Serum Vascular Endothelial Growth Factor in Breast Cancer: Its Relation with Cancer Type and Estrogen Receptor Status

Kamal Heer, Harish Kumar, John R. Read, John N. Fox, John R. T. Monson, and Michael J. Kerin

ABSTRACT

Purpose: Angiogenesis is essential for tumor growth. Vascular endothelial growth factor (VEGF) is one of the most potent angiogenic cytokines. In breast cancer, tumor VEGF has been shown to have a good correlation with relapse-free survival. The aim of this study was to determine the relation of serum VEGF levels to the various indices of breast cancer and known tumor markers carcinoembryonic antigen and CA15.3.

Experimental Design: Preoperative serum VEGF levels were determined in 200 women with breast cancer and compared with serum VEGF levels in 88 healthy female controls.

Results: The serum VEGF levels of the cancer patients as a group were significantly elevated compared with those of the controls (P < 0.0005). VEGF levels were elevated in patients with invasive cancer of ductal/no specific type, ductal carcinoma in situ, and estrogen receptor (ER)-positive tumors. Patients with lobular carcinoma and ER-negative tumors had serum VEGF levels comparable with those in the controls. VEGF was more sensitive than CA15.3 and carcinoembryonic antigen in detecting breast cancer and known tumor markers carcinoembryonic antigen and CA15.3.

Conclusions: Preoperative serum VEGF detects breast cancer with a sensitivity of 62.1%. The relationship to cancer type and ER status may have future therapeutic implications. Additional long-term studies are required to determine the prognostic significance of serum VEGF.

INTRODUCTION

It is well known that to grow beyond the size of 2–3 mm, it is essential for a tumor to acquire new blood vessels. This process of neovascularization is known as angiogenesis (1). VEGF is one of the most potent angiogenic cytokines. It causes mitosis of endothelial cells and increases blood vessel permeability. This increased permeability results in the extravasation of macromolecules such as fibrinogen, which provide an extraluminal meshwork over which endothelial cells can organize and tumor cells can migrate (2). Unlike other cytokines, VEGF is a selective cytokine, acting exclusively on vascular endothelial cells (3). Indeed, it has been shown that other mediators of neovascularization including interleukins, oncoproteins, and some growth factors may produce their effects by altering the expression of VEGF, suggesting that VEGF may be the final common pathway for all pathological in vivo angiogenesis (4).

VEGF is expressed by a wide variety of tumors, both in vitro and in vivo. We have previously shown that preoperative levels of VEGF in the serum can predict the stage of colorectal cancer (5). In breast cancer, intratumoral VEGF and microvessel density significantly correlate with decreased relapse-free survival (6). The aims of this study were to determine the relationship between preoperative serum VEGF and the various prognostic indices of breast cancer and to compare serum VEGF with two established tumor markers for breast cancer, namely, CEA and CA15.3.

PATIENTS AND METHODS

This was a prospective study involving 200 consecutive patients undergoing surgery for breast cancer. After obtaining informed verbal consent, a preoperative blood sample was taken from each patient for serum VEGF, CEA, and CA15.3 analysis. Patients who received neoadjuvant therapy (including tamoxifen) were excluded. Eighty-eight healthy females were also recruited as controls for serum VEGF.

VEGF Analysis. Blood (7 ml) was collected in a plain tube and allowed to clot for half an hour, after which it was centrifuged at 3000 rpm for 10 min. The serum was separated and stored at −80°C until a batch analysis for VEGF(165) (R&D Systems, Oxford, United Kingdom) was performed. This assay uses the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for VEGF is used to capture the VEGF, and an enzyme-linked monoclonal antibody specific for VEGF is used for quantification. Each standard and sample was assayed in duplicate. The minimal detectable amount of serum VEGF by this kit is 9.0 pg/ml, and the maximum undiluted

3 The abbreviations used are: VEGF, vascular endothelial growth factor; ER, estrogen receptor; DCIS, ductal carcinoma in situ; CEA, carcinoembryonic antigen; UICC, International Union Against Cancer; IQR, interquartile range; NOS, no specific type.

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amount detected is 2000 pg/ml. The serum may be appropriately diluted if higher levels need to be measured. The intra- and interassay range of variation of the kit are 4.5–6.7% and 6.2–8.8%, respectively. During the course of this study, r2, i.e., the coefficient of linearity (which shows the linear correlation between the measured absorbance and known amounts of standards), varied between 0.994 and 0.999.

**CEA and CA15.3 Analysis.** These assays were performed in Castle Hill Hospital laboratory using AxSYM Micro-particle Enzyme Immunoassays from Abbott Laboratories, Diagnostics Division (CEA, Dainabot, Tokyo Japan; CA15.3, Abbott Park, IL). In our laboratory, a cutoff level of 5 ng/ml is used for CEA, and a cutoff level of 30 units/ml is used for CA15.3.

**Tumor Staging.** A single pathologist reported on the resected tumor specimens using the UICC tumor-node-metastasis (TNM) classification. All patients underwent a metastatic screen consisting of liver function tests and a chest X-ray. A bone scan was performed on all patients with node-positive or locally advanced disease.

**Statistical Analysis.** Statistical analysis was performed with SPSS software for Windows [SPSS (UK) Ltd., Surrey, United Kingdom]. The Mann-Whitney U test for two independent samples, ANOVA, and Spearman’s correlation were used. Most descriptive statistics are reported as median and IQRs (the numerical difference between the 25th and 75th centiles).

**RESULTS**

**Controls.** The mean serum VEGF in the 88 controls was 201.7 pg/ml (median, 167.5 pg/ml; IQR, 101.5–245.3 pg/ml). We found that age did not influence the expression of serum VEGF when the controls were grouped according to the decade of their age (P = 0.35).

**Breast Cancer Patients.** The distribution of cancer patients is shown in Table 1. The serum VEGF levels of the cancer patients as a group were significantly elevated compared with those of the controls (median, 305.9 pg/ml; IQR, 156.7–451.6 pg/ml; P < 0.0005). The sensitivity of serum VEGF in detecting any breast cancer was 62.1%, and the specificity was 74% using a VEGF level of 241.02 pg/ml as the cutoff value. This represents the upper limit of the 99% confidence interval of the mean.

**CA15.3.** Levels were available in 177 of the 200 patients. The mean level was 20.9 units/ml (median, 16.0 units/ml; IQR, 10.5–23.0 units/ml). The sensitivity of CA15.3 in detecting breast cancer in this study was 13.5%, with a quoted specificity of 97.42%, according to the manufacturer’s data.

**CEA.** Results were available in 165 of the 200 patients. The mean level of CEA was 4.84 ng/ml (median, 2.0 ng/ml; IQR, 1.0–3.0 ng/ml). The sensitivity of CEA in detecting breast cancer in this study was 10.3%, with a quoted specificity of 95.4% from the manufacturer’s data.

**Type of Cancer.** Patients with histological cancer of ductal or NOS had a mean serum VEGF of 352.6 pg/ml that was significantly elevated compared with controls (P < 0.0005). Patients with lobular carcinoma had serum levels that were not significantly higher than those of controls (mean, 208.5 pg/ml; P = 0.76), whereas patients with DCIS (mean, 487.8 pg/ml) had significantly elevated serum VEGF levels that were not only significantly elevated compared with controls (P < 0.0005) but were also significantly elevated compared with those of patients with ductal (P = 0.04) and lobular carcinoma (P = 0.001). The elevation of VEGF levels in patients with ductal/NOS cancer compared with those in patients with lobular carcinoma was also significant (P = 0.01; Table 1). ANOVA showed a significant difference in VEGF distribution within the groups [NOS, lobular carcinoma, and DCIS (P = 0.0006)], whereas no difference was seen for CA15.3 (P = 0.2) or CEA (P = 0.4).

**ER Status.** ER-positive tumors had serum VEGF levels (mean, 341.5 pg/ml) that were significantly elevated when compared with those of controls (P < 0.0005) and patients with ER-negative tumors (mean, 258.9 pg/ml; P = 0.05). Serum VEGF in ER-negative tumors was not significantly elevated compared with controls (P = 0.24; Table 2). Using ANOVA, the levels of CA15.3 and CEA did not show any difference between ER-positive and -negative tumors (P = 0.32 and 0.25, respectively).

**UICC Stages.** Serum VEGF was elevated in all UICC stages compared with controls. Levels in patients with stage IV tumors (mean, 833.4 pg/ml) were significantly elevated as compared with patients with stages I, II, and III tumors (mean, 331.5, 360.0, and 295.7 pg/ml, respectively; Table 3). Within each stage, the serum VEGF levels in ductal/NOS carcinomas and ER-positive tumors were significantly elevated as compared with those of controls, whereas patients with lobular and ER-negative cancers had serum VEGF levels comparable to those of controls.

**Nodal Status.** Lymph node positivity did not appear to influence serum VEGF levels, which were comparable in node-negative and node-positive tumors (Mann-Whitney U test, P = 0.8; Spearman’s correlation, p = 0.016 (not significant).

**Other Parameters.** Serum VEGF levels did not show any significant correlation with tumor grade (P = 0.23), DCIS nuclear grade (P = 0.8), the number of positive lymph nodes (P = 0.55) or the absence or presence of intratumoral lymphatic or blood vessel cancer cell permeation (P = 0.57; Table 4).

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**Table 1** Median and IQR of serum VEGF in different types of cancer

<table>
<thead>
<tr>
<th>Type</th>
<th>Median VEGF (pg/ml)</th>
<th>IQR</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>167.5</td>
<td>101.5–245.3</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Ductal/NOS cancer</td>
<td>303.8</td>
<td>155.6–438.4</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Lobular cancer</td>
<td>178.1</td>
<td>109–290.7</td>
<td>0.76</td>
</tr>
<tr>
<td>DCIS</td>
<td>449.7</td>
<td>321.03–709.5</td>
<td>&lt;0.0005</td>
</tr>
</tbody>
</table>

* P value compared to controls; four tumors were excluded because cytology did not allow diagnosis of type.

**Table 2** Median and IQR serum VEGF according to tumor ER status (P value compared to controls)

<table>
<thead>
<tr>
<th>ER status</th>
<th>Median VEGF (pg/ml)</th>
<th>IQR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>167.5</td>
<td>101.5–245.3</td>
<td></td>
</tr>
<tr>
<td>ER negative</td>
<td>194.2</td>
<td>133.1–392.7</td>
<td>0.24</td>
</tr>
<tr>
<td>ER positive</td>
<td>297.8</td>
<td>155.7–438.6</td>
<td>&lt;0.0005</td>
</tr>
</tbody>
</table>

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 **CA15.3**. Levels were available in 177 of the 200 patients. The mean level was 20.9 units/ml (median, 16.0 units/ml; IQR, 10.5–23.0 units/ml). The sensitivity of CA15.3 in detecting breast cancer in this study was 13.5%, with a quoted specificity of 97.42%, according to the manufacturer’s data.
DISCUSSION

VEGF is a heparin-binding homodimeric glycoprotein which, by alternate splicing of the VEGF gene, may exist in one of four forms, namely, VEGF_{165} (the most abundant form, soluble, and tissue bound), VEGF_{121} (soluble), VEGF_{165} (bound), and VEGF_{186} (extremely rare, bound form). Each of these forms has similar biological activity. The ELISA kit used to detect VEGF in the serum in this study is specific for VEGF_{165} but will also detect VEGF_{121}. Thus, the detected levels are indicative of the total circulating level of VEGF. Our series of serum VEGF in controls represents levels in a Western population. A previous study by Yamamoto et al. (7) on a Japanese population used an ELISA specific for VEGF_{121}. They reported much lower range and mean values. This may be explained by the different, less abundant isof orm they detected or may be due to inter racial differences. The age range of our control group was 22–79 years, although we found that age has little bearing on serum VEGF levels.

Serum VEGF is significantly raised in ductal but not in lobular carcinoma. This finding agrees with that of Dvorak et al. (8), who showed that of the several varieties of carcinoma tissues they examined, only lobular carcinoma of the breast and papillary carcinoma of the bladder failed to reveal significant VEGF mRNA expression. This appears to corroborate the evidence that the tumor is the main source of VEGF measured in the serum. This has implications not only in understanding the pathogenesis and progression of lobular carcinoma but may also play a role in the management of this form of cancer in the future. Experimental studies have shown that on inoculation with tumor cells, animals treated with neutralizing antibodies against VEGF show an inhibition of tumor and metastasis growth compared with untreated animals (9). This has opened the realm of antiangiogenic treatment as a form of adjuvant therapy. A difference in the angiogenic response of different types of breast cancer would allow selection of patients for whom such adjuvant therapy may be appropriate, similar to the selection of patients for tamoxifen therapy on the basis of their ER status.

The finding of highly elevated serum VEGF levels in patients with DCIS is of great interest. Immunostaining of cancers has shown that the areas of increased microvessel density correspond very closely to the tumors areas showing high VEGF expression (6). DCIS is usually present in a wide field, unlike the localized center of invasion of a ductal carcinoma, and it is possible that a wide area of neovascularization may be responsible for the high VEGF levels noted. VEGF not only increases vascular permeability, it is also chemotactic for macrophages. These tumor-associated macrophages in turn produce increased levels of VEGF: thus, increased neovascularization would imply increased up-regulation of VEGF. Indeed, in their study of the expression of angiogenic factors in DCIS and carcinoma, Brown et al. (10) have shown that the formation of vascular stroma with high levels of VEGF preceded invasive stages. Thus, in the future, serum VEGF may be of use in the clinical scenario of equivocal cytological or radiological findings in diagnosing DCIS.

This is the first study to show a correlation between serum VEGF and ER positivity. Similar serum studies of basic fibroblast growth factor, another angiogenic cytokine that acts synergistically with VEGF, showed no such correlation (11). The up-regulation of VEGF by estrogen has been shown in the rat uterus and human endothelial cancer cell lines (12, 13). We have noted the same observation with the breast cancer cell line MCF-7 (data not shown). There are numerous ways by which estrogen could induce VEGF expression. Because the VEGF promoter lacks steroid-responsive elements, the induction of the VEGF gene is thought to occur through indirect mechanisms, including the up-regulation of various oncogenes. In this context, the activation protein transcription factor complexes AP-1 and AP-2 have been implicated. Various factors including estrogen, protein kinase C, and cAMP can induce the expression of these gene complexes, which in turn can up-regulate VEGF expression (14). In ER-positive cell lines, estrogen activates the c-Src tyrosine kinase, which can then induce VEGF expression (15). The presence or absence of the ER depends on the specific genetic makeup of the individual tumor, and ER positivity implies a greater likelihood of estrogen responsiveness of the tumor. Thus, the genetic status of a tumor may determine whether or not estrogen can stimulate VEGF expression (13). This once again raises the issue of why ER-positive tumors have a better prognosis, i.e., is it solely due to their being more differentiated and thus capable of expressing the receptor, or is it due to their responsiveness to hormones? The mechanisms mentioned above would seem to imply that it may be the removal of these tumors and the initiation of antiestrogen therapy that decrease the angiogenic potential and thus improve the prognosis of these tumors. Tamoxifen, the most commonly used antiestrogenic adjuvant drug, is known to be antiangiogenic (16).

Our previous study of serum VEGF in colorectal cancer had shown good correlation between VEGF levels, cancer stage, and nodal status (5). Such a correlation has not been demonstrated in the present work. This may reflect the importance of the female hormonal milieu in the pathogenesis of breast cancer.

<table>
<thead>
<tr>
<th>Median VEGF (pg/ml)</th>
<th>IQR (pg/ml)</th>
<th>P (Mann-Whitney U test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls 88 167.5 101.5-245.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage I 68 279.2 139.9-435.4</td>
<td>&lt;0.0005</td>
<td></td>
</tr>
<tr>
<td>Stage II 71 298.2 170.1-425.2</td>
<td>&lt;0.0005</td>
<td></td>
</tr>
<tr>
<td>Stage III 28 295.5 122.0-399.5</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Stage IV 6 637.4 475.3-1089.6</td>
<td>&lt;0.0005</td>
<td></td>
</tr>
<tr>
<td>Node negative 89 290.7 143.8-434.2</td>
<td>&lt;0.0005</td>
<td></td>
</tr>
<tr>
<td>Node positive 69 303.4 162.4-421.6</td>
<td>&lt;0.0005</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Median and IQR of serum VEGF according to stage and lymph node status. (P value compared to controls)

<table>
<thead>
<tr>
<th>Prognostic indices</th>
<th>Spearman’s correlation (ρ)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nodal metastasis</td>
<td>0.016</td>
<td>0.84</td>
</tr>
<tr>
<td>Grade of tumor</td>
<td>-0.092</td>
<td>0.23</td>
</tr>
<tr>
<td>DCIS nuclear grade</td>
<td>-0.023</td>
<td>0.8</td>
</tr>
<tr>
<td>Lymphovascular permeation</td>
<td>-0.043</td>
<td>0.57</td>
</tr>
<tr>
<td>Histological type</td>
<td>-0.025</td>
<td>0.73</td>
</tr>
<tr>
<td>ER status</td>
<td>-0.15</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Table 4: Spearman’s correlation of serum VEGF with various prognostic indices
The correlation of serum VEGF with ER status would appear to support this. Long-term follow-up studies are required to answer the questions of whether preoperative serum VEGF levels are of prognostic significance, as has been shown for tumor VEGF levels, and whether serum VEGF will be useful in detecting early recurrence in breast cancer.

We have shown that serum VEGF has a much higher sensitivity of 62.1% in detecting breast cancer than both the presently used tumor markers, CA15.3 (13.6%) and CEA (10.3%), with a specificity of 74%. Thus, there may be a place to add serum VEGF to the preoperative diagnostic armamentarium, especially in cases of difficult decisions between benign changes and DCIS on mammography.

In conclusion, this study shows that serum VEGF is raised in patients with breast cancer. More importantly, the relation of VEGF with cancer type and ER status may not only throw light on tumor biology but may also have therapeutic implications in the future.

REFERENCES
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