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Factors affecting measurement of optic parameters by time-resolved near-infrared spectroscopy in breast cancer

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Abstract. The purpose of this study was to evaluate the effects of the thickness and depth of tumors on hemoglobin measurements in breast cancer by optical spectroscopy and to demonstrate tissue oxygen saturation (SO_2) and reduced scattering coefficient (μ_s') in breast tissue and breast cancer in relation to the skin-to-chest wall distance. We examined 53 tumors from 44 patients. Total hemoglobin concentration (tHb), SO_2, and μ_s' were measured by time-resolved spectroscopy (TRS). The skin-to-chest wall distance and the size and depth of tumors were measured by ultrasonography. There was a positive correlation between tHb and tumor thickness, and a negative correlation between tHb and tumor depth. SO_2 in breast tissue decreased when the skin-to-chest wall distance decreased, and SO_2 in tumors tended to be lower than in breast tissue. In breast tissue, there was a negative correlation between μ_s' and the skin-to-chest wall distance, and μ_s' in tumors was higher than in breast tissue. Measurement of tHb in breast cancer by TRS was influenced by tumor thickness and depth. Although SO_2 seemed lower and μ_s' was higher in breast cancer than in breast tissue, the skin-to-chest wall distance may have affected the measurements. [DOI: 10.1117/1.JBO.23.2.026010]

Keywords: breast cancer; hemoglobin; tissue oxygen saturation; reduced scattering coefficient.

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1 Introduction

Diffuse optical spectroscopy with near-infrared light can assess tumor angiogenesis based on tissue hemoglobin concentrations. The concentrations of oxygenated and deoxygenated hemoglobin can be calculated from the absorption coefficient at multiple wavelengths. As optical spectroscopy is not expensive and can be repeated without ionizing radiation, this technique is promising for assessment of the effects of neoadjuvant chemotherapy for breast cancer.[1,2]

In the measurement of the absorption coefficient of breast cancer by optical spectroscopy, the surrounding mammary glands and fat are inevitably included in the measurement. Furthermore, chest wall muscles may be included when the skin-to-chest wall distance is small. We have demonstrated that hemoglobin measurement in breast cancer by time-resolved spectroscopy (TRS) was influenced by the chest wall muscles.[3] Tumor size and depth may also affect the measurement because the path length of light in the tumor and relative amount of light that reaches the tumor are different among tumors depending on their size and depth.

Although changes in tissue oxygen saturation (SO_2) may indicate the effectiveness of chemotherapy,[4,5] there is no consensus on the SO_2 level in breast cancer. The reduced scattering coefficient (μ_s') is another optical parameter that provides information on tissue structure and composition.[6] However, these indices may depend on the skin-to-chest wall distance.

The purpose of this study was to evaluate the effects of tumor size and depth on hemoglobin measurement in breast cancer and to report SO_2 and μ_s' in breast tissue and in breast cancer in relation with the skin-to-chest wall distance.

2 Materials and Methods

2.1 *Near-Infrared Time-Resolved Spectroscopy*

Optical properties of normal breast tissue and cancer were measured using a single-channel near-infrared TRS system (TRS-20SH, Hamamatsu Photonics K.K., Hamamatsu, Japan). This system uses a time-correlated single-photon counting method for acquiring the temporal response profiles from the tissue against optical impulse inputs and enables quantitative analysis of light absorption and scattering in tissue by the photon diffusion theory.[7] An ultrashort pulsed laser light source (PLP, Hamamatsu Photonics K.K.) emits light pulses at three different wavelengths (758, 795, and 833 nm) with an average power of 200 μW, full width at half maximum (FWHM) of <100 picoseconds (ps), and repetition frequency of 5 MHz. The detector section of this system consisted of a photomultiplier tube (H7422P-50MOD, Hamamatsu Photonics K.K.) followed by constant fraction discriminators, time-to-amplitude converters, A/D converters, and histogram memories. The instrumental response function of the system was approximately 320-ps FWHM. The three light sources

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emitted a pulse on a time series, and the three-wavelength optical pulses were guided into one optical fiber via a fiber coupler (NTT Advanced Technology Corp., Kawasaki, Japan). Each single optical fiber (GC200/250L, Fujikura Ltd., Tokyo, Japan) used for light irradiation had a numerical aperture (NA) of 0.25 and a core diameter of 200 μm. The optical bundle fiber (SCHOTT MORITEX Corp., Asaka, Japan) used to collect the light had an NA of 0.26 and a bundle diameter of 3 mm. The nonlinear least-squares method was used to fit the solution of the time-domain photon diffusion equation with a semi-infinite homogeneous model to the observed temporal profiles. The zero boundary condition of the reflectance mode was employed. The time resolution of the temporal profile was 10 ps, and the data acquisition time was 3 s. The distance from the light source to the detector was 3 cm. The absorption coefficient and reduced scattering coefficient were obtained at each wavelength. The data over the time range from −560 to 5440 ps were selected for fitting. The reduced chi-square value was used to evaluate fitting accuracy. Our fitting process was successfully implemented if this value was within the range of 0.8 to 1.2.[8] The concentrations of oxygenated and deoxygenated hemoglobin were calculated from the absorption coefficient at each wavelength by the least-squares method after subtracting water and lipid absorption in breast tissue. We assumed that water content was 18.7% and lipid content was 66.1%.[9] The concentration of tHb was the sum of the concentrations of oxygenated hemoglobin and deoxygenated hemoglobin.

2.2 *Ultrasonography*

An ultrasonography (US) system (EUB-7500, Hitachi Medical Corporation, Tokyo, Japan) with a linear probe (EUP-L65, Hitachi Medical Corporation) was used. The probe had a frequency range of 6 to 14 MHz. A spectroscopic probe was attached to the US probe.[3]

2.3 *Patient Characteristics*

We examined 86 patients with breast lesions between June 2013 and April 2016. We excluded 32 patients who had undergone chemotherapy or hormonal therapy. Three patients in whom the final diagnosis was not cancer were excluded. We excluded two patients just after biopsy and two patients just after subcutaneous injection of a radiopharmaceutical around the tumor for sentinel lymph node biopsy. We also excluded two patients whose tumors were undetectable on US and one patient in whom ultrasound images were not recorded. Finally, 44 patients were included in this study. All patients were female and their median age was 52.3 years, with a range of 33 to 78 years. Twenty patients were premenopausal and 24 patients were postmenopausal. The patients underwent TRS and US scan at least 12 days after core needle biopsy. Six patients had multiple lesions, and a total of 53 lesions were evaluated. Forty tumors were found to be invasive ductal cancer, 12 tumors were ductal carcinoma *in situ*, and one tumor was invasive lobular carcinoma. The mean maximum transverse diameter of the tumors was 19.5 mm, with a range of 7 to 61 mm. The study protocol was approved by the Ethical Review Committee of Hamamatsu University School of Medicine. All patients signed a written informed consent form.

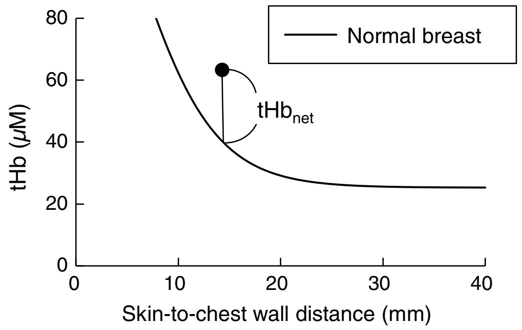

Fig. 1 tHb$_{net}$ was defined as the difference between tumor tHb and tHb in normal breast tissue. The reference curve of the tHb concentration in normal breast tissue as a function of the skin-to-chest wall distance for pre- and postmenopausal patients was derived from the previous study.[3]

2.4 *TRS and US Scan Protocol*

After the spectroscopic probe that was attached to the US probe was placed on the skin just above the tumor, the absorption coefficient and reduced scattering coefficient at three wavelengths were measured simultaneously. Tumor thickness, depth, and skin-to-chest wall distance were measured by US. The distance from the skin to the anterior surface of the tumor was defined as the depth. The distance between the anterior and posterior surface of the tumor was defined as the thickness. The chest wall was defined as the surface of the major pectoral muscle or anterior serratus muscle. The default setting of the maximal depth in our US system was 36 mm, and we were not able to measure the skin-to-chest wall distance in two normal breasts and four tumor-containing breasts. They were excluded from the analysis using the correlation coefficient between optical parameters and the skin-to-chest wall distance.

The optical parameters of normal breast tissue were measured in the contralateral breast at the corresponding area. Although the number of tumors examined was 53, the number from normal breasts was 46. The number of the measurement points in the normal side was less than the number of tumors in the patients who had multiple lesions.

Net tHb (tHb$_{net}$) was defined as the difference between tumor tHb and tHb in normal breast tissue (Fig. 1). The reference curve of the tHb concentration in normal breast tissue as a function of the skin-to-chest wall distance for pre- and postmenopausal patients was derived from the previous study.[3]

2.5 *Statistical Analysis*

Statistical examinations were performed using Microsoft Excel 2013 (Microsoft Corporation, Redmond, Washington) and StatFlex version 6.0 (Artech Co., Ltd., Osaka, Japan).

The Pearson's correlation coefficient was calculated to compare the relationship between tHb and tumor thickness, tHb$_{net}$ and tumor thickness, tHb and tumor depth, tHb$_{net}$ and tumor depth, tumor depth and skin-to-chest wall distance, SO_2 and the skin-to-chest wall distance, μ_s' and body mass index (BMI), and μ_s' and the skin-to-chest wall distance. Linear regression analyses were carried out for each pairing. The differences in SO_2 and μ_s' between tumor and normal breast tissues were determined using nonparametric Mann–Whitney U tests. Differences with $P < 0.05$ were considered significant.

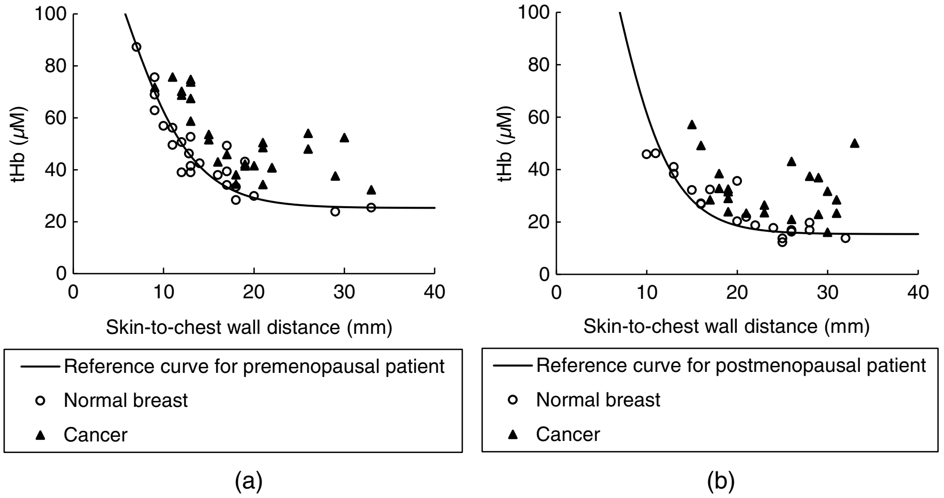

Fig. 2 (a) In premenopausal patients, tHb in normal breast tissue was plotted near the reference curve ($n = 24$) and tHb in all 27 tumors was plotted above the curve. (b) In postmenopausal patients, tHb in normal breast tissue was plotted near the reference curve ($n = 20$) and tHb in all 22 tumors was plotted above the curve.

3 Results

3.1 *Total Hemoglobin Concentration*

tHb in normal breast tissue was plotted near the reference curve in both pre- and postmenopausal patients. tHb in breast cancer was plotted above the curve in both pre- and postmenopausal patients [Figs. 2(a) and 2(b)].

Although no correlation was found between tHb and tumor thickness for all the tumors, a positive correlation was found for tumors with a skin-to-chest wall distance larger than 21 mm [Figs. 3(a) and 3(b)]. There was also a positive correlation between tHb_{net} and tumor thickness [Fig. 3(c)]. There was a negative correlation between tHb and tumor depth and between tHb_{net} and tumor depth [Figs. 4(a) and 4(b)]. The correlation coefficient for tHb was stronger than for tHb_{net}. The tumor

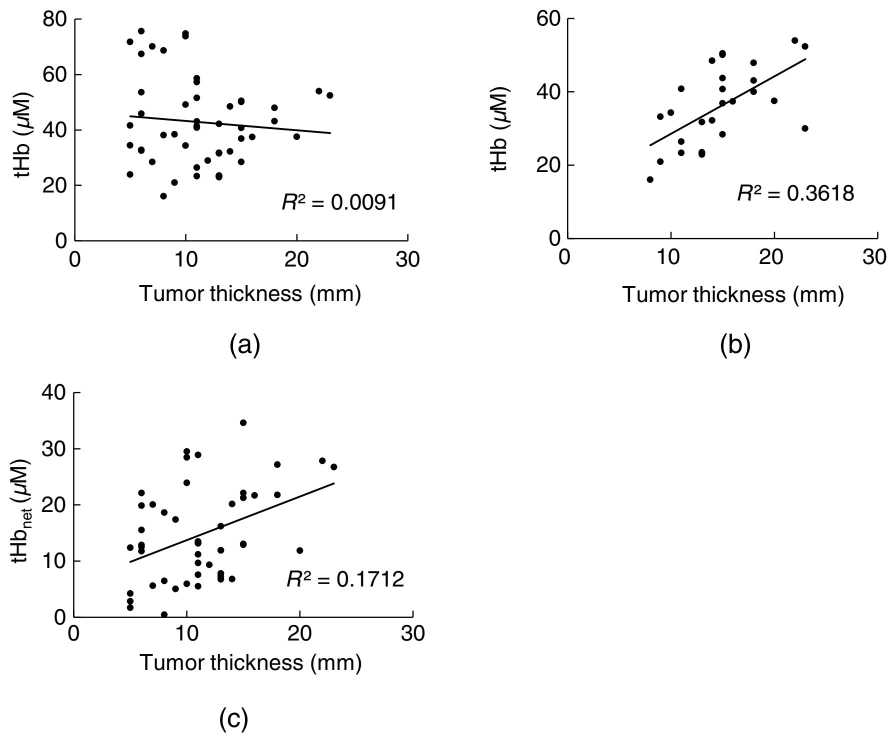

Fig. 3 (a) The tHb in tumors was plotted against the tumor thickness. No correlation was found between tHb and tumor thickness ($n = 49$, $R^2 = 0.01$). (b) In tumors with a skin-to-chest wall distance larger than 21 mm, a positive correlation was found between tHb and tumor thickness ($n = 27$, $R^2 = 0.36$, $P < 0.001$). (c) There was a positive correlation between tHb_{net} and tumor thickness ($n = 49$, $R^2 = 0.17$, $P = 0.003$).

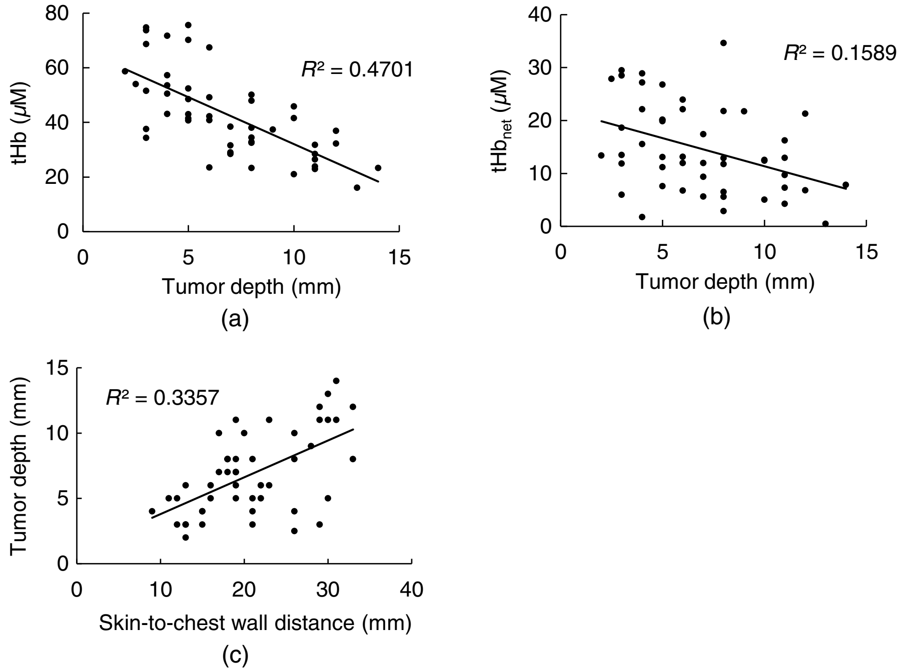

Fig. 4 (a) There was a negative correlation between tHb and tumor depth ($n = 49$, $R^2 = 0.47$, $P < 0.001$). (b) A negative correlation between tHb_{net} and tumor depth remained after calculating tHb_{net} ($n = 49$, $R^2 = 0.16$, $P = 0.005$). (c) There was a positive correlation between tumor depth and the skin-to-chest wall distance ($n = 49$, $R^2 = 0.34$, $P < 0.001$).

depth was small in tumors with a small skin-to-chest wall distance [Fig. 4(c)].

3.2 *Tissue Oxygen Saturation*

SO_2 in normal breast tissue decreased when the skin-to-chest wall distance decreased [Fig. 5(a)]. The mean SO_2 in normal breast tissue was $78.1\% \pm 2.9\%$ (mean $\pm$ SD). To minimize the effects of the chest wall, SO_2 in breast with a skin-to-chest wall distance larger than 21 mm was analyzed. The tumor SO_2 was lower than normal breast tissue SO_2, although the difference was not significant [Fig. 5(b)].

3.3 *Reduced Scattering Coefficient*

We reported results on μ_s' at a wavelength of 795 nm (μ_{s795}'). In normal breast tissue, a negative correlation was found between μ_{s795}' and BMI [Fig. 6(a)]. A negative correlation was also found between μ_{s795}' and age (data not shown). There was a negative correlation between μ_{s795}' and the skin-to-chest wall distance [Fig. 6(b)]. The μ_{s795}' in tumors was higher than that in normal breast tissue when the skin-to-chest wall distance was larger than 21 mm [Fig. 6(c)].

4 Discussion

The tHb was higher in patients with thick tumors when the skin-to-chest wall distance was larger than 21 mm. As the length of the light path through tumors was long in thick tumors, the tHb measurement was likely to be high. In a previous study, we proposed a subtraction method to assess the hemoglobin concentration, in which the skin-to-chest wall distance was taken into consideration.[3] There was a positive correlation between the tHb_{net} and tumor thickness for all tumors, including patients

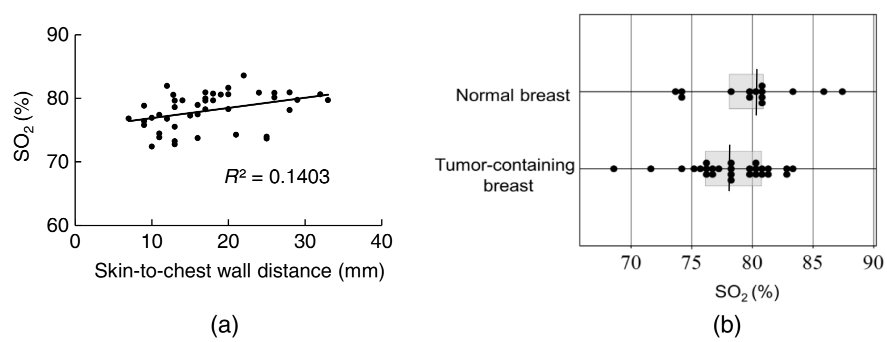

Fig. 5 (a) In normal breast tissue, there was a positive correlation between SO_2 and the skin-to-chest wall distance ($n = 44$, $R^2 = 0.14$, $P = 0.01$). (b) In breast tissue with a skin-to-chest wall distance larger than 21 mm, the tumor SO_2 ($n = 27$) was lower than breast tissue SO_2 ($n = 14$), although the difference was not significant ($P = 0.221$). Bars and boxes represent the median and interquartile range, respectively.

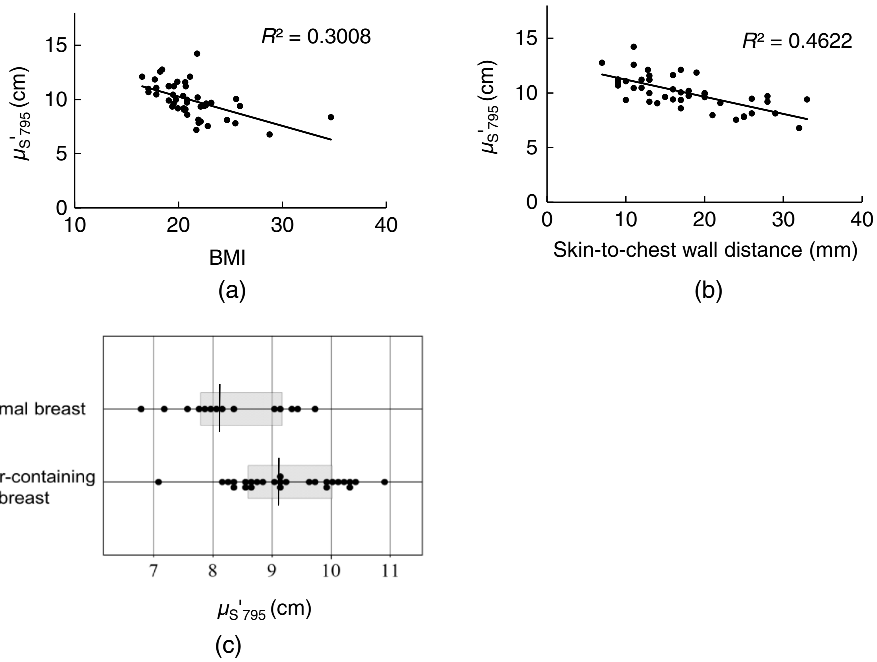

Fig. 6 (a) The μ'_{s795} in normal breast tissue decreased when the BMI increased ($n = 46$, $R^2 = 0.30$, $P < 0.001$). (b) In normal breast tissue, a negative correlation was found between μ'_{s795} and the skin-to-chest wall distance ($n = 44$, $R^2 = 0.46$, $P < 0.001$). (c) In breast tissue with a skin-to-chest wall distance larger than 21 mm, the μ'_{s795} in tumors ($n = 27$) was higher than that in normal breast tissue ($n = 14$) ($P = 0.005$). Bars and boxes represent the median and interquartile range, respectively.

with a small skin-to-chest wall distance, suggesting that tHb_{net} is a good indicator of tHb for tumors irrespective of the skin-to-chest wall distance.

In measurements with a reflection-type probe, the detection sensitivity of the optical measurement had a limit in the depth direction.[10] When the tumor was located deep in the breast, tHb measurements were low because of low sensitivity. tHb demonstrated a stronger correlation with tumor depth than tHb_{net}. In tumors where the skin-to-chest wall distance was small, the tumor was located near the skin [Fig. 4(c)]. In such situations, tHb measurements may be artificially high, which caused a stronger correlation of tHb with tumor depth.

The usefulness of tHb_{net} is under investigation in clinical settings. We assessed the therapeutic response to neoadjuvant chemotherapy for breast cancer by spectroscopy and found that tHb_{net} reflected therapeutic response more precisely than tHb.

SO_2 tended to be lower in breast cancer than in normal breast tissue when the skin-to-chest wall distance was larger than 21 mm, although the difference was not significant. There has been controversy over SO_2 in breast cancer. Chance et al.[11] reported that SO_2 in cancer was lower, whereas Grosenick et al.[12] and Choe et al.[13] reported that SO_2 in cancer was not lower than that in normal breast tissue. SO_2 in breast tissue decreased when the skin-to-chest wall distance was small [Fig. 5(a)]. SO_2 in the antebrachial muscle measured by TRS was reported as $73.9\% \pm 2.8\%$ (mean $\pm$ SD).[14] SO_2 in normal breast tissue measured in this study was $78.1\% \pm 2.9\%$ (mean $\pm$ SD), and SO_2 can be affected by chest wall muscles.

The μ'_s in normal breast tissue was low in patients with high BMI and in older patients, which is in accordance with the results of previous studies.[15,16] Adipose tissue has a lower μ'_s than glandular tissue.[17] A low μ'_s may reflect high amounts of fat in older patients and in patients with a high BMI. The μ'_s in normal breast tissue increased when the skin-to-chest wall distance decreased, and the μ'_s in muscles is reported to be widely distributed.[18] The μ'_s in muscles can be higher than in mammary glands and the measurement of μ'_s in breast tissue may also be influenced by chest wall muscles. It was reported that the μ'_s was high in tumors compared with that in normal breast tissue.[12,13,19] In this study, the μ'_s was higher in tumors than in normal breast tissue when the skin-to-chest wall distance was larger than 21 mm.

In conclusion, tumor thickness and depth, in addition to chest wall muscles, influenced measurement of tHb by TRS in breast cancer. Our proposed indicator, tHb_{net}, reflected the influence of tumor thickness and depth accurately, suggesting it as a good indicator of tHb irrespective of the skin-to-chest wall distance. The measurement of SO_2 and μ'_s was also affected by the chest wall. The effects of the chest wall on the measurement of optical properties by TRS should be considered.

Disclosures

The authors declare that they have no conflicts of interest.

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