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Ultrasound-Guided Diffuse Optical Tomography for Predicting and Monitoring Neoadjuvant Chemotherapy of Breast Cancers: Recent Progress

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Abstract

In this manuscript, we review the current progress of utilizing ultrasound-guided diffuse optical tomography (US-guided DOT) for predicting and monitoring neoadjuvant chemotherapy (NAC) outcomes of breast cancer patients. We also report the recent advance on optical tomography systems toward portable and robust clinical use at multiple clinical sites. The first patient who has been closely monitored before NAC, at day 2, day 8, end of first three cycles of NAC, and before surgery is given as an example to demonstrate the potential of US-guided DOT technique.

Keywords

near infrared imaging; ultrasound-guided optical imaging; ultrasound; breast cancer treatment monitoring; dual-modalities

Introduction

Preoperative or neoadjuvant chemotherapy (NAC) is frequently used in treating patients with locally advanced breast cancers as well as in patients whose cancers are resectable but not amenable to breast conserving surgery.^{1–3} Complete eradication of invasive tumor cells in the primary tumor bed following neoadjuvant therapy strongly correlates with improved disease-free survival and overall survival, particularly in estrogen receptor negative disease.⁴ Furthermore, clinical trials in the NAC setting are increasingly being utilized for the study of new agents and novel therapeutic strategies in breast cancer using pathological complete response (pCR), a surrogate marker for survival, as the primary endpoint.⁵

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Declaration of Conflicting Interests

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Classifying breast cancers into molecular subtypes has significantly improved our understanding of the preoperative chemotherapy outcome and has helped guide selection of treatment.^{6–9} Recent studies have established that basal-like or triple-negative breast tumors (estrogen receptor negative [ER–], progesterone receptor negative [PR–], and human epidermal growth factor receptor 2 negative [HER2–] and HER2 positive tumors respond best to cytotoxic^{8,9} and HER2-directed regimens,¹⁰ respectively. In particular, in HER2 positive breast cancer, the NAC approach yielded great successes. The dual HER2 blockade with trastuzumab and pertuzumab recently have showed the highest pCR rates ever reported.³ In triple-negative breast cancers, the NAC approach yielded much higher rates of pCR than for other breast tumor types. However, more than half of triple-negative breast cancer (TNBC) patients do not achieve a pCR and have a very poor prognosis.¹¹ Recent studies have also shown that luminal A subtype (ER+ and HER2– and low tumor grade or low-proliferative phenotype) tumors exhibit lower sensitivity to standard cytotoxic-based regimens.¹² With the advance toward personalized treatments, accurate response prediction becomes more critical to optimize treatment selections and may therefore improve survival.

Conventional methods for monitoring response to NAC include physical examination, ultrasound (US), and mammography. These methods have been shown to be modestly useful in their assessment of tumor response because of chemotherapy-induced fibrosis.^{13–16} There is evidence that contrast-enhanced magnetic resonance imaging (MRI) could be superior to standard clinical assessment methods in determining tumor response to NAC.^{17,18} Positron emission tomography (PET) using [18F]-fluoride has been reported for its potential role in assessing early metabolic changes that may correlate with final pathological response.^{19–22} However, both contrast-enhanced MRI and PET require the injection of contrast agents and are costly for repeated use during treatment.

In the past decade, optical tomography and optical spectroscopy using near infrared (NIR) diffused light has demonstrated great potential in the assessment of tumor vasculature and oxygen consumption responses to NAC.^{23–34} The NIR technique utilizes intrinsic hemoglobin contrast that is directly related to tumor angiogenesis, a key process required for tumor growth and metastasis. When multiple wavelengths are used, the optical absorptions at these wavelengths can be measured, and the proportions of oxygenated hemoglobin (oxyHb) and deoxygenated hemoglobin (deoxyHb) can be calculated, which are correlated with tumor oxygen metabolism and treatment resistance. Optical systems are low cost and safe with no ionizing radiation and are ideally suited for repeated use at clinical settings. Recently, significant progress has been made on early prediction of NAC using optical spectroscopy or tomography. In a study using NIR spectroscopy, 11 patients were monitored pretreatment and within one week of initial treatment.²³ DeoxyHb decreased within the first week in pathologically confirmed responders, whereas no significant change was found in non-responders. In addition, the measured total hemoglobin (tHb) decreased in all responders. Another study of 23 patients reported by the same group found a statistically significant increase, or flare, in oxyHb in responders on day 1, in contrast with a lack of flare or decrease in oxyHb noted on day 1 in non-responders.²⁸ Additional study reported by Ueda et al. has shown that the pretreatment or baseline tumor oxygen saturation $SO_2 = \text{oxyHb}/\text{tHb} \times 100$ levels in pCR patients were higher than those in non-pCR patients.³¹

Recently, a study reported by Jiang et al. has shown that pretreatment tHb measured by diffuse optical spectroscopic tomographic imaging predicts breast tumor response to NAC.³⁴

We have developed a dual-modality approach by using co-registered US to guide light illumination, reception, and also image reconstruction.^{25,29,35,36} This approach overcomes the problem of poor lesion localization of light due to optical scattering and improves reconstruction accuracy of lesions by using a priori lesion depth and size information obtained from US images. Recently, a multi-year study using US-guided Optical Tomography has completed. A total of 32 patients who were undergoing NAC were assessed pretreatment, at the end of every treatment cycle and before surgery.³⁰ The significant findings from this group of patients are (a) pretreatment tumor hemoglobin content (tHb, oxyHb, and deoxyHb) predicts patient pathological response to NAC, (b) the percentage of tHb changes (% tHb) normalized to the pretreatment level can be used to further identify responders from non-responders at the early treatment cycles (2–3 weeks after the initiation of NAC), (c) combining widely used tumor pathologic variables with hemoglobin functional parameters obtained before the initiation of NAC, a substantially improved prediction can be achieved using a logistic prediction model.³⁷

To validate these results at multiple sites, we have developed three US-guided NIR diffuse optical tomography (DOT) systems. The system is compact, portable, and robust in clinical operation. This paper is focused on reviews of the NAC prediction using US-guided DOT technique, advances of NIR system development, and the first patient result that obtained from the new prototype system.

Materials and Method

Recent Progress on NIR System Development

NIR system as an add-on unit to commercial US—In the past, we have developed two frequency domain DOT prototype systems that were used with commercial US systems in clinical studies. The first prototype system consisted of 12 pairs of laser diodes of wavelengths 780 and 830 nm and eight parallel channel photomultiplier tube (PMT) detectors.³⁵ Each pair of dual-wavelength laser diodes delivered the light through an optical coupler to a hand-held probe of 10 cm diameter, and all parallel detectors received the reflected light simultaneously via optical light guides mounted on the probe. The laser diodes were modulated at 140MHz and the detected signals were mixed with the 140.02 MHz reference signal and were further amplified and filtered at 20 KHz for processing. A commercial US array transducer was located in the middle of the probe for guiding the localization of breast lesions and image reconstruction. The second prototype system consisted of four laser diodes of wavelengths 740, 780, 808, and 830nm, which were sequentially switched by 4×1 and 1×9 optical switches to nine source positions on the handheld probe.³⁰ The detection included 10 PMT detectors and associated parallel electronics. The reflected light was coupled to the detectors via optical light guides mounted on the probe. The initial promising clinical results obtained from these prototype systems have been reported in several publications.^{25,29,30,36,38,39}

In both prototypes, a pair of National Instrument Data Acquisition (DAQ) boards and a desktop PC were used for DAQ and display, which has contributed to the bulkiness of the system in clinical studies. In our upgraded system, we have designed a compact DAQ system using a field programmable gate array (FPGA) to communicate with a laptop PC. The DAQ consists of two AD7609 ICs (Analog Device) and one SPARTAN 3E FPGA. Each AD7609 has eight simultaneously differential input channels of 200 KHz sampling speed and 18 bit resolution. The SPARTAN 3E FPGA has a USB 2 full-speed port for FPGA configuration and data transfers to the laptop PC. Besides DAQ, the FPGA is used to control the two optical switches of 4×1 and 1×9 for sequentially selecting one of the four wavelengths (740, 780, 808, 830nm) and one of the nine source positions on the hand-held probe. 14 PMT detectors and parallel electronic channels were used for parallel DAQ. Figure 1 shows a photograph of the upgraded NIR system used at the University of Connecticut (UCONN) Health Center. A commercial US transducer can be readily plugged into the hand-held probe for simultaneous US and optical imaging.

Imaging Reconstruction

In the data processing, the intralipid data sets were first loaded to calculate the system parameters for calibration, then the contralateral normal breast was measured and data processed to estimate background tissue optical properties. The average properties were used to compute a weight matrix for image reconstruction. The perturbation or scattered field was calculated as the difference between the reference data and the lesion data. By solving the inverse problem, the absorption distribution inside the medium can be estimated.

Born approximation was used to relate the scattered field U_{sc} measured at the probe surface to absorption variations in each volume element within the sample. In the Born approximation, the scattered field measured at source (s) and detector (d) is related to the weight W and the absorption change, μ_a , inside the medium. By discretizing the imaging volume into N voxels, the matrix form of image reconstruction is given by

$$[U_{sd}]_{M \times 1} = [W]_{M \times N} [\Delta\mu_a]_{N \times 1}, \quad (1)$$

where M is the total number of source–detector pairs, and W is the weight matrix that describes the distribution of diffuse wave in the homogeneous medium and characterizes the measurement sensitivity to different voxels inside the medium. To solve the unknown absorption distribution, the inverse problem is formulated as an optimization problem as $\min \|U_{sd} - W \mu_a\|^2$, where $\|\cdot\|$ is the Euclidean norm. The conjugate gradient method is used to solve the inverse problem. Because the total number of source–detector pairs (M) on the probe is much smaller than the total number of voxels in the medium, the inverse problem is underdetermined and ill-posed. However, US is excellent in providing information about the target depth and approximate size. We have integrated the target information from US and developed the dual-mesh imaging reconstruction algorithm.⁴⁰ Briefly, the entire tissue volume is segmented based on initial co-registered US measurements into a lesion region L and a background region B . The image reconstruction matrix is given as,

$$[\mathbf{U}_{sd}]_{M \times 1} = [\mathbf{W}_L, \mathbf{W}_B]_{M \times N} [\mathbf{M}_L, \mathbf{M}_B]_{N \times 1}^T, \quad (2)$$

where $\mathbf{W}_L$ and $\mathbf{W}_B$ are weight matrices for lesion and background regions, respectively, and $[\mathbf{M}_L] = [\int_{1L} \mu_a(r) d^3r, \dots, \int_{NL} \mu_a(r) d^3r]$ and $[\mathbf{M}_B] = [\int_{1B} \mu_a(r) d^3r, \dots, \int_{NB} \mu_a(r) d^3r]$ are total absorption distributions of lesion and background regions, respectively. The absorption distributions can be obtained by dividing the total absorption with the voxel sizes of lesion and background, respectively.

A fast or near real-time data acquisition, data processing, and image reconstruction is the key step to bring DOT into clinics for on-site diagnosis by physicians. To reach this goal, a C++ user interface was developed to combine both DAQ and processing. After the USB communication between the laptop PC and the DAQ board is established, the US images were continuously captured from the video port of the commercial US system and the lesion depth and target sizes read into the PC for guiding optical imaging reconstruction.

Logistic Regression Model for Predicting NAC Response

Recently, we have developed a Logistic regression model by integrating pretreatment or baseline hemoglobin parameters obtained from the US-guided DOT system and patient pathological criteria of tumor type, grade, mitotic counts, and tumor receptor status of ER, PR, and HER2 to predict the treatment response before NAC is given. The logistic regression is a statistical modeling approach that can be used to describe the relationship of several predictor variables $X_1, X_2, \dots, X_k$ to a dichotomous response variable Y , where Y is coded as 1 (responder) or 0 (non-responder) for its two possible categories.⁴¹ The model can be written in a form that describes the probability of occurrence of one of the two possible outcomes of Y as follows:

$$\Pr(Y=1|X_1, X_2, \dots, X_k) = \frac{1}{1 + \exp\left(-\left(\beta_0 + \sum_{n=1}^k \beta_n X_n\right)\right)}. \quad (3)$$

The estimated outputs (probability) for each set of predictor variables range from 0 to 1. Given the data on $Y, X_1, X_2, \dots, X_k$, the unknown parameters $\beta_n, n = 0, 1, \dots, k$ can be estimated using the maximum likelihood method. In Zhu et al., we have estimated and validated 13 logistic models and their prediction power using combinations of 13 sets of predictor variables of tumor pathological variables, hemoglobin predictor variables of tHb, oxyHb and deoxy only, tHb and oxyHb, tHb and deoxyHb, without tumor pathological variables and with these variables.³⁵ The ductal carcinoma was coded as 1, mixed ductal and lobular carcinoma was coded as 1, and the lobular carcinoma was coded as 0. The tumor triple-receptor negative, HER2, ER status were coded as follows: 1 for triple negative and 0 otherwise, 1 for HER2+ and 0 for HER2-, and 0 for ER+ and 1 for ER-. The Matlab logistic regression function glmfit was used to compute the coefficients $\beta_n, n = 0, 1, \dots, k$, and glmval was used to predict the response from these coefficients for the training set. The

same coefficients obtained from the training set were used to predict the response for the testing set.

As reported in Zhu et al., we have demonstrated that the tHb combined with the tumor pathological variables is the best predictor, and the oxyHb combined with pathological variables is the second best predictor.³⁵ The addition of tHb or oxyHb significantly improves the prediction sensitivity, negative predictive value (NPV), and the area under the receiver operating characteristic (ROC) curves (AUC) as compared with using tumor pathological variables alone.

Patients

The study protocol was approved by institutional review boards of UCONN Health Center and Hartford Hospital and was federal Health Insurance Portability and Accountability Act (HIPPA) compliant. Written informed consent was obtained from all patients. The final pathologic response was assessed utilizing the Miller–Payne system,⁴² in which pathologic response is divided into five grades based on comparison of tumor cellularity between pre-neoadjuvant core biopsy and definitive surgical specimen. The grading is as follows: grade 1, no change or some minor alteration in individual malignant cells but no reduction in overall cellularity; grade 2, a minor loss of tumor cells but overall high cellularity, up to 30% reduction of cellularity; grade 3, between an estimated 30% and 90% reduction in tumor cellularity; grade 4, a marked disappearance of more than 90% of tumor cells such that only small clusters or widely dispersed individual cells remain (almost pCR); and grade 5, no invasive malignant cells identifiable in sections from the site of the tumor (pCR). The Miller–Payne grade 4 and 5 patients were grouped as responders and grades 1 to 3 as non-responders.

Results and Discussion of US-Guided DOT on NAC Prediction

Summary of Early Results of NAC Prediction

In our early data reported in Zhu et al.,³⁰ there were 20 Miller–Payne grade 1 to grade 3 tumors and 15 grade 4 to grade 5 tumors. For the Miller–Payne grade 4 to grade 5 group, the mean maximum tHb was 110.8 $\mu\text{mol/L} \pm 34.6$ (standard deviation), whereas for the grade 1 to grade 3 group, the mean maximum tHb was 75.7 $\mu\text{mol/L} \pm 18.8$ ($p = 0.002$). The mean difference of maximum was 35.1 $\mu\text{mol/L}$ (95% confidence interval [CI] = [14.5 $\mu\text{mol/L}$, 55.7 $\mu\text{mol/L}$]). However, the significance was diminished at the end of treatment cycles 1 to 3 because the mean tHb level was reduced in grades 4 to 5 while the mean level did not change in the grade 1 to grade 3 group (see Figure 2a).

To assess each patient response, the tHb obtained before treatment is taken as the baseline and the percentage %tHb normalized to the baseline is used to quantitatively evaluate the tumor blood volume changes during chemotherapy. %tHb based on maximum was calculated and the results for the two groups based on maximum are given in Figure 2(b). Statistical significance was achieved at the end of cycle 1. For grade 1 to grade 3 group, %tHb was 110.5% ± 28.3 , whereas for the grade 4 to grade 5 group, %tHb was 88.0% ± 18.0 .

The mean difference was 22.5% ($p = 0.009$) and the 95% CI was [6.0%, 39.0%]. The significance remained high at the end of cycles 2 ($p < 0.001$) and 3 ($p < 0.001$).

Using the Logistic regression model given in Equation (3) and all patients' data reported in Zhu et al.³⁰ as a training set, we obtained ROC curves of treatment prediction using (a) tumor pathological variables only; (b) tumor pathological variables and pretreatment or baseline tHb concentration; (c) tumor pathological variables, baseline tHb and first cycle of tHb changes, tHb%; (d) tumor pathological variables, baseline tHb, and first three cycles of tHb%. Figure 3 shows the corresponding ROC curves and the AUCs are 87.9%, 92.5%, 95.7%, and 100%, respectively. Addition of baseline tHb and tHb% assessed at the end of the first cycle and first three cycles to standard tumor pathological variables has significantly improved the prediction of patient pathologic response to NAC.

First Patient Result Obtained from Upgraded US-Guided DOT System

To validate the early results summarized in Section “Summary of Early Results of NAC Prediction,” we are currently conducting a trial at three clinical sites. The first patient who entered the new trial in March 2014 was a 37-year-old woman with an invasive mammary carcinoma of mixed lobular and ductal features. The histological grade and nuclear grade were 3, and mitotic counts per 10 field high magnification was 9 and the Nottingham score was 8 out of 9. The receptor status was ER positive, PR positive, and HER2 positive. The pretreatment MRI image, co-registered US image, and the corresponding DOT tHb map are given in Figure 4(a) to (c). The tHb map is obtained in seven slices from left to right and top to bottom corresponding to depths of 0.5 cm to 3.5 cm from the skin surface to chest wall. Each slide is given in x - y spatial dimensions of 9×9 cm. The largest diameter of the tumor measured from MRI images was 3.6 cm and from US images was 2.5 cm. The tHb distribution provides functional information of tumor angiogenesis distribution and also quantitative levels. The tumor vascular distribution is mainly distributed at the periphery of the mass, which is often seen in aggressive high-grade tumors.³⁹ Quantitatively, the tHb level measured from the average maximum of several tHb images at the tumor site was $81.02 \mu\text{mol/L}$. Using this baseline, tHb level and all pathological variables as the inputs to the logistical regression model with $\beta_n, n = 0, 1, \dots, k$ estimated from Figure 3 (blue curve), the output of the model is 0.71, which is well above the threshold of 0.5 and predicts that this patient would have a complete or near complete pathological response that is Miller–Payne grade 4 or 5. The patient was treated with dual HER2 blockade of trastuzumab and pertuzumab for six cycles of total 18 weeks. The post-treatment MRI image, US, and DOT tHb map are given in Figure 4(d) to (f). There was no residual tumor seen in MRI images. The US image revealed a smaller mass that can be identified by the metal clip and tHb map revealed no vascular contrast. The final surgery report revealed a grossly identified mass of 5.5 cm at location of 10–2 o'clock. Microscopically, there were scattered tumor cells of ductal and lobular features within the scar tissue. The largest residual focus measured 1.25 mm. The Miller–Payne grade was four.

To assess the early changes during NAC, we have monitored the patient at day 2, day 8, and end of cycles 1, 2, and 3. Figure 5 shows five sets of co-registered US images and tHb maps obtained at these time points, and Figure 6 is the quantitative tHb level calculated from

average maximum tHb levels with the average taken from several tHb images at the tumor site at each time point. It is interesting to note that at day 2, the periphery distribution of tHb is similar to the pretreatment pattern; however, 24% higher tHb content was observed, which may be due to initial tumor vascular response to NAC treatment. At day 8, the tHb map has changed to more centralized distribution, but the level is still 12% higher than that at the pretreatment level. At the end of cycle 1, the tHb level dropped to 73% of the pretreatment level. At the end of cycles 2 and 3, the tHb is in the background level with no vascular contrast. Using tumor pathological variables, baseline tHb, and %tHb measured at the end of cycle 1 as inputs to the logical regression model with $\beta_{n,n} = 0, 1, \dots, k$ estimated from Figure 3 (magenta), the output is 0.97, which predicts the complete and near complete pathological response with a significantly high confidence. If additional %tHb measurements at the end of first three cycles are used, the output is 1.0, which yields a perfect prediction of patient's response to NAC.

Summary

In this manuscript, we have reviewed the recent progress on utilizing US-guided DOT for predicting and monitoring patients' pathological response to NAC. We have reported the result of the first patient who has entered the new trial designed to validate the results that combining pretreatment tHb content as well as tHb changes measured at early treatment cycles with standard pathological variables determined at the core biopsy can substantially improve the prediction accuracy of patients' pathological response to NAC and therefore guide the selection of personalized treatment plan. Ongoing studies are aimed at increasing patient numbers and utilizing this technology in trials with more tumor-specific targeted therapy. In the future, one might adopt a strategy where breast cancer patients treated in the neoadjuvant setting who were determined to be early non- or poor-responders by using our technique could be offered alternate chemotherapeutic regimens given the unlikelihood of a pathologic complete response.

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Figure 1.

The upgraded NIR system currently used at the University of Connecticut Health Center. The commercial US transducer is readily plugged into the combined probe that houses all source and detector fibers for light delivery and collection. The co-registered US images are captured by a video capture card and are used to guide the light illumination and optical imaging reconstruction. NIR = near infrared. US = ultrasound.

Figure 2. (a) Mean maximum tHb in micromoles per liter in two MP grades 1 to 3 and grades 4 to 5 obtained at pretreatment (cycle 0) and end of treatment cycles 1 to 3 of NAC. (b) % tHb in MP grades 1 to 3 and grade 4 to 5 obtained at pretreatment (cycle 0) and end of treatment cycles 1 to 3 of NAC.³⁰ tHb = total hemoglobin; MP = Miller–Payne; NAC = neoadjuvant chemotherapy.

Figure 3.

ROC curves of training data set obtained from Zhu et al.³⁰ Utilizing tumor pathological variables only, the AUC is 87.9%; combining tumor pathological variables and pretreatment tHb, the AUC is 92.5%; combining tumor pathological variables and first cycle of total hemoglobin changes, the AUC is 95.7%; combining tumor pathological variables and first three cycles of total hemoglobin changes, AUC is 100%. The 95% confident interval is also given in the figure. ROC = receiver operating characteristic; AUC = area under the ROC curve; tHb = total hemoglobin; CI = confidence interval.

Figure 4.

A 37-year-old woman with an invasive mammary carcinoma located at 2 o'clock position of her left breast. (a) Baseline or pretreatment MRI image, (b) US image, and (c) tHb map. tHb map revealed periphery distribution, which is often seen in aggressive carcinomas. (d) Post-treatment MRI image, (e) US image and (f) tHb map. MRI indicated no residual tumor, and tHb map showed no vascular contrast. The tHb map is obtained in seven slices from left to right and top to bottom corresponding to depths of 0.5 cm to 3.5 cm from skin surface to chest wall. Each slide is given in $x-y$ spatial dimensions of 9×9 cm. US = ultrasound; MRI = magnetic resonance imaging; tHb = total hemoglobin.

Figure 5.

Co-registered US images and tHb maps obtained at five time points. Day 2, 8, and cycle 1 US images are zoomed into the depth range of 0 to 4 cm while cycle 2 and 3 US images are zoomed in to the range of 0 to 2.5 cm to better visualize the tumor. US = ultrasound; tHb = total hemoglobin.

Figure 6.

Quantitative tHb level calculated from average maximum tHb levels with the average taken from several tHb images at the tumor site at each time point. tHb = total hemoglobin; NAC = neoadjuvant chemotherapy.