● *Original Contribution*

VASCULARITY CHANGE AND TUMOR RESPONSE TO NEOADJUVANT CHEMOTHERAPY FOR ADVANCED BREAST CANCER

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Abstract—For advanced breast cancer with severe local disease (ABC) (stage III/IV), neoadjuvant chemotherapy improves local control and surgical outcome. However, about ~20 to 30% of advanced cancers show either no or poor response to chemotherapy. To prevent unnecessary treatment, a capability of predicting clinical response to neoadjuvant chemotherapy of ABC is highly desirable. Vascularity index (VI) of breast cancers was derived from the quantification results in 30 ABC patients by using power Doppler sonography. Power Doppler sonography evaluation was performed every one to two weeks during chemotherapy. The overall response rate for 30 advanced patients tested was 70%, when 50% or more reduction in tumor size was the objective clinical response. Chemotherapy response was unrelated to the original tumor size ($p = 0.563$) or chemotherapy agents used ($p = 0.657$). The median VI for all 30 patients was 4.99%. The response rates for hypervascular tumors vs. hypovascular tumors, based on initial median value, were 86.7% and 53.3%, respectively ($p = 0.109$). The average VIs in responders and nonresponders were $7.67 \pm 4.77\%$ and $4.01 \pm 3.82\%$ ($p = 0.052$). There was a tendency for responders who have a relatively high initial vascularity. The VI change in responder group shows a pattern of initial increasing in vascularity followed by decreasing in vascularity. All patients (17/17) with a VI increment of $>5\%$ during chemotherapy had good chemotherapy response, whereas in patients with a VI increment of $<5\%$, the response rate was 30.8% (4/13) ($p < 0.001$). For patients with a peak VI of $>10\%$ during chemotherapy, the response rate was 94.1% (16/17). However, in patients with a peak VI of $<10\%$, the response rate was 38.5% (5/13) ($p = 0.001$). This prediction was made mostly within one month (25.47 ± 12.96 d for VI increments $>5\%$ and 25.44 ± 12.41 d for VI increased to $>10\%$). In the meantime, the differences in size reduction shown in B-mode sonography were insignificant between responders and nonresponders (patient group with VI increment $>5\%$, $p = 0.308$; patient group with peak VI $>10\%$, $p = 0.396$). In conclusion, we propose that VI as determined by using power Doppler sonography is a good and inexpensive clinical tool for monitoring vascularity changes during neoadjuvant chemotherapy in ABC patients. Two parameters—VI increment $>5\%$ and peak VI $>10\%$ —are potential early predictors for good responses to neoadjuvant chemotherapy within one month in patients with ABC. (E-mail: kingjen@ha.mc.ntu.edu.tw) © 2008 Published by Elsevier Inc. on behalf of the World Federation for Ultrasound in Medicine & Biology.

Key Words: Advanced breast cancer (ABC), Angiogenesis, Increasing in vascularity, Neoadjuvant chemotherapy, Power Doppler sonography, Vascularity index (VI).

INTRODUCTION

Most breast cancers are thought to be systemic disease at diagnosis (Bonadona et al. 1991; Smith et al. 1991). Adju-

vant chemotherapy is necessary for 80% of patients in Taiwan and can substantially improve disease-free and overall survival. For advanced breast cancer (ABC) with severe local disease (stage III/IV), preoperative chemotherapy improves local control and surgical outcome. About 20 to 30% of ABCs show either no or poor response to chemotherapy (Bonadona et al. 1991; Smith et al. 1991). To this subgroup of patients, early prediction of tumors in

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response to neoadjuvant chemotherapy is desirable. In this report, we intend to address a clinical diagnosis tool in monitoring the efficacy of neoadjuvant chemotherapy for ABC. This is important to both save money and spare patients' suffering during ineffective therapy.

Increased tumor vascularity may be a surrogate marker for prediction of tumor response to chemotherapy (Cosgrove et al. 1990, 1993). The research evidence shows that tissue necrosis induced by cytotoxic agents would release many kinds of angiogenic factors (Shaked et al. 2006). Increased vascularity is essential for the growth and metastasis of solid tumors (Folkman et al. 1963). Several studies have reported that increased tumor vascularity is an independent prognostic indicator in primary breast carcinoma (Toi et al. 1993; Weidner et al. 1991, 1992). Another possible mechanism was found in animal model that chemotherapy induces acute recruitment of bone marrow-derived circulating endothelial progenitor cells to tumors to enhance tumor vascularity (Shaked et al. 2006).

With Doppler sonography, tumor vascularity can be assessed *in vivo* (Dixon et al. 1992). Doppler sonography showed high sensitivity for predicting complete histologic response (Caruso et al. 1995; Lagalla et al. 1998; Singh et al. 2005). After neoadjuvant chemotherapy, Doppler sonography was more sensitive in disclosing the presence of active neoplastic tissue than conventional histopathological results (Huber et al. 2000; Vallone et al. 2005). In previous reports (Kedar et al. 1994; Roberto et al. 1998), decreased tumor vascularity at the end of treatment indicates good response, whereas increased or unchanged vascularity indicates no response. In 40% of these patients, the Doppler changes appeared four weeks before a size reduction was detectable using B-mode ultrasonography (Kedar et al. 1994). Thus, Doppler flow imaging can be helpful in both assessing and predicting the response of breast cancer to medical treatment.

Power Doppler sonography has shown a higher sensitivity in the detection of blood flow than conventional color Doppler sonography (Martinoli et al. 1998). It can be used as a tool for quantification of tumor vascularity *via* the color pixels of vessels scanned by power Doppler ultrasound (Cosgrove et al. 1990, 1993). Vascularity index (VI) is an ultrasound parameter for quantitatively evaluating *in vivo* increased vascularity. It offers the advantage of covering the entire tumor section for signal acquisition, *i.e.*, avoiding the signal bias may be from selected portions of tumor section. Consequently, the color signal in a given tumor section could be quantified. One of our reports has pointed out that the vascularity index could be used to differentiate the nature of neck lymph nodes (Wu et al. 1998). It has a good correlation with status of lymph node metastasis in cervical carcinoma (Cheng et al. 1999). It is also a good

preoperative indicator of recurrence and patient survival in colon cancer (Chen et al. 2000). In addition, it is an indicator of the efficacy of thalidomide in unresectable hepatocellular carcinoma (Hsu et al. 2005). The aim of this study is to evaluate the usefulness of VI of power Doppler sonography as a predictor for tumor response to neoadjuvant chemotherapy in locally advanced breast cancer. This will allow clinicians to consider the modification of the treatment protocol at the early stage of chemotherapy based on Doppler findings.

MATERIALS AND METHODS

Patients and chemotherapy

Thirty consecutive ABC patients (TNM stage IIB, III and IV), who received preoperative chemotherapy for tumor reduction or disease control from June 21, 2000 until June 3, 2002 at National Taiwan University Hospital (NTUH), were recruited for this study. The diagnosis of ABC was made according to the American Joint Committee on Cancer (AJCC)'s definition of breast cancer staging. These patients consisted of two at stage IIB, 19 at stage III and nine at stage IV, respectively. Eight patients had T4 disease documented by the pathologists and one patient with inflammatory breast cancer (T4 days) was included.

Intravenous chemotherapy was given for three weeks per cycle in these patients. The first seventeen patients who joined the Paclitaxel-based neoadjuvant chemotherapy clinical trial, received Paclitaxel (Phyxol, Sinphar Pharmaceutical, Taipei, Taiwan) 175 mg/m² by 3-h IV infusion immediately followed by cisplatin 50 mg/m² by 24-h IV infusion on day 1 of a three-week cycle. Eleven patients received Epirubicin (Pharmorubicin, Pfizer Pharmaceuticals, New York City, NY, USA) 90 mg/m², cyclophosphamide 600 mg/m² and 5-Fluorouracil 500 mg/m² on day 1 every three weeks. The remaining two stage IV patients received Vinorelbine (Navelbine, Glaxo Wellcome, Inc., Research Triangle Park, NC, USA) 25 mg/m² on day 1 and day 8 as chemotherapy regimen based on patient's choice. All patients gave informed consent according to guidelines approved by the Institutional Review Board of NTUH.

Sonography evaluation

For each case, power Doppler sonography (HDI 5000; Advanced Technology Laboratories, Bothell, WA, USA) with 5- to 12-MHz broad band linear array transducers (Transducer model: L12-5 50 mm Broadband Linear Array, multifocal zone, 8 transmit zones), along with conventional 2-D sonography were performed before incision biopsy, on day 8 and day 15 during therapy and/or before mastectomy.

Fig. 1. Chemotherapy and evaluation protocol. *Sonography evaluation; #chemotherapy was given on day 1; ¹MRM = Modified radical mastectomy; ²BCT = Breast conserving treatment; ³Bx = incision biopsy.

Evaluation of chemotherapy response

Tumor response was decided after three cycles of chemotherapy in all patients (*i.e.*, 9 to 12 weeks after the initiation of chemotherapy). At the time of decisive evaluation, the clinical response was classified as complete response (CR), partial response (PR), stable disease (SD) or progressive disease (PD) according to standard Union International Contre le Cancer (UICC) criteria (Hayward *et al.* 1977). CR was defined as the disappearance of all clinical evidence of the tumor including the axillary site. PR was defined as a reduction of 50% or more in the cross section area on B-mode sonography, without the appearance of new lesions. SD was defined as a decrease of <50% in the cross section area or an increase of <25%, without the appearance of new lesions. Any measured or estimated increase >25% or the appearance of new lesions was defined as PD. The patients were classified into two groups: the responder, who was classified as CR or PR, and the nonresponder, who was classified as SD or PD. All these data and other clinical information were analyzed, integrated and correlated.

Sonographic technique and quantification of vascular density in power Doppler images

The scanner used was a power Doppler ultrasound HDI 5000 model (Advanced Technology Laboratories). Settings of the power Doppler sonography were standardized for the highest sensitivity in the absence of apparent noise. They were standardized with a medium wall filter, pulsed repetition at frequency of 1000 Hz, moderate-to-long persistence and a slow sweep technique (a technique for small vessels within breast tumor detection. We recorded the Doppler signals of them after the signals became maximized and stabilized that required a slow mode in scanning the signal using the standard transducer of HDI 5000 model). Under these conditions, the lowest possible measurable velocity was claimed below 5 cm/s. Focusing depth

was set between 0.5 and 4 cm. Each tumor was scanned thoroughly, and a tangential scanning was made to avoid the nonrepresentative section. The power Doppler window was set to cover the whole tumor on the screen. If the maximal tumor diameter was larger than the width of sonography probe, the chosen power Doppler window should be filled with tumor and show partial image of the tumor. The tumor section was then scanned carefully in all directions. To avoid section bias, we recorded sections with possible maximal power Doppler signals. At least three sections were recorded during each evaluation. They were quantified after evaluation and the tumor section with subjectively maximal power Doppler signals was captured and stored for later analysis. Each tumor was scanned at least three times. These tumor sections with maximal power Doppler signals were available for quantitative analysis. After the examination, the previously stored images were retrieved on the monitor. The tumor margin was contoured using a cursor. Quantification of the vascular color signals within the demarcated tumor area was then automatically

Fig. 2. Definition of vascularity index (VI) = the number of colored pixels within the tumor section (area defined by fine dots)/the number of total pixels in that particular tumor section (area defined by bigger dots).

Fig. 3. The typical pattern of vascularity index change and tumor size reduction during chemotherapy for responders. (a) The patient with initial VI less than median 4.99% (hypovascular tumor). (b) The patient with initial VI greater than median 4.99% (hypervascular tumor). Vascularity index (VI) = the number of colored pixels within the tumor section/the number of total pixels in that particular tumor section.

performed by special software (Encomate; Electronic Business Machine Co., Ltd., Taipei, Taiwan). The results were expressed as the VI (the number of colored pixels within the tumor section/the number of total pixels in that particular tumor section). For each tumor, the maximum of the VI of representative tumor sections was used for statistical analysis. VI increment was defined as the change from baseline value to peak VI. More detailed description of VI quantification has been documented (Chen et al. 2000 and 2005).

Data analysis

Continuous variables were described as mean $\pm$ standard deviation. The nominal variables were demonstrated as frequency and percentage. Patients were grouped according to their VI status, tumor size and treatment regimen. The difference between two groups was tested by using Student's *t*-test for the variable with interval scale, or by using Fisher's exact test for the variable with nominal scale. All analyses were per-

Fig. 4. The typical pattern of VI variation and tumor size reduction during chemotherapy for nonresponders. (a) The patient with initial VI less than median 4.99% (hypovascular tumor). (b) The patient with initial VI greater than median 4.99% (hypervascular tumor). Vascularity index (VI) = the number of colored pixels within the tumor section/the number of total pixels in that particular tumor section.

formed using SAS software, version 9.1 (SAS Institute, Inc., Cary, NC, USA). Statistical significance was set at $\alpha = 0.05$ level for all analyses.

RESULTS

Sonographic evaluation was performed every one to two weeks during chemotherapy according to the stan-

dard protocol for the clinical trial of chemotherapy (Fig. 1). The quantitative measurement for vascularity in tumor is shown in Fig. 2. Among 30 patients, 21 were responders to chemotherapy, eight remained stable in their disease and one developed progressive disease. The overall response rate was 70%. The mean VI of these 30 cases was $6.57 \pm 6.63\%$. The medium VI was 4.99%.

Table 1. Chemotherapy response in related to VI change during neoadjuvant chemotherapy

Feature	No. of patients (%)	Responder	Non-responder	p-value (Fisher)
No. of patients (in %)	30 (100.0%)	21 (70.0%)	9 (30.0%)	
VI Increment				
>5%	17 (56.7%)	17 (100.0%)	0 (0.0%)	<0.001
≤5%	13 (43.3%)	4 (30.8%)	9 (69.2%)	
Peak VI				
>10%	17 (56.7%)	16 (94.1%)	1 (5.8%)*	0.002
≤10%	13 (43.3%)	5 (38.5%)	8 (61.5%)	

Vascularity index (VI) = the number of colored pixels within the tumor section/the number of total pixels in that particular tumor section.

* VI decreased after chemotherapy.

Table 2. The average time interval needed to get 5% VI increment or >10% VI in chemotherapy responders

VI measured after neoadjuvant chemotherapy	No. of Responders	Time interval required for tumor VI increment
VI increment >5%	17	25.47 ± 12.96d
Peak VI >10%	9*	25.44 ± 12.41d

Vascularity index (VI) = the number of colored pixels within the tumor section/the number of total pixels in that particular tumor section.

* Nine patients with original VI <10%.

The typical vascularity change in chemotherapy responder shows a pattern of initially increasing in vascularity followed by decreasing in vascularity (Fig. 3). The VI profile in nonresponders did not show evident fluctuation (Fig. 4). All patients (17/17) with a VI increment of >5% during chemotherapy had good chemotherapy response, whereas in patients with a VI increment of <5%, the response rate was 30.8 % (4/13) ($p < 0.001$) (Table 1). In patients showing a peak of VI >10% during chemotherapy, the response rate was 94.1 % (16/17). However, in patients with a peak VI of <10%, the response rate was 38.5 % (5/13) ($p = 0.002$) (Table 1).

In 17 patients with a VI increment >5%, the average time interval required to reach this cutoff point is 25.47 ± 12.96 d. The size reduction in cross section area at this time point was $27.35 \pm 18.34\%$. For nine responsive patients with original VI of <10%, their VI increased to >10% around 25.44 ± 12.41 days after chemotherapy. The size reduction at this time point was $26.19 \pm 18.28\%$ (including all 16 responders). In non-responsive patients, the size reduction on day 25 ± 5 after the first course of chemotherapy is $20.44 \pm 10.15\%$. At this time point, the size reduction in cross section area between responders with a VI increment of >5% and nonresponders showed no statistical significance ($p = 0.308$). The size reduction between patients with a peak VI of >10% and nonresponders showed no statistical significance either ($p = 0.396$) (Tables 2 and 3).

The effects of both original tumor properties and chemotherapy agents to chemotherapy response among ABC patients were also analyzed (Table 4). There was no significance in the response rates between both responder and nonresponder when they were grouped into hypervascular and hypovascular tumors based on the initial median VI ($p = 0.109$). Mean original VI between responders and nonresponders were different but not significant ($p = 0.052$) (Table 5). The estimated corresponding cut surface area for tumor with its largest diagonal diameter of 3 cm was 7.06 cm^2 ($1.5 \text{ cm} \times 1.5 \text{ cm} \times \pi = 7.06 \text{ cm}^2$). The response rates in larger tumors (area >7 cm²) and smaller tumors (area <7 cm²) were

73.6% and 63.6%, respectively ($p = 0.687$). Seventeen patients received neoadjuvant chemotherapy with Paclitaxel; the response rate was 64.7%. The response rate of patients who received Epirubicin-based treatment was 72.7%. Two patients received Vinorelbine as neoadjuvant agent and had partial response. The response rates between patients receiving Paclitaxel or Epirubicin-based neoadjuvant chemotherapy showed no statistical difference ($p = 0.999$) (Table 4).

DISCUSSION

Over the last decade, the use of neoadjuvant (pre-operative) chemotherapy has emerged as the standard of care for patients with locally advanced breast cancer. However, two large randomized trials proved that the survival rates were equivalent in patients receiving pre-operative or postoperative chemotherapy (Clahsen et al. 1996; Mamounas 1998). The shortcoming of current clinical practice is time consuming because the decision making is based on the pathologic response at the end of chemotherapy.

This is the first study that shows the role of power Doppler in monitoring neoadjuvant chemotherapeutic response. Therefore, it may shorten the time required for alternative therapeutic decision making when a subset of locally advanced breast cancer has poor response to certain chemotherapy strategies.

The mean VI of these 30 cases was $6.57 \pm 6.63\%$. The median VI was 4.99%. The overall response rate was 70%.

This response rate was compatible with other reported series (Smith et al. 2002). With quantitative measurement and sequential observation, we observed the vascularity change during chemotherapy of ABC. We have noticed that the typical trajectory of VI in chemotherapy responder revealed increasing vascularity with

Table 3. The percentage of size reduction in cross section area on day 25 ± 5 after first course of chemotherapy in responders whose VI increment was >5%, responders whose peak VI was >10% and nonresponders

Feature	No. of patients	Tumor size reduction (%) in cross section area on day 25 ± 5	
VI increment >5%	17	$27.35 \pm 18.34\%$	$p = 0.308^*$
Peak VI >10%	16	$26.19 \pm 18.28\%$	$p = 0.396^\dagger$
Nonresponders	9	$20.44 \pm 10.15\%$	

Vascularity index (VI) = the number of colored pixels within the tumor section/the number of total pixels in that particular tumor section.

* Comparison of size reduction percentage between responders whose VI increment was >5% and nonresponders.

† Comparison of size reduction percentage between responders whose peak VI was >10% and nonresponders.

Table 4. The statistical evaluation of the differential effects on chemotherapy responses in ABC patients based on initial VI, original tumor section area and chemotherapy agents, respectively

Feature	No. of patients (%)	Responder	Nonresponder	<i>p</i> -value (Fisher)
The number of patients(in percentage)	30 (100%)	21 (70%)	9 (30%)	
Initial VI*				0.109
Hypervascularity*	15 (50.0%)	13 (86.7%)	2 (13.3%)	
Hypovascularity	15 (50.0%)	8 (53.3%)	7 (46.7%)	
Original tumor section area				0.687
>7cm [†]	19 (63.3%)	14 (73.7%)	5 (26.3%)	
≤7cm ²	11 (37.8%)	7 (63.6%)	4 (36.4%)	
Chemotherapy agent				Paclitaxel vs. Epirubicin 0.999
Paclitaxel	17 (56.6%)	11 (64.7%)	6 (35.3%)	
Epirubicin	11 (36.7%)	8 (72.7%)	3 (27.3%)	
Vinorelbine	2 (6.7%)	2 (100%)	0 (0.0%)	

Vascularity index (VI) = the number of colored pixels within the tumor section/the number of total pixels in that particular tumor section.

* Hypervascularity: VI >4.99% (median).

† Tumor with 3cm in diameter, cut surface area $1.5\text{cm} \times 1.5\text{cm} \times \pi = 7.06\text{ cm}$.

the initiation of neochemotherapy followed by decreasing vascularity (Fig. 3). We postulated that after initiation of chemotherapy, abundant angiogenic factors such as vascular endothelial growth factor (VEGF), released from apoptotic and necrotic breast cancer cells, resulted in profoundly increasing vascularity. After peak vascularity, the vascularity decreased gradually at a later stage of chemotherapy because of fewer angiogenic factors released by the remaining tumor cells that are chemotherapy sensitive. The time course survey of vascularity changes detected by power Doppler sonography (Fig. 3) did stop before the time of appearance for the final clinical outcome as described by Kedar *et al.* (1994) and Roberito *et al.* (1998), which showed decreased tumor vascularity to the baseline correlated with improved clinical outcome. Instead, the key finding was that increased tumor vascularity appeared during early neoadjuvant chemotherapy. In addition, we found all patients with a VI increment of >5% during chemotherapy had good chemotherapy response (*i.e.*, 100%) (Table 1). This amplitude of vascular change can be easily visible by the experienced sonographer, even without the aid of computation. We also observed in patients with a peak VI of >10% during chemotherapy that, although the majority of their VI increment was <5%, the response rate was 94% (Table 1). A high vascularity profile during chemotherapy indicates good response to the treatment (Fig. 3, Tables 1, 2 and 3). These findings can be valuable surrogate markers in clinical practice based on two essential rationales.

Detection of chemotherapy response within one month based on vascularity profile makes power Doppler clinically applicable. Based on the angiogenic factors-related hypothesis (Furstenberger *et al.* 2006), we expected the angiogenic phase in ABC is an early event during chemotherapy. We found in patients with a VI increment of >5% or a peak VI of >10% after chemo-

therapy, the time to reach the cutoff point is 25.47 ± 12.96 d and 25.44 ± 12.41 d, respectively (Table 2). However, at these time points the tumor size reduction showed no statistical difference between responders and nonresponders (Table 4). This proved that the detectable vascularity change in Doppler sonography is a better indicator than the size reduction observed in B-mode ultrasonography.

There was no significance in response rate when they were grouped into hyper- and hypovascularity categories ($p = 0.109$) (Table 4). However, we noticed that mean initial VI for responders was $7.67 \pm 4.77\%$ compared with the $4.01 \pm 3.82\%$ in nonresponders. This difference has reached borderline significance ($p = 0.05$). Thus, the initial VI of the tumor might be a useful indicator of the future response to chemotherapy (Table 5). This is contradictory to previous report (Boonjunwetwat *et al.* 2005). We proposed that hypervascular tumor provided more accessible vessels for receiving chemotherapy agents. Therefore, more tumor cells can readily contact with chemotherapy drug *via* surrounding blood vessels. However, the accessibility of tumors to the drug(s) is not the solely determining factor to chemotherapy response. In our unpublished data, we observed

Table 5. The difference in mean original VI between responders and nonresponders of neoadjuvant chemotherapy in thirty ABC patients

Patient responses	No. of patients	Original VI	
Responder	21	$7.67 \pm 4.77\%$	$p = 0.052$
Nonresponder	9	$4.01 \pm 3.82\%$	

Vascularity index (VI) = the number of colored pixels within the tumor section/the number of total pixels in that particular tumor section.

≤VI decreased after chemotherapy

that VEGF level surged after chemotherapy and fluctuated during the course of chemotherapy. This preliminary observation supported the finding that VEGF is a key coplayer in chemotherapy-induced neovascularity increment (Furstenberger et al. 2006). On the contrary, we found that VEGF was detected to be the basal level after surgery. This evidence favors the hypothesis that VEGF may come from tumor related factors either from the tumor itself or their supporting matrix, which was removed after operation (Furstenberger et al. 2006).

Notably, a recent study revealed that the combination of bevacizumab and Paclitaxel in previously untreated metastatic breast cancer (MBC) showed a statistically significant benefit in terms of overall response rate (28.2% vs. 14.2%, $p < 0.0001$), progression-free survival (PFS) (10.97 months vs. 6.11 mo, $p < 0.001$) and overall survival (OS) (HR = 0.674, $p = 0.01$), in favor of the combination arm (Miller et al. 2003). In our chemotherapy-responsive ABC patients, increasing vascularity is an early event during neoadjuvant chemotherapy. This observation may provide the insight, at least in part, that the combined cytotoxic and antiangiogenesis therapy has the synergistic effect on disease control of ABC. However, phase III studies of chemotherapy plus bevacizumab in chemotherapy pretreated metastatic breast and colon cancer failed to show a benefit in these patients in terms of PFS and OS (Chen et al. 2004; Miller et al. 2005). Our surveillance showed a decreased vascularity at the later stage of chemotherapy. It is reasonable to expect that the tumor is less angiogenic-sensitive in the later course of chemotherapy. This observation supports the insignificant additive effect of antiangiogenic drug to chemotherapy in heavily treated metastatic cancers, which were likely chemotherapy resistant. Therefore, the effect of antiangiogenic drugs may be more prominent in the adjuvant setting than in the chemotherapy-pretreated tumors, which the angiogenic activities of tumors had diminished through pretreatment.

The major advantage of power Doppler sonography over other current methodologies in the field is for its efficiency in clinical vascularity determination. Low detection sensitivity is one of the most difficult problems of Doppler ultrasound. However, using power Doppler ultrasound, only a minimal frequency shift and a minimal number of intravascular reflecting particles are necessary for sufficient measurement and for increasing the sensitivity of detection (Taylor et al. 1988). Power Doppler ultrasound, in particular, is not angle dependent whenever a Doppler shift is detected. It is neither velocity dependent nor prone to aliasing (Kook et al. 1999; Milz et al. 2001; Raza and Baum 1997; Rubin et al. 1994). The power Doppler mode depicts the intensity or energy of the Doppler signals (integrated amplitude under spectral Doppler curve) for a time period. Power Doppler reduces

the temporal resolution and the visualization of vessel pulsatility. It does not show the direction or velocity of blood flow. But it depicts longer segments of smaller vessels as a result of holding the pixels colored for a longer time period. Therefore, it enables the examiner to get a more complex survey of intratumoral vascular structure than by conventional color Doppler. This is potentially useful, especially in breast cancer, because the vascular network of malignant tumors is characterized by a high capillary density of vessels, vessel-loops, arteriovenous shunts and chaotic vessel branching (Birdwell et al. 1997; Less et al. 1991; Martinoli et al. 1998; Milz et al. 2001; Weidner et al. 1991). Besides, power Doppler sonography has a significantly higher correlation with microvessel density when it is compared with 2-D color power Doppler sonography at baseline (Yang et al. 2002).

It is more sensitive in showing post-therapeutic changes of intratumoral blood flow by power Doppler sonography. Nevertheless, the role of power Doppler in changing therapeutic decisions has not been evaluated up to now despite its ability to improve the sensitivity in detection of blood flow compared with conventional color Doppler (Martinoli et al. 1998). Few papers address the correlation between Doppler ultrasound parameters and neoadjuvant chemotherapy response in breast cancer. Most of them are observational without any clinical predictive perspective. After two-point comparison of tumor vascularity before chemotherapy initiation and after surgery, all the papers concluded that decreased tumor vascularity indicates good response and increased or unchanged vascularity indicates no response at all (Kedar et al. 1994; Roberito et al. 1998; Roubidoux et al. 2005). However, increasing vascularity was observed in three to four weeks after initiation of chemotherapy for frequent monitoring of tumor vascularity in a one- to two-week interval (Fig. 3), and it could not be observed by two-point comparison in previous reports. Furthermore, their methodologies were lack of quantitative measurement of Doppler signals, such as VI, which provides more objective comparison.

In neoadjuvant context, the traditional Doppler sonography parameters are less consistent and less informative. They include maximum flow velocity ($V_{\max}$), pulsatility index (PI) and resistivity index (RI). First, the number of vessels, RI, PI and $V_{\max}$ fail to provide accurate differentiation of benign and malignant breast tumors (Chao et al. 1999). Second, in tumors with plentiful blood supply, vessel selection is highly subjective. In the pilot study, we observed the big variation of RI, PI and $V_{\max}$ of different vessels within a tumor (data not shown). Third, the impact of neovascularization after chemotherapy is hard to evaluate objectively with these conventional settings.

From our study we found that quantitative power Doppler ultrasound is a useful tool in monitoring chemotherapy response of ABC. Compared with other imaging modalities, computed tomography (CT) or dynamic magnetic resonance imaging (MRI) yielded a compound signal from blood flow, transcapillary diffusion and interstitial perfusion. It provided more sensitive imaging of increased vascularity, especially under contrast medium administration. Although MRI has superior sensitivity and more hemodynamic information, it is time consuming, less accessible and escalating in price. With slightly inferior precision, power Doppler ultrasound is cheaper, repeatable, readily available and has no radiation. It could be applied in a larger scale. However, it was examiner dependant, machine dependant and time consuming in the initial learning period. To avoid evaluation bias, this entire study was performed by a single investigator using the same ultrasound machine under consistent ultrasound settings.

In summary, neoadjuvant chemotherapy has been the standard treatment of ABC. The number of patients with ABC at initial diagnosis is gradually decreasing because most of the breast cancer diagnosed today is detected in its early stage. ABC is a heterogeneous disease with a dismal problem in afflicted women, and the efficacy of chemotherapy for ABC is unpredictable. Power Doppler VI was an effective measure in monitoring response of neoadjuvant chemotherapy for ABC in this study. Given the complex biology underlying response to chemotherapy, one should not expect any single factor to be sufficiently predictive to guide therapy. In this preliminary study, in spite of small sample size and multiple regimens of chemotherapy, measurement of vascularity response to chemotherapy turned out to be an important strategy in breast cancer treatment, and this deserves further investigation in a larger patient population. Although this research finding was in our hands about five years ago, the solid scientific foundation of this finding was found recently by others only in an animal model (Shaked *et al.* 2006), and HDI 5000 with 12-MHZ linear probe of power Doppler ultrasound is still one of the most updated models worldwide. Therefore, we suggest the clinical application in the future to look for the differences and the parameters, which may influence the VI as well as the best time interval for measuring increased vascularity during neoadjuvant chemotherapy upon updated treatment protocols and more recent advanced tools applied.

CONCLUSION

We propose that the VI, as determined by using power Doppler sonography, is a good and inexpensive clinical tool for monitoring vascularity changes during

neoadjuvant chemotherapy in ABC patients. Two parameters—VI increment >5% and peak VI >10%—are potential early predictors for good responses to neoadjuvant chemotherapy within one month in patients with ABC.

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