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Breast Cancer

Monitoring Serum VEGF in Neoadjuvant Chemotherapy for Patients with Triple-Negative Breast Cancer: A New Strategy for Early Prediction of Treatment Response and Patient Survival

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Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. Triple-negative breast cancer • Neoadjuvant chemotherapy • VEGF • Pathological response

Abstract

Background. This study aimed to investigate the clinical utility of serum biomarker changes during neoadjuvant chemotherapy (NAC) for triple-negative breast cancer (TNBC).

Methods. A total of 303 patients with TNBC were included in this study. Serum samples were taken at three time points during NAC: baseline, prior to the third cycle, and prior to surgery. Luminex multibiomarker panel for 29 serum biomarkers was used to detect their correlation with NAC response. The predictive and prognostic value of each selected biomarker was then studied.

Results. Vascular endothelial growth factor (VEGF) was the only biomarker that correlated with treatment response, with a decreasing trend in pCR patients relative to non-pCR patients ($p < .001$). Univariable and multivariable analyses revealed that the relative change in VEGF prior to the third cycle of NAC had a remarkable predictive value for both pCR and pathological nonresponse with high sensitivity and specificity. VEGF was also independently correlated with disease-free survival.

Conclusion. Our findings indicate that monitoring serum VEGF could help identify patients with different responses at an early time point of NAC and at varying risk of disease relapse. Serum VEGF may also serve as an alternative to traditional response-evaluating methodologies in tailoring and modifying the NAC strategy for both operable and advanced TNBCs. ***The Oncologist*** 2018;23:1–9

Implications for Practice: Neoadjuvant chemotherapy (NAC) followed by definitive surgery is a standard of care for locally advanced breast cancer. The identification of sensitive responders to neoadjuvant therapy is highly significant for breast cancer, especially triple-negative breast cancer (TNBC). Results of this study indicate that the monitoring of serum vascular endothelial growth factor (VEGF) could identify patients with favorable or poor responses at an early time point of NAC. Furthermore, the prediction power of VEGF was better than traditional response-evaluating methods. VEGF might serve as a complement or alternative to traditional imaging-based response-evaluating methodologies in tailoring systemic treatment strategies for both operable and advanced TNBCs.

Introduction

Neoadjuvant chemotherapy (NAC) followed by definitive surgery is a standard of care for locally advanced breast cancer and an option for early-stage breast cancer to increase the chance of breast-conserving surgery. The use of NAC has also provided insight into tumor biology and differential responses to treatment. The main goal of NAC is to achieve pathological complete response (pCR), because most patients with pCR have favorable outcomes [1, 2], especially those with triple-negative breast cancer (TNBC). TNBC, which lacks the expression of estrogen receptor (ER), progesterone receptor (PgR), and human epidermal growth factor receptor-2 (HER2), accounts for 15%–20% of all breast cancers and has an aggressive tumor biology [3]. Compared with other breast cancer subtypes, TNBC

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has a higher response rate to NAC; however, this advantage is not clearly translated into an improved overall survival [4]. This "TNBC paradox" indicates that TNBC is a heterogeneous disease comprising subtypes with different biological behaviors and clinical outcomes. Thus, a more specific NAC strategy combining effective regimens and clinically relevant models for TNBC individuals is needed.

With numerous neoadjuvant therapy regimens available and many more treatments emerging, the identification of sensitive responders to NAC is highly significant. Numerous biomarkers (e.g., tumor size, node status, Ki-67, HER2) and imaging-based metrics (e.g., magnetic resonance imaging [MRI] and positron emission tomography) have been investigated for the prediction of pCR [5, 6]; however, most efforts with traditional biomarkers measured prior to chemotherapy lack accuracy, and most efforts focusing on monitoring changes in morphological characteristics are indicative only of a late-stage response [7–10]. Therefore, a growing number of investigators have begun to focus on serum markers that may predict response to therapy. These biomarkers include cancer antigens, inflammatory factors, and growth factors; are easily measured at different time points compared with traditional pathological biomarkers; and are better indicators of change in biological nature compared with imaging-based metrics. However, few successful efforts have been made. A small sample study containing 44 patients established a predictive model based on a multibiomarker panel to differentiate between treatment response groups with high sensitivity and specificity [11], indicating the potential of utilizing serum biomarkers in a neoadjuvant setting.

Platinum agents, such as cisplatin and carboplatin, are DNA-damaging agents that are active in breast cancer [12]. The use of platinum agents, in addition to standard NAC, has potential advantages, particularly in the TNBC subgroup of breast cancer. In recent years, NAC with carboplatin and taxane has proven to be well tolerated and yielded favorable responses in both *BRCA*-associated and wild-type TNBC, with an overall pCR rate of 55% [13]. Thus, there is a clear need to develop and apply a model of early response evaluation referring to this specific treatment regimen. In this study, we aimed to investigate the clinical utility of serum biomarker changes during neoadjuvant paclitaxel plus carboplatin for TNBCs using a multibiomarker panel and to identify a predictor of treatment response at an early time point.

Subjects, Materials, and Methods

Study Population

Patients in this study were retrospectively selected according to inclusion and exclusion criteria reported previously [14]. Women with large operable (primary invasive tumor T3) or local advanced breast cancer were candidates, whereas male breast cancer, stage IV breast cancer, and inflammatory breast cancer were not included. All patients were confirmed with triple-negative invasive breast cancer by core needle biopsy and were treated with weekly paclitaxel plus carboplatin (PC) followed by surgical resection at Shanghai Cancer Hospital between January 2009 and July 2015. TNBC was defined as invasive breast cancer that was ER negative, PgR negative, and HER2 negative (HER2−). The cutoff values for ER positivity and PR positivity were 1% of positive tumor cells with nuclear staining. HER2 was evaluated as 0, 1+, 2+, or 3+ using circumferential membrane-bound staining. Positivity for HER2 was considered as 3+ using immunohistochemistry (IHC) or as positive on fluorescence in situ hybridization (FISH), whereas cases with 0 to 1+ or 2+ using IHC but without FISH detection were regarded as HER2−.

The NAC regimen was paclitaxel (80 mg/m^2) and carboplatin (area under the curva [AUC], 2 mg × min/mL) on days 1, 8, and 15 of a 28-day cycle for six cycles. No other anticancer treatments, including chemotherapy, radiation therapy, or endocrine therapy, were permitted before surgery. Following neoadjuvant therapy, patients received the appropriate surgical removal of their primary breast tumor and axillary lymph node dissection. Subsequently, patients with pCR received two additional cycles of PC, whereas those who failed to reach pCR received three cycles of anthracycline-containing chemotherapy. The use of radiation therapy was at the discretion of the treating radiologist and was based on disease status before NAC.

Response Evaluation

Clinical response was evaluated based on MRI examinations after two NAC cycles in accordance with RECIST version 1.1 [15]. Clinical complete response (cCR) was defined as no clinical evidence of tumor in the breast and lymph nodes. Partial response (cPR) was defined as a greater than 30% reduction in the greatest tumor diameter. A reduction of less than 30% or an increase of up to 20% in the greatest tumor diameter was regarded as clinical stable disease (cSD), whereas an increase of more than 20% in the greatest tumor diameter or the appearance of new disease was regarded as clinical progressive disease (cPD). Pathological response was based on the Miller-Payne (MP) grading system [16]. pCR was defined as no residual invasive cancer in either the breast or lymph nodes. Patients with ductal carcinoma in situ only were also considered pCR responders. We also defined pathological nonresponse (pNR) as MP 1 and 2 disease in the breast.

Serum Samples and Immunoassay

Serum samples were obtained prior to the start of NAC, prior to the third cycle of NAC, and prior to surgery. Blood was drawn using standard phlebotomy procedures and was collected without anticoagulant. Blood was allowed to coagulate for up to 2 hours at room temperature. Sera were separated by centrifugation, immediately aliquoted, frozen, and stored at −80°C. No more than two freeze-thaw cycles were allowed for any sample. Cytokine profiles were determined using the Human Cytokine/Chemokine Magnetic Bead Panel protocol from the MILLIPLEX Map Kit

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(HCYTOMAG-60K-PX29, Billerica, MA). The concentrations of interleukin-10 (IL-10), IL-12P40, IL-12P70, IL-13, IL-15, IL-17A, IL-1RA, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, interferon-γ (IFN-γ), IFN-α2, eotaxin, macrophage chemoattractant protein-1, epidermal growth factor, tumor necrosis factor-α (TNF-α), TGF-β, vascular endothelial growth factor (VEGF), interferon-induced protein 10, granulocyte macrophage colony-stimulating factor, granulocyte colony-stimulating factor, macrophage inflammatory protein-1α (MIP-1α), and MIP-1β were determined using standard procedures recommended by the manufacturer. Plates were run on the MagPix machine (Luminex, Austin, TX), and data were collected using Luminex xPONENT software (version 5.1). Median fluorescent intensity data were analyzed using a five-parameter logistic or spline curve-fitting method to calculate cytokine/chemokine concentrations in samples. The intra-assay variability was 1.6%–7.2%. The VEGF level in each sample in the validation set was measured by enzyme-linked immunosorbent assay (ELISA) using a standard protocol. Serum samples were centrifuged at 15,000 × g for 5 minutes to remove precipitate and subsequently loaded onto SearchLight plates (LightArray Biomarker Systems, Wuxi, People's Republic of China) with standard protein controls. Samples and standards were incubated at room temperature for 1 hour shaking at 950 rpm. Plates were washed three times using a plate washer (BioTek Instruments, Inc., Winooski, VT), biotinylated secondary antibody was added, and plates were incubated for 30 minutes. After washes, streptavidin-HRP was added, plates were incubated for 60 minutes and washed again, and TMB soluble substrate was added. Images were taken within 5 minutes. All data represent the average of triplicate measures. The intra-assay variability was 9.4%. The antibody was purchased from Abcam (ab32152, Cambridge, MA).

Statistical Analysis

Analysis of variance with replicate measures was used to evaluate the significance of alterations in serum biomarker levels at separate treatment time points between treatment response groups. The *p* value was adjusted by Bonferroni correction. The chi-squared test was used to evaluate the relationships between patient characteristics and pathological response. Multivariable analyses for predicting treatment response were performed using a logistic regression model with the forward selection of variables. Disease-free survival (DFS) was calculated from the date of surgery to the date of disease relapse (local or distant relapse or death from any cause). Patients without events or death were censored at the last follow-up. Uni- and multivariable survival analyses were performed using the Cox regression model. Survival curves were estimated using the Kaplan-Meier method, and the log-rank test was used to test for differences between groups. All statistical tests were two-sided, and *p* values less than 0.05 were considered significant. All analyses were performed with SPSS (version 19.0, SPSS Company, Chicago, IL).

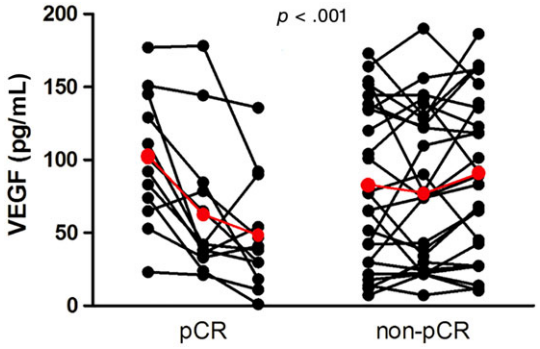

Figure 1. Correlation of VEGF alteration and neoadjuvant chemotherapy (NAC) response in 37 patients of the "filter set." The time points of measuring VEGF level are prior to NAC, prior to the 3rd cycle of NAC, and prior to surgery. A decreasing trend of VEGF level is observed in pCR patients relative to non-pCR patients ($p < .001$). Mean values of VEGF level at different time points are shown as red dots.
Abbreviations: pCR, pathological complete response; VEGF, vascular endothelial growth factor.

Results

Multiplex Analysis of Serum Levels

In total, 37 patients with TNBC were randomly selected as the "filter set" for the multiplex analysis of serum levels. Twelve of the 37 patients demonstrated pCR. Serum samples were analyzed using a 29-biomarker panel at three time points: prior to the start of NAC, prior to the third cycle of NAC, and prior to surgery. The biomarkers included cancer antigens, growth/antigenic factors, metastasis-related molecules, adhesion molecules, cytokines, and other proteins. Biomarker levels were compared at each treatment time point between pCR responders and nonresponders. Among all the biomarkers examined, only VEGF correlated with treatment response, with a decreasing trend in pCR patients relative to non-pCR patients (adjusted $p < .001$; Fig. 1). The mean level of VEGF in the pCR group before, during, and after NAC was 100.31 pg/mL, 65.58 pg/mL, and 48.95 pg/mL, respectively, whereas the mean level of VEGF in the non-pCR group before, during, and after NAC remained stable (82.91, 81.53, and 89.36 pg/mL, respectively). However, we did not detect any significant correlation between serum levels at any time point and pathologic response in other biomarkers.

VEGF Level in the Validation Set

To verify the clinical value of VEGF, we built a validation set including a total of 303 patients with TNBC. Similar to the filter set, serum VEGF was also measured at three different time points. The median VEGF level prior to the start of NAC, prior to the third cycle of NAC, and prior to surgery was 96.80 pg/mL, 96.45 pg/mL, and 90.00 pg/mL, respectively. The change in VEGF was calculated as relative change at two time points: (a) prior to the 3rd cycle, $(VEGF^{baseline} - VEGF^{third\ cycle})/VEGF^{baseline} \times 100\%$, the median change was 1.1% (range, −195.5% to 75.2%); and (b) prior to surgery, $(VEGF^{baseline} - VEGF^{surgery})/VEGF^{baseline}$

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× 100%, the median change was 7.4% (range, −240.3% to 98.65%). The correlation between VEGF level and multiple patient characteristics was analyzed, and we found no significant differences in patient age, menopausal status, tumor size, node status, histology, and Ki-67 according to different VEGF level.

VEGF and Treatment Response

To enhance its value in predicting pCR in clinical practice, only the VEGF levels at baseline and prior to the third cycle were included in this assay. The value of the VEGF change prior to the third cycle was also examined. Correlations between pCR and other clinical and pathological variables, including patient age, menopausal status, primary tumor size, node status, stage, Ki-67 value, and clinical response (early response evaluated prior to the third cycle), were also determined (Table 1). The VEGF category in Table 1 was defined according to the tertile cutoff points. Of the 303 patients, 103 (34.0%) experienced pCR after completion of NAC. Table 1 shows the results of the chi-squared test and multivariable logistic regression analysis for pCR predictors. The relative change in VEGF independently correlated with pCR as a continuous variable ($p < .001$; odds ratio, 1.034; 95% confidence interval [CI], 1.022–1.047). Ki-67 expression at baseline and clinical response were also independent predictors of pCR ($p = .018$ and $p = .004$, respectively). We then generated receiver operating characteristic (ROC) curves to identify the efficacy of predictors (Fig. 2A). The AUC of VEGF change was the largest (AUC, 0.788; 95% CI, 0.732–0.845; $p < .001$) among all the variables examined.

Predictors of pNR were then studied using a similar procedure. Nonresponse was defined as a mild treatment response evaluated by the MP grading system (grade 1/2). In all, 53 patients (17.5%) were identified as nonresponders. Table 2 shows the results of univariable and multivariable analyses. VEGF change (odds ratio, 0.985; 95% CI, 0.974–0.996; $p < .001$), Ki-67 ($p = .004$), and clinical response ($p = .005$) independently correlated with pNR. ROC curves also showed that VEGF change was the best predictor, with an AUC of 0.862 (95% CI, 0.806–0.918; $p < .001$; Fig. 2B).

In summary, the relative change in VEGF prior to the third cycle of NAC showed a remarkable predictive value for both pCR and pNR. Using tertile VEGF values as cutoffs, patients could be categorized into three groups: VEGF-reduced (>13%), VEGF-stable (−10%~13%), and VEGF-elevated (less than −10%), as shown in Figure 3. Patients in the VEGF-reduced group would have a trebled possibility of achieving pCR relative to other patients (67.0%; 95% CI, 57.6%–76.4% versus 19.8%; 95% CI, 11.8%–27.8% of VEGF-stable and 15.7%; 95% CI, 8.5%–22.9% of VEGF-elevated), whereas patients in the VEGF-elevated group would have a high possibility of treatment failure relative to those with a stable or reduced level of VEGF (42.2%; 95% CI, 32.4%–51.9% versus 6.9%; 95% CI, 1.9%–12.1% of VEGF-stable and 3.0%; 95% CI, 0.0%–6.0% of VEGF-reduced). The Ki-67 level at baseline was also a valuable predictor of treatment response. However, early clinical response exhibited a relatively poor predictive value of both pCR and pNR to NAC.

VEGF and Patient Survival

The median follow-up time for all patients was 45 months. Among the 103 pCR patients, only 4 developed disease recurrence or metastasis. However, non-pCR responders had a relatively poor survival, with 65 (32.5%) cases of event or death.

Among the 200 non-pCR responders, univariable survival analysis was performed to assess the prognostic value of each variable. Change in VEGF was defined as a relative change in VEGF level prior to surgery. Residual tumor size ($p < .001$), residual node involvement ($p < .001$), vascular invasion ($p = .035$), residual tumor Ki-67 ($p = .001$), VEGF prior to surgery ($p < .001$), and VEGF change ($p = .010$) were significant predictors of DFS and were entered into the multivariable Cox regression model with forward selection. However, the VEGF level at baseline and prior to the third cycle did not correlate with DFS ($p = .650$ and $p = .309$, respectively). Patient age, menopausal status, primary tumor size, primary node status, primary Ki-67, and residual tumor grade were not significant variables. In the Cox model (Table 3), the VEGF level prior to surgery showed an independent prognostic value of DFS (hazard ratio, 1.008; 95% CI, 1.003–1.014; $p = .004$). Residual node involvement ($p = .002$) and Ki-67 value ($p = .010$) were also independent predictors of patient outcome. Better survival was more frequently observed in patients with a lower VEGF level prior to surgery, a lower Ki-67 value, and fewer involved nodes. The distributions of survival curves by categorical VEGF are shown in Figure 4 (log-rank test; $p < .001$). All patients were categorized into three groups according to the tertile level of VEGF prior to surgery. The observed 3-year DFS of the three groups was 85%, 63%, and 53%, respectively. Survival curves of VEGF at baseline and prior to the third cycle and VEGF change prior to the third cycle and prior to surgery are shown in supplemental online Figure 1.

Discussion

Pathological complete response following NAC implies the absence of residual invasive disease and strongly correlates with prolonged patient survival [1, 2]. Compared with other breast cancer subtypes, TNBC has a relatively higher possibility to achieve pCR; however, this advantage is not clearly translated into an improved overall survival because of the poor outcomes of non-pCR responders [4]. Therefore, the early identification of sensitive responders would have definitive value to base therapeutic decisions for TNBC. Because current prediction methods using measurements of clinical, pathological, and radiological responses lack necessary precision, we focused on serum biomarkers that are rarely investigated in this field. In this study, we screened multiserum biomarkers and identified VEGF as a new biomarker for pCR prediction. A significant decreasing trend in VEGF level was observed in pCR responders only;

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Table 1. Patient characteristics and observed pCR

Characteristics	Number of patients	Number of pCR (%)	Chi-squared test *p* value	Multivariable *p* value
Age, y			.564	NS
<40	60	23 (38.3)		
40–59	194	66 (34.0)		
60+	49	14 (28.6)		
Menopausal status			.817	NS
Premenopausal	159	55 (34.6)		
Postpenopausal	144	48 (33.3)		
Tumor size at baseline			.026	NS
T2	150	62 (41.3)		
T3	100	28 (28.0)		
T4	53	13 (24.5)		
Node status at baseline			.917	NS
–	52	18 (34.6)		
+	251	85 (33.9)		
Histology at baseline			.263	NS
Invasive ductal carcinoma	224	82 (36.6)		
Invasive (mixed) carcinoma	62	17 (27.4)		
Others	17	4 (23.5)		
Ki-67 expression at baseline			<.001	.018
<20%	107	22 (20.6)		
≥20%	196	81 (41.3)		
VEGF at baseline, pg/mL			.005	NS[a]
Low (<81.49)	103	23 (22.3)		
Intermediate (81.49–117.15)	101	37 (36.6)		
High (>117.15)	99	43 (43.4)		
VEGF prior to 3rd cycle, pg/mL			<.001	NS[a]
Low (<82.96)	100	41 (41.0)		
Intermediate (82.96–114.18)	102	44 (43.1)		
High (>114.18)	101	18 (17.8)		
VEGF change prior to the 3rd cycle			<.001	<.001[a]
Elevated (less than −10%)	102	16 (15.7)		
Stable (−10% to 13%)	101	20 (19.8)		
Reduced (>13%)	100	67 (67.0)		
VEGF at surgery			.003	NS[a]
Low (<56.80)	101	46 (45.5)		
Intermediate (56.80–118.17)	101	34 (33.7)		
High (>118.17)	101	23 (22.8)		
Clinical response at the third cycle			.172	.004
CR/PR	102	40 (39.2)		
SD/PD	201	63 (31.3)		

[a]VEGF was studied in the multivariable analysis as continuous variable.

Abbreviations: CR, complete response; NS, not significant; pCR, pathological complete response; PD, progressive disease; PR, partial response; SD, stable disease; VEGF, vascular endothelial growth factor.

however, the VEGF level remained relatively stable in non-responders. These findings suggest that changes in circulating VEGF levels may be a useful indicator of treatment response in the neoadjuvant setting.

Furthermore, if an accurate prediction of response/non-response can be made early in a patient's treatment, the regimen could be modified accordingly; this is known as the response-guided treatment strategy, which may avoid

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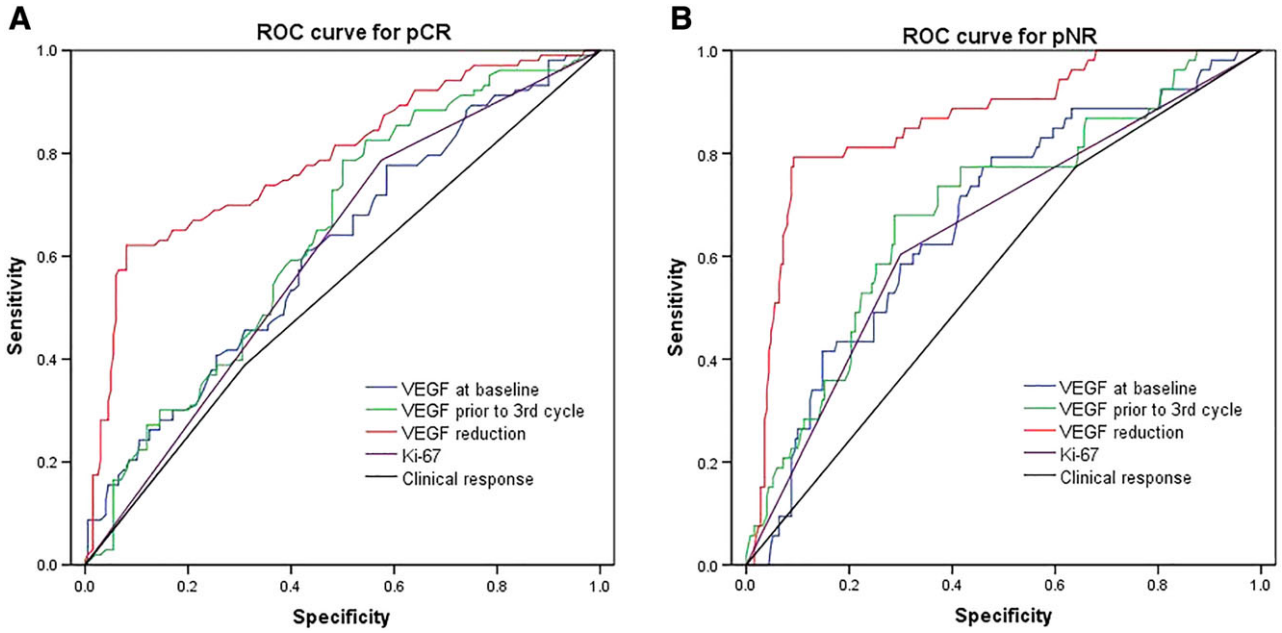

Figure 2. ROC curves for predictors of pCR and pNR. **(A):** The area under the curve (AUC) of VEGF change (red line) was 0.788 (95% confidence interval [CI], 0.732–0.845; $p < .001$) **(B):** The AUC of VEGF change (red line) was 0.862 (95% CI, 0.806–0.918; $p < .001$).
Abbreviations: pCR, pathological complete response; pNR, pathological nonresponse; ROC, receiver operating curve; VEGF, vascular endothelial growth factor.

unnecessary treatment-related toxicities and provide a better survival, regardless of pCR [17]. For predicted well-responders, additional NAC cycles with the same regimen are recommended; however, for predicted nonresponders, an alternative regimen may be necessary [17]. Because an early evaluation of treatment response is critical for modifying the NAC strategy, in the present study, we provide univariable and multivariable analyses of pCR responders with variables that can only be evaluated prior to or at an early course of NAC. The relative change in VEGF prior to the third cycle independently correlated with pCR as a continuous variable. It is also an independent predictor of nonresponse, which was defined as MP grade 1 and 2. Compared with Ki-67 and clinical response, ROC curves demonstrated that VEGF is a better response predictor. Our study also demonstrated that patients in the VEGF-reduced group would have a high possibility of achieving pCR relative to other patients (67.0% vs. 19.8% of VEGF-stable and 15.7% of VEGF-elevated), whereas patients in the VEGF-elevated group have a high possibility of treatment failure relative to those with a stable or reduced level of VEGF (42.2% vs. 6.9% of VEGF-stable and 3.0% of VEGF-reduced). Our findings also suggest that serum VEGF measurement may play a utility role in the response evaluation at any time point among the whole NAC period, considering its decreasing trend in patients with high chemosensitivity. Thus, early detection of a VEGF change may suggest an additional treatment course with the same regimen, whereas an elevated VEGF level is indicative of an alternative regimen or suspension.

Remarkably, this approach represents a significant departure from existing models of response monitoring by using imaging-based metrics, also known as clinical response evaluation. Although several studies have indicated that MRI is an effective tool for predicting the response to NAC [18], the accuracy was lower when pCR was more rigorously defined [18] and varies with tumor subtype [19]. More importantly, the clinical response often lacks accuracy in the early prediction of pathologic response to neoadjuvant therapy [20]. In our study, we found a relatively poor relationship between clinical response and pathological response, with an AUC of 0.539 for pCR and 0.567 for pNR. This result suggests that VEGF is a better alternative for imaging evaluation in response monitoring during NAC.

Previous studies have examined the value of biomarkers such as CA 15-3, MMP-2, MMP-9, EGFR, HER2, IL-6, and IL-8 in predicting the response to NAC for breast cancer [11, 21, 22]. However, the results of these investigations have been inconsistent. The multibiomarker panel examined in the present study included 29 common biomarkers. Although several have already been investigated, definite relationships have not yet been reported. Among these biomarkers, VEGF is one of the most potent angiogenic cytokines. It causes mitosis of endothelial cells and increases blood vessel permeability [23] and has been shown to have a good correlation with relapse-free survival in breast cancer [24]. Because of the significant effects of VEGF on tumor angiogenesis and vascular permeability, inhibitors of VEGF signaling have become an important research focus in the development of antitumor therapies and enhancement of cancer immunotherapy [25]. To our knowledge, the value of VEGF we identified and validated in a large patient cohort in a neoadjuvant setting herein has not been previously described. The mechanism of this relationship is unclear. It is reported that circulating levels of VEGF correlate with the levels of circulating endothelial

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Table 2. Patient characteristics and observed pNR

Characteristics	Number of patients	Number of pNR (%)	Chi-squared test *p* value	Multivariable *p* value
Age			.226	NS
<40	60	9 (15.0)		
40–59	194	39 (20.1)		
≥60	49	5 (10.2)		
Menopausal status			.334	NS
Premenopausal	159	31 (19.5)		
Postmenopausal	144	22 (15.3)		
Tumor size at baseline			.726	NS
T2	150	24 (16.0)		
T3	100	18 (18.0)		
T4	53	11 (20.8)		
Node status at baseline			.244	NS
–	52	12 (23.1)		
+	251	41 (16.3)		
Histology at baseline			.892	NS
Invasive ductal carcinoma	224	40 (17.8)		
Invasive (mixed) carcinoma	62	11 (17.7)		
Others	17	2 (11.8)		
Ki-67 expression at baseline			<.001	.004
<20%	107	32 (29.9)		
≥20%	196	21 (10.7)		
VEGF at baseline, pg/mL			.011	NS*
Low (<81.49)	103	29 (28.2)		
Intermediate (81.49–117.15)	101	17 (16.8)		
High (>117.15)	99	7 (7.1)		
VEGF prior to 3rd cycle, pg/mL			<.001	NS*
Low (<82.18)	100	11 (11.0)		
Intermediate (82.18–115.10)	102	11 (10.8)		
High (>115.10)	101	31 (30.7)		
VEGF change prior to 3rd cycle			<.001	<.001*
Elevated (less than −10%)	102	43 (42.2)		
Stable (−10% to 13%)	101	7 (6.9)		
Reduced (>13%)	100	3 (3.0)		
VEGF at surgery			<.001	NS*
Low (<56.80)	101	8 (7.9)		
Intermediate (56.80–118.17)	101	12 (11.9)		
High (>118.17)	101	33 (32.7)		
Clinical response at 3rd cycle			.062	.005
CR/PR	102	12 (11.8)		
SD/PD	201	41 (20.4)		

[a]VEGF was studied in the multivariable analysis as continuous variable.

Abbreviations: CR, complete response; NS, not significant; PD, progressive disease; pNR, pathological nonresponse; PR, partial response; SD, stable disease; VEGF, vascular endothelial growth factor.

cells in patients with cancer [26]. VEGF derived from tumor-associated macrophages may also result in local, transient vascular permeability and tumor cell intravasation [27]. Thus, the chemotherapy-induced change in VEGF may be attributed to interactions between chemotherapy, tumor biology, and the immune microenvironment of the host.

We also demonstrated that Ki-67 is an independent predictive and prognostic biomarker in the NAC setting. The clinical value of Ki-67 has been reported previously

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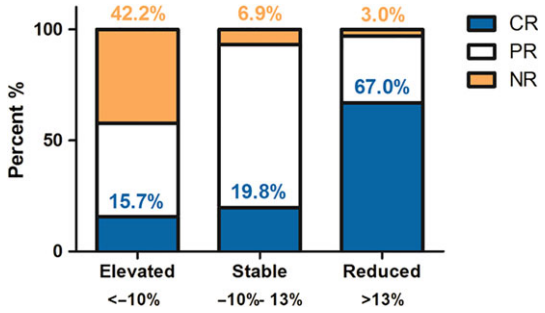

Figure 3. Possibility of observed pathological response according to vascular endothelial growth factor (VEGF) change. Using tertile VEGF values as cutoffs, patients could be categorized into three groups: VEGF-reduced (>13%), VEGF-stable (−10% to 13%), and VEGF-elevated (less than −10%). The patient in the VEGF-reduced group has a high pathological complete response (pCR) rate of 67.0% and a low pathological nonresponse (pNR) rate of 3.0%, whereas the patient in the VEGF-elevated group has a high pNR rate of 42.2%, and a low pCR rate of 15.7%.
Abbreviations: CR, complete response; NR, nonresponse; PR, partial response.

Table 3. Multivariable survival analysis for DFS

	DFS	
Variable	**HR (95% CI)**	***p* value**
Residual tumor size, cm, 0–2 vs. 2–5 vs. >5		NS
Residual LNs involved		.002
0	Ref.	
1–3	1.091 (0.459–2.595)	
4+	2.706 (1.247–5.872)	
Vascular invasion, yes vs. no		NS
Post-NAC Ki-67, <20% vs. ≥20%	2.076 (1.194–3.609)	.010
VEGF change prior to surgery: continuous	1.008 (1.003–1.014)	.004
VEGF change prior to surgery: continuous		NS

Abbreviations: CI, confidence interval; DFS, disease-free survival; HR, hazard ratio; LN, lymph node; NAC, neoadjuvant chemotherapy; NS, not significant.

and indicates that the change in Ki-67 correlates with treatment response and residual tumor Ki-67 correlates with patient survival [7, 28–30]. Similarly, we found that the VEGF level prior to surgery, but not the alteration in VEGF, has a prognostic value in non-pCR responders. Because pCR responders would have a relatively favorable outcome [1, 2], the utility of VEGF is not restricted to early measurement for response evaluation. It may also play an important role in predicting patient survival. Considering the inconvenience of tumor biopsy during NAC, we believe that the monitoring of VEGF, which is easily detected in blood samples, has a better practical value than Ki-67. The NAC regimen used in this study was paclitaxel and carboplatin, which has proven to be well tolerated and yielded

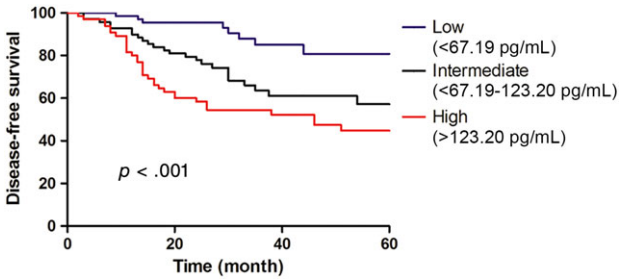

Figure 4. Cumulative disease-free survival of patients according to vascular endothelial (VEGF) levels after neoadjuvant chemotherapy. High expression of VEGF was significantly correlated to an unfavorable outcome ($p < .001$).

favorable responses in both *BRCA*-associated and wild-type TNBC [13]. Thus, the present study provides new evidence of response prediction to platinum agents, which is of great clinical significance in the treatment of TNBC.

There are several limitations to the analysis presented. First, only 37 patients were included in our filter set, and it is only possible for us to analysis 29 biomarkers, which might cause the limitation of our findings. The variability in processing can also influence the measurement of several analytes, particularly those that are affected by quality of serum. Further validations on the accuracy of multiplex serum biomarkers analyses in patients who underwent chemotherapy might be needed. Furthermore, the different protocols of ELISA in different labs might influence the clinical utility of VEGF. The predictive and prognostic value of VEGF in neoadjuvant setting also needs to be validated in a separate cohort of patients using the same assay and methods of analysis, because this study only included a single training set. It is also necessary to test the value of VEGF in neoadjuvant setting for other breast cancer subtype with other NAC regimens.

Conclusion

We demonstrated a new strategy for response prediction and evaluation for TNBC patients undergoing chemotherapy. The monitoring of serum VEGF could help identify patients with favorable or poor responses at an early time point of NAC, which allows for the modification of NAC regimens. In non-pCR responders, it is also correlated with a risk of relapse or death. In future prospective studies, VEGF may also serve as a complement or alternative to traditional imaging-based response-evaluating methodologies in tailoring systemic treatment strategies for both operable and advanced TNBCs.

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patients gave their written informed consent before inclusion in this study.

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Collection and/or assembly of data: Ruo-Xi Wang, Ying Zhou
Data analysis and interpretation: Sheng Chen, Liang Huang
Manuscript writing: Ruo-Xi Wang
Final approval of manuscript: Ruo-Xi Wang, Sheng Chen

Disclosures

The authors indicated no financial relationships.

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