Published in final edited form as:

Cancer Causes Control. 2009 April ; 20(3): 375–386. doi:10.1007/s10552-008-9252-4.

VASCULAR ENDOTHELIAL GROWTH FACTOR AND BREAST CANCER RISK

Katherine W. Reeves^{1,2,*}, Roberta B. Ness², Roslyn A. Stone³, Joel L. Weissfeld^{2,4}, Victor G. Vogel⁵, Robert W. Powers^{6,7}, Francesmary Modugno², and Jane A. Cauley²

¹Department of Public Health, School of Public Health and Health Sciences, University of Massachusetts, Amherst, Amherst, Massachusetts

²Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania

³Department of Biostatistics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania

⁴University of Pittsburgh Cancer Institute, Pittsburgh, Pennsylvania

⁵Department of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania

⁶Department of Obstetrics & Gynecology and Reproductive Biology, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania

⁷Magee-Womens Research Institute, Pittsburgh, Pennsylvania

Abstract

Vascular endothelial growth factor (VEGF) is a key factor in angiogenesis and is important to carcinogenesis. Previous studies relating circulating levels of VEGF to breast cancer have been limited by small numbers of participants and lack of adjustment for confounders. We studied the association between serum VEGF and breast cancer in an unmatched case-control study of 407 pre- and postmenopausal women (N=203 cases, N=204 controls). Logistic regression was used to model breast cancer risk as a function of natural log transformed VEGF levels adjusted for age, Gail score, education, physical activity, history of breastfeeding, serum testosterone, and hormone therapy use. The majority of the population was postmenopausal (67.6%) and the average age was 56 years; age and menopausal status were similar among cases and controls. Geometric mean VEGF levels were non-significantly higher in cases (321.4 pg/mL) than controls (291.4 pg/mL; $p=0.21$). In a multivariable model the odds of breast cancer was 37% higher for women with VEGF levels ≥ 314.2 pg/mL compared to those with levels below 314.2 pg/mL, albeit not significantly ($p=0.16$). There was no interaction between VEGF and menopausal status ($p=0.52$). In this case-control study VEGF was not significantly associated with breast cancer risk in pre- and postmenopausal women.

Keywords

Angiogenesis; breast neoplasms; premenopausal; postmenopausal

*To whom correspondence should be addressed: 410 Arnold House 715 North Pleasant Street Amherst, MA 01003 Phone: (413) 577-4298 Fax: (413) 545-1645 kwreeves@schoolph.umass.edu.

The authors have no conflicts of interest to report.

INTRODUCTION

An estimated 182,460 women in the United States will be diagnosed with breast cancer in 2008 [1]. Breast cancer risk factors include age, family history, and nulliparity, yet it remains difficult to predict which women will develop the disease. The ability of existing prediction models for breast cancer, such as the Gail model [2-4], to discriminate between women who will and will not be diagnosed with breast cancer is limited [5]. Little is known about biological factors that may increase breast cancer risk. Biological risk factors that are measured in blood or urine could be used to refine current risk prediction models and may enhance our ability to identify women likely to develop breast cancer [5].

Angiogenesis is the process of forming new blood vessels. Tumor growth is dependent on angiogenesis [6, 7]. Without vascularization tumors are unable to grow beyond 1-2 mm³ [8]. While angiogenesis is a multi-factorial, tightly controlled biological process, vascular endothelial growth factor (VEGF) is a primary promoter of angiogenesis [9]. VEGF is a potent mitogen with specificity for endothelial cells [9] and the ability to act on cancer cells [10]. VEGF has many functions, including promoting endothelial cell mitogenesis and survival, stromal degradation, and vascular permeability [10]. VEGF occurs in six different isoforms due to alternative splicing of the VEGF gene [11, 12]; the 121 and 165 isoforms, which are secreted as soluble proteins, are the most abundant [11, 12].

In vitro and animal studies provide strong evidence of an association between VEGF and breast cancer, though studies in humans are not definitive. Numerous studies have reported that plasma or serum VEGF levels are increased among women with breast cancer compared to those without [13-25]. These studies, however, are limited by small sample sizes, incomplete description of the study populations, and/or failure to control for potential confounders. Thus, the true association between VEGF levels and breast cancer remains unclear. Further, estrogen is known to be important in breast cancer development, and *in vitro* evidence indicates that estrogen may upregulate VEGF mRNA and protein expression [26-31]. Few studies have investigated associations between VEGF and estrogen in humans, however.

We conducted an unmatched case-control study of serum VEGF levels in relation to breast cancer in an ancillary study to the Mammograms and Masses Study (MAMS). Our primary hypothesis was that serum VEGF levels would be positively associated with breast cancer in this population of pre- and postmenopausal women. We also investigated associations between VEGF and direct and indirect measures of estrogen exposure.

MATERIALS AND METHODS

Study population

MAMS is an unmatched case-control study of hormonal determinants of mammographic breast density [32]. Briefly, women were eligible for MAMS if they were age ≥ 18 and were receiving a) a breast biopsy, b) an initial surgical consultation after breast cancer diagnosis, or c) a routine screening mammogram. Exclusion criteria were prior cancer history other than non-melanoma skin cancer, alcohol intake >5 drinks per day, or weight <110 pounds or >300 pounds. Women were enrolled from 2001-2005 through mammography and surgical clinics operated by Magee-Womens Hospital, Pittsburgh, PA. Pathology reports were obtained for women recruited from biopsy and surgical clinics to ascertain their disease status (benign breast disease, in situ disease, or invasive cancer). Controls were recruited from women with negative findings on screening mammograms. Of the eligible respondents, 55% of cases and 55% of controls enrolled in MAMS. The MAMS study population consists

of 1,133 women, including 264 cases with in situ or invasive breast cancer, 313 women with benign breast disease, and 556 controls.

A subset of MAMS participants was selected for this investigation of VEGF and breast cancer. We included only breast cancer cases and healthy controls; participants with benign breast disease were excluded (N=313). Cases and controls were excluded from this analysis if they had no available mammogram (38 cases, 36 controls), were missing questionnaires (13 cases, 7 controls), had no available serum sample (5 cases, 5 controls), or if their blood draw was >14 days from enrollment (3 cases, 0 controls). After these exclusions 205 cases (66 premenopausal and 139 postmenopausal) and 508 controls (105 premenopausal and 403 postmenopausal) were eligible for the VEGF analysis. We included all 205 eligible cases and selected a simple random sample of 66 premenopausal and 139 postmenopausal controls from the 508 eligible controls. After completion of the VEGF analyses, three participants (1 control and 2 cases) were discovered to have a previous history of cancer and were excluded. The final 407 participants included 203 cases and 204 controls. The Institutional Review Board at the University of Pittsburgh approved this study, and all participants provided written informed consent.

Data Collection

Participants completed a self-administered questionnaire that collected data on demographic characteristics, current and lifetime data on medical conditions and procedures, medications including hormone therapy (HT) and oral contraceptives (OCs), reproductive history, family cancer history, physical activity, smoking, and alcohol use. A research nurse measured participants' height and weight using a stadiometer and a standard balance beam scale while participants wore light clothing and no shoes.

Participants gave a non-fasting, 40 mL sample of peripheral blood at enrollment. The blood draw was not timed with the menstrual cycle for premenopausal women. Premenopausal women reported the date of their last menstrual period and the expected date of their next menstrual period, and menstrual cycle phase was inferred from this information. All blood samples were processed immediately at the Magee-Womens Hospital Satellite Clinical Research Center and stored at -70°C .

Laboratory Assays

Samples were relabeled with dummy identifiers and randomly distributed throughout the boxes transferred to the laboratories. A random sample of 40 masked duplicates (including 10 each from premenopausal cases and controls and postmenopausal cases and controls) were randomly distributed throughout the boxes for a reliability sub-study. Samples were transferred packed in dry ice. All laboratory staff were masked to the identity, disease status, and demographic and risk factor characteristics of the samples.

VEGF was measured in serum by enzyme-linked immunosorbent assay (Quantikine® Human VEGF Immunoassay, R&D Systems, Minneapolis, MN) [33]. This assay measures the 121 and 165 isoforms of VEGF-A and has a minimum detectable concentration of <5 pg/mL. The coefficient of variation (CV) for the VEGF assay was 14.2% (15.3% for pre- and 12.9% for postmenopausal women).

Estradiol (E2), follicle stimulating hormone (FSH), sex hormone binding globulin (SHBG), and testosterone (T) were measured in serum at the Clinical Ligand Assay Service Satellite (CLASS) Laboratory at the University of Michigan, School of Public Health. E2 was measured with a modified, off-line ACS:180 (E2-6) immunoassay (Bayer Diagnostics Corp, Tarrytown, NY) [34]. This assay has a detectable range of 1 – 250 pg/mL. FSH was measured with a two-site chemiluminescence (sandwich) immunoassay [35, 36]. This assay

measures FSH concentrations from 0.3 – 200 mIU/mL. SHBG was measured using a competitive immunoassay run on Bayer Diagnostic's ACS:180 automated analyzer using chemiluminescent technology [35]. The detectable range for SHBG is 1.95 to 250 nM. Total T was measured using a modification of the ACS:180 total T assay to measure with greater precision samples in the low ranges found in women in peri- and postmenopause [37]. The limit of detection of this assay is <5.15 ng/dL. CVs were 42.3% for E2 (32.0% for pre- and 50.6% for postmenopausal women), 5.5% for FSH (6.1% for pre- and 4.8% for postmenopausal women), 14.6% for SHBG (11.5% for pre- and 17.2% for postmenopausal women), and 13.6% for T (15.6% for pre- and 11.3% for postmenopausal women). The high CV for E2 was related to the low concentrations of E2 observed in postmenopausal women; in the analyses we categorized E2 into quartiles based on the control distribution because this provided more reliable data.

Statistical Analysis

Descriptive statistics were calculated for participant characteristics and the laboratory measures. Biological measures below the detection limit for the assay were reset to the stated detection limit (N=41 for E2, N=4 for T). A natural log transformation was applied to all biological measures to improve normality. Distributions of variables were compared by disease status using two-sample t tests for continuous variables and chi-square tests for categorical variables. We evaluated whether VEGF levels differed by recruitment site for controls using analysis of variance.

Variables associated with breast cancer or VEGF in previous studies were evaluated for their association with VEGF: age (continuous), race (white, other), educational level (high school, >high school), BMI (<25 kg/m², 25–<30 kg/m², 30 kg/m²), alcohol intake in year prior to enrollment (none, <12 g/d, 12 g/d), current alcohol use (no, yes), smoking status (never, former, current), cumulative physical activity in metabolic equivalent (MET)-h/wk (0, 0.1 – 10, 10.1), age at menarche (<13, 13), menstrual cycle regularity (no, yes, sometimes), menopausal status (premenopausal, postmenopausal without hysterectomy, postmenopausal with hysterectomy), age at menopause (premenopausal, <50, 50), menstrual cycle phase (luteal, follicular, unknown, postmenopausal), ever pregnant (no, yes), number of live births (none, 1, 2, 3), age at first pregnancy lasting >6 months (no live births/pregnancy lasted <6 months, <20, 20–24, 25–29, 30), breastfeeding history (not applicable, no, yes), breast cancer family history (no, yes), cancer family history (no, yes), Gail score (<1.66%, 1.66%), previous breast biopsy (no, yes), diabetes (no, yes), myocardial infarction history (no, yes), heart disease history (no, yes), HT use (never, former, current), OC use (never, former, current), E2 (quartiles), FSH (quartiles), SHBG (quartiles), and T (quartiles).

Categorizations of these variables were based on common cutpoints (e.g. BMI) or on the original response categories with cells with small counts collapsed (e.g. age at menarche). Quartiles for E2, FSH, SHBG, and T were based on the distribution of these hormones among controls. VEGF was dichotomized based on the median level among the controls (<314.2 pg/mL, 314.2 pg/mL). Bivariate associations between dichotomous VEGF and these variables among control participants were assessed using chi-square tests or analysis of variance. Fisher's exact test was used in instances where the expected cell count was <5.

Logistic regression was used to evaluate the association between VEGF and breast cancer adjusting for relevant covariates. VEGF was modeled in separate regressions as a continuous, natural log transformed variable and as a dichotomous variable. Dummy variables were created for categorical variables as appropriate. The aforementioned variables were evaluated for inclusion as potential confounders, using backward selection based on likelihood ratio tests. All covariates with a p value <0.10 from a likelihood ratio test were retained in the model. Fractional polynomials were used to assess the assumption that continuous variables were linear in the logit scale; these analyses indicated that this

assumption was met for the continuous variables. The Hosmer-Lemeshow test was used to assess goodness of fit. Potentially influential observations were identified as those having significant influence on model deviance (as assessed by Hosmer-Lemeshow's delta deviance test) or parameter estimates (as assessed by Pregibon's delta beta test). Likelihood ratio tests were used to evaluate the significance of hypothesized interactions by comparing the model including the interaction term to the main effects model.

All analyses were repeated among subgroups defined by menopausal status. Stata version 10.0 was used for all analyses (Stata Corporation, College Station, TX). Two-sided p values 0.05 were considered statistically significant, with no adjustment for multiple comparisons.

RESULTS

The 407 participants comprising the study population are described in Table 1. The mean age of both cases and controls was 56 ($p=0.99$). The majority of the participants was non-Hispanic white, though participants of other ethnicities were somewhat more common among controls than cases (7.4% versus 2.5%, $p=0.02$). Compared to controls, cases exercised less ($p<0.001$), were less likely to have breastfed ($p=0.05$), had a higher mean Gail score ($p=0.02$), and were more likely to be current users of HT ($p<0.001$). Of the 203 cases, 52 (25.6%) had in situ disease and 151 (74.4%) had invasive cancer.

Distributions of VEGF and the measured reproductive hormones are summarized in Table 2. The geometric mean of serum VEGF among cases (321.4 pg/mL) was non-significantly higher than that among controls (291.4 pg/mL, $p=0.21$). There was no significant difference in the geometric mean of serum VEGF between in situ (328.6 pg/mL) and invasive cases (319.0 pg/mL; $p=0.81$). We observed no significant difference in serum VEGF among controls by recruitment site ($p=0.32$; data not shown). Similar VEGF levels were observed within the subgroups of pre- and postmenopausal women (data not shown). No significant differences were observed between cases and controls for the geometric means of E2 (15.5 versus 12.6 pg/mL, $p=0.18$), FSH (62.9 mIU/mL versus 55.5 mIU/mL, $p=0.31$), and SHBG (51.6 nM versus 49.3 nM, $p=0.45$). Geometric mean T levels were higher among cases (32.8 ng/dL) compared to controls (28.1 ng/dL; $p=0.01$). When restricted to premenopausal women only, geometric mean FSH levels were significantly higher among cases (20.7 mIU/mL) than controls (13.4 mIU/mL; $p=0.05$). Among postmenopausal women only, E2 levels were significantly higher among cases than controls (geometric mean 9.5 pg/mL versus 6.8 pg/mL, respectively, $p=0.02$; data not shown). Other hormone distributions were similar between these two subgroups.

Participants with high VEGF were more likely to have relatively high or relatively low levels of FSH ($p=0.04$; Table 3). None of the other characteristics considered was significantly associated with serum VEGF levels among controls. Characteristics related to breast cancer risk, such as the Gail score, age at menopause, parity, and HT use, were similar among women with VEGF levels at or above the median level of controls (314.2 pg/mL) compared to those with lower levels. Results were similar in analyses restricted to premenopausal or postmenopausal women (data not shown).

In unadjusted and age-adjusted logistic regression analyses, associations between serum VEGF and breast cancer were not significant (Table 4). In a model adjusted for age, Gail score, education, physical activity, history of breastfeeding, serum T, and HT use, continuous VEGF was non-significantly positively associated with breast cancer (OR 1.21 per unit increase in $\ln(\text{VEGF})$, 95% CI 0.91 – 1.59). Figure 1 illustrates this association by displaying odds ratios for specific levels of VEGF calculated from the continuous logistic regression model. For example, the odds of breast cancer were increased by 16% for a

woman with a serum VEGF level of 691.4 pg/mL compared to a similar woman with a VEGF level of 291.4 pg/mL, albeit non-significantly.

In the comparable multivariable regression model with dichotomous VEGF, women with VEGF levels ≥ 314.2 pg/mL had a non-significant 37% increase in the odds of breast cancer (OR 1.37, 95% CI 0.88 – 2.12), compared to women with VEGF < 314.2 pg/mL (Figure 1). Results were similar in a regression including cases with invasive cancer only (OR 1.41, 95% CI 0.88 – 2.27; $p=0.16$). Further, results were attenuated but remained non-significant in a regression excluding six cases reporting tamoxifen use at enrollment (OR 1.17, 95% CI 0.89 – 1.55). The magnitude of the association between dichotomous VEGF and breast cancer was similar among premenopausal (OR 1.40, 95% CI 0.64 – 3.07) and postmenopausal women (OR 1.28, 95% CI 0.74 – 2.22). There was no indication of an interaction between VEGF and menopausal status ($p=0.52$). Additionally, no significant interactions were observed between VEGF dichotomized at the control median and Gail score ($p=0.41$), E2 ($p=0.62$), or T ($p=0.88$).

In exploratory analyses we investigated whether HT use affected the association between VEGF and breast cancer (Table 4). The logistic regression models were refit within subgroups defined by HT use (never, past, and current users). In multivariable models with dichotomous VEGF, no association between VEGF and breast cancer was observed among never HT users (OR 1.23, 95% CI 0.68 – 2.22) or current HT users (OR 1.09, 95% CI 0.31 – 3.87). VEGF was positively ($p=0.08$) associated with breast cancer among past HT users (OR 2.28, 95% CI 0.86 – 5.68). The interaction between VEGF and HT use status was not statistically significant ($p=0.45$) in this model.

We identified four influential observations (3 controls and 1 case) in the multivariable regression with dichotomous VEGF. In a sensitivity analysis excluding these four observations, the increase in breast cancer risk for women with VEGF greater than the median was similar to that observed in the full population and remained non-statistically significant (OR 1.45, 95% CI 0.92 – 2.27, $p=0.11$).

DISCUSSION

We found that increased serum VEGF levels were positively, but not significantly, associated with breast cancer in this case-control study. A woman with a serum VEGF level greater than the control median of 314.2 pg/mL, for example, had 37% higher odds of breast cancer compared to an otherwise similar woman with a serum VEGF level below 314.2 pg/mL ($p=0.16$). Among premenopausal women the strength of the association was somewhat greater (OR 1.40, 95% CI 0.64 – 3.07) than that among postmenopausal women (OR 1.28, 95% CI 0.74 – 2.22) though neither association achieved statistical significance. A formal test for interaction revealed that the association between VEGF and breast cancer was not significantly different between pre- and postmenopausal women ($p=0.52$). Due to limited power to detect significance of such an interaction, future studies should examine whether the association between VEGF and breast cancer might be greater among premenopausal women.

The role of VEGF in angiogenesis and carcinogenesis is complex and likely involves interaction with multiple pathways. Of particular interest to breast cancer development is the possibility that hormones may regulate VEGF. Numerous *in vitro* studies report that estrogen increases VEGF mRNA and/or protein expression by breast cancer cells [26–31], although some do not support this finding [38, 39]. The selective estrogen receptor modulator tamoxifen may decrease VEGF expression [27, 28, 40], though this finding is not consistent [30, 39]. Many studies report that progestin exposure increases VEGF expression

[38, 41, 42], though at least one found no effect [43]. Both natural and synthetic progestins are able to increase VEGF expression *in vitro*, and the synthetic progestin medroxyprogesterone acetate (MPA) is reported to have the strongest effect on VEGF expression [38, 41]. MPA is commonly used in postmenopausal HT, and it has been suggested that the strong effect of MPA on VEGF expression may at least partially explain the increased breast cancer risk observed in women using combination estrogen and progestin preparations versus those using estrogen alone [41].

Few human studies have investigated associations between hormones and VEGF in relation to breast cancer risk. A small study reported high correlations between VEGF in breast tissue and plasma E2 ($r=0.81$, $p<0.0001$) and between VEGF and E2 both measured in breast tissue ($r=0.67$, $p=0.004$) [44]. We observed no significant association between serum VEGF and serum E2 among healthy controls, though women with VEGF levels 314.2 pg/mL were somewhat more likely to be in the lowest quartile of E2. Differences in the study populations and in the medium in which VEGF and E2 were measured preclude a direct comparison between these studies and may explain the discrepant results. We also observed a stronger, positive association between VEGF and breast cancer among past HT users. This association was not apparent among the small number of current HT users, and a formal test for an interaction between VEGF and HT use was not significant. Though exploratory, our results provide little evidence that steroid hormones are associated with VEGF.

Our results are in contrast to previous reports finding positive and statistically significant associations between serum or plasma VEGF and breast cancer [13-25, 45]. For example, Heer et al. [20] reported that in a sample of 196 incident breast cancer cases and 88 healthy controls, the median serum VEGF level was higher in cases (305.9 pg/mL) than in controls (167.5 pg/mL; $p<0.0005$). Though our results agree with prior reports in terms of the direction of the association, we did not find such associations to be statistically significant. These previous studies, however, only performed comparisons of mean or median VEGF levels between cases and controls, and did not examine associations between VEGF and breast cancer in a multivariable context as we have done.

Further, there are important differences in our study population as compared to previous reports. Previous studies all included fewer than 100 healthy female controls, and most failed to describe how such controls were selected [13, 14, 18-20, 24]. Control participants in MAMS were recruited from healthy women seeking a screening mammogram. Our study population consisted of pre- and postmenopausal women ranging in age from 35 to 84. Many previous studies have failed to adequately describe the age [13, 16, 20, 22-25] or menopausal status [13-17, 19-21, 23-25, 45, 46] of their study populations, making a direct comparison to the results observed in our well-characterized population difficult. The control VEGF distribution in our population differed substantially from previous reports. The arithmetic mean serum VEGF among controls was 387.5 pg/mL in our population, but ranged from 77.0 pg/mL to 230.0 pg/mL in other studies [13, 14, 23, 24]. Similarly, the median serum VEGF among our controls was 314.2 pg/mL compared to medians ranging from 17.0 pg/mL to 261.1 pg/mL reported in other studies [17-21, 47]. This may reflect the higher sensitivity of the assay used here, the influence of a selection bias within our study, or may indicate that serum VEGF levels among healthy women are higher and more variable than previously thought.

An important limitation to our study is the low statistical power. Although we estimated *a priori* that we would have greater than 80% power to detect a difference of 60 pg/mL in mean serum VEGF between cases and controls, the variability of VEGF in our study population far exceeded that observed in the study used as a basis for power calculations [13]. In fact, *a posteriori* power calculations showed that we had only 24% power to detect a

difference between the mean values of serum VEGF we observed among cases and controls, which limited our ability to detect true differences in VEGF these groups.

The measurement of VEGF in serum rather than plasma may also limit our study. It has been demonstrated that platelets release VEGF into serum during routine specimen processing [13, 47-51] and that serum VEGF levels are highly correlated with the platelet count [18, 52]. For these reasons, many investigators have stated that plasma is the preferred specimen for measuring circulating VEGF [49-51]. Serum and plasma VEGF levels have been found to correlate significantly with one another in some studies [18, 53], however, and some investigators still regard serum as the appropriate specimen for circulating VEGF measurements [54]. In our study, blood samples were processed identically for both cases and controls, so any effects of time between collection and processing or of our handling and storage methods which might affect release of VEGF from platelets into the serum should have affected cases and controls equally. We found that VEGF levels among controls did not vary by recruitment site, though it might be reasonable to suspect that specimen handling procedures may have differed slightly by recruitment site. Additionally, a study by McDowell et al. demonstrated that samples from women with early breast cancer and from healthy female controls showed no difference in the amount of VEGF released into the serum per platelet [55], lending credibility to our results. Further, platelets have been demonstrated to be important to tumor growth, angiogenesis, and metastasis [54, 56]. Thus the VEGF released from platelets into serum might reflect bioavailable VEGF which could impact tumor development. Though it is possible that serum may not be the optimal specimen for measurement of circulating VEGF, serum VEGF does appear to be relevant to tumor biology.

The relevance of circulating VEGF to tumor VEGF has also been questioned. Some studies have reported no correlation between serum VEGF and VEGF in tumor tissue [19, 50, 57]. Others have suggested, however, that measuring extracellular tumor levels of VEGF is more relevant than measuring tissue levels due to posttranslational activation of VEGF [44]. In fact, one study did report a weak but positive correlation between serum VEGF and VEGF in breast tumor cytosols ($r=0.32$, $p<0.05$) but no correlation between serum VEGF and VEGF in the tumor tissue ($r=0.12$, $p>0.05$) [58]. The relationship between circulating and tumor VEGF warrants further investigation.

Other limitations include the case-control design, which precludes temporal inferences. However, case-control studies are an important step towards recommending prospective studies. VEGF levels were assessed at a single point in time. The observed levels may not be representative of a participant's usual levels and could reflect recent changes in general health or medication use. The extensive data collected in MAMS allowed for statistical control of these variables and reduced potential confounding. Only a small percentage of MAMS participants were non-white, so our results may not apply to women of other races or ethnicities. Further, MAMS participants were better educated and less likely to smoke as compared to women from Allegheny County overall [32, 59], indicating a possible volunteer bias.

Our results show that circulating VEGF may not distinguish breast cancer cases from controls. These results should be confirmed with additional epidemiological studies with control for confounders. Although we found no statistically significant differences in VEGF levels between breast cancer cases and controls, we did find that levels were higher among cases. VEGF has demonstrated importance in breast cancer development and in prognosis. Thus it remains important to investigate whether VEGF levels measured prospectively predict subsequent breast cancer development.

Acknowledgments

This work was supported in part by National Institutes of Health grants P20 CA103730, R25-CA57703, K07-CA80668 and R21-CA95113 to Dr. Modugno; by Department of Defense grant DAMD17-02-1-0553 to Dr. Modugno and by Pennsylvania Department of Health grant P2777693 to Dr. Modugno. Additional support was provided by funds received from the National Institutes of Health/National Center for Research Resources/General Clinical Research Center Grant MO1-RR000056 and by a small grant from the Department of Epidemiology, University of Pittsburgh to Dr. Reeves.

REFERENCES

1. American Cancer Society. Cancer Facts & Figures 2008. American Cancer Society, Inc; Atlanta: 2008.
2. Gail MH, Costantino JP, Bryant J, et al. Weighing the risks and benefits of tamoxifen treatment for preventing breast cancer. *J Natl Cancer Inst.* 1999; 91:1829–1846. [PubMed: 10547390]
3. Costantino JP, Gail MH, Pee D, et al. Validation studies for models projecting the risk of invasive and total breast cancer incidence. *J Natl Cancer Inst.* 1999; 91:1541–1548. [PubMed: 10491430]
4. Gail MH, Brinton LA, Byar DP, et al. Projecting individualized probabilities of developing breast cancer for white females who are being examined annually. *J Natl Cancer Inst.* 1989; 81:1879–1886. [PubMed: 2593165]
5. Rockhill B, Spiegelman D, Byrne C, Hunter DJ, Colditz GA. Validation of the Gail et al. model of breast cancer risk prediction and implications for chemoprevention. *J Natl Cancer Inst.* 2001; 93:358–366. [PubMed: 11238697]
6. Folkman J, Klagsbrun M. Angiogenic factors. *Science.* 1987; 235:442–447. [PubMed: 2432664]
7. Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med.* 1971; 285:1182–1186. [PubMed: 4938153]
8. Folkman J. What is the evidence that tumors are angiogenesis dependent? *J Natl Cancer Inst.* 1990; 82:4–6. [PubMed: 1688381]
9. Kushlinskii NE, Gershtein ES. Role of vascular endothelial growth factor during breast cancer. *Bull Exp Biol Med.* 2002; 133:521–528. [PubMed: 12447454]
10. Sledge GW Jr. Vascular endothelial growth factor in breast cancer: biologic and therapeutic aspects. *Semin Oncol.* 2002; 29:104–110. [PubMed: 12138404]
11. Ferrara N. The role of vascular endothelial growth factor in pathological angiogenesis. *Breast Cancer Res Treat.* 1995; 36:127–137. [PubMed: 8534862]
12. Stimpfl M, Tong D, Fasching B, et al. Vascular endothelial growth factor splice variants and their prognostic value in breast and ovarian cancer. *Clin Cancer Res.* 2002; 8:2253–2259. [PubMed: 12114428]
13. Benoy I, Salgado R, Colpaert C, Weytjens R, Vermeulen PB, Dirix LY. Serum interleukin 6, plasma VEGF, serum VEGF, and VEGF platelet load in breast cancer patients. *Clin Breast Cancer.* 2002; 2:311–315. [PubMed: 11899364]
14. Coskun U, Gunel N, Sancak B, et al. Significance of serum vascular endothelial growth factor, insulin-like growth factor-I levels and nitric oxide activity in breast cancer patients. *Breast.* 2003; 12:104–110. [PubMed: 14659339]
15. Hoar FJ, Lip GYH, Belgore F, Stonelake PS. Circulating levels of VEGF-A, VEGF-D and soluble VEGF-A receptor (sFlt-1) in human breast cancer. *Int J Biol Markers.* 2004; 19:229–235. [PubMed: 15503825]
16. Dirix LY, Vermeulen PB, Pawinski A, et al. Elevated levels of the angiogenic cytokines basic fibroblast growth factor and vascular endothelial growth factor in sera of cancer patients. *Br J Canc.* 1997; 76:238–243.
17. Salven P, Perhoniemi V, Tykka H, Maenpaa H, Joensuu H. Serum VEGF levels in women with a benign breast tumor or breast cancer. *Breast Cancer Res Treat.* 1999; 53:161–166. [PubMed: 10326793]
18. Adams J, Carder PJ, Downey S, et al. Vascular endothelial growth factor (VEGF) in breast cancer: comparison of plasma, serum, and tissue VEGF and microvessel density and effects of tamoxifen. *Canc Res.* 2000; 60:2898–2905.

19. Granato AM, Nanni O, Falcini F, et al. Basic fibroblast growth factor and vascular endothelial growth factor serum levels in breast cancer patients and healthy women: useful as diagnostic tools? *Breast Cancer Res.* 2004; 6:R38–45. [PubMed: 14680499]
20. Heer K, Kumar H, Read JR, Fox JN, Monson JR, Kerin MJ. Serum vascular endothelial growth factor in breast cancer: its relation with cancer type and estrogen receptor status. *Clin Cancer Res.* 2001; 7:3491–3494. [PubMed: 11705867]
21. Salven P, Manpaa H, Orpana A, Alitalo K, Joensuu H. Serum vascular endothelial growth factor is often elevated in disseminated cancer. *Clin Cancer Res.* 1997; 3:647–651. [PubMed: 9815732]
22. Wu Y, Saldana L, Chillar R, Vadgama JV. Plasma vascular endothelial growth factor is useful in assessing progression of breast cancer post surgery and during adjuvant treatment. *Int J Oncol.* 2002; 20:509–516. [PubMed: 11836562]
23. Yamamoto Y, Toi M, Kondo S, et al. Concentrations of vascular endothelial growth factor in the sera of normal controls and cancer patients. *Clin Cancer Res.* 1996; 2:821–826. [PubMed: 9816236]
24. Donovan D, Harmey JH, Toomey D, Osborne DH, Redmond HP, Bouchier-Hayes DJ. TGF beta-1 regulation of VEGF production by breast cancer cells. *Ann Surg Oncol.* 1997; 4:621–627. [PubMed: 9416408]
25. Zaman K, Driscoll R, Hahn D, et al. Monitoring multiple angiogenesis-related molecules in the blood of cancer patients shows a correlation between VEGF-A and MMP-9 levels before treatment and divergent changes after surgical vs. conservative therapy. *Int J Cancer.* 2006; 118:755–764. [PubMed: 16114015]
26. Buteau-Lozano H, Ancelin M, Lardeux B, Milanini J, Perrot-Applanat M. Transcriptional regulation of vascular endothelial growth factor by estradiol and tamoxifen in breast cancer cells: a complex interplay between estrogen receptors alpha and beta. *Canc Res.* 2002; 62:4977–4984.
27. Garvin S, Nilsson UW, Dabrosin C. Effects of oestradiol and tamoxifen on VEGF, soluble VEGFR-1, and VEGFR-2 in breast cancer and endothelial cells. *Br J Canc.* 2005; 93:1005–1010.
28. Lee JE, Chung K-W, Han W, et al. Effect of estrogen, tamoxifen and epidermal growth factor on the transcriptional regulation of vascular endothelial growth factor in breast cancer cells. *Anticancer Res.* 2004; 24:3961–3964. [PubMed: 15736439]
29. Maity A, Sall W, Koch CJ, Oprysko PR, Evans SM. Low pO₂ and beta-estradiol induce VEGF in MCF-7 and MCF-7-5C cells: relationship to in vivo hypoxia. *Breast Cancer Res Treat.* 2001; 67:51–60. [PubMed: 11518466]
30. Ruohola JK, Valve EM, Karkkainen MJ, Joukov V, Alitalo K, Harkonen PL. Vascular endothelial growth factors are differentially regulated by steroid hormones and antiestrogens in breast cancer cells. *Mol Cell Endocrinol.* 1999; 149:29–40. [PubMed: 10375015]
31. Takei H, Lee E-S, Jordan VC. In vitro regulation of vascular endothelial growth factor by estrogens and antiestrogens in estrogen-receptor positive breast cancer. *Breast Cancer.* 2002; 9:39–42. [PubMed: 12196720]
32. Reeves KW, Gierach GL, Modugno F. Recreational physical activity and mammographic breast density characteristics. *Cancer Epidemiol Biomarkers Prev.* 2007; 16:934–942. [PubMed: 17507619]
33. Quantikine Human VEGF Immunoassay. [cited December 27, 2006] Catalog number DVE00. Available from: <http://www.rndsystems.com/pdf/dve00.pdf>
34. England BG, Parsons GH, Possley RM, McConnell DS, Midgley AR. Ultrasensitive semiautomated chemiluminescent immunoassay for estradiol. *Clin Chem.* 2002; 48:1584–1586. [PubMed: 12194939]
35. Randolph JF Jr. Sowers M, Gold EB, et al. Reproductive hormones in the early menopausal transition: relationship to ethnicity, body size, and menopausal status. *J Clin Endocrinol Metab.* 2003; 88:1516–1522. [PubMed: 12679432]
36. Santoro N, Crawford SL, Allsworth JE, et al. Assessing menstrual cycles with urinary hormone assays. *Am J Physiol Endocrinol Metab.* 2003; 284:E521–530. [PubMed: 12441312]
37. Sowers MF, Beebe JL, McConnell D, Randolph J, Jannausch M. Testosterone concentrations in women aged 25–50 years: associations with lifestyle, body composition, and ovarian status. *Am J Epidemiol.* 2001; 153:256–264. [PubMed: 11157413]

38. Mirkin S, Wong BC, Archer DF. Effect of 17 beta-estradiol, progesterone, synthetic progestins, tibolone, and tibolone metabolites on vascular endothelial growth factor mRNA in breast cancer cells. *Fertil Steril*. 2005; 84:485–491. [PubMed: 16084894]
39. Bogin L, Degani H. Hormonal regulation of VEGF in orthotopic MCF7 human breast cancer. *Canc Res*. 2002; 62:1948–1951.
40. Garvin S, Dabrosin C. Tamoxifen inhibits secretion of vascular endothelial growth factor in breast cancer in vivo. *Canc Res*. 2003; 63:8742–8748.
41. Hyder SM, Chiappetta C, Stancel GM. Pharmacological and endogenous progestins induce vascular endothelial growth factor expression in human breast cancer cells. *Int J Cancer*. 2001; 92:469–473. [PubMed: 11304678]
42. Liang Y, Hyder SM. Proliferation of endothelial and tumor epithelial cells by progestin-induced vascular endothelial growth factor from human breast cancer cells: paracrine and autocrine effects. *Endocrinology*. 2005; 146:3632–3641. [PubMed: 15845615]
43. Scott PA, Gleadle JM, Bicknell R, Harris AL. Role of the hypoxia sensing system, acidity and reproductive hormones in the variability of vascular endothelial growth factor induction in human breast carcinoma cell lines. *Int J Cancer*. 1998; 75:706–712. [PubMed: 9495238]
44. Dabrosin C. Positive correlation between estradiol and vascular endothelial growth factor but not fibroblast growth factor-2 in normal human breast tissue in vivo. *Clin Cancer Res*. 2005; 11:8036–8041. [PubMed: 16299233]
45. Nishimura R, Nagao K, Miyayama H, et al. Higher plasma vascular endothelial growth factor levels correlate with menopause, overexpression of p53, and recurrence of breast cancer. *Breast Cancer*. 2003; 10:120–128. [PubMed: 12736564]
46. Teh SH, Hill ADK, Lee AWS, et al. Raised plasma endostatin levels correlate inversely with breast cancer angiogenesis. *J Surg Res*. 2004; 116:165–171. [PubMed: 14732364]
47. McIlhenny C, George WD, Doughty JC. A comparison of serum and plasma levels of vascular endothelial growth factor during the menstrual cycle in healthy female volunteers. *Br J Canc*. 2002; 86:1786–1789.
48. Kusumanto YH, Dam WA, Hospers GAP, Meijer C, Mulder NH. Platelets and granulocytes, in particular the neutrophils, form important compartments for circulating vascular endothelial growth factor. *Angiogenesis*. 2003; 6:283–287. [PubMed: 15166496]
49. Verheul HM, Hoekx K, Luyckx-de Bakker S, et al. Platelet: transporter of vascular endothelial growth factor. *Clin Cancer Res*. 1997; 3:2187–2190. [PubMed: 9815613]
50. Byrne GJ, McDowell G, Agarawal R, Sinha G, Kumar S, Bundred NJ. Serum vascular endothelial growth factor in breast cancer. *Anticancer Res*. 2007; 27:3481–3487. [PubMed: 17972505]
51. Banks RE, Forbes MA, Kinsey SE, et al. Release of the angiogenic cytokine vascular endothelial growth factor (VEGF) from platelets: significance for VEGF measurements and cancer biology. *Br J Cancer*. 1998; 77:956–964. [PubMed: 9528841]
52. Salgado R, Benoy I, Bogers J, et al. Platelets and vascular endothelial growth factor (VEGF): a morphological and functional study. *Angiogenesis*. 2001; 4:37–43. [PubMed: 11824377]
53. Bachelot T, Ray-Coquard I, Menetrier-Caux C, Rastkha M, Duc A, Blay JY. Prognostic value of serum levels of interleukin 6 and of serum and plasma levels of vascular endothelial growth factor in hormone-refractory metastatic breast cancer patients. *Br J Canc*. 2003; 88:1721–1726.
54. Lee JK, Hong YJ, Han CJ, Hwang DY, Hong SI. Clinical usefulness of serum and plasma vascular endothelial growth factor in cancer patients: which is the optimal specimen? *Int J Oncol*. 2000; 17:149–152. [PubMed: 10853032]
55. McDowell G, Temple I, Li C, et al. Alteration in platelet function in patients with early breast cancer. *Anticancer Res*. 2005; 25:3963–3966. [PubMed: 16309184]
56. Trikha M, Nakada MT. Platelets and cancer: implications for antiangiogenic therapy. *Semin Thromb Hemost*. 2002; 28:39–44. [PubMed: 11885024]
57. Lantzsch T, Hefler L, Krause U, et al. The correlation between immunohistochemically-detected markers of angiogenesis and serum vascular endothelial growth factor in patients with breast cancer. *Anticancer Res*. 2002; 22:1925–1928. [PubMed: 12168895]

58. Gershtein ES, Shcherbakov AM, Alieva SK, Anurova OA, Laktionov KP, Kushlinskii NE. Vascular endothelial growth factor in tumor tissue and blood serum from patients with breast cancer. *Bull Exp Biol Med.* 2003; 135:85–88. [PubMed: 12717522]
59. Centers for Disease Control and Prevention (CDC). Behavioral Risk Factor Surveillance System Survey Data: Selected Metropolitan/Micropolitan Area Risk Trends. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention; Atlanta, Georgia: 2005.

Figure 1. Estimated odds ratios and 95% confidence intervals for breast cancer by serum VEGF level estimated from a multivariable logistic regression model, N=402^{a,b}

^aOdds ratios are adjusted for age, Gail score, education, physical activity, history of breastfeeding, serum testosterone, and hormone therapy use

^bThe set of dark, solid bars on the left display odds ratios calculated for 200 pg/mL increments of serum VEGF relative to the control population geometric mean value of 291.4 pg/mL. The two lighter, hatched bars on the right display odds ratios for serum VEGF when dichotomized at the control population median of 314.2 pg/mL.

Table 1

Characteristics of the study population by breast cancer status, N=407

Characteristic	Cases N (%)	Controls N (%)	P value ^a
Age, years; mean (SD)	56.5 (10.3)	56.5 (10.1)	0.99
<50	56 (27.6)	56 (27.5)	0.56
50-59	73 (36.0)	81 (39.7)	
60-69	51 (25.1)	40 (19.6)	
70	23 (11.3)	27 (13.2)	
Ethnicity			0.02
White	198 (97.5)	189 (92.7)	
Other	5 (2.5)	15 (7.4)	
Education level			0.001
High school	66 (32.5)	37 (18.1)	
Greater than high school	137 (67.5)	167 (81.7)	
Body mass index, kg/m ² ; mean (SD)	27.8 (5.8)	27.9 (6.2)	0.84
Normal, <25 kg/m ²	76 (37.8)	76 (37.3)	0.99
Overweight, 25-<30 kg/m ²	64 (31.8)	66 (32.4)	
Obese, ≥30 kg/m ²	61 (30.4)	62 (30.4)	
Physical activity, MET h/wk			<0.001
0	54 (26.6)	20 (9.8)	
0.1 – <10	63 (31.0)	72 (35.3)	
10	86 (42.4)	112 (54.9)	
Age at menarche, years			0.99
<13	105 (52.2)	106 (52.2)	
13	96 (47.8)	97 (47.8)	
Menopausal status			0.09
Premenopausal	66 (32.5)	66 (32.4)	
Postmenopausal without hysterectomy	82 (40.4)	100 (49.0)	
Postmenopausal with hysterectomy	55 (27.1)	38 (18.6)	
Age at menopause, years			0.12
Premenopausal	66 (33.5)	66 (32.8)	
<50	70 (35.5)	55 (27.4)	
50	61 (31.0)	80 (39.8)	
Number of live births			0.36
None	41 (20.2)	48 (23.5)	
1	19 (9.4)	28 (13.7)	
2	73 (36.0)	68 (33.3)	
3	70 (34.5)	60 (29.4)	
History of breastfeeding			0.05
Not applicable	41 (20.3)	48 (23.5)	
No	88 (43.6)	65 (31.9)	
Yes	73 (36.1)	91 (44.6)	

Characteristic	Cases N (%)	Controls N (%)	P value ^a
Previous breast biopsy	51 (25.5)	25 (12.3)	0.001
First degree relative with breast cancer	39 (19.2)	28 (13.8)	0.14
Gail score; mean (SD)	1.71 (1.08)	1.49 (0.67)	0.02 ^b
< 1.66%	126 (62.4)	140 (69.0)	0.16
1.66%	76 (37.6)	63 (31.0)	
Hormone therapy use status			<0.001
Never	108 (53.7)	115 (56.4)	
Former	45 (22.4)	69 (33.8)	
Current (within previous 3 months)	48 (23.9)	20 (9.8)	
Oral contraceptive use status			0.44
Never	64 (34.0)	66 (34.6)	
Former	121 (64.4)	118 (61.8)	
Current	3 (1.6)	7 (3.7)	

^aP values from t tests for continuous variables and chi square tests for categorical variables

^bFrom t test with unequal variances

Abbreviations used: SD, standard deviation; MET, metabolic equivalent

Table 2

Summary statistics of serum levels of vascular endothelial growth factor (VEGF), estradiol (E2), follicle stimulating hormone (FSH), sex hormone binding globulin (SHBG), and testosterone (T) in the study population by breast cancer status, N=407^a

	N	Mean	SD	Geometric Mean	Median	25 th – 75 th Percentiles
<i>Cases</i>						
VEGF, pg/mL	202 ^b	415.7	287.8	321.4	341.7	190.8 – 579.4
E2, pg/mL	203	36.8	56.2	15.5	13.7	6.9 – 38.7
FSH, mIU/mL	203	97.3	66.7	62.9	101.0	30.7 – 138.0
SHBG, nM	203	62.9	45.0	51.6	49.2	34.4 – 73.4
T, ng/dL	203	38.0	22.0	32.8	34.1	24.6 – 44.9
<i>Controls</i>						
VEGF, pg/mL	204	387.5	293.3	291.4	314.2	180.5 – 510.9
E2, pg/mL	204	44.5	82.8	12.6	11.2	4.1 – 40.9
FSH, mIU/mL	204	92.4	66.2	55.5	88.8	31.4 – 138.9
SHBG, nM	204	58.2	33.8	49.3	51.6	35.3 – 73.1
T, ng/dL	204	32.9	18.9	28.1	29.2	18.4 – 42.2

^aP values from t tests comparing cases to controls on natural log transformed values: VEGF, p=0.21; E2, p=0.18; FSH, p=0.31; SHBG, p=0.45; T, p=0.01

^bVEGF could not be measured in one case due to insufficient sample volume

Abbreviations used: VEGF, vascular endothelial growth factor; E2, estradiol; FSH, follicle stimulating hormone; SHBG, sex hormone binding globulin; T, testosterone

Table 3

Bivariate associations between serum VEGF level and personal characteristics among controls, by VEGF level, N=204

Characteristic	VEGF <314.2 pg/mL N (%)	VEGF ≥ 314.2 pg/mL N (%)	P value ^a
Age, years			0.96
<50	29 (28.4)	27 (26.5)	
50-59	39 (38.2)	42 (41.2)	
60-69	21 (20.6)	19 (18.6)	
70	13 (12.8)	14 (13.8)	
Ethnicity			0.79
White	95 (93.1)	94 (92.2)	
Other	7 (6.9)	8 (7.8)	
Education level			0.20
High school	22 (21.6)	15 (14.7)	
Greater than high school	80 (78.4)	87 (85.3)	
Body mass index, kg/m ²			0.20
Normal, <25 kg/m ²	35 (34.3)	41 (40.2)	
Overweight, 25-<30 kg/m ²	39 (38.2)	27 (26.5)	
Obese, ≥ 30 kg/m ²	28 (27.5)	34 (33.3)	
Physical activity, MET h/wk			0.49
0	9 (8.8)	11 (10.8)	
0.1 – <10	40 (39.2)	32 (31.4)	
10	53 (52.0)	59 (57.8)	
Age at menarche, years			0.63
<13	55 (53.9)	51 (50.5)	
13	47 (46.1)	50 (49.5)	
Menopausal status			0.67
Premenopausal	36 (35.3)	30 (29.4)	
Postmenopausal without hysterectomy	48 (47.1)	52 (51.0)	
Postmenopausal with hysterectomy	18 (17.7)	20 (19.6)	
Age at menopause, years			0.49
Premenopausal	36 (36.0)	30 (29.7)	
<50	24 (24.0)	31 (30.7)	
50	40 (40.0)	40 (39.6)	
Number of live births			0.65
None	27 (26.5)	21 (20.6)	
1	12 (11.8)	16 (15.7)	
2	35 (34.3)	33 (32.4)	
3	28 (27.5)	32 (31.4)	
History of breastfeeding			0.19
Not applicable	27 (26.5)	21 (20.6)	
No	36 (35.3)	29 (28.4)	

Characteristic	VEGF <314.2 pg/mL N (%)	VEGF ≥314.2 pg/mL N (%)	P value ^a
Yes	39 (38.2)	52 (51.0)	
Previous breast biopsy			0.83
No	90 (88.2)	89 (87.3)	
Yes	12 (11.8)	13 (12.8)	
First degree relative with breast cancer			0.43
No	86 (84.3)	89 (88.1)	
Yes	16 (15.7)	12 (11.9)	
Gail score			0.42
<1.66%	73 (71.6)	67 (66.3)	
1.66%	29 (28.4)	34 (33.7)	
Hormone therapy use status			0.64
Never	59 (57.8)	56 (54.9)	
Former	35 (34.3)	34 (33.3)	
Current (within previous 3 months)	8 (7.8)	12 (11.8)	
Oral contraceptive use status			0.14 ^b
Never	36 (36.7)	30 (32.3)	
Former	61 (62.2)	57 (61.3)	
Current	1 (1.0)	6 (6.5)	
Serum E2 level, pg/mL			0.33
0.0 – <4.1	21 (20.6)	31 (30.4)	
4.1 – <11.2	29 (28.4)	21 (20.6)	
11.2 – <40.9	25 (24.5)	26 (25.5)	
40.9	27 (26.5)	24 (23.5)	
Serum FSH level, mIU/mL			0.04
0.3 – 31.4	20 (19.6)	31 (30.4)	
31.4 – <88.8	33 (32.4)	19 (18.6)	
88.8 – <138.9	28 (27.5)	22 (21.6)	
138.9	21 (20.6)	30 (29.4)	
Serum SHBG level, nM			0.94
1.95 – 35.3	26 (25.5)	25 (24.5)	
35.3 – 51.6	25 (24.5)	26 (25.5)	
51.6 – 73.1	27 (26.5)	24 (23.5)	
73.1	24 (23.5)	27 (26.5)	
Serum T level, ng/dL			0.17
5.2 – <18.4	21 (20.6)	30 (29.4)	
18.4 – <29.2	24 (23.5)	27 (26.5)	
29.2 – <42.2	32 (31.4)	19 (18.6)	
42.2	25 (24.5)	26 (25.5)	

^aP values from chi-square test

^bP value from Fisher's exact test

Abbreviations used: VEGF, vascular endothelial growth factor; MET, metabolic equivalent, E2, estradiol; FSH, follicle stimulating hormone; SHBG, sex hormone binding globulin; T, testosterone

Table 4

Estimated odds ratios of breast cancer by serum VEGF level in unadjusted, age adjusted, and multivariable adjusted logistic regression models^a

	Unadjusted			Age adjusted			Multivariable adjusted ^b		
	N	OR (95% CI)	P Value	N	OR (95% CI)	P Value	N	OR (95% CI)	P Value
<i>Total sample</i>									
Continuous VEGF ^c	406	1.17 (0.91 – 1.50)	0.22	406	1.17 (0.91 – 1.50)	0.21	402	1.21 (0.91 – 1.59)	0.19
Categorical VEGF, split at median	406	1.22 (0.83 – 1.80)	0.32	406	1.22 (0.83 – 1.80)	0.32	402	1.37 (0.88 – 2.12)	0.16
<i>Menopausal status</i>									
Premenopausal	132	1.44 (0.73 – 2.86)	0.30	132	1.46 (0.73 – 2.91)	0.29	131	1.40 (0.64 – 3.07)	0.40
Postmenopausal	274	1.13 (0.70 – 1.81)	0.62	274	1.13 (0.70 – 1.82)	0.62	271	1.28 (0.74 – 2.22)	0.38
<i>Hormone therapy use</i>									
Never user	223	1.13 (0.67 – 1.92)	0.64	223	1.12 (0.66 – 1.90)	0.66	223	1.23 (0.68 – 2.22)	0.50
Past user	114	1.70 (0.79 – 3.65)	0.18	114	1.74 (0.81 – 3.77)	0.16	112	2.28 (0.86 – 5.68)	0.08
Current user (within previous 3 months)	67	0.83 (0.28 – 2.39)	0.72	67	0.84 (0.29 – 2.43)	0.74	67	1.09 (0.31 – 3.87)	0.90

^a Odds ratios comparing individuals with VEGF ≥ 314.2 pg/mL to those with VEGF < 314.2 pg/mL unless otherwise specified

^b Adjusted for age, Gail score, education, physical activity, history of breastfeeding, serum testosterone, HT use

^c Odds ratios in this row are for a 1 unit increase in ln(VEGF) beyond the control population mean of 5.67 (219.4 pg/mL in the observed scale)

Abbreviations used: OR, odds ratio; CI, confidence interval