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Clinical Cancer Research

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Imaging, Diagnosis, Prognosis

Clinical Cancer Research

Functional Imaging Using Diffuse Optical Spectroscopy of Neoadjuvant Chemotherapy Response in Women with Locally Advanced Breast Cancer

Hany Soliman[1,4], Anoma Gunasekara[4], Mary Rycroft[4], Judit Zubovits[2], Rebecca Dent[3], Jacqueline Spayne[1], Martin J. Yaffe[4,5], and Gregory J. Czarnota[1,4,5]

Abstract

Purpose: Functional imaging with tomographic near-infrared diffuse optical spectroscopy (DOS) can measure tissue concentration of deoxyhemoglobin (Hb), oxyhemoglobin (HbO2), percent water (%water), and scattering power (SP). In this study, we evaluated tumor DOS parameters and described their relationship to clinical and pathologic outcome in patients undergoing neoadjuvant therapy for locally advanced breast cancer.

Experimental Design: Ten patients were enrolled and intended to undergo five scans each. Scans were taken up to 3 days before treatment and at 1, 4, and 8 weeks after neoadjuvant treatment before surgery. Changes in volume of interest weighted tissue Hb, HbO2, %water, and SP corresponding to the tumor were compared with clinical and pathologic response.

Results: All patients' tumor volumes of interest were significantly different compared with background tissue for all parameters. Five patients had a good pathologic response. Four patients were considered nonresponders. One patient initially did not respond to chemotherapy but, after a change in chemotherapy, had a good response. In the five patients with a good response, the mean drop in Hb, HbO2, %water, and SP from baseline to the 4-week scan was 67.6% (SD = 20.8), 58.9% (SD = 20.3), 51.2% (SD = 28.3), and 52.6% (SD = 26.4), respectively. In contrast, the four nonresponders had a mean drop of 17.7% (SD = 9.8), 18.0% (SD = 20.8), 15.4% (SD = 11.7), and 12.6% (SD = 10.2) for Hb, HbO2, %water, and SP, respectively.

Conclusions: Responders and nonresponders were significantly different for all functional parameters at the 4-week scan, except for %water, which approached significance. Thus, DOS could be used as an early detector of tumor response. *Clin Cancer Res; 16(9); 2605–14. ©2010 AACR.*

Breast cancer is the second leading cause of cancer-related mortality in women in North America. In 2007, there were an estimated 262,810 *in situ* and invasive cases of breast cancer in the United States and Canada (1, 2). There locally advanced breast cancer (LABC) represents 5% to 20% of all newly diagnosed breast cancers (3). This diagnosis typically includes tumors >5 cm or involving the skin or chest wall, or inflammatory breast cancer. It also includes patients with supraclavicular, infraclavicular, internal mammary, or fixed or matted axillary lymph nodes. All stage III and a subset of stage IIB (T3N0) tumors are usually considered locally advanced (4).

Women with LABC, owing to the extensive nature of the disease, typically have poor outcomes in terms of local and systemic control. Long-term survival is ~50% (5). The emerging standard treatment for LABC involves multimodality treatment with neoadjuvant chemotherapy. Although there is no evidence to suggest that there is a survival benefit of neoadjuvant chemotherapy when compared with adjuvant treatment, some studies have shown an overall survival benefit for those patients who achieve a pathologic complete response after neoadjuvant chemotherapy compared with patients without a complete response (6–11). An advantage of neoadjuvant chemotherapy is that it decreases tumor bulk and may allow breast-conserving therapy. Finally, assessment of the effect of chemotherapy on gross disease may indicate the potential response of distant micrometastases—something that is not possible in the adjuvant setting (12).

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

This study is a prospective evaluation of the use of functional tomographic near-infrared diffuse optical spectroscopy in monitoring tumor response in patients undergoing neoadjuvant treatment for locally advanced breast cancer. We show that as early as 4 weeks, diffuse optical spectroscopy parameters of oxygenated hemoglobin, deoxygenated hemoglobin, percent water, and scattering power are predictors of final whole-mount mastectomy histopathology. Early identification of treatment response in patients undergoing neoadjuvant therapy can potentially improve clinical outcomes if ineffective treatment is changed to a more efficacious treatment.

Traditional methods of monitoring tumor response, including clinical palpation, X-ray mammography, ultrasonography, and magnetic resonance imaging (MRI), have been typically used as anatomic measures of disease. Conventional computed tomography uses ionizing radiation, produces artifact due to cardiac and breathing motion, and lacks fine detail in spatial resolution (13). It has not been used yet in routine clinical practice for the detection or monitoring of breast tumor responses. X-ray mammography is commonly used for disease detection but is not as useful in the presence of fibrotic tissue (14) or high mammographic density (15). In addition, some studies show its poor correlation with pathology (10, 16). Mammography has not found use in disease treatment monitoring. Standard anatomic ultrasound is not very sensitive for disease detection. Offering high resolution and high sensitivity, anatomic MRI is the standard in most cancer centers for monitoring LABC. However, it remains relatively costly and no standard dynamic contrast-enhanced criteria of response have yet emerged. Yeh et al. (17) found that palpation, X-ray mammography, ultrasound, and MRI showed agreement with pathologic response in 19%, 26%, 35%, and 71% of cases, respectively. On its own, clinical palpation is used routinely in clinic but remains a relatively subjective and inaccurate method of assessing tumor response and has been found to correlate poorly with pathology (14).

The need for a noninvasive and inexpensive imaging modality to both diagnose disease and monitor treatment response has given recent new enthusiasm to optical imaging. Optical imaging in breast cancer can be traced as far back as 1929 when light was first used with breast tissue to observe absorption patterns (18). Due to low resolution in the breast and poor differentiation between tumor and benign tissue, this method did not gain clinical interest. However, in recent decades with advances in computing, in addition to source and detection technology, this imaging modality has been revisited (19).

Near-infrared (IR) diffuse optical spectroscopy (DOS) is a noninvasive three-dimensional tomographic technique that quantitatively measures near-IR absorption and scattering spectra across tissue. There are three types of DOS systems: "continuous wave," "time domain," and "frequency domain." Continuous wave systems emit light at constant amplitude and measure its attenuation, whereas time domain systems emit short picosecond-range pulses of light. Measured temporal distributions of the photons are used to infer the optical properties of breast tissue. Finally, frequency domain systems emit light continuously but the amplitude is modulated. Amplitude decay and phase shift are used to infer the optical properties of the breast tissue (20). Through calculations, optically derived functional images of the major absorption chromophores of oxyhemoglobin (HbO2), deoxyhemoglobin (Hb), and percent water (%water) can be obtained. Although functional imaging with DOS in comparison with conventional imaging modalities provides less spatial resolution, it can potentially provide valuable functional information shortly after the initiation of cancer treatment.

Angiogenesis, tumor cell proliferation, and hypoxia are known factors involved in tumor growth that alter HbO2, Hb, %water, and lipid measurements (21). In a review of

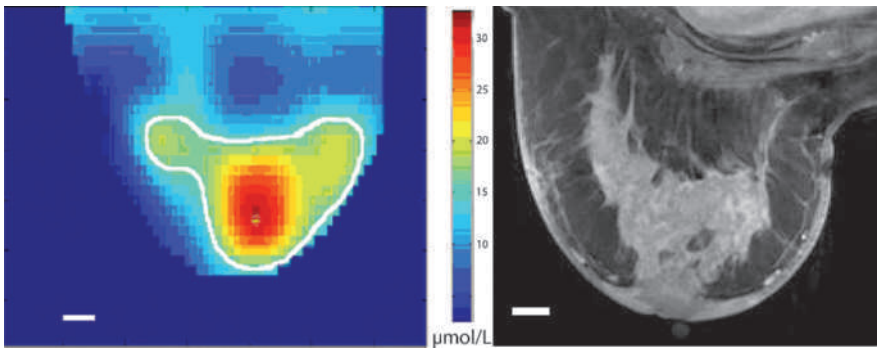

Fig. 1. Breast tumor on functional DOS and MRI. Left, transverse tomographic slice with measurements of HbO2. Red, area of high HbO2; blue, area of low HbO2. The white line represents the boundaries of the VOI. The threshold value defining the boundary of the VOI was 14.5 μmol/L. Right, MR image of the same patient with a 5.5 × 6.5 × 7.5 cm invasive ductal carcinoma. Scale bar, 1 cm.

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DOS breast imaging, ~2,000 women were evaluated from multiple studies, with ~85% of breast lesions detectable. Breast lesions were found to contain approximately twice the concentration of Hb compared with background tissue. Breast tissue optical parameters [Hb, HbO2, %water, %lipid, and scattering power (SP)] varied with age, body mass index, premenopausal or postmenopausal status, and fluctuations in the menstrual cycle. Despite this, it remains difficult to distinguish benign (such as fibroadenomas) from malignant tissue (20).

Given its limitations in spatial resolution and difficulty in using DOS as a screening tool, interest has shifted to the use of DOS in monitoring treatment response. LABC is an obvious candidate for study because of the use of neoadjuvant treatment and the pathologic data that are available after surgery. Multiple studies, primarily case reports (22–26), have been published that show the utility of DOS in predicting early tumor response to neoadjuvant treatment. In the study here with 10 patients, we report to our knowledge the first study that relates functional DOS parameters (Hb, HbO2, %water, and SP) at multiple times during neoadjuvant therapy to clinical and final whole-mount pathologic outcome.

Our ultimate goal is to identify early optical parameters of response that predict for a good clinical response. In cases where there is a predicted poor response, chemotherapy regimens could be changed earlier (after weeks rather than months) based on functional imaging. We show here a concordance between early parameters from functional imaging within 4 weeks and clinical/histopathologic response. We show that optical parameters could be used to discriminate between patients who responded to chemotherapy and those who did not.

Table 1. Patient characteristics

Characteristic	Value
Mean age	50 years (range, 38-64)
Menstrual status before treatment	
Premenopausal	6 patients
Postmenopausal	4 patients
Neoadjuvant treatment	
Chemoradiotherapy	3 patients
Epirubicin and docetaxel	2 patients
AC + T	2 patients
FEC + D	1 patient
Sunitinib/trastuzumab→ docetaxel/trastuzumab/ pamidronate	1 patient
Docetaxel/carboplatinum/ trastuzumab	1 patient
Mean maximum tumor size	7.7 ± 2.4 cm (range, 5-11.2)
Tumor histology	
Lobular carcinoma	2 patients
Ductal carcinoma	8 patients
Hormone receptor (estrogen or progesterone receptor)	
Positive	7 patients
Negative	3 patients
Mean body mass index	25.2 ± 7.0
Her-2-neu	
Positive	4 patients
Negative	6 patients
Triple negative (estrogen, progesterone, and Her-2-neu)	2 patients
Grade	
1	0 patients
2	10 patients
3	0 patients

Abbreviations: AC, Adriamycin/cyclophosphamide; T, trastuzumab; FEC, 5-fluourouracil/epirubicin/cyclophosphamide; D, docetaxel.

Table 2. Differences between tumor and normal tissue

Parameter	Mean tumor	Mean normal	*P*
Hb (μmol/L)	10.23 ± 2.47	6.41 ± 1.30	<0.0001
HbO2 (μmol/L)	23.82 ± 5.85	14.01 ± 5.15	<0.0001
%water	0.52 ± 0.17	0.15 ± 0.10	<0.0001
SP	1.57 ± 0.45	0.55 ± 0.28	<0.0001

Materials and Methods

Protocol. This study was conducted in accordance with research ethics approval. Ten patients with LABC were recruited to take part in this study. Informed consent was obtained and five scans were planned for each patient. The first scan took place before the start of neoadjuvant therapy, and a subsequent three scans were to take place at 1, 4, and 8 weeks after the start of treatment. The final scan was scheduled after completion of neoadjuvant therapy before surgery. The timing of scans was intentionally weighted toward the beginning of neoadjuvant therapy to investigate early optical markers of response.

DOS images were obtained with the patient lying prone with the pendant breast suspended and stabilized between polymethylmethacrylate plates in an aquarium. Optical compensation medium with optical properties similar to breast tissue was added to the aquarium. DOS images were acquired with the SoftScan platform (Advanced Research Technologies) and took ~1 hour per study.

All patients were biopsied to confirm a cancer diagnosis before treatment. Baseline imaging of these patients included a clinical MRI of the breast and metastatic workup as necessary as part of the institutional standard of care for such patients. Patients were followed clinically in a

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Table 3. Individual patient results

Patient no.	Neoadjuvant treatment	MRI dimensions of tumor pretreatment (AP × ML × SI)	MRI dimensions of tumor presurgery (AP × ML × SI)
1	Chemoradiotherapy	5 × 4.5 × 4.5	3.0 × 3.4 × 5.5
2	Sutent/Herceptin→docetaxel/trastuzumab/pamidronate	11.2 × 7.3 × 8.3	3.3 × 1.4 × 2.1
3	Epirubicin and docetaxel	6.1 × 7.8 × 3.3	3.7 × 6.2 × 2.8
4	Chemoradiotherapy	10 × 6*	No palpable disease
5	Epirubicin and docetaxel	11 × 6.3 × 5.5	10.6 × 7.7 × 7.1
6	AC + D	7.8 × 4.6 × 5.5	0.5 × 0.5 × 0.5
7	AC + D	7.5 × 6.5 × 5.5	6.5 × 3.5 × 2.7
8	Chemoradiotherapy	5.1 × 5.5 × 4.5	4.4 × 3.2 × 3.9
9	Docetaxel/carboplatinum/trastuzumab	4.8 × 3.1 × 5.5	2.8 × 0.8 × 3.9
10	FEC + D	10.2 × 7.2 × 6.8	4.3 × 2.3 × 1.9

NOTE: Results with anatomic MRI imaging indicated a mean volume change of 72 ± 70% (mean ± SD) from pretreatment to presurgery in pathologic nonresponders and a mean volume change of 16 ± 19% (mean ± SD) in pathologic responders. The difference between the two groups based on anatomic MRI was not significant (P = 0.18).
*No MRI was done; measurements are based on clinical and mammographic assessment.

routine fashion by an oncologist blinded to the DOS data. An MRI was obtained before mastectomy. Clinical examination was done at each follow-up, and an assessment of tumor size was recorded. Assessment of clinical response, duration, and changes in therapy was made blinded to DOS information. Pathology was examined after mastectomy on full-mount pathology slides (27), and data on tumor size, grade, histologic subtype, and tumor response were recorded. Pathologists were blinded to DOS data, and vice versa.

All studies were examined in the same manner by the same pathologist (J.Z.), with expertise in reading whole-mount mastectomy slides, who interpreted all LABC pathology. The slides were examined for residual tumor size, content of cellularity, and whether residual cells seemed to exhibit a chemotherapy effect or seem viable. Tumors were categorized as having either a complete pathologic response, good pathologic response, or minimal pathologic response. Complete response was defined as not having any residual invasive or *in situ* tumor in the pathologic specimen. Good pathologic response meant an over 50% diminishment in size (compared with pretreatment size) with a significant decrease in cellularity (≤10% cells seem invasive and viable). Minimal pathologic response referred to a small change in tumor size (enlargement or diminishment to at best 50%).

Instrument. The acquisition platform used in the study was a time-resolved, optical imaging device used to measure photon migration through the breast in the near-IR range (28). The laser emission assembly was composed of four individual pulsed semiconductor diode lasers (LDH-P, PicoQuant) operating at 690, 730, 780, and 830 nm with a pulse duration (full width at half-maximum) of <150 picoseconds, an average output of 0.5 mW when driven at 20 MHz (PDL 808, PicoQuant), and an oscillator module to synchronize the drivers. The pulses were time multiplexed (12.5 ns) through a single fiber on the emission side of the breast. Photons were collected through five optical fibers positioned on a mobile detector head in an "M" configuration on the collection side of the breast and detected by a photomultiplier (H7422P-50, Hamamatsu). Patients were positioned prone on a tabletop with a pendant breast scanned in an aquarium in a raster pattern. The optical compensation medium was an oil-in-water emulsion that mimicked average optical properties of the human breast (coefficient of absorption = 0.04 cm^{-1} and coefficient of scatter = 10 cm^{-1}). The acquired data were reconstructed, and tomographic functional images were created from the optical parameters.

Data analysis. Transverse tomographic images of the breast in a craniocaudal direction were reconstructed from scattering data. Image reconstruction was carried out to provide sagittal and coronal views. The DOS system provided three-dimensional images of the breast with measurements of HbO2, Hb, %water, and SP with a 3 mm by 3 mm voxel resolution.

The tumor was identified in the pretreatment DOS images for each of the functional parameters and verified using information from each patient's clinical exam, mammogram, ultrasound, and MRI with regard to tumor size and location. For HbO2, Hb, %water, and SP, the tumor

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Table 3. Individual patient results (Cont'd)

Pathologic dimensions of tumor (AP × ML × SI)	Notes	Response
1.8 × 4 × 4.5	Some response but significant *in situ* tumor present	Weak pathologic response
N/A	Clinically good response with second chemotherapy regimen	Initial poor clinical response then good clinical response with change in chemotherapy
5.2 × 7 × 1.8	Minimal response to chemotherapy	Minimal pathologic response
1 × 1 × 0.7	Very small volume of disease remaining	Good pathologic response
4.5 × 4 × 9	Minimal response to chemotherapy	Minimal pathologic response
2 × 7 × 3	Despite nests of tumor occupying a large volume, very minimal tumor cellularity overall	Good pathologic response
8.9 × 7.7 × 3	Tumor cellularity remains high	Minimal pathologic response
2.5 × 4.8 × 3.6	Numerous small foci of invasive disease; only 10% of tumor is invasive	Good pathologic response
2 × 6 × 3	Very small nests of cells, very marked response	Good pathologic response
1.7 × 6 × 4.5	Invasive tumor is present as single cells only, rare groups of cells	Good pathologic response

visually corresponded quite well with the areas of highest measured values. A volume of interest (VOI) for HbO2, Hb, %water, and SP on all of the DOS scans was created by obtaining a threshold value that was a fraction of the maximum measured value. This threshold value was adjusted such that the VOI corresponded in size and location as close as possible to the actual tumor as known through other imaging and clinical exam (see Fig. 1). The threshold value was kept consistent across all of the studies, at each time point, for the same optical parameter for each patient. Mean measured values in the VOI of each of the functional parameters were obtained.

Results

Patient characteristics. Ten patients were recruited to this study. Seven patients had all five scans as planned. Two patients underwent four scans each and one patient had three scans. Pathology results were available for all but one patient. The one patient without pathology developed bone metastases and did not undergo a mastectomy. All but one patient had pretreatment and presurgery MRI. Patient, tumor, and treatment characteristics are presented in Table 1.

Patients received a variety of neoadjuvant treatment regimens. Three patients received treatment under a study at our institution receiving weekly docetaxel (25 mg/m^2) concurrent with radiotherapy including the breast, axilla, and supraclavicular area of 50 Gy in 25 fractions over 5 weeks. One patient had docetaxel (75 mg/m^2) and carboplatinum (AUC, 6), with trastuzumab (6 mg/kg). Another patient had 5-fluourouracil (500 mg/m^2), epirubicin (100 mg/m^2), and cyclophosphamide (500 mg/m^2) followed by docetaxel (100 mg/m^2; 5-fluourouracil/epirubicin/cyclophosphamide + docetaxel). Two patients received Adriamycin (60 mg/m^2) and cyclophosphamide (600 mg/m^2) followed by paclitaxel (175 mg/m^2; Adriamycin/cyclophosphamide + trastuzumab). Another two patients received epirubicin (90 mg/m^2) and docetaxel (75 mg/m^2). The final patient received sunitinib (37.5 mg) and trastuzumab (4 mg/kg, day 1, and then 2 mg/kg weekly) initially, but this was changed after three cycles to docetaxel (75 mg/m^2), trastuzumab (4 mg/kg, day 1, and then 2 mg/kg weekly), and pamidronate (90 mg). Doses were adjusted and treatment was delayed under the medical oncologist's discretion depending on toxicity and response.

Image measurement. Reconstructed images of optical absorption and scattering at each of the four wavelengths were produced as above, and images of the functional parameters, Hb, HbO2, %water, and SP, were generated. VOIs were placed in the area of the tumor and over adjacent normal tissue to extract quantitative measurements on all of the DOS studies. Table 2 displays the differences between tumor and the background tissue for each of the functional parameters. All parameters showed a significant difference compared with the background normal breast tissue.

Clinical and pathologic response to neoadjuvant therapy. No patient in this study had a complete response. Table 3 displays patients' initial tumor size, the treatment regimen, and pathologic results. Patient 1 had a weak partial response with the tumor initially measuring 5.0 × 4.5 × 4.5 cm [anterior/posterior (AP) × medial/lateral (ML) × superior/inferior (SI)] on MR before treatment with final surgical pathology revealing a 4.5 × 4.0 × 1.8 cm lesion after chemoradiotherapy. Despite having almost no invasive disease left behind, there was significant *in situ* tumor remaining. Patients 3, 5, and 7 were considered minimal pathologic responders; the tumors on initial MR measured

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6.1 × 7.8 × 3.3 cm, 11.0 × 6.3 × 5.5 cm, and 7.5 × 6.5 × 5.5 cm, respectively. Final surgical pathology revealed minimal response with the tumors remaining relatively unchanged in size. Patients 4, 6, 8, 9, and 10 had good pathologic responses. Although the volume occupied by the remaining lesion on final pathology was in some cases large, the appearance of decreased tumor cellularity and only scattered islands of cells indicated a good response (as defined in Materials and Methods).

Patient 2 was a unique case. This 38-year-old female presented with metastatic disease in the bone. Initial MR revealed a right-sided invasive ductal carcinoma measuring 11.2 × 7.3 × 8.3 cm. The patient was started on sunitinib/trastuzumab chemotherapy and, after three cycles, was noted clinically to be getting worse with a right-sided axillary node increasing in size. When symptoms were observed, a whole-body bone scan was done and metastatic bone progression was noted. The patient's chemotherapy was then changed to docetaxel/pamidronate/trastuzumab. Symptomatically and clinically, the patient began to improve almost immediately. On clinical exam, the large breast mass had subsequently decreased in dimensions to 3 cm × 3 cm (ML × SI).

DOS response to neoadjuvant therapy. For each patient, the mean measured value for the functional parameter in the VOI was multiplied by its volume. Figure 2 shows the changes in parameters resulting from neoadjuvant treatment as a percentage of the initial value, in both a typical nonresponder (patient 5) and a typical responder (patient 6). Alternatively, this was carried out on the basis of the volume occupied by each functional parameter/chromophore alone with nearly identical results (Supplementary Fig. S1).

A representative whole-mount pathology slide illustrating the pathologic response is also presented. Patients 1, 4, 6, 8, and 9 showed significant decreases in HbO2, Hb,

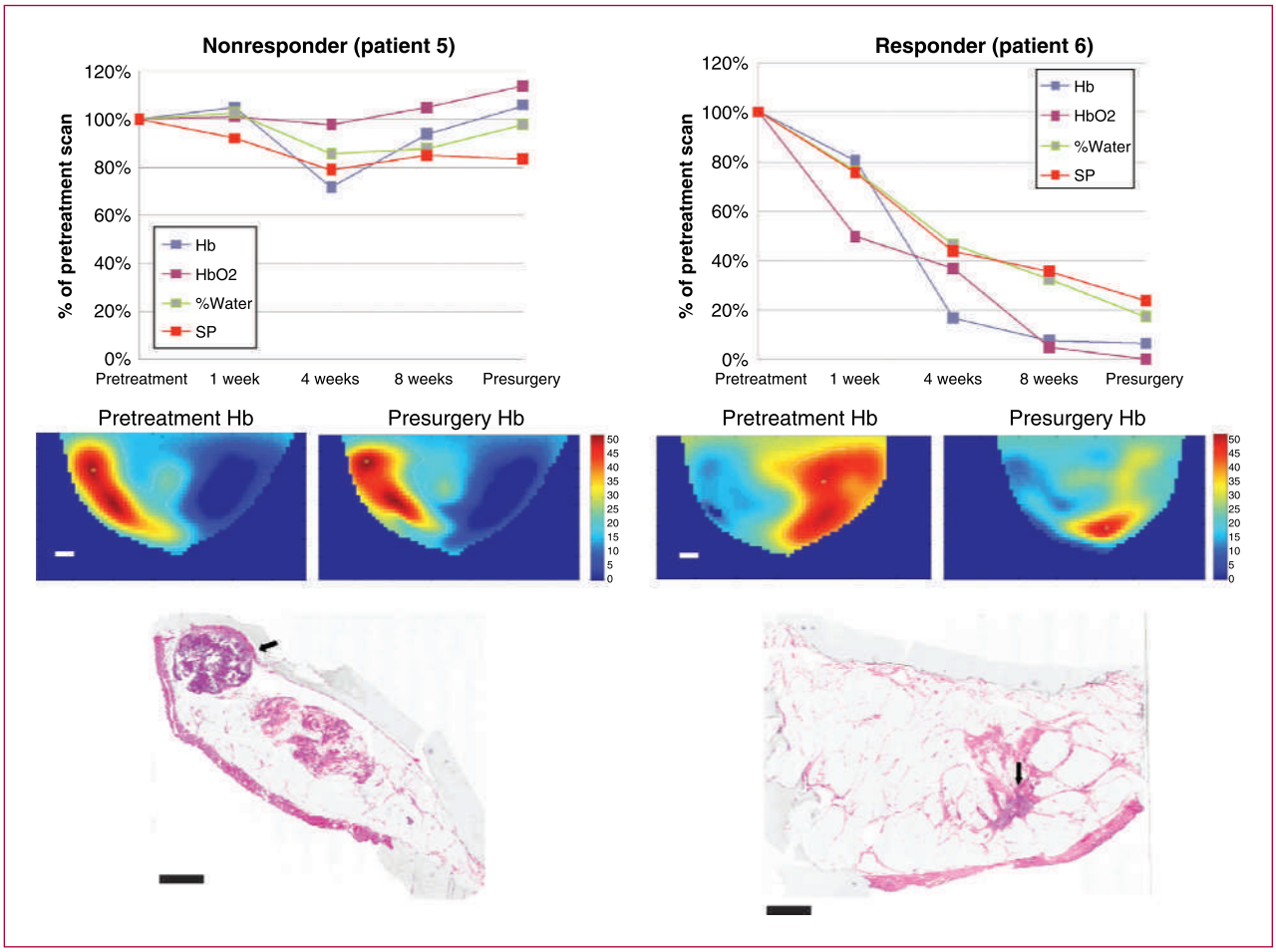

Fig. 2. Responders versus nonresponders for each of the optical parameters as measured in breast tumors. The graphs illustrate the %change measured within tumor volume plotted over time for a typical nonresponder (patient 5) and responder (patient 6). The corresponding pretreatment and presurgery DOS images of the Hb are also shown along with one whole-mount slide of the pathologic specimen for each of the responder and nonresponder. Note that in the nonresponder, there is minimal change in the Hb from pretreatment to presurgery. The area of residual disease in the nonresponder and responder is identified by the small black arrow. In the responder, there was only a few nests of cells in three pathology slices surrounded by fibrosis. The remaining slices were clear of disease. Scale bars, 1 cm.

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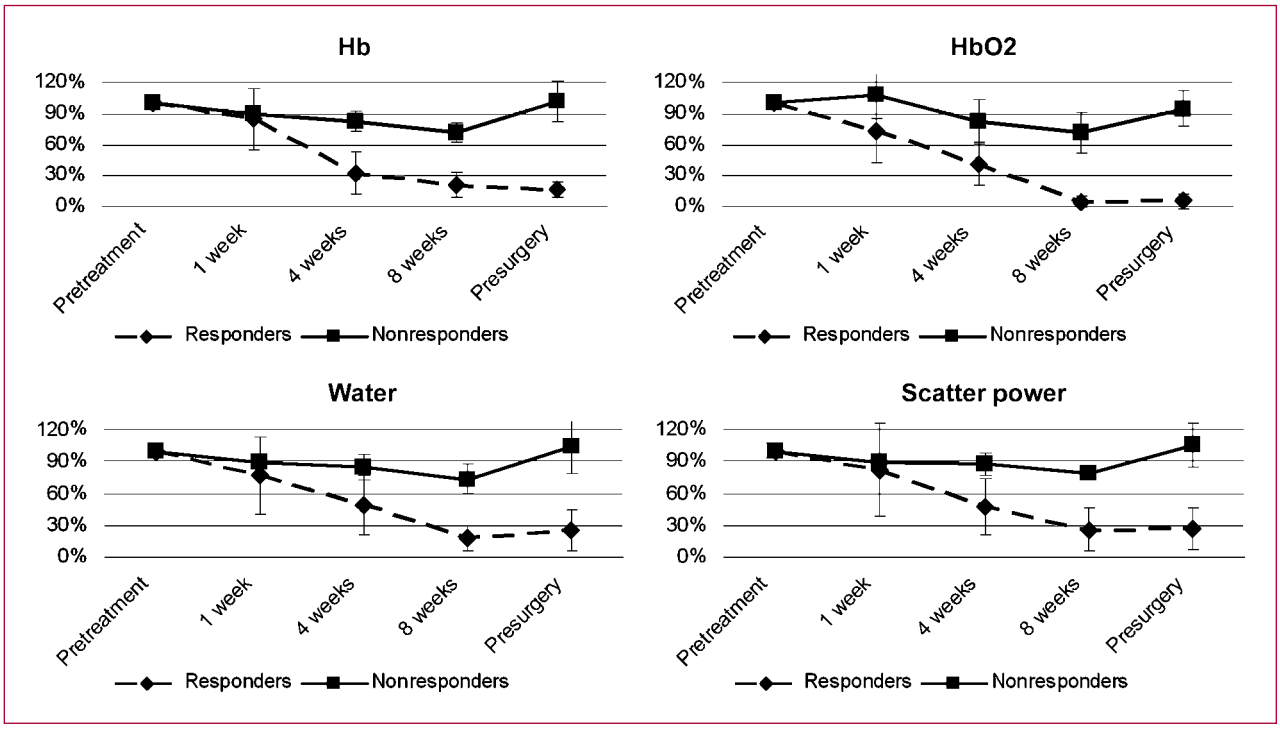

Fig. 3. Changes in functional DOS parameters with treatment as measured in breast tumors. The four graphs illustrate each of the functional parameters plotted as a %change from pretreatment of responders versus nonresponders at 4 weeks of time within breast tumors. Bars, SE. At 4 weeks, HbO2, Hb, %water, and SP were all lower for responders compared with nonresponders. All differences were statistically significant, except for %water, which approached significance. The measured values within adjacent normal tissue were relatively invariant for the functional parameters. For instance, the mean variability for the %water parameter was 2.21% (SE) for all patients and for the SP parameter was 2.56%. The remaining parameters were comparable.

%water, and SP compared with values before treatment. Clinically and pathologically, these patients were determined to be good partial responders, except for patient 1. Patients 3, 5, and 7 were clinical and pathologic nonresponders, and they exhibited only a minimal response to the functional parameters HbO2, Hb, %water, and SP. Patient 2 exhibited an initial increase in all the parameters. However, after the change in chemotherapy in this patient before the 8-week scan, there seemed to be a significant decrease in the optically derived parameters.

The DOS parameters corresponded well with the clinical and pathologic outcomes of these patients. Figure 3 displays graphs of each of the parameters for patients who were clinical and pathologic responders (patients 4, 6, 8, 9, and 10) versus the nonresponders (patients 3, 5, and 7). The graphs for the responders and nonresponders seem to separate at ~4 weeks after initiation of neoadjuvant treatment. Table 4 presents the values as a percentage of the pretreatment scan for each of the optical parameters at 4 weeks of time. The responders and nonresponders were significantly different (using a two-sided t test) at 4 weeks of time for HbO2 ($P = 0.049$), Hb ($P = 0.012$), and SP ($P = 0.050$) and approached significance for %water ($P = 0.081$).

A repeated-measures analysis was done using a general linear mixed-model statistical analysis. The mean percent change for Hb in responders was −41.9% (SE = 2.4) versus nonresponders of −12.0% (SE = 3.1). This difference was significant ($P < 0.001$). The mean percent change for HbO2 in responders was −51.6% (SE = 5.8) versus nonresponders of −10.0% (SE = 7.5). This difference was also significant ($P = 0.005$). The mean percent change for HbO2 in responders was −43.8% (SE = 6.8) versus nonresponders of −12.0% (SE = 8.7) and significant ($P = 0.028$). The mean percent change for SP in responders was −41.6% (SE = 7.4) versus nonresponders of −7.0% (SE = 9.5). This difference was in addition significant ($P = 0.028$).

Discussion

Standard treatment for LABC usually involves the use of neoadjuvant chemotherapy. Patients with a complete pathologic response have significantly higher disease-free and overall survival rates compared with nonresponders (6–11). The need for a straightforward, inexpensive, and noninvasive method of early treatment monitoring is essential to ensure that patients receive appropriate treatment. Traditionally, tumor response has been assessed clinically or radiographically through changes in tumor size, which may take several weeks to months to manifest, despite a positive response. The corollary is that patients may continue on treatment that is ineffective because response assessment with clinical or radiographic means is dependent on late-occurring anatomic changes. DOS is

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gaining new enthusiasm in breast imaging, and studies, primarily case reports (22–26), have shown its feasibility for monitoring neoadjuvant treatment in LABC. The largest trial (11 patients), with patients receiving neoadjuvant chemotherapy with Adriamycin and cyclophosphamide, with or without taxane, showed statistically significant changes in Hb (33 ± 7%), HbO2 (27 ± 15%), and %water (11 ± 15%) in pathology-confirmed responders compared with parameters measured before treatment (22). Patients in that study had a scan before treatment (mean of 1.8 days before chemotherapy) and 1 week after the start of the first cycle of chemotherapy. In that study, six patients were considered responders to neoadjuvant therapy and the rest were nonresponders.

In our study of 10 patients with LABC, we were able to use each of the DOS optical parameters to identify an area that corresponded to the tumor detected with MRI, clinical exam, and other imaging. Measurements of all the optical parameters differed between tumor and background breast tissue. We have shown using DOS that as early as 4 weeks after therapy initiation, responders and nonresponders, as finally evaluated by whole-mount pathology, can be differentiated. One patient (patient 1) had significant changes in the DOS parameters despite a weak pathologic response. In that patient, there was a significant diminishment in her invasive disease with almost none left, which was consistent with what the optical parameters indicated. However, there was a significant amount of residual *in situ* carcinoma left in place, which caused the pathologist to rate the response as weak. We suspect that the optical parameters changed coincident with the changes in the invasive component but were potentially insensitive to the presence of ductal carcinoma *in situ*, as this would only affect the linings of the breast ducts and possibly not have an effect on HbO2 and Hb.

The patients in this study intentionally received different types of chemotherapy. Results were obtained after normalizing for age, body mass index, premenopausal or postmenopausal status, and fluctuations in the menstrual cycle (20). The results were statistically significant at 4 weeks after the commencement of therapy, and we believe that this makes the method generally applicable, as many types of therapy induced the same response in optical parameters, which corresponded to tumor response. The results were generally consistent and independent of how tumor cell death was induced. The patient numbers used (n = 10) were appropriate given the magnitude of response in the optical measures that were obtained. Pathologic complete response can exceed 40% in hormone receptor–negative patients and is lower in hormone receptor–positive patients (29–32). Our patient population exhibited more aggressive disease with no complete response.

Patient chemotherapy in LABC is already to a degree risk adaptive (33–35) and customized based on individual clinical circumstances in women with LABC. All 10 patients received a taxane drug. The majority of patients (8 of 10) did receive a combined anthracycline and taxane regimen, presently considered the clinical standard for such women with cancer, or combined radiation and a taxane. We believe that DOS changes *in vivo* are surrogates of cell death occurring from such exposures and how death is induced, so long as it is induced, is less important to the optical changes than having death induced. We hope that using a "real-life" patient population in such a study better potentially ascertains the use of the imaging technology under investigation.

Other functional imaging modalities have been used to assess treatment response. Positron emission tomography, using radiopharmaceuticals such as fluorodeoxyglucose (FDG PET), has been shown to detect and stage breast cancer with high sensitivity and specificity (36–39). In a study by Kim et al. (40), 50 patients with LABC receiving neoadjuvant treatment were assessed for the correlation between peak standardized uptake values of FDG PET with clinical and pathologic outcome. There was a statistically significant difference in the reduction of peak standardized uptake values between pathologic complete responders, partial responders, and nonresponders. However, there was no detected difference between the different groups using clinical assessment. Investigations of FDG PET as a tool for treatment monitoring in multiple cancer sites are ongoing, but the cost to produce the radionuclides can limit its widespread use. Dynamic contrast-enhanced MRI can be used to measure tissue microvasculature and is sensitive to blood volume and vascular permeability.

Table 4. Results of statistical analysis

Optical parameter	Responders at 4 weeks	Nonresponders at 4 weeks	*P*
HbO2	33.6%	82.0%	0.03
Hb	29.6%	82.3%	0.0044
%water	40.4%	84.6%	0.60
SP	39.3%	87.4%	0.036

NOTE: The table shows the percentage at 4 weeks of the pretreatment value for all of the functional parameters in responders and nonresponders. At 4 weeks, HbO2, Hb, %water, and SP were all lower for responders compared with nonresponders. All differences were statistically significant, except for %water, which approached significance.

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It can monitor the effectiveness of a variety of treatments, including chemotherapy, hormonal manipulation, radiotherapy, and novel therapeutic approaches such as the use of antiangiogenic drugs (41). However, no standard quantification approach has yet emerged. Prospective studies comparing the various quantification approaches are needed (42). Finally, contrast-enhanced ultrasound using microbubbles can measure and assess intravascular volume and, therefore, tumor response. However, approval of microbubble contrast agents for this indication is still pending. Other imaging modalities are being developed but remain mainly experimental at present (43).

DOS is an encouraging functional imaging modality because of its relatively low cost. There are a number of related challenges remaining. Identifying the boundaries of the tumor VOI on DOS tomographic images is not yet standardized and could show considerable variation from one method to the next. In this work, we have defined the VOI on the initial scan and found the minimum value for each functional parameter to be used as a cutoff value to define the tumor VOI for each subsequent scan. This provided for a standardized approach to assess response for each patient. Alternatively, combining the superior spatial resolution of other imaging, such as MRI or ultrasound, with DOS imaging may be useful.

For all the functional parameters tested, the pathologic responder and nonresponder groups could be differentiated at 4 weeks of time; this continued for the 8-week and presurgery scans where the groups remained separated. At 1 week, the responders and nonresponders were not statistically significantly different. With these large tumors, there may be no significant response at 1 week for these patients or greater patient numbers may be required. However, ascertainment of response or lack thereof at 4 weeks into several months of therapy could spare women months of ineffective therapy and permit more effective salvage regimens to be undertaken in a timely manner after one to two cycles of chemotherapy. Nevertheless, results, with the majority of patients receiving a present-day clinical standard of combined anthracycline and taxane regimen, indicated that DOS was able to measure statistically significant changes in women who had pathologic responses compared with those who did not.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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