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Purpose:
To assess initial breast tumor hemoglobin (Hb) content before the initiation of neoadjuvant chemotherapy, monitor the Hb changes at the end of each treatment cycle, and correlate these findings with tumor pathologic response.

Materials and Methods:
The HIPAA-compliant study protocol was approved by the institutional review boards of both institutions. Written informed consent was obtained from all patients. Patients who were eligible for neoadjuvant chemotherapy were recruited between December 2007 and May 2011, and their tumor Hb content was assessed by using a near-infrared imager coupled with an ultrasonography (US) system. Thirty-two women (mean age, 48 years; range, 32–82 years) were imaged before treatment, at the end of every treatment cycle, and before definitive surgery. The patients were graded in terms of their final pathologic response on the basis of the Miller-Payne system as nonresponders and partial responders (grades 1–3) and near-complete and complete responders (grades 4 and 5). Tumor vascularity was assessed from total Hb (tHb), oxygenated Hb (oxyHb), and deoxygenated Hb (deoxyHb) concentrations. Tumor vascularity changes during treatment were assessed from percentage tHb normalized to the pretreatment level. A two-sample two-sided t test was used to calculate the P value and to evaluate statistical significance between groups. Bonferroni-Holm correction was applied to obtain the corrected P value for multiple comparisons.

Results:
There were 20 Miller-Payne grade 1–3 tumors and 14 grade 4 or 5 tumors. Mean maximum pretreatment tHb, oxyHb, and deoxyHb levels were significantly higher in grade 4 and 5 tumors than in grade 1–3 tumors (P = .005, P = .008, and P = .017, respectively). The mean percentage tHb changes were significantly higher in grade 4 or 5 tumors than in grade 1–3 tumors at the end of treatment cycles 1–3 (P = .009 and corrected P = .009, P = .002 and corrected P = .004, and P < .001 and corrected P < .001, respectively).

Discussion:
These findings indicate that initial tumor Hb content is a strong predictor of final pathologic response. Additionally, the tHb changes during early treatment cycles can further predict final pathologic response.

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Preoperative or neoadjuvant chemotherapy is used in treating patients with locally advanced breast cancers as well as patients whose cancers are resectable but are not amenable to breast-conserving surgery (1–2). Histologic documentation of tumor response to preoperative chemotherapy is correlated with clinical outcome. An absence of residual tumor cells in the primary tumor bed after neoadjuvant therapy is strongly correlated with improved disease-free survival and overall survival (3). Surprisingly, neoadjuvant chemotherapy does not improve survival in the majority of patients who demonstrate lesser degrees of pathologic response. This is due in part to the standard “one treatment fits all” way clinical trials have been performed in the past. With the trend toward personalized treatment, accurate prediction of responses becomes more critical and may therefore improve survival.

Several tumor markers are routinely used to predict treatment outcome and select therapy (4–6). Classifying breast cancers into subgroups on the basis of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2/neu) status has improved our understanding of tumor response and has helped guide tailored treatment (7–8). However, these subgroup analyses are imperfect; within and among these subgroups, the response to chemotherapy varies widely. Furthermore, the group of “triple-negative” (ER/PR/HER2-negative) tumors does not, as yet, allow targeted therapy. Therefore, it is important to predict outcome and assess early tumor response, so treatment regimens can be appropriately tailored.

Conventional methods, including clinical examination, ultrasonography (US), and mammography have been shown to be moderately useful in the assessment of tumor response (9). Contrast material–enhanced magnetic resonance (MR) imaging has been used to assess breast cancers before treatment and prior to surgery for treatment and surgical planning (10–11). Fluorine 18 fluorodeoxyglucose positron emission tomography (PET) has been shown to demonstrate early metabolic changes that may correlate with final pathologic response (12). However, both MR imaging and PET require the injection of contrast agents and are costly for repeated use during treatment.

In the past decade, optical tomography and spectroscopy with near-infrared (NIR) diffused light have demonstrated great potential in the initial diagnosis of a tumor (13–22) and in the assessment of the tumor vasculature’s response to neoadjuvant chemotherapy (23–28). The NIR technique uses the intrinsic contrast of hemoglobin (Hb), which is directly related to tumor angiogenesis development, a key process required for tumor growth and metastasis. Cost effectiveness, portability, lack of ionizing radiation, and the lack of need for contrast agents make NIR systems ideal for repeated use in clinical settings. Tomographic imaging with a pure NIR light technique is challenging because of poor lesion localization owing to light scattering. We have developed a US-guided NIR imaging technique that utilizes US to localize the light illumination and guide image reconstruction (18,21,29–30). In a pilot study, we demonstrated the feasibility of using this technique to monitor tumor Hb changes during neoadjuvant therapy.

### Advances in Knowledge

- Pretreatment breast tumor hemoglobin (Hb) content is a strong predictor of response to neoadjuvant chemotherapy: For the near-complete and complete responder group (Miller-Payne grades 4 and 5), the mean maximum total Hb (tHb) was 107.9 μmol/L ± 33.9 (standard deviation) and the mean average tHb was 72.2 μmol/L ± 24.5, whereas for the nonresponder and partial responder group (grades 1–3), the mean maximum tHb was 75.7 μmol/L ± 18.8 and the mean average tHb was 51.0 μmol/L ± 13.9 (P = .005 and P = .009, respectively).

- The percentage of tHb changes normalized to the pretreatment level can be used to predict pathologic tumor response at early treatment cycles; the differences between the two responder groups were statistically significant at the end of cycles 1–3 (corrected P = .009, corrected P = .004, and corrected P < .001, respectively).

### Implications for Patient Care

- The near-infrared technique with US localization of breast tumors can potentially be used to manage neoadjuvant chemotherapy by helping predict pathologic response even before the initiation of treatment.

- Monitoring of tumor response during early treatment cycles further enhances its predictive utility, potentially allowing the personalization of chemotherapy regimens.
chemotherapy (25). The purpose of this study was to evaluate initial tumor Hb content and early Hb changes during neoadjuvant chemotherapy as predictors of final tumor pathologic response with a larger patient pool.

Materials and Methods

Patients

The study protocol was approved by the institutional review boards of both institutions and was compliant with the terms of the Health Insurance Portability and Accountability Act. Written informed consent was obtained from all patients. Forty patients who were referred for neoadjuvant chemotherapy to two medical oncologists (P.A.D. and S.H.T.) were recruited from December 2007 to May 2011 at two hospitals. Two patients did not complete the study, as a result of a change in their treatment plan or a desire to withdraw from the study. Three patients underwent their first NIR US examination several days after the initiation of chemotherapy because of scheduling problems. The other 33 patients were imaged with NIR US prior to initiation of chemotherapy, at the end of every treatment cycle during chemotherapy, and prior to definitive surgery. Of these 33 patients, three were excluded from data analysis: One had inflammatory breast cancer with no measurable vascular content, another was an elderly patient with an HER2-positive tumor who was treated with weekly sequential chemotherapy for 6 weeks longer than the remainder of the patients in the HER2-positive group, and the third patient had undergone core biopsy 3 days before pretreatment NIR US measurements.

Among the 32 patients from whom data were collected and analyzed (Table 1), 20 were treated with dose-dense doxorubicin, cyclophosphamide, and paclitaxel. This cohort was monitored at the end of each 2-week treatment cycle. The following groups of patients were monitored at the end of each 3-week treatment cycle: Patients treated with docetaxel and cyclophosphamide (n = 4); patients treated with docetaxel and carboplatin with trastuzumab (HER2 positive, n = 5); and patients treated according to the National Surgical Adjuvant Breast and Bowel Project B4-0 trial protocol, who received doxorubicin, cyclophosphamide, docetaxel, and bevacizumab (an antiangiogenic agent), with (n = 2) or without (n = 1) capecitabine.

Among the 32 patients (mean age, 48 years; range, 32–82 years), six underwent the first NIR US study before core biopsy, while 26 patients were imaged after core biopsy, with an average interval of 22 days (median, 22 days; range, 9–49 days). The first cycle of neoadjuvant chemotherapy was after the initial NIR US study (median, 0 days; range, 0–44 days). The average interval between posttreatment NIR US and surgery was 8 days (median, 8 days; range, 0–35 days). During treatment, the NIR US studies were performed on the same day before scheduled chemotherapy unless scheduling or weather problems interfered (median, 0 days; range, 0–4 days). Among the 32 patients, 16 had a metal clip inserted before chemotherapy to mark the site of biopsy and to guide subsequent US imaging and surgical resection. Among the 32 patients, 26 underwent both pre- and posttreatment MR imaging. Four patients underwent pretreatment MR imaging but not posttreatment MR imaging, either because they underwent early surgery after clinical evidence showed disease progression or because they decided on mastectomy. One patient transferred from another hospital after core biopsy did not undergo pre-treatment MR imaging, and one patient did not undergo MR imaging at all because of claustrophobia. The median interval between the pretreatment MR imaging examination and the first treatment was 17 days (range, 1–41 days); the median interval between the posttreatment MR imaging examination and surgery was 21 days (range, 3–49 days).

The histologic type of cancer was IDC in 21 patients; ILC in five patients; and invasive mammary carcinoma with mixed ductal and lobular features, or IDC/ILC, in six patients. Two patients had two distinct tumor masses in the same breast with similar characteristics. Invasive carcinoma within the pretreatment core biopsies was graded by using the Nottingham histologic score. Testing for ER, PR, and HER2/neu immunohistochemistry was performed on formalin-fixed, paraffin-embedded core biopsy tissue. ER and PR statuses were scored by using the modified San Antonio scoring system, where total scores range from 0 to 8 and scores of 0–2 are considered negative, a score of 3 is equivocal, and scores of 4 or greater are positive. Testing for HER2/neu gene amplification was performed by using the fluorescence in situ hybridization technique. The results were reported as the ratio of HER2/neu to CEP17. A ratio greater than 2.2 is considered positive for amplification of this gene, a ratio of less than 1.8 is considered negative, and a ratio between 1.8 and 2.2 is scored as equivocal. All assays were performed on pretreatment core biopsy samples.

Pathologic Response Assessment

Pathologic response was assessed by applying the Miller-Payne grading criteria to definitive surgery specimens in comparison with initial core biopsy samples. Two breast pathologists (A.R., with 27 years of experience, and P.U.H., with 10 years of experience) individually evaluated most cases in their respective hospitals. In three complex cases, hematoxylin-eosin–stained slides were reviewed by both pathologists in consultation, and a consensus was reached.

In the Miller-Payne system (31), pathologic response is divided into five grades on the basis of comparison of tumor cellularity between pre–neoadjuvant therapy core biopsy specimens and definitive surgical specimens as follows: A grade of 1 indicated no change or some alteration in individual malignant cells but no reduction in overall cellularity (pathologic nonresponse); a grade of 2, minor loss of tumor cells but still high overall cellularity, of up to 30% (pathologic partial response); a grade of 3, an estimated reduction in tumor cells of between 30% and 90% (pathologic partial response); a grade of 4, a marked disappearance...
of tumor cells such that only small clusters or widely dispersed individual cells remained, with more than 90% loss of tumor cells (almost pathologic complete response); and a grade of 5, no malignant cells identifiable in slices from the site of the tumor and only vascular fibroelastic stroma remaining, often containing macrophages (however, ductal carcinoma in situ may be present) (pathologic complete response).
US and NIR System and Imaging

US examinations were performed with either an IU/22 unit with an L12 linear transducer (Philips Medical Systems, Bothell, Wash) or a Sequoia unit with a 15L8 linear transducer (Acuson, Mountain View, Calif). Two NIR systems with identical designs were used at the two hospitals, and details about these systems have been given previously (32). Briefly, the probe consists of the commercial US transducer located in the middle, with source and detector light guides (optical fibers) distributed at the periphery. Four laser diodes with 740-, 780-, 808-, and 830-nm optical wavelengths were sequentially switched to nine positions on the probe, while the reflected light was coupled by the light guides to 10 parallel detectors. The entire NIR data acquisition interval was less than 5 seconds. For each patient, US images and optical measurements were acquired simultaneously before treatment at multiple locations, including the lesion region and a normal region of the contralateral breast in the same quadrant as the lesion. The optical data acquired in the normal contralateral breast were used as a reference for calculating the background optical absorption and reduced scattering coefficients that were used in the image reconstruction of the lesions.

The US and optical measurements were repeated at the end of each treatment cycle and before the surgery.

Details of the optical imaging reconstruction algorithm with experimental validation have been described elsewhere (33). Briefly, the NIR reconstruction takes advantages of US localization of lesions and segments the imaging volume into a region of interest (ROI) and background nonlesion regions. Because the spatial resolution of diffused light is poorer than that of US, the ROI is chosen to be at least two to three times larger than that seen by using US in the x- and y-axis dimensions. In this study, because of the large tumor size, the ROI in the x- and y-axis dimensions was chosen to be 10 cm, which is the size of the probe in most cases. In addition, because the depth localization of diffused light is very poor, a tighter ROI in the depth dimension was set by using coregistered US. For each patient, the same size ROI obtained from the pretreatment US examination was used for processing all data sets obtained at different treatment cycles. Therefore, the changes in tumor size seen with US during treatment had no major effect on NIR image reconstruction. Among the 32 patients, 11 had initially palpable tumors with ill-defined and heterogeneous pretreatment US images. For these patients, tumor sizes estimated from pretreatment MR images were used to assist the determination of the US ROI in the x- and y-axis dimensions. The ROI in the depth dimension was typically set from the top border of the ill-defined tissue pattern to the chest wall as seen with US.

The optical absorption distribution at each wavelength was reconstructed, and total Hb (tHb) concentration, as well as oxygenated Hb (oxyHb) concentration and deoxygenated Hb (deoxyHb) concentration, was computed from absorption maps at the four wavelengths (34). Maximum and average tHb, oxyHb, and deoxyHb were measured, and the average was computed within the volumetric zone exceeding 50% of the maximum value. For each patient imaged at each cycle, average values of maximum and average that were obtained from several quality NIR images at the tumor location were used to characterize the tumor. Data with patient motion as evaluated by using two coregistered US images before and after each NIR measurement were excluded from averaging. To assess each patient’s response, the tHb obtained before treatment was taken as the baseline, and the percentage tHb normalized to the baseline was used to quantitatively evaluate the tumor vascular changes during chemotherapy. One author (Q.Z., with 12 years of experience in US and optical imaging) performed the optical imaging.

MR Imaging, US, and Measurements

MR images were obtained by using an MR imaging unit (Vista 1.5 T or Avanto 1.5 T, Siemens, Erlangen, Germany; Excite 1.5 T, GE Healthcare, Waukesha, Wis) with three-dimensional flash dynamic acquisition with subtraction. Pre- and posttreatment images were available in 26 patients. The size of the tumor was measured on pre- and posttreatment images in the craniocaudal, transverse, and anteroposterior dimensions by two radiologists (E.B.C., with 23 years of experience, and M.K., with 13 years of experience) for cases from their respective hospital. The percentage ratio of the largest dimension of posttreatment measurements to the largest dimension of pretreatment measurements was used to compute the percentage reduction.

Twenty-one patients had well-defined tumors at US, and their tumor sizes were measured by US technologists in consultation with attending radiologists. The percentage ratio of the largest dimension of each posttreatment measurement to the largest dimension of pretreatment measurement was used to compute the percentage reduction in tumor size, which was noted as percentage reduction at US. For the 11 patients with initially palpable lesions but ill-defined and heterogeneous US images, pretreatment tumor sizes were estimated at the time of imaging. The tumor location at each treatment cycle was tracked by using previous US images as references. The tumor clock position, distance of the tumor to the nipple, and depth were documented for each case. Additionally, the tumor posterior shadowing, color Doppler profile, surrounding tissue structures, and metal clip position were also reviewed and used to help identify the tumor for each subsequent measurement. Seven of 20 Miller-Payne grade 1–3 tumors and nine of 14 grade 4 or 5 tumors had metal clips marking the biopsy sites.

Statistical Analysis

A two-sample two-sided t test was used to calculate statistical significance for comparisons between groups, with P < .05 considered to indicate a statistically significant difference. When comparing percentage tHb and percentage reduction at US at different treatment cycles, the Bonferroni-Holm correction was applied to obtain the corrected P.
value for the number of treatment cycles. Bonferroni-Holm correction ranks all P values from the smallest to the largest and evaluates statistical significance on the basis of $P(i) < \alpha/(n - i + 1)$ or $P(i) = (n - i + 1) \cdot P(i) < \alpha$, where $\alpha$ is .05, $n$ is the total number of comparisons, $i$ starts from 1 (corresponding to the smallest $P$ value) to $n$ (corresponding to the largest $P$ value), and $P(i)$ is the corrected $P$ value. Both the prediction accuracy and the specificity based on pretreatment maximum tHb were determined by selecting an optimal threshold by using the receiver operating characteristic curve that provided the best compromise between sensitivity and specificity. Pearson correlation was performed between each tumor’s Miller-Payne grade and the pretreatment maximum and average tHb, oxyHb, and deoxyHb; tumor Nottingham score and mitotic index at core biopsy, and pretreatment tumor size at MR imaging (Table E1 [online]). When we compared the predictive value of pretreatment tHb, oxyHb, and deoxyHb with the predictive value of Nottingham score and mitotic index at core biopsy, pretreatment maximum tHb and deoxyHb achieved the highest predictive value ($r = 0.531$ and $r = 0.538$, $P = .001$), followed closely by tumor Nottingham score ($r = 0.531$, $P = .001$, Table 2). We found no correlation between pretreatment tumor size at MR imaging ($P = .552$) or US ($P = .417$) and Miller-Payne grade (Table 2).

We also evaluated prediction accuracy and specificity on the basis of pretreatment maximum tHb levels in grade 1–3 and grade 4 or 5 tumors (Fig 2). For each group, three tumor types (IDC, IDC/ILC, and ILC) were grouped together. However, there were no ILC tumors in the Miller-Payne grade 4 or 5 group. An optimal threshold between 89 and 91 yielded a situation in which 11 of 14 grade 1–3 tumors were below it. The prediction accuracy was 80%, and the specificity was 80%. The mean maximum tHb

### Table 2

<table>
<thead>
<tr>
<th>Statistic</th>
<th>Maximum/Average tHb</th>
<th>Maximum/Average OxyHb</th>
<th>Maximum/Average DeoxyHb</th>
<th>Nottingham Score</th>
<th>Mitotic Count (per 10 High-Power Fields)</th>
<th>Largest Tumor Dimension at MR Imaging*</th>
<th>Largest Tumor Dimension at US†</th>
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<td>Correlation coefficient</td>
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<td>0.456/0.436</td>
<td>0.538/0.523</td>
<td>0.531</td>
<td>0.407</td>
<td>0.111</td>
<td>0.174</td>
</tr>
<tr>
<td>P value</td>
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<td>.007/.010</td>
<td>.001/.001</td>
<td>.001</td>
<td>.017</td>
<td>.552</td>
<td>.417</td>
</tr>
</tbody>
</table>

* In 30 patients with available pretreatment MR images.
† In 21 patients with 22 tumors with well-defined margins on pretreatment US images.

### Results

There were 20 grade 1–3 tumors and 14 grade 4 or 5 tumors. For grade 4 or 5 tumors, the mean maximum tHb was 107.9 μmol/L ± 33.9 (standard deviation), and the mean average tHb was 72.2 μmol/L ± 24.5, whereas for grade 1–3 tumors, the mean maximum tHb was 57.5 μmol/L ± 18.8, and the mean average tHb was 51.0 μmol/L ± 13.9 ($P = .005$ and $P = .009$, respectively). The mean differences in maximum and average tHb were 32.2 μmol/L (95% confidence interval [CI]: 11.2, 53.2 μmol/L) and 21.2 μmol/L (95% CI: 3.9, 36.4 μmol/L), respectively. However, there was no significant difference at the end of treatment cycles 1–3 (Fig 1).

Before treatment, oxyHb and deoxy-Hb were significantly different between grade 1–3 and grade 4 or 5 tumors ($P = .008$ and $P = .017$, respectively), but this was not true in subsequent measurements (Table E1 [online]). When we compared the predictive value of pretreatment tHb, oxyHb, and deoxyHb with the predictive value of Nottingham score and mitotic index at core biopsy, pretreatment maximum tHb and deoxyHb achieved the highest predictive value ($r = 0.531$ and $r = 0.538$, $P = .001$), followed closely by tumor Nottingham score ($r = 0.531$, $P = .001$, Table 2).
Multiple NIR data sets were obtained at the tumor sites, and average tHb values were used to characterize each tumor. The mean standard deviations of pretreatment maximum tHb values of grade 1–3 and grade 4 or 5 tumors were 5.9 and 4.9 µmol/L, respectively. Thus, on average, 4.5% (4.9/107.9) to 7.8% (5.9/75.7) changes could be encountered in repeated imaging by using the hand-held probe. The mean standard deviations of subsequent measurements were in a similar range—for example, the corresponding values were 5.6 and 5.9, 6.1 and 5.2, and 5.2 and 6.1 µmol/L at the ends of cycles 1–3 for grade 1–3 and grade 4 or 5 tumors, respectively.

Percentage tHb based on maximum and average tHb was calculated, and the results for the two groups based on the maximum are given in Figure 3 and in Table E2 (online). Statistical significance was achieved at the end of cycle 1. For grade 1–3 tumors, percentage tHb change was 109.8% ± 27.7, whereas for grade 4 or 5 tumors, percentage tHb change was 87.9% ± 17.9. The mean difference was 21.9% (P = .009 and corrected P = .009), and the 95% CI was 5.9%, 37.9%. The significance remained high at the end of cycles 2 and 3 (P = .002 and corrected P = .004 and P < .001 and corrected P < .001, respectively). The percentage tHb changes in three patient groups in different treatment regimens are given in Appendix E1 (online). An example of complete pathologic response is shown in Figure 4, and examples of complete and partial pathologic response can be found in Figures E1–E4 (online).

The percentage changes in size at US of the largest dimension of grade 1–3 and grade 4 or 5 tumors were measured in 21 patients with 22 tumors that were well-defined at US (Table E2 [online]). For grade 1–3 tumors (n = 11), percentage reduction in size at US was 82.7% ± 28.1, 66.0% ± 15.6, and 59.2% ± 13.0 at the end of cycles 1–3, respectively; whereas for grade 4 or 5 tumors (n = 11), percentage reduction in size at US was 74.8% ± 17.4, 55.5% ± 19.0, 41.6% ± 16.4, respectively. The P values were .437, .172, and .02, respectively.
and corrected *P* values were .0437, .344, and .06, respectively. At the end of cycle 3, the difference between the two groups approached statistical significance.

The pretreatment MR imaging measurements of the largest dimension were 5.0 cm ± 1.8 and 4.7 cm ± 2.2, and the posttreatment measurements were 2.7 cm ± 1.5 and 0.7 cm ± 1.1, for grade 1–3 tumors and grade 4 or 5 tumors, respectively. The percentage reduction was 53.0% ± 21.3 for grade 1–3 tumors and 21.1% ± 28.2 for grade 4 or 5 tumors (*P* = .004).

**Discussion**

In the past 5 years, optical tomography and spectroscopy have been explored by several groups (23–28) for potential use in assessing chemotherapy response. A recent study (27) involving NIR spectroscopy measured percentage changes in oxyHb, deoxyHb, water, and optical scattering normalized to pretreatment values at 1, 4, and 8 weeks after neoadjuvant chemotherapy for 10 patients. All functional parameters differed significantly in responders and nonresponders at the 4-week examination, except for percentage water change. In our previous study (25), we imaged 11 patients by using the NIR US imager before treatment; after chemotherapy cycles 2, 4, and 6; and prior to surgery. We found a noticeable difference in tHb changes at the end of cycle 2 between responders and nonresponders. This study, with its larger patient pool, showed that percentage tHb over the first three treatment cycles was statistically different between Miller-Payne grade 4 or 5 tumors and grade 1–3 tumors. Furthermore, the pretreatment maximum and mean average tHb levels were significantly higher in the tumors with the best pathologic response (grade 4 or 5) than in the tumors with modest or no response to chemotherapy (grades 1–3). This suggests that hypervascular tumors respond to standard cytotoxic, herceptin-based, and antiangiogenic therapies better and in a more substantive way than do hypovascular tumors, perhaps because of the better access of chemotherapeutic...
agents to the cancer cells. Given the relative hypovascularity of most ILCs, it is not surprising that there were no pathologic complete responses in this subgroup. Although US is useful in monitoring chemotherapy responses in patients with well-defined tumor margins, the early assessment of tumor size response is not quite possible at early treatment cycles 1–3.

A closely related result was reported by Kuo et al (35), who assessed power Doppler US measurements obtained in 30 patients. They found that there was a tendency for response in tumors with relatively high initial vascularity. In a study (23) involving NIR spectroscopy, deoxyHb was found to decrease within the 1st week of chemotherapy in responders. In another study, oxyHb was found to increase in responders on day 1 (28). We obtained the statistical significance of oxyHb and deoxyHb levels at pretreatment between the two responder groups. However, oxyHb and deoxyHb are not independent predictors, as their levels vary directly with the tHb level. Pearson correlation revealed that pretreatment tHb and deoxyHb have the highest predictive value for pathologic response. The results of our study and those of others cited have substantial implications for the use of NIR-measured tumor Hb content in predicting response to chemotherapy.

There were some limitations to this study. First, the majority of the patients were referred for neoadjuvant chemotherapy after core biopsy, and hence the baseline NIR US imaging examination was performed after the initial biopsy. A bruise or hematoma caused by prior biopsy could have had some effect on pretreatment NIR measurements. Second, patients treated with different regimens were pooled together to assess early response. Antiangiogenic therapy has a more profound effect on tumor early response. Antiangiogenic therapy was pooled together to assess early response. Antiangiogenic therapy is a strong predictor of response to chemotherapy. The technical limitations of the US-guided NIR technique include the accuracy of reconstructed optical absorption coefficients and the longitudinal repeatability of the measurements. For a large high-contrast phantom target of 5 cm, about 55%–65% reconstruction accuracy on target absorption can be achieved (33). Because the average pretreatment tumor sizes of the two responder groups were similar, the underreconstruction should affect light quantification in both groups similarly. Therefore, the comparison of pretreatment and early treatment Hb levels between the two responder groups should be minimally affected. Additionally, because the same size ROI obtained in each patient at pretreatment was used for reconstructions at all subsequent treatment cycles, the underreconstruction should have had a minimal effect on the percentage tHb, which was normalized to the pretreatment level. The longitudinal repeatability of reconstructed phantom absorption coefficient is about 5%–10%. In a recent study (34), we validated the estimated oxygen saturation (SO2), computed as SO2 = oxyHb/tHb · 100, by using a PO2 electrode as a reference, and the difference was less than 8% for blood phantoms of various oxygen conditions and sizes located at different depths.

In conclusion, our initial findings indicate that pretreatment tumor Hb content is a strong predictor of response to neoadjuvant chemotherapy and that percentage tHb changes normalized to the pretreatment level can be used to further identify responders and nonresponders at early treatment cycles. In the genomic era of personalized medicine, where monitoring of early responses for outcome prediction becomes crucial, our NIR technology could prove valuable.

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References


5. Bakha EA, El-Sayed ME, Green AR, et al. Biologic and clinical characteristics of breast...


