Vascular Endothelial Growth Factor Independently Predicts the Efficacy of Postoperative Radiotherapy in Node-Negative Breast Cancer Patients

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ABSTRACT

Purpose: Vascular endothelial growth factor (VEGF) is a mediator of angiogenesis and is up-regulated under hypoxic conditions. Hypoxic tumors are known to exhibit resistance to radiotherapy. We investigated the association between VEGF levels in tumor tissue and the effect of radiotherapy for relapse-free survival (RFS) and overall survival (OS) in node-negative breast cancer.

Experimental Design: The study was performed on 489 patients; 221 patients received postoperative radiotherapy as part of the breast-conserving therapy (BCT), and 268 patients were treated by mastectomy only. VEGF levels were measured using a quantitative ELISA. None of the patients received adjuvant systemic therapy. The median follow-up was 64 months (range, 2–149) after BCT and 59 months (range, 2–117) after mastectomy. Correlations with well-known prognostic factors were studied, and univariate and multivariate survival analyses were performed.

Results: Only in the BCT group, high VEGF levels (equal or above the median level) predicted a reduced RFS and OS in univariate survival analysis (P = 0.004 and P = 0.028, respectively), implying that patients with high VEGF levels have less benefit from BCT. This was seen as a significant interaction between local treatment and VEGF for the total population for RFS (P = 0.012) and OS (P = 0.004). The interaction between local treatment and tumor size was also significant for both RFS (P = 0.046) and OS (P = 0.019) in the multivariate analysis.

Conclusions: These results show that, in node-negative patients, both tumor size and VEGF content predict for a reduced efficacy of postoperative radiotherapy as part of BCT, indicating that the choice of local treatment of these patients can also be modified based on tumor VEGF content.

INTRODUCTION

The growth of solid tumors and their metastatic spread is angiogenesis dependent (1, 2). Angiogenesis that results in tumor microvascularity is an acknowledged early requirement for both tumor growth and dissemination (3). The change to the angiogenic phenotype may be caused by overexpression of a number of endothelial growth factors, such as vascular endothelial growth factor (VEGF; Ref. 2). VEGF works as a principal mediator of normal and pathological angiogenesis (4) and is secreted by a wide variety of cell types, including neutrophils, platelets, and tumor cells (5–8). VEGF is produced by the above mentioned cell types in response to hypoxia and inflammation, and by malignant cells that have undergone genetic changes (9, 10). Previous studies have shown the prognostic value of VEGF in patients with primary breast cancer (11–18).

Koukourakis et al. (19) showed a possible association between tumor angiogenesis and the effects of radiation. The efficacy of radiotherapy depends on tumor oxygenation and hypoxia, and, therefore, indirectly on the blood supply of the target tissue, and is less effective in tissues with poor vascularization (20, 21). Interestingly, the efficacy of radiotherapy in tumors with high angiogenesis is also low. It is suggested that this might be because of the antiapoptotic properties of angiogenic factors (22–24).

Studies assessing the predictive value of markers of angiogenesis on the effect of radiotherapy in patients with different malignancies [e.g., cervix (25), prostate (26), and head and neck squamous cell (27–30) carcinoma] have produced conflicting data.

There is only one study showing the predictive value of VEGF on outcome of radiotherapy for breast cancer (14). However, in that study, only patients treated with radiotherapy were included. Therefore, it is not possible to conclude whether the results were attributable to an association between radiotherapy and VEGF (predictive impact) or attributable to an intrinsic prognostic impact of VEGF on survival.

The aim of the present study was to investigate the predictive impact of VEGF in primary breast cancer with regard to adjuvant radiotherapy. For this purpose, patients who were treated with breast-conserving therapy (BCT) including postoperative radiotherapy and patients who were treated with a modified radical mastectomy without radiotherapy were both included. All included patients had node-negative primary invasive breast cancer and were not treated with adjuvant systemic therapy.
PATIENTS AND METHODS

Patients. A series of 1325 patients with operable breast cancer who underwent resection of their primary tumor between January 1987 and December 1996 were selected by the availability of frozen tissue in our tumor bank. This bank contains frozen tumor tissue of patients with breast cancer from nine different hospitals of the Comprehensive Cancer Center East in the Netherlands, because in the past the measurement of estrogen receptor (ER) and progesterone receptor (PgR) levels, by means of the ligand-binding assay, was centrally done for these hospitals. The clinical data were collected retrospectively from these nine hospitals. To determine the prognostic value of VEGF in irradiated and nonirradiated patients, node-negative breast cancer patients who were not treated with adjuvant systemic therapy were selected (n = 561). All patients underwent an axillary lymph node dissection to determine whether they were node negative. The patients included in the current study were included in the group of patients that were studied previously by Maders et al. (18). In that previous study, we investigated the prognostic impact in node-negative breast cancer patients who did not receive adjuvant systemic treatment (n = 576). After that study was reported, questions rose regarding what the exact influence of additional radiotherapy in some of these patients might have been. For that reason, we decided to analyze the outcome of the patients of the previous study who were treated with breast-conserving surgery and radiotherapy versus those with a modified radical mastectomy without radiotherapy. Patients who were treated with a modified radical mastectomy were selected by not having an indication for postoperative radiotherapy. As a consequence, patients with pT3 and pT4 tumors were not included in this study, nor were patients who did not receive radiotherapy as part of the BCT. The tumors of the investigated patients had a maximum size of 5 cm, not invading skin or thoracic wall (pT1 and pT2). Twenty-two patients with distant metastases at the time of diagnosis and six with distant metastases within 1 month after surgery were excluded from the analysis. Patients with previous diagnosis of carcinoma, with the exception of basal cell skin cancer, were also excluded (n = 15), as were patients with bilateral breast cancer (n = 10) and patients with only carcinoma in situ (n = 19). Of total, 489 patients were considered assessable, 221 of whom were treated with BCT and 268 who were treated with a modified radical mastectomy. All patients who had a lumpectomy with axillary lymph node dissection received postoperative radiotherapy to the breast as part of the BCT. A lumpectomy was considered complete when there were no tumor cells in the inked border of the surgical specimen. If the margins were not free of carcinoma, a re-resection or breast ablation was performed. The BCT group was followed for a median time of 64 months (range, 2–149). The patients who were treated with a mastectomy had a median follow-up time of 59 months (range, 2–117) after primary surgery. Categorical distributions of baseline characteristics in all patients and in BCT patients versus mastectomy patients are listed in Table 1.

During follow-up, 44 (20%) of the 221 patients who were treated with BCT had a recurrence (15 local, 27 distant metastases, and 4 both). Eight patients had a secondary primary tumor after the primary breast tumor. Fifteen patients died (12 confirmed breast cancer related and 3 unknown). Of the 268 patients who were treated with a modified radical mastectomy, 45 (17%) showed evidence of relapse of disease during follow-up. The first relapses observed were local recurrence in 8 patients, distant metastasis in 30 patients, and both in 7 patients. There were nine patients who had a secondary primary tumor after the primary breast tumor. Nineteen patients died as a result of breast cancer, whereas 15 patients died without evidence of disease at last follow-up. The secondary tumors were not considered as failures.

Radiotherapy. Patients receiving BCT were treated with postoperative radiotherapy. In general, this consisted of a dose of 45–50 Gy at 2–2.25 Gy/fraction for a total period of 4.5–5 weeks (4–6 megavolt photons) to the whole breast. An additional boost was given on the primary tumor bed to a total dose of 64–66 Gy with electrons or photons again at 2–2.25 Gy/fraction in 6.5–7 weeks to 29 patients.

Tumor Tissue Processing. After surgery, a representative part of the tumor specimen was selected by the pathologist, frozen in liquid nitrogen, and sent to the department of Chemical Endocrinology. The primary breast cancer biopsies were stored in liquid nitrogen and pulverized in a frozen state with a microdismembrator, as recommended by the European Organisation for Research and Treatment of Cancer for analyzing ER and PgR (31). The tissue powders were suspended in European Organization for Research and Treatment of Cancer buffer containing 20 mM K₂HPO₄/KH₂PO₄, 1.5 mM K₂EDTA, 3 mM sodium azide, 10 mM monothioglycerol, and 10% (v/v) glycerol/water (pH 7.4) and centrifuged at 800 × g for 20 min at 4°C. The supernatants were collected and subjected to additional centrifugation for 1 h at 100,000 × g for 4°C. Part of the high-speed supernatants obtained (cytosols) were used for measurement of ER and PgR levels by ligand-binding assay as described previously (32), and the remaining cytosols were stored at −80°C. The protein concentrations were determined by the method of Lowry et al. (33) using BSA as standard.

VEGF Assay. VEGF levels were determined in the primary breast tumor cytosols with an ELISA developed by our department for the Receptor and Biomarker Group of the European Organization for Research and Treatment of Cancer. The assay measures VEGF₁₆⁵ and VEGF₁₂₁, the main isoforms of VEGF. Details of the assay, including specificity and performance, have been described previously (34).

To increase the sensitivity of the VEGF assay, the horseradish peroxidase-labeled goat antirabbit-detecting antibody was replaced by a goat antirabbit alkaline phosphatase conjugate (A-3687; Sigma Chemical Co., St. Louis, MO). 4-Methylumbelliferyl phosphate (M-6491; Molecular Probes, Eugene, OR) in 10% diethanoamone in liquid nitrogen, and sent to the department of Chemical Endocrinology. The primary breast cancer biopsies were stored in liquid nitrogen and pulverized in a frozen state with a microdismembrator, as recommended by the European Organisation for Research and Treatment of Cancer for analyzing ER and PgR (31). The tissue powders were suspended in European Organization for Research and Treatment of Cancer buffer containing 20 mM K₂HPO₄/KH₂PO₄, 1.5 mM K₂EDTA, 3 mM sodium azide, 10 mM monothioglycerol, and 10% (v/v) glycerol/water (pH 7.4) and centrifuged at 800 × g for 20 min at 4°C. The supernatants were collected and subjected to additional centrifugation for 1 h at 100,000 × g for 4°C. Part of the high-speed supernatants obtained (cytosols) were used for measurement of ER and PgR levels by ligand-binding assay as described previously (32), and the remaining cytosols were stored at −80°C. The protein concentrations were determined by the method of Lowry et al. (33) using BSA as standard.

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Data Analysis. The median value of VEGF in the total group of patients was used as the cutoff value in the statistical analyses when analyzing VEGF as a dichotomized variable. The baseline characteristics in the BCT group and the group of patients treated with mastectomy were compared by the use of cross-tabs and the Pearson χ² test.

To analyze interrelations between VEGF and various traditional parameters, Spearman rank correlations (rₚ) were calculated for continuous variables, and the Kruskal-Wallis test was used for ordered variables.
Survival curves were generated using the method of Kaplan and Meier (35). For the univariate survival rate analysis, relapse-free survival (RFS) time (defined as the time from surgery until the diagnosis of recurrent disease) and overall survival (OS) time (defined as the time between date of surgery and death by any cause) were used as follow-up parameters. The survival curves only include the first 96 months of follow-up because of the rapidly declining number of patients thereafter. After a certain period of observation, patients are frequently redirected to their general practitioner for check-ups and mammography and cease to belong to the outpatients collective of our breast cancer clinic. Patients with events after 96 months were censored at 96 months.

Cox univariate regression analysis was used in the analysis of the associations between the different variables and RFS and OS (36). Multivariate Cox regression modeling with stepwise removal of nonsignificant factors was used to assess the contributions of several clinicopathological factors to RFS and OS time. The likelihood ratio test was used to perform the stepwise removal. An interaction variable was entered to establish whether VEGF had a predictive value for radiotherapy success according to a previously reported method (37, 38). Because of missing values, numbers do not always add up to the total of 489. All computations were done with the SPSS statistical package (release 10.0.5, November 1999). Two-sided $P < 0.05$ was considered to be statistically significant.

RESULTS

Baseline Characteristics by Surgical Therapy

Table 1 shows the results of the baseline characteristics of all patients and of the patients who were treated with BCT (with radiotherapy) and those treated with mastectomy (without radiotherapy). The patients in the BCT group were younger ($P < 0.001$) and had statistically significant more tumors with a size $\leq 2$ cm (pT1; $P < 0.001$) compared with patients who were treated with mastectomy. There was no statistically significant difference between the two groups of patients with respect to menopausal status, histology of the invasive breast cancers, histological grade, and the ER/PgR status.

Distribution of VEGF

VEGF concentrations were measured in all cytosolic samples of the primary breast cancers studied. In this series, a wide
range of concentrations of VEGF in cytosol, ranging from 0.00 to 48.03 ng/mg protein, was observed with a log-normal distribution. The median cytosolic VEGF level in the total group of patients was 0.53 ng/mg protein. This value was used as a cutoff value to enable the analysis of VEGF as a categorized variable (low, \(<0.53\) ng/mg protein; high, \(\geq 0.53\) ng/mg protein). The proportion of patients with VEGF values above the median value of VEGF was significantly lower in patients who were treated with BCT compared with patients who were treated with mastectomy (Table 1; \(P = 0.001\)). The levels of VEGF ranged from 0.00 to 48.03 ng/mg protein, with a median value of 0.44 ng/mg protein in the BCT group, and from 0.00–11.58 ng/mg protein, with a median value of 0.60 ng/mg protein in the group of patients that was treated with mastectomy.

Correlations

In the total group of patients, the tumor levels of VEGF were associated with age (higher levels at younger age, \(P = 0.077\)), tumor size (higher levels in larger tumors, \(P = 0.029\)), histological grade (higher levels in poorer differentiated tumors, \(P = 0.057\)), and with ER and PgR status (higher in hormone-receptor-negative tumors (ER: \(r_s = -0.134\), \(P = 0.004\); PgR: \(r_s = -0.137\), \(P = 0.003\)).

Survival Rate Analyses

Patients Treated with BCT. The 5-year RFS was 88% for patients with low VEGF levels and 72% for those with high VEGF levels (Fig. 1A; \(P = 0.004\)). For OS, the 5-year rates were 98 and 90%, respectively (Fig. 1B; \(P = 0.028\)). There was not only a significant association between higher VEGF levels and poor RFS observed when VEGF was used as a dichotomized variable (\(P = 0.004\)) but also when used as a log-transformed continuous variable (\(P = 0.003\)). Similarly, high VEGF levels were associated with a poor OS, both when analyzed as a dichotomized variable (\(P = 0.028\)) and as a log-transformed continuous variable (\(P = 0.016\)).

Patients Treated with Modified Radical Mastectomy. The survival rate analyses for the group of patients who were treated with a radical mastectomy showed no significant relationship between VEGF levels and RFS when VEGF was used as a dichotomized variable or as a log-transformed continuous variable (data not shown). Similarly, high VEGF levels were not associated with a poor OS, neither when analyzed as a dichotomized variable nor as a log-transformed continuous variable (data not shown).

Cox Analysis

In the Cox univariate analysis, younger age, larger tumor size, negative ER and PgR status, and high VEGF levels were significantly associated with a poor RFS and OS. In addition, histological grade showed borderline significance with RFS, and local treatment was significantly associated with OS (Tables 2 and 3). There was also a significant association between higher VEGF levels and poor RFS and OS when VEGF was used as a log-transformed continuous variable (\(P = 0.016\) and \(P = 0.009\), respectively).

Cox multivariate regression analysis was performed to determine whether VEGF had a predictive impact with regard to type of treatment. The combined prognostic value of the established clinicopathological factors (age, histological grade, tumor size, and ER and PgR status) as well as VEGF and local treatment were assessed in a model by Cox multivariate regression analysis. The additive value of interaction terms [local treatment (BCT or mastectomy) with VEGF and local treatment with tumor size] was assessed by an additional round of reverse stepwise regression analysis.

The main result is the significant interaction between local treatment and the variable VEGF for both RFS and OS (Tables 2 and 3). This implies that patients with high VEGF levels have a higher risk for reduced RFS and OS after BCT. The interaction between local treatment and tumor size was also significant for both RFS and OS in the multivariate analysis (Tables 2 and 3). This implies that patients with larger tumors have increased benefit from modified radical mastectomy, which is to be expected. Together with both interaction terms, age and PgR were significantly associated with both RFS and OS, whereas local treatment was only significantly associated with OS. Similar findings were seen when VEGF was added to the basic multivariate model as log-transformed continuous variables instead of categorized variables (data not shown). A similar result was...
found when all aforementioned analyses were performed, with the exclusion of the 29 patients treated with an additional boost.

**DISCUSSION**

To our knowledge, this is the first study in which a predictive impact of VEGF in primary breast cancer with regard to efficacy of adjuvant radiotherapy has been investigated with a control group not treated by radiotherapy. We demonstrated that, in patients treated with breast-conservative surgery and adjuvant radiotherapy, high VEGF levels predicted for a reduced RFS and OS. RFS, rather than OS, should be considered the primary end point of the study because OS is also influenced by (systemic) therapy for metastatic disease or other causes of death. Patients with high VEGF levels benefited less strongly from radiotherapy than those with low levels. This effect was seen as a significant interaction between local treatment and VEGF for the total population for both RFS and OS. As high VEGF was not associated with a worse survival in the no-radiotherapy group, high VEGF seems to predict for a reduced efficacy of postoperative radiotherapy in patients with node-negative breast cancer.

VEGF is reported to be of prognostic value in breast cancer (11–18). Furthermore, VEGF has been reported to be an adverse predictive factor in advanced breast cancer patients with regard to the efficacy of systemic endocrine or chemotherapy (39). There is, however, only little information available concerning the importance of angiogenesis and hypoxia-related parameters, such as VEGF, in predicting the probability of treatment success in patients with a primary cancer of the breast treated with radiotherapy. In case such an association between VEGF and efficacy of radiotherapy does exist, it may have confounded previous results on prognostic impact, because in a number of the aforementioned studies, radiotherapy was administered to at least part of the patients (11–13, 15–18). For example, we previously showed that a high VEGF level was independently associated with both a reduced RFS and OS in 574 patients with node-negative breast cancer who were not treated with adjuvant systemic therapy (18). But, of note, 50% of the patients in that study were treated with additional radiotherapy after primary surgery for various reasons. Also, in three other previous studies on the prognostic value of VEGF, 45–60% of the patients was treated with postoperative radiotherapy (11, 13, 15). The percentage of patients that received additional radiotherapy after primary surgery was not reported in the two remaining studies (12, 16).

It was rather unexpected to observe a 5-year RFS of only 72% for patients with high VEGF levels treated with radiotherapy compared with 82% for patients with high VEGF levels not
treated with radiotherapy, especially because tumor size was significantly smaller in the first group (median, 1.8 cm). This may be confounded by younger age in the first group. Yet, it can be hypothesized that in patients with a small tumor but a high VEGF level, a mastectomy is preferred above a lumpectomy, followed by radiotherapy. In the multivariate analysis, an interaction between local treatment and tumor size was also included. This interaction was significant for RFS and OS, with HRs of 0.30 and 0.10, respectively. This implies that patients with larger tumors have increased benefit from modified radical mastectomy, which is to be expected. In the multivariate analysis, there seems to be a stronger interaction between VEGF and local treatment \((P = 0.012)\) than for tumor size and local treatment \((P = 0.046)\), suggesting mainly a biological interaction. In contrast, tumor size may be more pragmatic in use.

Because we evaluated the predictive impact of VEGF with regard to efficacy of adjuvant radiotherapy, it would have been worthwhile to also use local relapse as an end point. Unfortunately, the number of patients with local recurrences was too small \((n = 21)\) to perform reliable exploratory analyses. Of note, the impact of radiotherapy on local control has been demonstrated to improve OS also \((40-43)\).

Table 3  Cox univariate and multivariate analysis of overall survival in all patients

<table>
<thead>
<tr>
<th>Factor</th>
<th>Univariate analysis</th>
<th>Multivariate analysisa</th>
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<tr>
<td></td>
<td>(P)</td>
<td>HRb (95% CI)b</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>&lt;0.001</td>
<td>1</td>
</tr>
<tr>
<td>(\leq 40)</td>
<td></td>
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<tr>
<td>41–55</td>
<td>0.07 (0.02–0.25)</td>
<td>0.06 (0.01–0.32)</td>
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<tr>
<td>56–70</td>
<td>0.33 (0.15–0.73)</td>
<td>0.22 (0.08–0.65)</td>
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<tr>
<td>(&gt; 70)</td>
<td>0.61 (0.27–1.41)</td>
<td>0.81 (0.28–2.36)</td>
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<tr>
<td>Histological grade</td>
<td>0.185</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>3.00 (0.39–23.25)</td>
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<tr>
<td>III</td>
<td>4.87 (0.65–36.80)</td>
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<tr>
<td>Tumor size</td>
<td>0.013</td>
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<tr>
<td>pT1</td>
<td>1</td>
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<tr>
<td>pT2</td>
<td>2.08 (1.17–3.71)</td>
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<tr>
<td>ER statusc</td>
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<tr>
<td>Negative</td>
<td>1</td>
<td>0.36 (0.20–0.65)</td>
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<tr>
<td>Positive</td>
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<tr>
<td>PgR statusc</td>
<td>&lt;0.001</td>
<td></td>
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<tr>
<td>Negative</td>
<td>1</td>
<td>0.27 (0.15–0.48)</td>
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<tr>
<td>Positive</td>
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<td>VEGF levelsd</td>
<td>0.013</td>
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<tr>
<td>Low</td>
<td>1</td>
<td>2.19 (1.18–4.05)</td>
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<tr>
<td>High</td>
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<td>Local treatment</td>
<td>0.002</td>
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<tr>
<td>BCTe</td>
<td>1</td>
<td>0.36 (0.19–0.70)</td>
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<tr>
<td>Mastectomy</td>
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<tr>
<td>Interaction, local treatment (\times) VEGF</td>
<td></td>
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<tr>
<td>Either or both 0</td>
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<tr>
<td>Both 1</td>
<td>29.22 (3.02–282.76)</td>
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<tr>
<td>Interaction, local treatment (\times) tumor size</td>
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<tr>
<td>Either or both 0</td>
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<tr>
<td>Both 1</td>
<td>0.10 (0.01–0.68)</td>
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a The final multivariate model with all the factors known included 462 patients.
b Hazard ratio (95% confidence interval) of univariate and multivariate analysis.
c Cutoff points used for estrogen receptor (ER) and progesterone receptor (PgR), 10 fmol/mg protein.
d Low, \(<0.53\) ng/mg protein; high, \(\geq0.53\) ng/mg protein.
e BCT, breast-conserving therapy.

Because the present study should be regarded as a pilot study, it was not the intention to find an optimal cutoff value for VEGF. From a biological point of view, such an arbitrary assignment might be inappropriate. Furthermore, if the total data set would have been used to find the optimal cutoff value, an independent data set still would be needed to validate this cutoff value \((44)\). Additional studies are needed to determine the optimal cutoff value for clinical use.

The three most important mechanisms for failure of radiotherapy are intrinsic radioresistance \((45, 46)\), rapid tumor cell proliferation \((47–49)\), and tumor cell hypoxia \((19, 20, 50)\).
Hypoxia has been shown to up-regulate VEGF, both at the mRNA and the protein level, in tumor cell lines and around necrotic foci of tumors (51–53). In the present study, we showed that a high tumor tissue VEGF level acts as an independent predictor of reduced RFS as well as OS in patients subjected to locoregional radiotherapy for breast cancer. An explanation for these results could be that VEGF in tumor tissue reflects tumor cell hypoxia, which is known to make cells more resistant to radiotherapy. Therefore, it is not certain whether VEGF is a true independent predictor or whether it is a surrogate marker for hypoxia with radioresistance as a consequence.

Several other studies have assessed the predictive value of angiogenesis, in patients with other malignancies treated with radiotherapy, with conflicting results (25–30). Révéz et al. (25) reported that high vascular density is associated with a better survival in irradiated cervix tumors. Hall et al. (26) showed that microvessel density (MVD) in irradiated prostate cancer was correlated with other bad prognostic factors. Low MVD is reported to be an independent predictor of complete remission in head and neck squamous cell carcinoma after radiotherapy (27), whereas in another study OS was worse in patients with head and neck squamous cell carcinoma who had very low MVD scores (28). Furthermore, Giatromanolaki et al. (29) reported that patients with squamous cell carcinoma of the head and neck area with intermediate vascular density showed a better complete response rate after induction chemotherapy or after concurrent chemoradiotherapy and better RFS and OS than in patients with tumors with low and high vascular density (29). Aebersold et al. (30) showed that VEGF expression was not predictive for complete remission after radiotherapy in patients with squamous cell carcinomas of the oropharynx (30). But, again, none of these studies included a control group not treated with radiotherapy.

In conclusion, the results of our study indicate that breast cancer patients with high primary tumor levels of VEGF have reduced benefit from adjuvant radiotherapy. An interaction between tumor size and local treatment was also established. Additional research should focus on the most optimal local treatment strategy for this specific group of patients. These patients may preferably be treated by radical mastectomy to circumvent the need for complementary radiotherapy. From the present data, it is not possible to select one parameter above the other, for use in daily clinical practice. Additional prospective studies are warranted to investigate which parameter is clinically more relevant and to determine its optimal cutoff value.

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