

# Optical Mammography Using Diffuse Optical Spectroscopy for Monitoring Tumor Response to Neoadjuvant Chemotherapy in Women with Locally Advanced Breast Cancer

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## Abstract

**Purpose:** Diffuse optical spectroscopy (DOS) has the potential to enable monitoring of tumor response during chemotherapy, particularly in the early stages of treatment. This study aims to assess feasibility of DOS for monitoring treatment response in HER2-negative breast cancer patients receiving neoadjuvant chemotherapy (NAC) and compare DOS with tumor response assessment by MRI.

**Experimental Design:** Patients received NAC in six cycles of 3 weeks. In addition to standard treatment monitoring by dynamic contrast enhanced MRI (DCE-MRI), DOS scans were acquired after the first, third, and last cycle of chemotherapy. The primary goal was to assess feasibility of DOS for early assessment of tumor response. The predictive value of DOS and DCE-MRI compared with pathologic response was assessed.

**Results:** Of the 22 patients, 18 patients had a partial or complete tumor response at pathologic examination, whereas

4 patients were nonresponders. As early as after the first chemotherapy cycle, a significant difference between responders and nonresponders was found using DOS (HbO<sub>2</sub> 86% ± 25 vs. 136% ± 25,  $P = 0.023$ ). The differences between responders and nonresponders continued during treatment (halfway treatment, HbO<sub>2</sub> 68% ± 22 vs. 110% ± 10,  $P = 0.010$ ). Using DCE-MRI, a difference between responders and nonresponders was found halfway treatment ( $P = 0.005$ ) using tumor volume measurement calculations.

**Conclusions:** DOS allows for tumor response assessment and is able to differentiate between responders and nonresponders after the first chemotherapy cycle and halfway treatment. In this study, DOS was equally effective in predicting tumor response halfway treatment compared with DCE-MRI. Therefore, DOS may be used as a novel imaging modality for (early) treatment monitoring of NAC. *Clin Cancer Res*; 21(3); 577–84. ©2014 AACR.

## Introduction

Neoadjuvant chemotherapy (NAC) has been established as the standard-of-care treatment for locally advanced inoperable breast cancer, and is increasingly being used for patients with operable cancer (1, 2). A pathologic complete response (pCR) has been consistently shown to be associated with increased long-term survival (2). A significant number of patients, however, is unresponsive to NAC or even experience tumor growth under NAC (3, 4). A key advantage of NAC is the opportunity to assess

response during treatment as a predictor of final pathologic response, with the potential to modify therapy. Therefore, the early knowledge of response to NAC is essential for providing the optimal treatment strategy.

Currently, response of the tumor to NAC is most often monitored by a combination of clinical examination and conventional imaging techniques, such as mammography, ultrasound, or dynamic contrast enhanced MRI (DCE-MRI). However, clinical examination and these imaging techniques are often unable to objectively assess treatment response during the course of treatment (5). Moreover, the correlation between MRI and pCR assessment is limited (6–8). Therefore, novel, noninvasive imaging techniques are needed to improve early monitoring response of the tumor to NAC. In addition to DCE-MRI and PET modalities, optical imaging has received new interest as a noninvasive and nonionizing technique to assess tumor response (9–14).

Diffuse optical spectroscopy (DOS) uses near-infrared light to provide quantitative spectral information about tissue absorption and scattering properties (15, 16). These optical properties of tissue can be used to assess tissue microstructure and functional parameters, such as oxygenated hemoglobin, deoxygenated hemoglobin, relative oxygen desaturation, and water and lipid composition. Hypoxia, blood flow, oxygen saturation,

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### Translational Relevance

Advances in neoadjuvant chemotherapy (NAC), in particular during treatment of breast cancer, have elicited the need for noninvasive treatment monitoring techniques. This prospective study shows the successful use of optical imaging using diffuse optical spectroscopy (DOS) for tumor response monitoring during NAC in patients with locally advanced breast cancer. Using DOS, it was possible to differentiate between responders and nonresponders as early as after the first chemotherapy cycle. Moreover, in this study, the predictive power of DOS was higher compared with conventional MRI treatment monitoring in our group of patients with HER2-negative breast cancer. These results show that this technique may be used as a novel imaging modality for treatment monitoring to assist patient tailored medicine.

and hemoglobin concentration are correlated to tumor response (17). As DOS is noninvasive and does not require contrast agents, it is a promising modality for frequent measurements of tumor response.

Multiple studies have assessed DOS for monitoring NAC treatment in patients with breast cancer (9–14). These studies have suggested that DOS may provide clinically useful information on tumor response on NAC treatment. In previous studies, patients using different chemotherapeutic regimes were included. This study aims to assess the feasibility and predictive power of DOS for monitoring treatment response in patients with breast cancer receiving NAC directly after the start of NAC until surgery. Moreover, this study compares the tumor response assessment by DOS to tumor response assessment by MRI.

### Materials and Methods

This prospective, single-arm, single-center study was approved by the Medical Ethics Committee of the Leiden University Medical Center (Leiden, the Netherlands) and was performed in accordance with the ethical standards of the Helsinki Declaration of 1975. Inclusion criteria were patients with breast cancer with HER2 negative tumors larger than 2 cm or HER2-negative tumors with lymph node metastases, without distant metastases and eligible for neoadjuvant treatment. Patients with known allergies to materials used in the DOS apparatus, prior breast surgery or chemotherapy or radiation therapy were excluded. Patients were included in the period from March 2011 to October 2012. All included patients gave informed consent and the acquired data were anonymized.

#### Clinical trial

Patients received TAC (docetaxel, doxorubicin, and cyclophosphamide) with or without zoledronic acid in six cycles of 3 weeks. Patients were mainly enrolled in the context of the NEOZOTAC trial (NCT01099436). Standard monitoring of treatment response of the tumor to NAC was based on clinical examination before each cycle and three dynamic contrast enhanced MRI (DCE-MRI) scans (before NAC, before fourth cycle, before surgery) performed on a 1.5 Tesla system (Philips Medical Systems). In addition, patients were scheduled for four optical mammo-

graphies using DOS (before NAC, before second cycle, before fourth cycle, before surgery). DOS was performed using a commercially available breast imaging system (Softscan; Softscan Healthcare Group) as described previously (18). Briefly, Softscan is a bed-based imaging system on which the patient has to lie down with the breast inserted into an aquarium filled with optical compensation media (OCM). OCM is an oil-in-water emulsion that mimics average optical properties of the human breast. It is used to minimize light reflections at the breast surface to improve image quality. The system consists of four individual pulsed diode lasers operating at 690, 730, 780, and 830 nm. Light is collected by a mobile detector in a 1 cm-X constellation composed of five optical fibers and detected by a photomultiplier. The breast is scanned in approximately 10 to 20 minutes. The count by the detector was time correlated with the synchronization signal provided by the laser system driver. Using the measured absorption and scattering, an accurate estimate of the oxyhemoglobin (HbO<sub>2</sub>), deoxyhemoglobin (Hb), total hemoglobin (HbT), and scattering amplitude (SA) and power (SP) can be obtained. Water (H<sub>2</sub>O) and %lipids have low, but non-negligible absorption coefficients at the higher NIR wavelengths and were estimated as well. Patients were positioned into the Softscan aperture under guidance of a radiology technician. Breasts were scanned in the craniocaudal angle and the scanning area encompassed the whole breast. To ensure consistency, stabilizing plates were used to secure the breast in place. The acquired data were reconstructed using the software associated with the Softscan device, and three-dimensional (3D) tomographic images were created from the optical parameters with a typical voxel size of 3 × 3 × 7 mm<sup>3</sup> (Fig. 1).

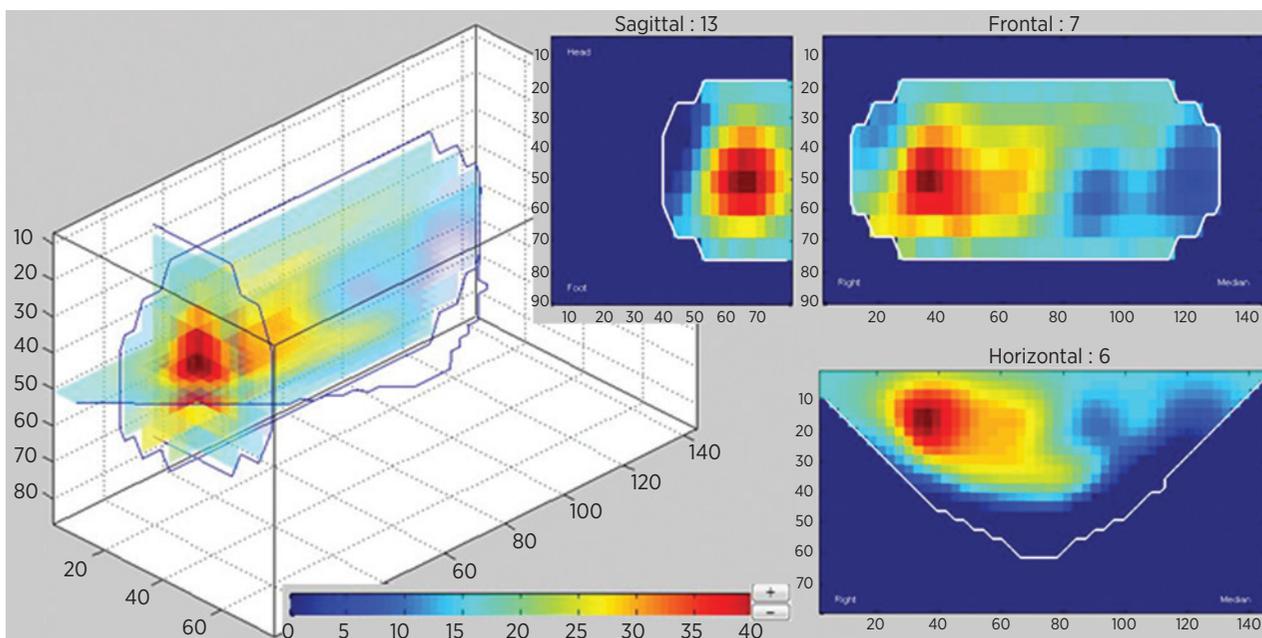
#### MRI- and pathologic assessment of tumor response to neoadjuvant chemotherapy

**DCE-MRI response assessment.** Tumor response to NAC on DCE-MRI was assessed in two ways: (i) according to the response evaluation criteria in solid tumors (RECIST 1.1) guidelines and (ii) by semiautomated measurement of tumor volume using a dedicated software program. All measurements were performed by a single observer (M.N.J.M. Wasser).

Following RECIST 1.1, tumor response was measured by changes in the longest diameter of the enhancing tumor target lesions. Complete reduction of the target lesions was termed as a complete response (CR). Partial response (PR) was deemed to have occurred if tumor size was reduced by at least 30%. Progressive disease (PD) was defined as an increase in tumor size of at least 20%. The remaining cases were considered to have stable disease (SD).

Measurement of tumor volumes on the subtracted 3D T1-weighted DCE-MR images was done using dedicated software (Vitrea Enterprise Suite version 6.6.3 software, Vital Images Inc.). Calculation of tumor volume consisted of semiautomated summing of all voxels with enhancement above background parenchymal enhancement.

**Pathology.** Following definitive surgery, the excised specimens were cut in multiple serial sections of approximately 5 mm, fixed in 10% formalin, paraffin embedded as tissue blocks, stained by hematoxylin and eosin, and evaluated by an experienced pathologist to determine the degree of pathologic tumor response of the primary breast lesion. Pathologic response was scored according to the Miller and Payne criteria (19).



**Figure 1.** Acquired DOS data of HbO<sub>2</sub> (μmol/L) was reconstructed to allow 3D analysis (BreastViewer 3.1).

#### Diffuse optical spectroscopy assessment of neoadjuvant chemotherapy

Tumor regions in each of the four DOS scans were manually annotated. The tumor in the baseline scan was annotated as an ellipsoid with axes of the sizes reported in the first radiologic assessment. The annotated region was centered on the maximum scatter amplitude in breast section containing the tumor based on conventional radiology. In DOS scans 2 to 4, an annotated region of the same size and shape as in the first scan was centered at the maximum scatter amplitude in these respective scans. Within each annotated region, the mean Hb and HbO<sub>2</sub> contents were measured in μmol/L. Hb and HbO<sub>2</sub> contents in follow-up scans were normalized by the baseline scan for inter-subject comparison. Subjects where the baseline scan was unreliable were completely excluded. Subjects where a follow-up scan was unreliable were removed from the respective groups. Criteria for unreliable scans were tumor on the edge of the scan or outside the scan and severe reconstruction artifacts in the breast section containing the tumor. Severe reconstruction artifacts were identified as physically improbable measurements, particularly zero SA and negative scatter power.

#### Patient and tumor characteristics

A total of 25 patients were initially included in the study. Two patients were excluded because of a failed baseline DOS scan and one patient due to a failed scan after one cycle. Patient and tumor characteristics of the 22 analyzed patients are summarized in Table 1. The median age was 50 years (range, 38–66), and median tumor size before NAC was 30 mm (range, 16–81). Of the patients, 6 patients had lobular carcinoma, 16 had ductal carcinoma, 20 had an ER-positive tumor, and 15 had a PR-positive tumor. All patients received a DOS scan before NAC and before the second cycle. In 2 patients, the DOS scan halfway therapy and

in 4 patients the DOS scan before surgery was not performed because of patient complications (not related to Softscan) or logistic reasons.

#### Data and statistical analysis

The primary goal of this study was to assess feasibility of DOS for assessment of tumor response to NAC based on pathologic response before the second NAC cycle. Failure of a missed first or second DOS scan resulted in exclusion from further DOS scans. All subjects were divided in two groups based on the Miller and Payne criteria: nonresponders (MP 1; i.e., 0% decrease of tumor cellularity after NAC) and (partial) responders (MP 2-5). For DOS time points, the changes in Hb and HbO<sub>2</sub> with respect to baseline were compared between the two groups and tested for significant differences using the Mann-Whitney *U* test. To test the predictive power of the DOS scans for identification of nonresponders, a linear logistic classifier was trained. For the DOS scans, HbO<sub>2</sub> was used as the feature. The classification performance was tested by training on 50% of the data, using the other 50% for testing. The classifier was evaluated by estimating an ROC. Because of the small number of patients, the training and testing were repeated 100 times with different permutations of training and test datasets. The average ROC was computed for final predictive power evaluation.

To compare DOS with DCE-MRI, the sensitivity and specificity of both RECIST and MRI volume measurements to predict tumor response (pathologic) to chemotherapy were assessed. The radiologic assessment by RECIST criteria was converted into a numerical ordering as: PD: 1, SD: 2, PR: 3 and CR: 4. Volume measurements after three and six cycles were normalized to the baseline tumor volume. Subjects were divided in two groups based on the same Miller and Payne criteria: nonresponders (MP 1) and (partial) responders (MP 2-5). The RECIST criteria and normalized volumes after three and six

**Table 1.** Patient and tumor characteristics

Characteristic	All patients (N = 22),	Responders (N = 18),	Nonresponders (N = 4),
	n	n	n
Age in years (median; range)	50 (38–66)	50 (38–59)	50 (46–66)
Menopausal status			
Premenopausal	16	13	3
Postmenopausal	6	5	1
Tumor histology			
Invasive ductal carcinoma	16	12	4
Invasive lobular carcinoma	6	6	0
Estrogen receptor status			
Positive	20	17	3
Negative	2	1	1
Progesterone receptor status			
Positive	15	12	3
Negative	7	6	1
HER receptor status			
Positive	0	0	0
Negative	22	18	4
Tumor size in mm (median; range)	30 (16–81)	33 (16–81)	29 (16–36)
Stage primary tumor <sup>a</sup>			
T1c	4	3	1
T2	13	10	3
T3	5	5	0

<sup>a</sup>Staging according to American Joint Committee on Cancer guidelines.

cycles were compared between the two groups and tested for significant differences using the Man–Whitney *U* test. Moreover, the predictive power of MRI was assessed. Both assessment by MRI using the RECIST 1.1 criteria and MRI volume were used to predict tumor response to NAC (MP criteria) by training a logistic classifier as for DOS.

Predictive power is estimated from classification results. The classification result is a trade-off between the desired fraction of true positives (nonresponders classified as nonresponders) and the accepted fraction of false positives (responders classified as nonresponders). The area under the curve (AUC) is a measure for the predictive power and is 1 for a perfect classifier (100% true positives for 0% false negatives), 0.5 for a random classification (similar to a coin-flip), and 0 for a completely wrong classification (0% true positives for 100% false negatives).

## Results

### MRI and pathologic assessment of tumor response

A summary of DCE-MRI response (RECIST criteria) and pathologic response (Miller and Payne criteria) measurements is given in Fig. 2. In 4 patients after three cycles and in 2 patients after six cycles, RECIST criteria could not be applied because of scattered presentation of the contrast-enhanced lesions and as in 1 patient no, MRI was obtained. Tumor volume could not be accurately measured in 1 patient after six cycles because of too scattered presentation.

MRI response measurements after three cycles (halfway treatment) indicated zero patients with PD, 5 patients with SD, 9 patients with a PR, and 4 patients with a CR.

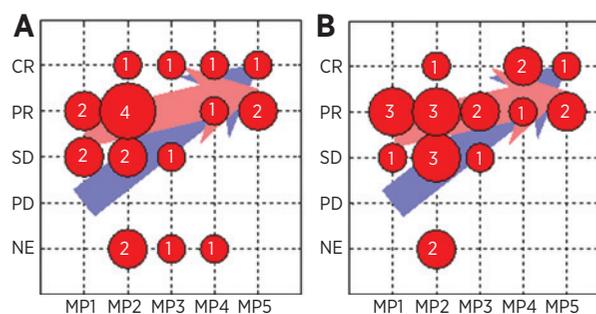
After six cycles and before surgery, MRI response measurements indicated 0 patients with PD, 5 patients with SD, 11 patients with a PR, and 4 patients with a CR.

The distribution of the pathologic response classification, following Miller and Payne, was as follows: grade 1, 4 patients; grade 2, 9 patients, grade 3, 3 patients; grade 4, 3 patients; grade 5, 3 patients.

### Comparison between responders and nonresponders

**Diffuse optical spectroscopy.** In all 22 patients, the tumor could be located on the optical mammography. The mean HbO<sub>2</sub> content (SDs between parentheses) for tumors at baseline were 25.9 (7.8) μmol/L for responders and 16.7 (3.8) μmol/L for nonresponders. Corresponding Hb content was 10.5 (3.8) μmol/L and 8.2 (0.8) μmol/L. These differences were nonsignificant: *P* = 0.064 (HbO<sub>2</sub>), *P* = 0.335 (Hb). In follow-up scans, interpatient variability remained and Hb and HbO<sub>2</sub> content (μmol/L) was not significantly different between responders and nonresponders: *P* > 0.500 for all follow-up scans.

However, the average Hb and HbO<sub>2</sub> contents relative to baseline in Table 2 show clear significant differences between responders and nonresponders that are distributed on the basis of pathologic criteria. The relative HbO<sub>2</sub> content (compared with baseline) is significantly lower in all three treatment stages: *P* = 0.023 after one cycle, *P* = 0.010 after three cycles, and *P* = 0.009 after therapy completion. Representative examples of DOS in

**Figure 2.**

Agreement between Miller and Payne (MP) criteria and radiology assessment by DCE-MRI using RECIST criteria after three cycles (A) and after six cycles (B) of NAC. The values indicate the patient count. The blue arrow denotes the expected relation, whereas the red arrow shows the linear regression between MP and DCE-MRI. MRI was assessed according to the RECIST criteria. NE, not evaluated.

**Table 2.** Differentiation between responders and nonresponders using DOS and MRI volumes

	DOS measurements			
	Baseline	1 cycle	3 cycles	6 cycles
Responder Hb (%)	100 (0)	91 (42)	76 (25)	83 (40)
Nonresponder Hb (%)	100 (0)	118 (11)	101 (3)	119 (39)
<i>U</i> test <i>P</i> value	1	0.109	0.076	0.233
Responder HbO <sub>2</sub> (%)	100 (0)	86 (25)	68 (22)	65 (20)
Nonresponder HbO <sub>2</sub> (%)	100 (0)	136 (25)	110 (10)	109 (18)
<i>U</i> test <i>P</i> value	1	<b>0.023</b>	<b>0.010</b>	<b>0.009</b>

	MRI volume measurements		
	Baseline	3 cycles	6 cycles
Responder	100 (0)	32 (23)	12 (24)
Nonresponder	100 (0)	78 (11)	65 (64)
<i>U</i> test <i>P</i> value	1	<b>0.005</b>	<b>0.011</b>

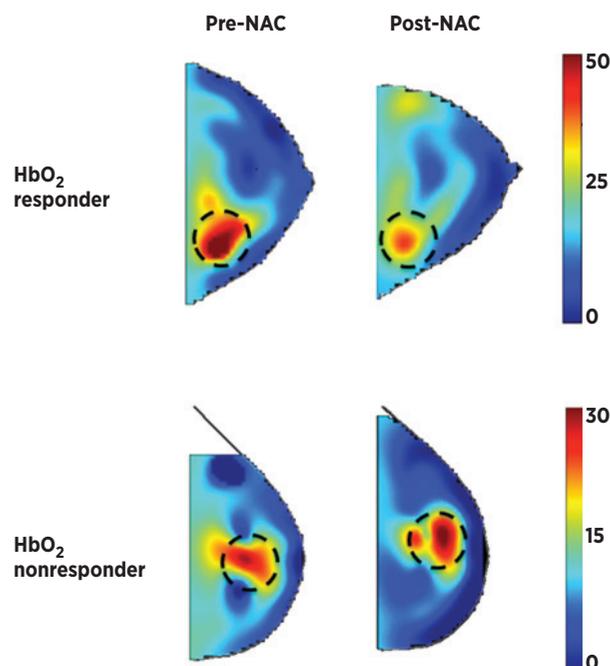
NOTE: Average Hb and HbO<sub>2</sub> content measured by DOS and tumor volumes measured by MRI relative to baseline for responders and nonresponders selected on the basis of pathology criteria. SDs are between parentheses. The Mann-Whitney *U* test *P* values estimate significance of the differences between the two groups. Bold text highlights *P* values indicating statistically significant differences ( $P < 0.05$ ).

responders ( $n = 18$ ; decrease of HbO<sub>2</sub> content) and nonresponders ( $n = 4$ ; stable or increasing HbO<sub>2</sub> content) are shown in Fig. 3. The average Hb content for responders is lower for all cycles, but this is not statistically significant.

**Dynamic contrast enhanced MRI.** The Mann-Whitney *U* test shows no significant difference in DCE-MRI assessment using RECIST for pathologic responders (MP 2-5,  $n = 18$ ) and nonresponders (MP 1,  $n = 4$ ) both after three cycles ( $P = 0.10$ ) and after six cycles ( $P = 0.77$ ). This is further illustrated in Fig. 2. After both three and six cycles, a weak trend is visible in which a better response seems to correspond to a higher Miller and Payne

ranking. This is, however, far from statistically significant. Correlation coefficients between Miller and Payne and RECIST assessment were 0.43 after both three and six cycles of NAC.

MRI volume measurements show statistically significant differences between responders and nonresponders after both three ( $P = 0.005$ ) and six cycles ( $P = 0.011$ ; Table 2). In both responders and nonresponders, the tumor has decreased halfway therapy and decreased even further in the scan before surgery. The decrease in tumor volume is much stronger in the group with responders. Correlations between normalized MRI volumes and normalized Hb/HbO<sub>2</sub> content after three cycles were 0.46 and 0.69 for Hb and HbO<sub>2</sub>, respectively, and 0.64 (Hb) and 0.70 (HbO<sub>2</sub>) after six cycles.

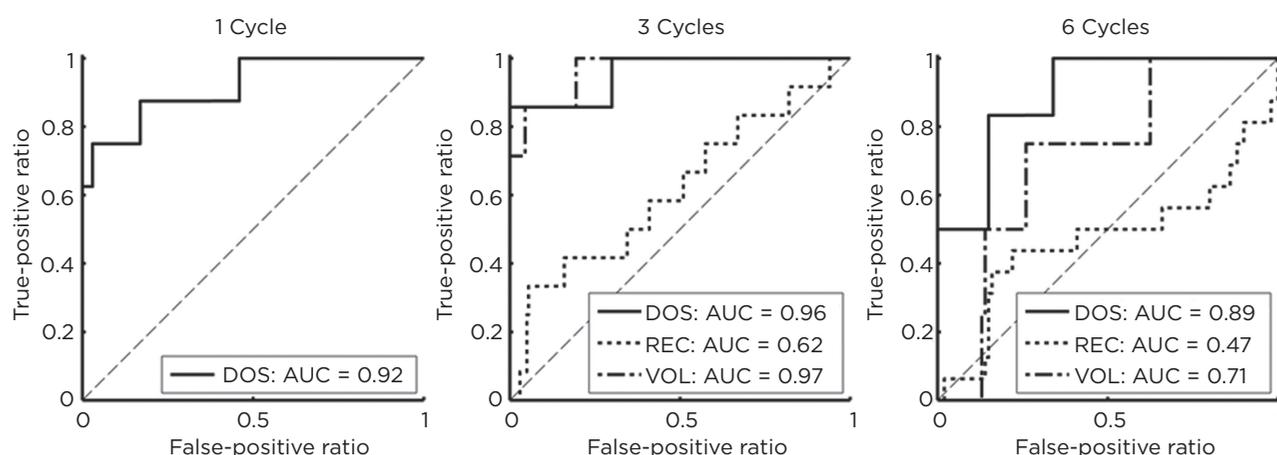


**Figure 3.** Transverse DOS images (after interpolation) before and after NAC of a typical responder, which shows a significant decrease of HbO<sub>2</sub> ( $\mu\text{mol/L}$ ) in the area of the tumor after NAC (top row), and nonresponder, which shows increase of HbO<sub>2</sub> in the area of the tumor after NAC (bottom row). The circle indicates where the tumor is located.

#### Predictive power of diffuse optical spectroscopy and MRI

The ROC curves in Fig. 4 show the prediction sensitivity and specificity for different trade-offs for DOS, RECIST, and DCE-MRI volumes normalized to baseline. For DOS assessment after only one cycle, accepting incorrect classification of 5% of the responders as nonresponders would correctly identify 75% of the nonresponders and allow them to change therapy. Similarly, accepting 10% incorrectly identified responders would correctly identify 87% of the nonresponders as responders.

The ROC curves show that DOS and normalized volume measurements have a comparable predictive power, both far better than RECIST. For example, predicting if a patient is a nonresponder after three cycles with a 20% false-positive rate is correct in 86% of the DOS scans and volume measurements, while this is only correct in 58% of the RECIST assessments. Considering the AUC, the predictive power for DOS is similar for all time points, with AUC values of 0.92, 0.96, and 0.89 after, respectively, one, three, and six cycles. The MRI volume AUC is 0.97 after three cycles and 0.71 after six cycles. The RECIST AUC is 0.62 after three cycles and only 0.47 after six cycles. These RECIST figures are much lower than that for DOS and MRI volume measurements, especially when taking into account that an AUC of 0.5 corresponds to a random prediction. The MRI volume measurements at six cycles have a decreased predictive power due to tumors that were nonresponders based on the MP criteria, but showed no contrast enhancement after six cycles. The large SD of 64 in Table 2 is indicative for the wide spread of nonresponding tumor volumes after six cycles.



**Figure 4.**

Predictive value of DOS and MRI-ROC curves for DOS and conventional radiology by MRI using RECIST (REC) and volume measurements (VOL) after one, three, and six cycles of NAC. The dashed diagonal shows a ROC for a completely random classification result with an AUC of 0.5.

## Discussion

The present study demonstrates the clinical use of DOS for treatment monitoring of NAC in patients with breast cancer. Treatment monitoring using DOS showed a significant difference in the physiologic tumor parameters between responders and nonresponders as early as after the first gift of NAC. Moreover, the predictive value of DOS to determine tumor response corresponded to MRI volume measurements after three cycles but appeared to outperform DCE-MRI evaluation based on RECIST.

DOS showed a decrease in Hb and HbO<sub>2</sub> content in the patients with a response of the tumor to NAC. We found this decrease to be only statistically significant for HbO<sub>2</sub>, but not for Hb. Similar results were obtained in previous studies (9–13, 20). In comparative studies between Hb and HbO<sub>2</sub> levels in tumor tissue and healthy tissue, tumor tissue shows nearly a 2-fold increased level of Hb and HbO<sub>2</sub> as a result of increased vascular supply (17). The decrease of Hb and HbO<sub>2</sub> levels observed in the study suggests a decrease of tumor tissue and an increase of nontumorous tissue in the region of interest (ROI) where the tumor was located. The reason we did not find a statistically significant decrease of Hb can be partly attributed to the small number of nonresponders ( $N = 4$ ), compared with the large number of responders. Under these circumstances, the Mann–Whitney  $U$  test has a strongly decreased sensitivity and only large group differences as in the relative HbO<sub>2</sub> changes were tested as statistically significant.

The absolute quantities of Hb and HbO<sub>2</sub> at baseline were varying greatly between tumors and did not show significant differences between potential responders and nonresponders. This large interpatient variability was also present in the follow-up scans, and responders and nonresponders could not be separated on the basis of absolute Hb and HbO<sub>2</sub> content ( $\mu\text{mol/L}$ ). Normalization with respect to the baseline scan is thus essential. Apart from Hb and HbO<sub>2</sub>, the two most important absorbers in NIR are water and lipids. The low absorption coefficients of the latter two, compared with Hb and HbO<sub>2</sub>, prevented reliable estimates, with frequent erroneous estimates (0% or 100% water/lipids content).

Correlating the normalized DOS parameters Hb, HbO<sub>2</sub> with normalized MRI volume measurements showed a strong positive relationship between changes in MRI volume and

changes in (de)oxyhemoglobin content. Although it is likely that tumor volume and oxygenation have a positive relationship, a confounder may be in the hemoglobin measurements where a constant volume is used to measure these parameters, while the tumor volume has decreased and additional normal tissue can be in the measurement volume. One should therefore be careful with interpreting the absolute hemoglobin measurements in relation to tumor viability.

In the current study, a homogenous HER2-negative group of patients with locally advanced breast cancer was used receiving the same NAC regime. It is important to validate DOS in a homogenous patient group as tumor variables such as HER2 status and tumor size have a significant effect on the optical properties of tumors. Positive HER2 tumors have a significant higher Hb level by DOS assessment (21). This is most likely related to angiogenesis as HER2 overexpression is associated with increased angiogenesis. As the difference between Hb levels between HER2-positive tumors and healthy tissue is larger compared with HER2-negative tumors, it can be expected that DOS assessment of NAC in HER2-positive tumors will result in even a more distinct difference between responders and nonresponders.

Moreover, in addition to NAC, neoadjuvant hormonal therapy is also increasing rapidly and has shown similar patient outcomes compared with NAC (22). Many different anticancer agents are available (chemotherapy, hormonal therapy, monoclonal agents), all have different antitumor and antiangiogenic effects. Therefore, when assessing early treatment response, it is crucial to use a homogenous treatment regime. As the therapeutic effect of hormonal therapy acts on a different mechanism compared with chemotherapy, validation of using DOS for treatment monitoring in neoadjuvant hormonal therapy is essential to provide wide clinical use of DOS in the treatment monitoring of patients with breast cancer (23).

With the increasing interest in neoadjuvant treatment, improved imaging modalities that can provide early prediction of tumor response will be required to select patient who may benefit from a different treatment. In case of locally advanced breast cancer, early detection of nonresponders could prevent unnecessary toxic NAC treatment and allow selecting patient who would benefit from a different neoadjuvant treatment regime or

early surgery. In the current study, after one cycle of NAC, DOS would correctly identify most nonresponders, allowing them to change treatment strategy early. Prediction accuracies were evaluated as AUCs of 0.92, 0.96, and 0.87 after one, three, and six cycles, respectively. The lower AUC after six cycles is due to a smaller number of available scans for classifier training. Previous studies have shown similar results showing specificity of 80% to 83% (9, 11). These data suggest that DOS is able to identify a large set of patients early during treatment that would benefit from a different treatment regime. However, it should be emphasized that prospective studies are necessary to validate these results.

In the current study, the predictive value of DOS to determine tumor response corresponded to MRI volume measurements after three cycles but appeared to outperform DCE-MRI evaluation based on RECIST. To date, DCE-MRI is the most accurate response assessment (24). However, this modality is not in all breast tumors equally reliable. Several breast tumor subtypes, such as HER2-negative and ER-positive breast tumors, are associated with reduced accuracy (25). This might explain the poor adequacy of MRI in our study, as our study population consisted almost completely of HER2-negative and ER-positive patients. The biologic explanation for this may be that HER2-negative breast cancer is associated with decreased angiogenesis, resulting in impaired tumor tissue perfusion of MRI contrast (26). Also, hormone receptor-positive tumors have a different growth pattern than hormone receptor-negative tumors, with less often (unifocal) mass-like lesions, making it more difficult to accurately determine the residual tumor diameter (8, 27).

Limitation of the used DOS device in our study is that determination of the correct ROI for the DOS was difficult because DOS provided limited anatomical context. Therefore, the ROI at baseline was partly based upon data of conventional mammography and MRI acquired before therapy. Localization of the ROI was not trivial during data analysis and incorrect ROI placement can lead to an underestimation of tissue changes and consequently decrease the sensitivity. A 5-mm displacement of the ROI introduces variations in Hb/HbO<sub>2</sub> estimates of about 2% for tumors of about 30 cm<sup>3</sup>, whereas for smaller tumors of approximately 5 cm<sup>3</sup>, estimates could differ by up to 8%. The possibility of merging MRI and DOS could therefore improve DOS assessment and provide more functional parameters to the MRI and further increase the sensitivity.

DOS is a relatively novel imaging modality in the field of breast cancer and is rapidly evolving by increasing spatial resolution and by increasing analytic techniques to provide more accurate assessment of functional parameters (28, 29). A recent study by Roblyer and colleagues indicated that DOS as early as the first day after NAC may possibly discriminate nonresponding from responding patients (30). Remarkably, this study found an increase in HbO<sub>2</sub> for responders, compared with nonresponders, while monitoring in the first week. Roblyer and colleagues hypothesized that this increase can be attributed to an acute inflammatory reaction in the responding tumors. Measurements with the same device as Roblyer and colleagues at mid-therapy and before surgery showed lower HbO<sub>2</sub> values for responders, comparable with our findings (30). We therefore hypothesize that after the acute inflammatory

reaction stops, the responding tumors have a lower metabolism and that our measurement after 3 weeks (one cycle) is measuring the same decrease in metabolism in the tumor as response to NAC as the measurements after three and six cycles. A future study with frequent measurements during the first 3 weeks after NAC therapy starts could provide further insight.

Moreover, several optical contrast agents are available, which may improve contrast and sensitivity (31, 32). Furthermore, DOS can be combined with other imaging modalities, such as ultrasound, for improved tumor localization accuracy (11). These developments will allow further improvement of DOS sensitivity and accuracy.

In conclusion, DOS allows early response monitoring of tumor tissue to chemotherapy and is able to differentiate between responders and nonresponders in early stages of therapy. Therefore, DOS could be used as a novel imaging modality for treatment monitoring of NAC to assist patient tailored medicine.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

### Authors' Contributions

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