Effectiveness of evaluating tumor vascularization using 3D power Doppler ultrasound with high-definition flow technology in the prediction of the response to neoadjuvant chemotherapy for T2 breast cancer: a preliminary report

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Abstract
The aim of this study was to evaluate the effectiveness of advanced ultrasound (US) imaging of vascular flow and morphological features in the prediction of a pathologic complete response (pCR) and a partial response (PR) to neoadjuvant chemotherapy for T2 breast cancer.

Twenty-nine consecutive patients with T2 breast cancer treated with six courses of anthracycline-based neoadjuvant chemotherapy were enrolled. Three-dimensional (3D) power Doppler US with high-definition flow (HDF) technology was used to investigate the blood flow in and morphological features of the tumors. Six vascularity quantization features, three morphological features, and two vascular direction features were selected and extracted from the US images. A support vector machine was used to evaluate the changes in vascularity after neoadjuvant chemotherapy, and pCR and PR were predicted on the basis of these changes.
The most accurate prediction of pCR was achieved after the first chemotherapy cycle, with an accuracy of 93.1% and a specificity of 85.5%, while that of a PR was achieved after the second cycle, with an accuracy of 79.3% and a specificity of 72.22%.

Vascularity data can be useful to predict the effects of neoadjuvant chemotherapy. Determination of changes in vascularity after neoadjuvant chemotherapy using 3D power Doppler US with HDF can generate accurate predictions of the patient response, facilitating early decision-making.

Keywords: breast cancer, neoadjuvant chemotherapy, 3D power Doppler ultrasound, tumor vascularization

(Some figures may appear in colour only in the online journal)

Introduction

Neoadjuvant chemotherapy results in tumor volume shrinkage, decreases the probability of relapse after surgery, and increases disease-free and overall survival for most patients with locally advanced breast cancer. Tumor eradication can be successfully achieved in approximately 3%–46% patients with breast cancer if the response is assessed by careful pathological examination of surgical specimens obtained after neoadjuvant chemotherapy (Shapiro and Stockman 2001).

Because the absence of residual invasive cancer in the pathological evaluation of resected breast specimens and lymph nodes after preoperative therapy shows the most significant association with a likelihood of benefit (Kaufmann et al. 2003, Prowell and Pazdur 2012), pCR is the primarily used therapeutic endpoint after disease-free and overall survival. However, the current clinical practice in this regard is time consuming, because decision-making is based on the pathologic response at the end of chemotherapy. Therefore, a method for predicting the early response to neoadjuvant chemotherapy is necessary. pCR prediction helps in identifying individuals with low or no benefit from chemotherapy, consequently sparing them from the toxicity of inactive treatments and allowing the use of alternative approaches at an earlier stage.

Mammography and US have been the most common and routine examinations for breast cancer for a long time. Several benefits of US imaging, such as decreased cost, its noninvasive nature, and the safety of repeated examinations for the same lesion, increase the competition for other imaging modalities for clinical use. A previous review of studies on the prediction of the response to neoadjuvant chemotherapy using US imaging involved the comparison of features such as distortion, light and shade contrasts, and image graininess (Kuo et al. 2008). Another approach is manual measurement of the size of the residual lesion after neoadjuvant chemotherapy by a radiologist, which is used to predict the response. By adjusting data-related bias using statistics and the experience of the radiologist, a high degree of predictive sensitivity and specificity can be obtained. However, the disadvantage of these approaches is that the predictive performance is highly reliant on the viewing experience of the radiologist; furthermore, excessive manpower is required for image reading.

The characteristics of tumor vascularization can be a key factor for predicting the effects of neoadjuvant chemotherapy. Tumor vascularity is strongly correlated with the degree of

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5 pCR, pathologic complete response.

6 US, ultrasound.
malignancy (Folkman 1985, 1990); therefore, changes in this vascularity may predict pCR to neoadjuvant chemotherapy for breast cancer. Previously, color and Doppler US were the main tools for investigating the blood flow and solid directional flow in breast lesions (Raza and Baum 1997). With advances in transducer design and signal processing, diagnostic US technology has also shown great improvements. Recently, color flow display technology in 3D\(^7\) power Doppler US was developed to improve the visualization of small vessels in breast masses, and HDF\(^8\) technology additionally provides high-definition images and allows for stronger detection of vascular signals to assess tumor vascularization. Compared with conventional 2D color imaging in Doppler US, 3D color flow and HDF technology facilitates superior imaging of the vascular morphology and better discrimination of malignant breast tumors, because of the limited transverse and longitudinal planes provided by diagnostic breast sonography (Carson \textit{et al} 1997, Wright \textit{et al} 1998, Lee \textit{et al} 2002).

The various vascularization features imaged by 3D power Doppler US with HDF include the vascular directional flow and density, and the morphology established using this data helps in detailed observations of tumor vessels in a noninvasive manner. Because the transport ability of vessels is strongly related to the effects of chemotherapy (Mathew \textit{et al} 2009), tumor vascularization characteristics show potential as key factors in the evaluation of patient response to neoadjuvant chemotherapy. From these perspectives, we used 3D power Doppler US with HDF to investigate the solid blood flow and directional flow in breast tumors. Essential vascularization features such as vascular flow and direction in the tumor vessels were extracted and used to predict pCR to neoadjuvant chemotherapy through the application of an SVM\(^9\). Therefore, the goal of this study was to evaluate the effectiveness of advanced US imaging in the prediction of the response to neoadjuvant chemotherapy for T2 breast cancer.

Materials and methods

A flow chart of the procedures used in this study is shown in figure 1. These procedures were useful for determining appropriate chemotherapy strategies for patients showing pCR or a PR\(^10\) in the early stages of neoadjuvant chemotherapy.

Patients

This retrospective study was approved by the institutional review board, and informed consent was obtained from all patients before participation. From July 2007 to October 2010, 96 patients receiving treatment from one surgeon (one of the authors) were recruited. According to the treatment guidelines for breast cancer at our hospital, at least three regimens are administered: FEC\(^11\), EC + TH\(^12\), and EC + T\(^13\). Considering that different chemotherapy drugs play different roles in angiogenesis and may result in confusion among gathered vascular data, the enrolled patients were limited to those receiving anthracycline-based regimens to avoid difficulties in analysis or dissimilar predictions. After the exclusion of patients without a continuous sequence of US image sets (lack of US images between any of the six cycles) and those who received non-FEC regimens, including taxane-based and trastuzumab-targeted

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\(^7\) 3D, three-dimensional.
\(^8\) HDF, high-definition flow imaging.
\(^9\) SVM, support vector machine.
\(^10\) PR, partial response.
\(^11\) FEC, epirubicin + fluorouracil + cyclophosphamide.
\(^12\) EC + TH, epirubicin + cyclophosphamide & docetaxel + trastuzumab.
\(^13\) EC + T, epirubicin + cyclophosphamide & docetaxel.
therapy, 29 consecutive patients with T2 stage breast cancer who received neoadjuvant chemotherapy were selected. All enrolled patients received an anthracycline-based regimen every 3 weeks, for a total of six cycles.

Axillary staging was performed using fine needle aspiration cytology. Mastectomy specimens were formalin-fixed, paraffin-embedded, sectioned, and stained with hematoxylin–eosin (H&E). Histological examination confirmed infiltrated ductal carcinoma in 26 patients (89.66%), mixed carcinoma or mucinous adenocarcinoma in three (10.34%), and axillary lymph node metastases in 16 (55.17%).

The initial tumor size was determined by selecting the larger of the two maximum diameters on 3D-HDF US before neoadjuvant therapy, and the postneoadjuvant therapy tumor size was determined by the pathologist. The two maximum diameters were measured on each perpendicular view by an experienced physician (one of the authors). Most of the lesions were in the T2 stage with a size of 2–5 cm, thus conforming to the transducer parameters. The mean tumor size obtained was 3.2 × 2.8 cm. After the neoadjuvant regimen, the measured mean tumor size was 1.5 cm. The responses were classified according to RECIST\textsuperscript{14} criteria (Byrne and Nowak 2004). With regard to the response to neoadjuvant chemotherapy, six patients showed a complete response (20.69%), 14 a PR (48.28%), five stable disease (17.24%), and three progressive disease (10.34%).

ER\textsuperscript{15}, PgR\textsuperscript{16}, and HER2\textsuperscript{17} expressions were evaluated in surgical and core needle biopsy specimens using the Roche Ventana Benchmark XT autostainer for immunohistochemistry (IHC). ER expression was evaluated using the SP1 antibody clone, PgR expression was

\textsuperscript{14}RECIST, response evaluation criteria in solid tumors.
\textsuperscript{15}ER, estrogen receptors.
\textsuperscript{16}PgR, progesterone receptor.
\textsuperscript{17}HER2, human epidermal growth factor 2.
evaluated using the 1E2 antibody, and HER2 expression was evaluated using the 4B5 antibody (Ventana/Roche, Ventana Medical Systems, Tucson, AZ). ER and PR expressions were assessed using Allred scoring; a score of >3 indicated positivity. HER2 staining was scored as 0, 1+, 2+, or 3+. Tumors with a 3+ score were classified as HER2-positive; all others were classified as negative. With regard to the receptor status, 21 patients showed positivity for ER (72.41%), 28 showed positivity for PgR (96.55%), and 13 showed positivity for HER2 (44.83%). None of the patients showed triple-negative breast cancer. The outcomes of primary chemotherapy, expression of breast cancer biomarkers, and tumor sizes are summarized in table 1.

**US**

Figure 2 shows the complete course of neoadjuvant chemotherapy and the predefined time points for US image acquisition. Three time points were predefined: before core needle biopsy (P0), before chemotherapy cycle two (P1), and before cycle three (P2). Each US image was obtained prior to the injection of the chemotherapy drug. 3D power Doppler US imaging was performed using the GE Voluson 730 scanner equipped with the RSP 6–12 transducer (GE Healthcare, Zipf, Austria); a linear array broadband probe with a frequency of 6 to 12 MHz, a scan width of 37.5 mm, and a sweep angle of 5 to 29° was used for 3D volume scanning. A preinstalled 20 sweep angle and power Doppler settings with a moderate frequency, 0.9 kHz pulse repetition frequency, gain of −0.6, and ‘low 1’ wall motion filter were used for all women in this study. Each scanning procedure took approximately 10 to 15 s. If the patient’s chest wall movements were prominent on scanning, excessive artifacts occurred. The operator held the large 3D probe as steady as possible to minimize such artifacts. To prevent variations in the power Doppler color gain intensity, body habitus settings, and wall filters were maintained throughout the study.

**VOI extraction**

A physician with more than 10 years of experience in breast US image interpretation (one of the authors) manually determined the 3D contours of the tumor for each patient using a PMS system to obtain an approximate 3D contour. Six preliminary 2D contours were manually sketched on image slices in six axes at 0°, 30°, 60°, 90°, 120°, and 150° of VOI18; and these slides were used as references for 3D contour building. 3D contours of tumors on B-mode images were automatically estimated later in the PMS19 system. Figure 3 shows the method for manually sketching the 2D contours on the image slices at 30° (figure 3(a)), 60° (figure 3(b)) and 90° (figure 3(c)) and the use of these 2D slices to establish the 3D contours in the PMS system (figure 3(d)). The details of the PMS system are described in a previous study (Huang et al 2013).

When contours were sketched after the procedure, the PMS system automatically shows a tumor volume that is 3 mm³ greater than the original volume. This extended volume is also known as dilated volume. This is to minimize deviations in tumor contours sketched by physicians because of the blurred borders of tumors on US images. In our previous study, the tumor contour was repeatedly sketched three times by the same physician to reducing the errors (Stylianopoulos and Jain 2013). However, that is a time-consuming procedure and decreases

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18 VOI, volume of interest.
19 PMS, partial manual sketching.
Table 1. Characteristics of the 29 patients who received neoadjuvant chemotherapy.

| Case No. | Age (years) | Histology | Lymph nodes invaded | ER status | PgR status | HER2 status | Tumor grade (BR) | Tumor size (cm) | Post-chemotherapy tumor size (cm) | Chemotherapy | RECIST |
|----------|-------------|-----------|---------------------|-----------|------------|-------------|-----------------|----------------|----------------------------------|--------------|--------|
| 1        | 52          | IDC       | −                   | +         | +          | −           | 2               | 3.4 × 2.7       | 2.1                | FEC          | PR     |
| 2        | 66          | IDC       | −                   | −         | +          | −           | 2               | 2.7 × 3.2       | 2.5                | FEC          | SD     |
| 3        | 81          | IDC       | +                   | −         | +          | −           | ND              | 3.0 × 2.9       | 0                  | FEC          | CR     |
| 4        | 50          | IDC       | −                   | −         | +          | +           | 2               | 2.0 × 2.2       | 2.5                | FEC          | PD     |
| 5        | 50          | IDC       | +                   | +         | +          | +           | 3               | 3.9 × 4.1       | 0                  | FEC          | CR     |
| 6        | 47          | IDC       | +                   | +         | +          | −           | 2               | 4.7 × 3.8       | 4                  | FEC          | SD     |
| 7        | 49          | IDC       | +                   | +         | +          | −           | 3               | 4.8 × 3.3       | 0.5                | FEC          | PR     |
| 8        | 61          | IDC       | +                   | −         | +          | +           | 2               | 4.2 × 2.6       | 2.9                | FEC          | SD     |
| 9        | 46          | IDC       | +                   | +         | +          | −           | 2               | 4.1 × 3.4       | 0.6                | FEC          | PR     |
| 10       | 33          | IDC       | +                   | +         | +          | +           | 2               | 4.2 × 3.0       | 1.2                | FEC          | PR     |
| 11       | 47          | IDC & ILC | −                   | −         | +          | +           | 2               | 2.5 × 3.0       | 1.8                | FEC          | PR     |
| 12       | 71          | IDC       | +                   | +         | +          | +           | 2               | 4.7 × 3.8       | 2.5                | FEC          | PR     |
| 13       | 41          | IDC       | +                   | +         | +          | +           | 3               | 4.3 × 3.7       | 3.2                | FEC          | SD     |
| 14       | 53          | IDC       | −                   | −         | +          | −           | 2               | 3.6 × 2.9       | 1.1                | FEC          | PR     |
| 15       | 56          | IDC       | +                   | −         | +          | +           | 3               | 3.2 × 3.5       | 0.2                | FEC          | PR     |
| 16       | 52          | IDC       | −                   | +         | +          | +           | 2               | 3.7 × 3.1       | 2.5                | FEC          | PR     |
| 17       | 53          | IDC       | −                   | +         | +          | −           | 2               | 2.2 × 1.7       | 0.6                | FEC          | PR     |
| 18       | 69          | IDC       | +                   | +         | +          | −           | 2               | 2.4 × 3.5       | 4.9                | FEC          | PD     |
| ID  | Size | Type     | ER | PR | DCIS | PR  | CR  |  ER | PR  | DCIS | PR  |
|-----|------|----------|----|----|------|-----|-----|-----|-----|------|-----|
| 19  | 35   | MUC      | −  | +  | +    | −   | ND  | 2.4 | 2.6 | 2.5  | FEC | SD  |
| 20  | 50   | IDC      | +  | −  | −    | +   | 3   | 2.0 | 3.0 | 2.5  | FEC | PD  |
| 21  | 54   | IDC      | −  | +  | +    | −   | 1   | 2.9 | 2.8 | 0.8  | FEC | PR  |
| 22  | 45   | IDC      | −  | +  | +    | −   | 2   | 2.6 | 1.9 | 1.5  | FEC | SD  |
| 23  | 45   | IDC      | −  | −  | +    | −   | 3   | 4.1 | 2.5 | 0.8  | FEC | PR  |
| 24  | 54   | IDC      | +  | +  | +    | −   | 2   | 2.6 | 1.4 | 0    | FEC | CR  |
| 25  | 42   | IDC      | +  | −  | +    | −   | 3   | 2.4 | 2.3 | DCIS | EC  | CR  |
| 26  | 54   | IDC      | −  | +  | +    | +   | 2   | 2.5 | 2.3 | 0    | FEC | CR  |
| 27  | 47   | IDC      | +  | +  | +    | −   | 3   | 2.7 | 1.9 | 0.2  | EC  | PR  |
| 28  | 45   | IDC & MUC| +  | +  | +    | −   | 2   | 2.6 | 1.9 | 1.5  | EC  | PR  |
| 29  | 67   | IDC      | −  | −  | −    | +   | 3   | 2.8 | 3.0 | 0    | EC  | CR  |

**Abbreviations**: DCIS, ductal carcinoma in situ; ER, estrogen receptor; IDC, infiltrating duct carcinoma; ILC, invasive lobular carcinoma; MUC, mucinous adenocarcinoma. *with microinvasion of less than 0.1 cm.
the clinical efficiency, because each 3D US image includes over 180 slices of B-mode 2D US images. Relative to the exact tumor volume, the inner volume was derived from the sketched tumor contours. For more precise observation, two areas were separately processed for feature extraction.

The blue dotted line shows the sketched margin.
Image preprocessing

The considerable noise and speckles on US images complicate blood vessel identification. A suitable preprocessing procedure aims to reduce noise and preserve useful vascular information before extracting vascular centerlines.

The 3D Gaussian low-pass filters (Haddad and Akansu 1991) were used for preprocessing to smoothen the vascular intensity and facilitate further image processing procedures ($\Sigma = 0.65$). Weighted average filter was used for calculating the weighted values from the voxels in the mask based on Gaussian function, and each voxel generated on the basis of its surrounding. The HDF Doppler signals were converted into a binary vascular images by using the threshold algorithm (Shapiro and Stockman 2001), preserving the blood vessel contours as required (threshold $TH = 35$).

Binary vascular imaging always involves the formation of some cavities on the surface or within the vessels because of noise. The noise and disconnected segments can result in 3D blood vessel thinning and construct skeletons in incorrect structure. The proposed method utilized a $3 \times 3 \times 3$ cm cube as a template for the mathematical morphology. A double closing operation, i.e. two successive dilations followed by two successive erosions, was initiated to fill up the 3D vascular area. The color of voxels in the 3D HDF images represented the direction of blood flow ($0$–$127$ indicated blood flow toward the probe and $129$–$255$ indicated blood flow away from the probe), while the intensity represented the speed (intensity $> 128$ indicated greater speed). Therefore, the directional information for blood flow annotated with each point in the vascular area.

A parallel 3D 6-subiteration thinning algorithm (Palágyi and Kuba 1998) was used to directly extract vascular centerlines from elongated 3D binary objects and preserve the vascular topology on US images with noise reduction. This eliminated the skeletal blood vessels that contained less than four voxels, producing either curve skeletons or surface skeletons from 3D binary objects in $(26, 6)$-connectivity by repetitive iterations, until no more points needed to be eliminated.

Vascular feature extraction

The 3D HDF images contained three image channels including B-mode signal, reflection intensity of blood vessels, and the direction of blood flow. B-mode sonography was used as the draft while sketching the tumor volume; the channels for blood vessel reflection intensity and direction of blood flow were used for statistical analysis of the vascularization features on Doppler US imaging. Quantized vascularity and morphological and vascular directions were selected as meaningful tumor vessel data after assessment.

Three vascular quantization indices were exacted from the blood flow channels (Pairleitner et al 1999): ($VI_{20}$, which was the ratio between the color boxes and the total number of voxels in VOI, representing the vessel density in the defined volume; $FI_{21}$, which was the mean energy of the voxel per color, representing the average intensity of flow; and $VFI_{22}$, which was the mean color value for all voxels in the volume, representing the intensity of both vascularization and flow (Jarvela et al 2002). For further comparison, we divided $VI$, $FI$, and $VFI$ into two groups on the basis of their location in the dilated volume (d$VI$, d$FI$, and d$VFI$) or the inner volume (i$VI$, i$FI$, and i$VFI$). These indices were repeatedly measured to avoid intraobserver errors and guarantee reproducibility.

\(^{20}\) $VI$, vascularization index.
\(^{21}\) $FI$, flow index.
\(^{22}\) $VFI$, vascularization-flow index.
Three vascular morphological features were extracted from the blood vessel intensity image, including NT, NB, and SDVC. NT was also classified into dNT and iNT. With regard to the vascularization directivity features, sigma (σ) was a measure of abnormal blood vessel intensity, and entropy (ε) was a measure of disorders in the vascular direction. Higher entropy and sigma values represented a more severe disorder.

Classification analysis using SVM

Unlike traditional statistical methods, SVMs are nonlinear, nonparametric classification devices, particularly suitable for binary classification. They have shown a favorable performance in the field of medical diagnostics and several other fields. SVMs allow for good out-of-sample generalization after the selection of appropriate parameters C and γ, and it provides robust results through the selection of an appropriate generalization grade, even if some bias exists in the training set (Cristianini and Shawe-Taylor 2000, Smola and Schölkopf 2004, Sehgal et al 2006). The classification problem is solved by finding a hyperplane that can divide these eigenvalues, and this process is dependent on optimal mapping using a nonlinear mapping algorithm in the high-dimensional data space. These features were converted into vectors and input to the SVM classifier for evaluating the response to neoadjuvant chemotherapy.

In this study, C-SVC (Boser et al 1992) was chosen for the formulation using in SVM, and the open source machine learning library LibSVM (Chang and Lin 2011) was used as the implementation. Given training vectors \( x_i \in \mathbb{R}^n \) in two classes, and an indicator vector \( y_i \in \{1, -1\} \), than

\[
\begin{align*}
\min_{w,b,c} & \frac{1}{2} w'w + c \sum_{i=1}^l \varepsilon_i \quad \text{subject to} \quad y_i(w' \odot (x_i) + b) \geq 1 - \varepsilon_i
\end{align*}
\]

where \( \varepsilon_i > 0 \), \( C > 0 \), \( \odot (x_i) \) maps \( x_i \) into a higher-dimensional space. The classification problem is solved by the decision function as:

\[
f(x) = \left[ \sum_{i=1}^l y_i \alpha_i k(x_i, x) + b \right]
\]

where \( \alpha_i \) is the positive Lagrange multiplier, \( x_i \) are the support vectors (\( l \) in total), and \( k(x_i, x) \) is the function for convolution of the kernel. RBF kernel was chosen for the kernel function after the performance estimate; it defined as:

\[
k(x_i, y_i) = \exp(-\gamma \|x_i - x_j\|^2)
\]

where \( \gamma \in \mathbb{R}, \gamma \neq 0 \).

The vascular features of each US image included six quantization, three morphological, and two vascular direction features. Predictions were generated by combining both flow and morphological features. After the feature extraction procedure, the intensities of each vascular feature were turned into an eigenvalue. All eigenvalues were required for scaling, which refers to the compression of eigenvalues (two floating point numbers) between zero and one according to the numeric distribution of each feature to obtain better performance in classification. Because of multiple features, all eigenvalues constitute a high-dimensional data space.
Predictions were generated by the utilization of the extracted vascular features from the US images acquired at the predefined time points.

For evaluating the accuracy of prediction, receiver operating characteristic (ROC\(^{27}\)) analysis was performed and areas under the ROC curve (AUCs\(^{28}\)) (Obuchowski 2003) with k-fold cross-validation (Weiss and Kapouleas 1990) were generated. Five-fold cross-validation was used to measure the performance of each prediction model. By plotting the TPR\(^{29}\) and FPR\(^{30}\) fractions at various threshold settings, ROC analysis was used to evaluate the classifier output quality, and the proportion of AUC reflecting the overall performance of a diagnostic system was quantitatively measured. The open source python module and toolkit for machine learning scikit-learn (Pedregosa et al 2011) and its inner RBF-kernel C-SVC, which has functions similar to LibSVM, were used for plotting, simulation, and evaluation of performance (scripts were written by one of the authors).

**Results**

Figure 4 shows the ROC curves and accuracy values for the prediction of pCR and a PR at the three predefined time points (P0, P1, and P2, see figure 2). After SVM classifiers were trained and optimized, the best prediction efficiency for pCR was observed at P1, with an accuracy of 93.1\% (AUC, 0.8551, when \(C = 2048, \gamma = 0.0078125\)), followed by P0, with an accuracy of 89.655\% (AUC, 0.8788, when \(C = 2, \gamma = 0.5\)). Larger AUCs generally indicate better classification performance. In general, the specificity and sensitivity of pCR prediction were greater than those of PR prediction. The best prediction of a PR was achieved at P0, with an accuracy of 79.31\% (AUC, 0.7222, when \(C = 2, \gamma = 0.5\)). The prediction model used in this study showed high sensitivity for pCR prediction compared with that in other studies, possibly because of differences in feature selection strategies. In actual practice, superior pCR prediction appears to be more practical than superior PR prediction.

Flow and morphology are the major features observed on 3D Doppler US. Figure 5 shows the AUC and accuracy values for pCR prediction using flow features only and flow and morphology combination features. Although flow features are the predominant features for prediction, the flow and morphology combination features significantly increased the accuracy and AUC values. The most significant increase was observed at P1, when the accuracy increased by 18.1\% (figure 5(a)) and AUC increased by 0.277 (flow feature only: accuracy, 75\%; AUC, 0.5781, where \(C = 0.03125, \gamma = 0.0078125\); figure 5(b)). However, when the morphology features were used as the main features for prediction, most of the predictions were not significant (AUC < 0.6).

Left panel: patients with pCR, Right panel: patients with PR.

**Discussion**

Because of the small number of patients, limited chemotherapy regimens, and inclusion criteria for this study, a large dataset was difficult to acquire. Therefore, derivation of a highly predictive model based on a smaller dataset was challenging and required the accurate extraction of flow and morphology features and the selection of appropriate image preprocessing methods to facilitate feature extraction. When the data are not uniformly distributed or show

\(^{27}\) ROC, receiver operating characteristic.

\(^{28}\) AUC, area under the ROC curve.

\(^{29}\) TPR, true positive rate.

\(^{30}\) FPR, false positive rate.
an unknown distribution, SVM is a useful tool, with a disadvantage of the lack of transparency in prediction outcomes. Because pCR prediction may occur in a very high-dimensional data space, SVM cannot represent it as a simple parametric function.

Most vascular features extracted in this study were nonlinear data that were difficult to classify directly. SVM shows a distinct advantage of resolving this problem. The application of PCA\textsuperscript{31} to downgrade the data space from a high-dimensional to a bi-dimensional graph

\textsuperscript{31}PCA, principal component analysis.

Figure 4. Comparison of the prediction rates and areas under the curve (AUCs) between patients who showed a pathological complete response (pCR) and those who showed a partial response (PR). (a) and (b) Before the first chemotherapy (P0). (c) and (d) After the first chemotherapy (P1). (e) and (f) After the second chemotherapy (P2).
aids the observation of vascular features and shows that vascular features are slightly different between patients who show pCR and those who show other responses. In figure 6(a), the data points for pCR patients (green circles) are grouped and above all the other data points (blue ‘+’), easily dividing the two classes. After scaling the vector of features from zero to one to increase the contradistinction (figure 6(b)), the property described previously was also increased. This suggests that the vascular features of pCR patients have innate characteristics and can be used to distinguish pCR from a PR.
The data points for patients who exhibited a complete response (CR) are clustered and can be grouped in SVM. Green dots (CR): data points for patients who exhibited a pathological complete response (pCR). Blue dots (NCR): data points for all non-CR patients. X and Y axis: the scale of the first component and the second component after PCA.

Because of the different characteristics of the vascular features at the different time points and in the different prediction groups, through the adjustment of learning strategies, SVM can adapt the dataset to achieve good predictive results by the adjustment of parameters $C$ and $\gamma$. After the classifiers were trained and optimized, the sensitivity of prediction of the response to neoadjuvant chemotherapy was high in this study, with fewer limitations related to lesion size compared with sonography and mammography (Gruber et al 2013). Furthermore, the value was noninferior to the overall average values of 70% to 80% derived in related studies that used advanced imaging technologies such as PET\textsuperscript{32} and MRI\textsuperscript{33} (Park et al 2012, De Los Santos et al 2013).

There were 11 HER2-positive patients who received the FEC regimen instead of target-therapy, such as herceptin-based regimens. This is because trastuzumab-targeted therapy was administered only for metastatic breast cancer up to August 2010, according to the National Health Insurance policy in Taiwan. This indicates that the exclusion of HER2 positivity could have led to group selection bias and consequently decreased the accuracy of the findings of this study. The same SVM classifier was applied again, and the expression status of HER2 was added as the 13th feature for the evaluation of pCR prediction performance in P0 and P1 patients. The accuracy and AUC of pCR prediction was decreased after the addition of HER2 expression status (accuracy, 89.66% in P0 and 82.76% in P1; AUC, 0.6812 in P0 and 0.8478 in P1). Because these results were not superior to the previous ones, it was considered that the expression status of HER2 is not a predictor of the response to FEC-based neoadjuvant chemotherapy. A previous study also indicated an approximate accuracy of 40% for the prediction of the response to chemotherapy using the ER/PgR/HER2 expression status (Gianni et al 2005).

Conclusions

This study preliminarily demonstrates that vascularization data is useful for predicting the effects of neoadjuvant chemotherapy. However, several limitations exist, such as the small quantity of samples and the retrospective study design; therefore, the results need to be interpreted with caution. A larger sample quantity can decrease the bias in this model and increase the prediction performance in SVM. Also, a prospective study will help avoid bias and generate more accurate and efficient results. Future studies should include more vascularization data for more accurate and earlier prediction of the response to preoperative chemotherapy, which will further decrease the rate of unnecessary treatments.

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\begin{footnotesize}
\begin{itemize}
\item PET, positron emission tomography.
\item MRI, magnetic resonance imaging.
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