vandewalle B, Kelly PA, Djiane J. Effect of bromocriptin treatment on prolactin and steroid receptor levels in human breast cancer. Eur J Cancer Clin Oncol 1984; 20: 1363–1367.

[28] Pidgeon GP, Barr MP, Harmey JH, Foley DA, Bouchier-Hayes DJ. Vascular endothelial growth factor (VEGF) upregulates BCL-2 and inhibits apoptosis in human and murine mammary adenocarcinoma cells. Br J Cancer 2001; 85: 273–278.

[29] Salven P, Perhoniemi V, Tykka H, Maenpaa H, Joensuu H. Serum VEGF levels in women with a benign breast tumor or cancer. Breast Cancer Res Tr 1999; 53: 161–166.

[30] Sierra-Honigmann MR, Nath AK, Murakami C, Garcia-Cardena G, Papapetropoulos A, Sessa WC, Madge LA, Schechner JS, Schwabb MB, Polverini PJ, Flores-Riveros JR. Biological action of leptin as an angiogenic factor. Science 1998; 281: 1683-1686.

[31] Stattin P, Soderberg S, Hallmans G, Bylund A, Kaaks R, Stenman UH, Bergh A, Olsson T. Leptin is associated with increased prostate cancer risk: a nested case-referent study. J Clin Endocrinol Metab 2001; 86: 1341–1345.

[32] Toi M, Kondo S, Suzuki H, Yamamoto Y, Inada K, Imazawa T, Taniguchi T, Tominaga T. Quantitative analysis of vascular endothelial growth factor in primary breast cancer. Cancer 1996; 77: 1101–1106.

[33] Weidner N, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis-correlation in invasive breast carcinoma. N Engl J Med 1991; 324: 1–8.

[34] Yamamoto Y, Toi M, Kondo S, Matsumoto T, Suzuki H, Kitamura M, Tsuruta K, Taniguchi T, Okamoto A, Mori T, Yoshida M, Ikeda T, Tominaga T. Concentrations of vascular endothelial growth factor in the sera of normal controls and cancer patients. Clin Cancer Res 1996; 2: 821–826.