Association between Transillumination Breast Spectroscopy and Quantitative Mammographic Features of the Breast

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Abstract

Transillumination breast spectroscopy (TiBS) uses nonionizing optical radiation to gain information about tissue properties directly from the breast. TiBS measurements were obtained from 225 women with normal mammograms. Principal component analysis was used to reduce the spectral data set into four principal components and to generate four TiBS scores (t_1-t_4) for each woman. These components and scores represent light scattering, water, lipid, and hemoglobin content. Percent density, dense area, and nondense area were measured using Cumulus. The association between TiBS scores and quantitative mammographic features was analyzed using linear regression stratified by menopausal status and adjusted for body mass index. Among premenopausal women, t_1 and t_3 were significantly associated with percent density ($\beta_{t1} = -0.14$, $P = 0.04$; $\beta_{t3} = -2.43$, $P < 0.0001$), whereas t_2 and t_3 were significantly associated with dense area ($\beta_{t2} = -1.57$, $P < 0.0001$; $\beta_{t3} = -2.54$, $P < 0.0001$). Among postmenopausal women, t_1, t_3, and t_4 were significantly associated with percent density ($\beta_{t1} = -0.30$, $P < 0.0001$; $\beta_{t3} = -2.51$, $P < 0.0001$; $\beta_{t4} = 4.75$, $P < 0.0001$) and dense area ($\beta_{t1} = -0.19$, $P = 0.004$; $\beta_{t3} = -2.13$, $P = 0.002$; $\beta_{t4} = 5.02$, $P < 0.0001$). Scores t_2 and t_4 were also significantly correlated with age among postmenopausal women ($r_{t2} = 0.41$ and $r_{t4} = -0.36$). Given the association with quantitative mammographic features and tissue changes related to age and menopause, TiBS scores may prove useful as intermediate markers in studies of breast cancer etiology and prevention. (Cancer Epidemiol Biomarkers Prev 2008;17(5):1043–50)

Introduction

The radiologic appearance of the breast varies between women because of differences in tissue composition and in the X-ray attenuation properties of fat, epithelium, and stroma (1). Studies employing quantitative methods to assess the X-ray dense tissue of the breast (that is, mammographic density) have shown that women with dense tissue occupying ≥75% of the total breast area have a 4- to 6-fold higher risk of breast cancer in the next decade than women in the lowest category (<10%; refs. 2-14). A dose-response relationship between mammographic density and breast cancer risk has been shown (15). Although risk estimates associated with percentage density are those most often cited, the area of dense tissue alone was also associated with differences in risk of breast cancer in some studies (16, 17).

Because of its strong association with breast cancer and its relation to established risk factors for the disease (11), mammographic density is increasingly being used as an intermediate marker in studies both investigating the etiology of breast cancer and testing new preventive strategies (18, 19). However, mammography, which involves exposure to ionizing radiation, may not be appropriate for studies in young women or women at increased risk, such as high-risk mutation carriers, or for studies involving repeated assessments.

Visible light and near-infrared radiation-based transillumination breast spectroscopy (TiBS) is an inexpensive nonimaging, noninvasive technique that provides information about bulk breast tissue properties based on the spectral dependence of the probability of a photon to pass through breast tissue (20-23). Red and near-infrared light can penetrate ∼7 cm of breast tissue while permitting good-quality spectra carrying information on wavelength-dependent light scattering and absorption by water, lipids, and hemoglobins (oxyhemoglobin and deoxyhemoglobin; refs. 24-31). In breast tissue, stromal and epithelial tissues are characterized by increased scattering and water-associated absorption and concomitant decreased lipid associated absorption, larger total hemoglobin content, and lower oxygen saturation (31-33).

In a previous cross-sectional study of 292 women without radiologically suspicious lesions, we showed that TiBS scores (that is, *t* scores) derived from principal component analysis were highly associated with categorical mammographic density as assessed by a radiologist (23). In this article, we report on the association between TiBS scores and quantitative mammographic features of the breast (percent density, dense area, and nondense area) obtained from analogue films from 104 premenopausal and 121 postmenopausal women from the same cross-sectional study.

Received 10/5/07; revised 1/9/08; accepted 2/10/08.

Grant support: Congress Directed Medical Research Plan grant DAMD 17-00-1-0393 and Canadian Institute of Photonics Innovation.

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doi:10.1158/1055-9965.EPI-07-2658

Materials and Methods

Study Population. Study participants were recruited between March 1, 2000 and September 30, 2004 from the Marvelle Koffler Breast Imaging Centre at Mount Sinai Hospital. This study was approved by the Research Ethics Boards of the University of Toronto, Mount Sinai Hospital, and the University Health Network. Inclusion criteria were an analogue (film) standard screening mammogram within ~12 months before recruitment, revealing no radiologic suspicious lesions (n = 232). Exclusion criteria were prior fine-needle aspiration, core biopsies, or any other type of breast surgery, including breast reduction or augmentation, and any type of tattoos on the breast(s).

Information concerning participants' age, menopausal status, height, and weight were collected by means of a self-administered questionnaire. Postmenopausal status was defined as having had no menstrual period for at least 12 months before mammography. As five women were missing information on height and weight, their body mass index (BMI) could not be calculated and they were excluded from the present analysis. An additional two women were omitted as their BMI was >50.0 (that is, outliers). Hence, the data presented here are for 225 women with complete spectral and demographic information.

Optical Setup and Procedure. The instrumentation used to gather transillumination spectra was described previously in detail and is depicted in Fig. 1 (20-23). A 50 W halogen lamp served as broadband light source; 200 mW light of wavelength 550 to <1,300 λ was coupled into a 5-mm-diameter liquid light guide (Fibre Guide) placed in contact with the skin on top of the breast with minimal compression. Transmitted light was collected via a 7-mm-diameter optical fiber bundle (140 Si/Si fibers, 200 μm core diameter, numerical aperture: 0.36, P&P Optica) held coaxially and pointing toward the light source. A calliper provided the inter-optode distance in centimeters. A spectrophotometer (Kaiser) with holographic transillumination grating (15.7 rules/mm blazed at 850 nm) and a two-dimensional cryogenically cooled silicon CCD (Photometrics) provided wavelength-dependent detection at a resolution of better than 3 nm from 625 to 1,060 λ (n = 436 wavelengths). Hospital Grade Canada Standards Association certification and Health Canada Investigational New Device Class II approval were obtained for use of the instrument on volunteers.

All optical measurements were collected before quantification of mammographic features. For premenopausal women, all measurements were taken during the first week of the luteal phase of the menstrual cycle. Time in menstrual cycle was based on the woman's self-reported last menstrual bleeding and usual cycle length over ≥2 consecutive cycles. Additionally, in a subgroup of 63 premenopausal women, four weekly measurements (not timed to the menstrual cycle but covering it) were carried out. Measurements were taken in the dark, with the participant seated comfortably in an upright position and the breast resting on the support platform. For each woman, TiBS measurements were executed in four standardized positions (center, medial, distal, and lateral) on each breast in cranial caudal view, providing optical interrogation of different anatomical regions (20-23). The center position was in the midline closest to the sternum, the distal position was 2 cm posterior to the nipple, and the medial and lateral positions were 2 cm in from the border of the breast adjacent to the sternum and axilla, respectively. With the measurement of four positions on each breast, an ovoid shaped volume of ~23 mL for a 5-cm-thick breast is sampled (20-23). Estimates showed that 85% of the tissue contributes to the optical signal (20, 21). Temporal and spatial reproducibility of the optical measurements is good, as addressed previously (20, 21). The entire TiBS procedure takes ~15 min.

Spectral Processing and Principal Component Analysis. The methods used in spectral processing and analysis were described previously (20-23). Spectra were corrected for the system's wavelength-dependent signal transfer function using a transmission standard and divided by the interoptode distance (centimeters) and expressed in units of optical density per centimeter [OD cm^{-1}]. Principal component analysis (Matlab 12.1, MathWorks) was used to capture the variation in light scattering and absorption (that is, total attenuation in OD cm^{-1}) overall spectra (S_i) comprising the entire data set for the studied population and to generate a component score (t_{in}) for each identified principal component (p_n) for each measurement position on both breasts of each woman (34). The principal components are common to all spectra, whereas the component scores (that is, TiBS scores) represent each individual woman's spectra and indicate the contribution of each identified principal component to each spectrum (20-23). Each individual spectrum is a linear combination of the principal component spectra (p_n) multiplied by the respective component score (t_{in}), such that $S_i = t_{i1}p_1 + t_{i2}p_2 + t_{i3}p_3 + t_{i4}p_4 + \varepsilon$, where ε represents the residual error.

Nearly all spectral variation in the entire data set (~99.96%) is captured by four principal components (p_1-p_4) representing different tissue features, including light scattering by cellular and structural components and absorption by water, lipid, oxyhemoglobin, and deoxyhemoglobin, all of which are related to the amount of proliferating tissue in the breast (20-23). Each woman therefore had four individual TiBS scores (t_1-t_4) associated with each measurement position (center, medial, distal, and lateral) on each breast.

Quantification of Percent Density and Dense Area from Mammograms. All film mammograms in cranial-caudal view [n = 2 films (left and right) × 225 eligible volunteers] were digitized using a Lumisys Digital Scanner (Kodak) with a 12-bit gray scale resolution and a pixel pitch of 260 μm. Percent mammographic density [(dense area / total area) × 100], dense area, and nondense area (total area - dense area) were estimated from each digitized mammogram using a computer-assisted thresholding program (Cumulus; ref. 7, 35). For dense area, nondense area and total area pixels were converted into square centimeters using a factor of 0.000676. All images were read by two individuals (K.M.B. and L.L.) after training by an expert rater (N.B.). The reading of each mammogram was repeated twice for the entire data set by each trained rater with a period of at least 1 month separating each read. The reproducibility of mammographic measurements on duplicate readings (15% random repeat set) was high; the resulting intraclass correlation coefficients for the two trained raters for the final read were 0.96 and 0.93, respectively. Agreement between each trained rater and the expert for the final read was also high; interclass correlation coefficients were 0.92 and 0.93, respectively.

Statistical Analysis. Each TiBS score (t_1-t_4) was averaged over all measurement positions over both breasts (left and right) resulting in four global TiBS scores ($\overline{t_1}$-$\overline{t_4}$) for each woman, thereby providing a global assessment of the breast tissue similar to quantitative mammographic measures. Bilateral symmetry in the spectra and derived TiBS scores at corresponding measurement positions was shown previously (20, 21). Given the high correlation between raters and between readings from the right and left breasts ($r > 0.90$), the final mammography variable read results were averaged over the three raters (two trained and one expert) over both breasts (left and right) for each woman. A t test showed no significant difference between left and right percent density, dense area, and nondense area in the population under study ($P = 0.96$, 0.80, and 0.64, respectively).

To measure the association between the four continuous TiBS scores ($\overline{t_1}$-$\overline{t_4}$) and percent density, dense area, and nondense area, we employed univariate and multivariate linear regression analyses. As percent density, dense area, and nondense area were not normally distributed, we used a square root transformation, which resulted in more normal distributions. The distributions among the independent variables (TiBS scores, age, and BMI) were reasonably normal; hence, no other transformations were done. As BMI and age were considered potential confounders (others were not available), we looked at the TiBS scores both unadjusted and adjusted for continuous age and BMI (kg/m^2). Analyses were done separately for premenopausal and postmenopausal women. We further conducted stratified analyses according to months between mammography and TiBS measurements. Among 63 premenopausal women who came in for TiBS measurements over a single menstrual cycle (four visits, 1-week intervals), we examined whether the TiBS scores differed as a function of week as well as phase (follicular versus luteal) of the menstrual cycle. Statistical analyses were carried out using Statistical Analysis Systems (SAS Institutes) version 9.1 and P values < 0.05 were considered significant.

Results

Table 1 lists the descriptive statistics for age at TiBS, BMI (kg/m^2), quantitative mammographic features, and each TiBS score ($\overline{t_1}$-$\overline{t_4}$) for premenopausal and postmenopausal women. Premenopausal women had significantly higher percent density and larger dense area (both at $P < 0.0001$), whereas nondense area was significantly larger in postmenopausal women ($P < 0.0001$). Total breast area was similar in the two groups. As expected, age at TiBS measurements ($P < 0.0001$) differed between the two groups, but BMI did not. Mean global scores $\overline{t_1}$ ($P = 0.001$), $\overline{t_2}$ ($P < 0.0001$), and $\overline{t_4}$ ($P < 0.0001$) also differed significantly between premenopausal and postmenopausal women but $\overline{t_3}$ did not.

Correlation analysis showed a strong positive association between $\overline{t_1}$ and BMI in both premenopausal and postmenopausal women ($r = 0.61$ and 0.47, respectively; both at $P < 0.0001$). Among postmenopausal women, $\overline{t_2}$ showed a strong positive association with age ($r = 0.41$; $P < 0.0001$), whereas $\overline{t_4}$ was negatively associated with age ($r = -0.36$; $P < 0.0001$).

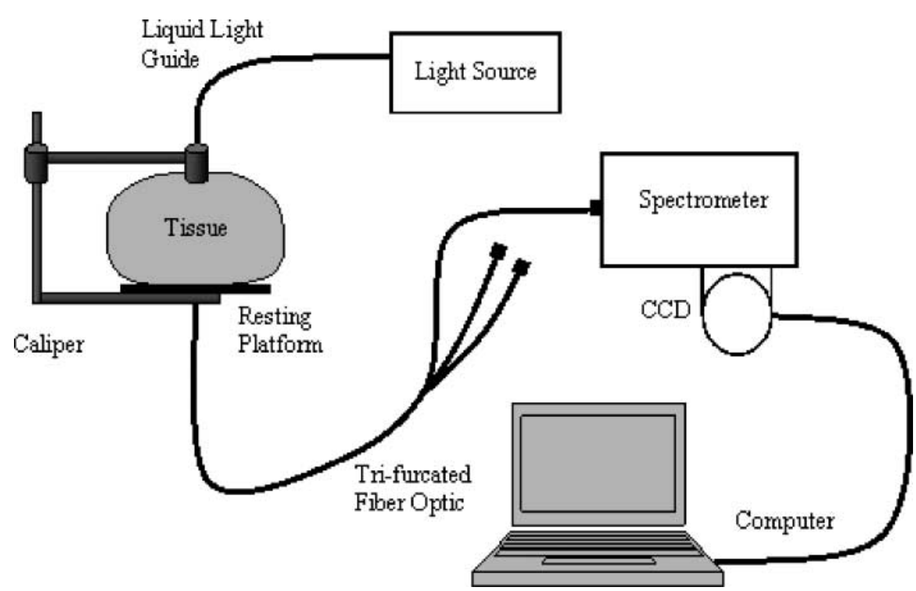

Figure 1. Components if TiBS setup comprising light source, light guide in contact with the tissue delivering 200 mW total optical power, and detection fiber bundle directing transmitted photons to the holographic spectrophotometer equipped with a liquid nitrogen cooled CCD.

Table 1. Mean and SD of percent mammographic density, dense area, nondense area, total area, age, BMI, and TiBS scores ($\overline{t_1}$ -$\overline{t_4}$) by menopausal status

	Premenopausal (n = 104)	Postmenopausal (n = 121)
Age (y), mean ± SD (range)	45.9 ± 4.1 (37-59)	55.4 ± 6.3 (42-75)
BMI (kg/m^2), mean ± SD (range)	25.8 ± 5.2 (18.0-42.6)	26.7 ± 5.1 (18.6-43.9)
Percent density, mean ± SD (range)	36.7 ± 18.9 (2.4-79.5)	23.3 ± 18.5 (0.2-67.8)
Dense area (cm^2), mean ± SD	48.8 ± 31.3 (6.1-157.8)	29.2 ± 24.0 (0.0-101.7)
Nondense area (cm^2), mean ± SD	100.6 ± 62.5 (11.7-277.5)	126.6 ± 68.5 (19.2-307.4)
Total area (cm^2), mean ± SD	149.4 ± 65.5 (35.6-310.1)	155.8 ± 62.5 (47.8-336.0)
$\overline{t_1}$, mean ± SD (range)*	−0.39 ± 3.31 (−9.24 to 5.08)	1.08 ± 3.07 (−7.88 to 6.42)
$\overline{t_2}$, mean ± SD (range)†	−0.11 ± 0.51 (−1.80 to 0.90)	0.12 ± 0.38 (−1.38 to 0.90)
$\overline{t_3}$, mean ± SD (range)‡	−0.02 ± 0.33 (−1.18 to 0.81)	−0.01 ± 0.31 (−1.18 to 0.97)
$\overline{t_4}$, mean ± SD (range)§	0.06 ± 0.18 (−0.73 to 0.42)	−0.06 ± 0.17 (−0.60 to 0.38)

*Decreasing $\overline{t_1}$ values suggest more light scattering and overall attenuation.
† Increasing $\overline{t_2}$ values suggest more lipid contribution; decreasing $\overline{t_2}$ values suggest larger hemoglobin (deoxyhemoglobin and oxyhemoglobin) contribution.
‡ Increasing $\overline{t_3}$ values suggest more lipid contribution; decreasing $\overline{t_3}$ values suggest more water and deoxyhemoglobin contributions.
§Increasing $\overline{t_4}$ values suggest more water and oxyhemoglobin contributions; decreasing $\overline{t_4}$ values suggest more lipid contribution.

Table 2 presents the unadjusted and adjusted (for age and BMI) associations between each TiBS score ($\overline{t_1}$ -$\overline{t_4}$) and percent density, dense area, and nondense area for premenopausal women. In the unadjusted analysis, TiBS scores $\overline{t_1}$ and $\overline{t_3}$ and BMI were significantly and inversely associated with percent density and significantly positively associated with nondense area; $\overline{t_2}$ and $\overline{t_3}$ $\overline{t_3}$ were significantly and inversely associated with dense area. In the adjusted analysis, TiBS scores $\overline{t_1}$ and $\overline{t_3}$ remained significantly and inversely associated with percent density and positively associated with nondense area. However, the association between $\overline{t_1}$ and both percent density and nondense area was weakened. TiBS scores $\overline{t_2}$ and $\overline{t_3}$ both remained significantly and inversely associated with dense area in the adjusted analysis.

Table 3 presents similar information for postmenopausal women. TiBS scores $\overline{t_1}$ and $\overline{t_3}$ and BMI were significantly and inversely associated with both percent density and dense area, and TiBS score $\overline{t_4}$ showed a significant positive association with these two measures. In addition, $\overline{t_2}$ was significantly negatively associated with dense area. With respect to nondense area, TiBS scores $\overline{t_1}$ and $\overline{t_3}$ and BMI were significantly and

Table 2. Unadjusted and adjusted (for age and BMI) regression coefficients (β) and SE for each individual TiBS score ($\overline{t_1}$ -$\overline{t_4}$) with percent density (square root transformation), dense area (square root transformation), and nondense area (square root transformation) for 104 premenopausal women

	Unadjusted				Adjusted*			
	β	SE	P	Individual R^2	β	SE	P	Individual R^2
Percent density								
$\overline{t_1}$ †	−0.24	0.05	<0.0001	0.21	−0.11	0.05	0.04	0.02
$\overline{t_2}$ ‡	−0.13	0.33	0.69	0.00	−0.46	0.28	0.11	0.01
$\overline{t_3}$ §	−2.45	0.44	<0.0001	0.23	−2.39	0.36	<0.0001	0.22
$\overline{t_4}$ ‖	0.93	0.94	0.32	0.01	1.08	0.80	0.18	0.01
Age (y)	0.01	0.04	0.86	0.00				
BMI (kg/m^2)	−0.17	0.03	<0.0001	0.28				
Dense area								
$\overline{t_1}$	0.05	0.06	0.43	0.01	0.12	0.08	0.16	0.02
$\overline{t_2}$	−1.32	0.40	0.001	0.10	−1.44	0.40	0.0005	0.11
$\overline{t_3}$	−2.75	0.58	<0.0001	0.18	−2.77	0.58	<0.0001	0.19
$\overline{t_4}$	1.03	1.20	0.40	0.01	1.07	1.20	0.37	0.01
Age (y)	0.06	0.05	0.22	0.01				
BMI (kg/m^2)	−0.03	0.04	0.45	0.01				
Nondense area								
$\overline{t_1}$	0.65	0.07	<0.0001	0.47	0.40	0.08	<0.0001	0.11
$\overline{t_2}$	−1.10	0.60	0.07	0.03	−0.38	0.45	0.40	0.01
$\overline{t_3}$	2.77	0.89	0.002	0.09	2.57	0.63	<0.0001	0.08
$\overline{t_4}$	−0.85	1.75	0.63	0.00	−1.17	1.28	0.36	0.01
Age (y)	0.07	0.08	0.35	0.01				
BMI (kg/m^2)	0.41	0.04	<0.0001	0.47				

*Adjusted for age (years) and BMI (kg/m^2).
† Decreasing $\overline{t_1}$ values suggest more light scattering and overall attenuation.
‡ Increasing $\overline{t_2}$ values suggest more lipid contribution; decreasing $\overline{t_2}$ values suggest larger hemoglobin (deoxyhemoglobin and oxyhemoglobin) contribution.
§Increasing $\overline{t_3}$ values suggest more lipid contribution; decreasing $\overline{t_3}$ values suggest more water and deoxyhemoglobin contributions.
‖Increasing $\overline{t_4}$ values suggest more water and oxyhemoglobin contributions; decreasing $\overline{t_4}$ values suggest more lipid contribution.

positively associated with this outcome, and $\overline{t_4}$ showed a negative association. After adjustment for age and BMI, TiBS scores $\overline{t_1}$, $\overline{t_3}$, and $\overline{t_4}$ remained significantly associated with percent density, and all four scores showed a significant association with dense area; TiBS score $\overline{t_4}$ was no longer associated with nondense area.

Table 4 displays the results of multivariate regression analysis for all TiBS score $\overline{t_1}$ to $\overline{t_4}$ and BMI with percent density, dense area, and nondense area for premenopausal and postmenopausal women. As age was not associated with any of the quantitative mammographic features in the unadjusted and adjusted analyses (Tables 2 and 3), it was excluded from further models.

In the multivariate analysis in premenopausal women, both $\overline{t_3}$ and BMI each explained the largest proportion of variability in percent density (22% and 28%, respectively), with the other variables explaining a very small amount. TiBS scores $\overline{t_2}$ and $\overline{t_3}$ remained significantly and inversely associated with dense area, with $\overline{t_3}$ accounting for the largest proportion of variation in this outcome (18%). TiBS scores $\overline{t_1}$ and $\overline{t_3}$ also remained significantly and positively associated with the nondense area, although BMI explained the largest proportion of variation in this outcome (46%). Once adjusted for BMI, the four TiBS scores together explained 30% of the variation in percent density, 31% of the variation in dense area, and 22% of the variation in nondense area, beyond the variation explained by BMI (total model R^2 - individual R^2 for BMI).

In the multivariate analysis among postmenopausal women, scores $\overline{t_1}$, $\overline{t_3}$, and $\overline{t_4}$ remained significantly associated with percent density and each score explained approximately the same proportion of variation in this outcome (13-14%). BMI was also significantly inversely associated with percent density. TiBS scores $\overline{t_1}$, $\overline{t_3}$, and $\overline{t_4}$ remained significantly associated with dense area, with $\overline{t_1}$ and $\overline{t_4}$ each accounting for the majority of the variation (14-15%). Although all four scores were significantly associated with nondense area, $\overline{t_1}$ and $\overline{t_3}$ explained most of the variation; BMI, however, accounted for the largest proportion of variation in nondense area (49%). Together, the four TiBS scores explained a fair amount of the variation in percent density (41%), dense area (39%), and nondense area (23%), respectively, after adjustment for BMI and excluding the variation explained by BMI.

The majority of women (~90%) had TiBS measurements performed within ~12 months of their mammogram, with a slightly higher proportion of postmenopausal women having TiBS done within 6 months (premenopausal, 17%; postmenopausal, 25%). However, stratified analyses by months between TiBS and mammography (<6, 6-12, and >12 mos.) in both premenopausal and postmenopausal women showed no evidence of a timing effect on the association between TiBS scores and mammographic features (data not shown). A repeated-measures analysis in 63 premenopausal women showed a significant correlation between TiBS scores measured 4 weeks apart ($\overline{t_1}$, r = 0.93; $\overline{t_2}$, r = 0.80; $\overline{t_3}$, r = 0.81; $\overline{t_4}$,

Table 3. Unadjusted and adjusted (for age and BMI) regression coefficients (β) and SE for each individual TiBS score ($\overline{t_1}$-$\overline{t_4}$) with percent density (square root transformation), dense area (square root transformation) and nondense area (square root transformation) for 121 postmenopausal women

	Unadjusted				Adjusted*			
	β	SE	P	Individual R^2	β	SE	P	Individual R^2
Percent density								
$\overline{t_1}$ †	−0.43	0.05	<0.0001	0.39	−0.31	0.05	<0.0001	0.15
$\overline{t_2}$ ‡	−1.02	0.50	0.04	0.03	−0.88	0.44	0.05	0.02
$\overline{t_3}$ §	−2.94	0.56	<0.0001	0.19	−2.53	0.47	<0.0001	0.13
$\overline{t_4}$ ‖	4.76	1.03	<0.0001	0.15	3.49	0.99	0.006	0.07
Age (y)	−0.05	0.03	0.10	0.02				
BMI (kg/m^2)	−0.22	0.03	<0.0001	0.28				
Dense area								
$\overline{t_1}$	−0.29	0.06	<0.0001	0.15	−0.22	0.07	0.003	0.06
$\overline{t_2}$	−1.63	0.53	0.003	0.07	−1.60	0.54	0.004	0.06
$\overline{t_3}$	−2.36	0.65	0.0004	0.10	−2.10	0.62	0.001	0.08
$\overline{t_4}$	5.20	1.13	<0.0001	0.15	4.67	1.22	0.0002	0.10
Age (y)	−0.04	0.03	0.19	0.01				
BMI (kg/m^2)	−0.14	0.04	0.0006	0.09				
Nondense area								
$\overline{t_1}$	0.65	0.07	<0.0001	0.40	0.38	0.07	<0.0001	0.11
$\overline{t_2}$	0.11	0.76	0.88	0.00	−0.07	0.57	0.90	0.00
$\overline{t_3}$	3.90	0.87	<0.0001	0.15	3.07	0.60	<0.0001	0.09
$\overline{t_4}$	−4.57	1.63	0.006	0.06	−1.98	1.30	0.13	0.01
Age (y)	0.04	0.05	0.36	0.01				
BMI (kg/m^2)	0.43	0.04	<0.0001	0.49				

*Adjusted for age (years) and BMI (kg/m^2).
† Decreasing $\overline{t_1}$ values suggest more light scattering and overall attenuation.
‡ Increasing $\overline{t_2}$ values suggest more lipid contribution; decreasing $\overline{t_2}$ values suggest larger hemoglobin (deoxyhemoglobin and oxyhemoglobin) contribution.
§Increasing $\overline{t_3}$ values suggest more lipid contribution; decreasing $\overline{t_3}$ values suggest more water and deoxyhemoglobin contributions.
‖Increasing $\overline{t_4}$ values suggest more water and oxyhemoglobin contributions; decreasing $\overline{t_4}$ values suggest more lipid contribution.

Table 4. Regression coefficients (β) and SE for TiBS scores ($\overline{t_1}$-$\overline{t_4}$) and BMI with percent density (square root transformation), dense area (square root transformation), and nondense area (square root transformation) in multivariate models by menopausal status

	β	SE	P	Individual R^2	Model R^{2}*
Premenopausal (n = 104)					
Percent density					
$\overline{t_1}$ †	−0.14	0.04	0.001	0.04	0.58
$\overline{t_2}$ ‡	−0.54	0.23	0.02	0.02	
$\overline{t_3}$ §	−2.43	0.33	<0.0001	0.22	
$\overline{t_4}$ ‖	1.55	0.65	0.02	0.02	
BMI (kg/m^2)	−0.13	0.03	<0.0001	0.28	
Dense area					
$\overline{t_1}$	0.09	0.07	0.17	0.01	0.33
$\overline{t_2}$	−1.57	0.37	<0.0001	0.09	
$\overline{t_3}$	−2.54	0.54	<0.0001	0.18	
$\overline{t_4}$	2.14	1.05	0.04	0.03	
BMI (kg/m^2)	−0.09	0.04	0.04	0.02	
Nondense area					
$\overline{t_1}$	0.44	0.07	<0.0001	0.13	0.68
$\overline{t_2}$	−0.35	0.37	0.35	0.00	
$\overline{t_3}$	2.93	0.54	<0.0001	0.09	
$\overline{t_4}$	−1.20	1.05	0.26	0.00	
BMI (kg/m^2)	0.23	0.04	<0.0001	0.46	
Postmenopausal (n = 121)					
Percent density					
$\overline{t_1}$	−0.30	0.04	<0.0001	0.14	0.70
$\overline{t_2}$	0.06	0.30	0.83	0.00	
$\overline{t_3}$	−2.51	0.36	<0.0001	0.13	
$\overline{t_4}$	4.75	0.66	<0.0001	0.14	
BMI (kg/m^2)	−0.09	0.02	0.0003	0.29	
Dense area					
$\overline{t_1}$	−0.19	0.06	0.004	0.15	0.40
$\overline{t_2}$	−0.69	0.47	0.14	0.01	
$\overline{t_3}$	−2.13	0.56	0.002	0.09	
$\overline{t_4}$	5.02	1.02	<0.0001	0.14	
BMI (kg/m^2)	−0.05	0.04	0.21	0.01	
Nondense area					
$\overline{t_1}$	0.39	0.06	<0.0001	0.10	0.72
$\overline{t_2}$	−1.02	0.43	0.02	0.01	
$\overline{t_3}$	3.04	0.52	<0.0001	0.08	
$\overline{t_4}$	−4.29	0.94	<0.0001	0.04	
BMI (kg/m^2)	0.27	0.04	<0.0001	0.49	

*Total model R^2 includes variation explained by all TiBS scores ($\overline{t_1}$-$\overline{t_4}$) and BMI.
† Decreasing $\overline{t_1}$ values suggest more light scattering and overall attenuation.
‡ Increasing $\overline{t_2}$ values suggest more lipid contribution; decreasing $\overline{t_2}$ values suggest larger hemoglobin (deoxyhemoglobin and oxyhemoglobin) contribution.
§Increasing $\overline{t_3}$ values suggest more lipid contribution; decreasing $\overline{t_3}$ values suggest more water and deoxyhemoglobin contributions.
‖Increasing $\overline{t_4}$ values suggest more water and oxyhemoglobin contributions; decreasing $\overline{t_4}$ values suggest more lipid contribution.

r = 0.70; all at P < 0.0001) and no significant difference in TiBS scores over menstrual cycle phase ($\overline{t_1}$, P = 0.70; $\overline{t_2}$, P = 0.64; $\overline{t_3}$, P = 0.09; $\overline{t_4}$, P = 0.85).

Discussion

This study complements earlier publications by our group (20-23) by examining the association between TiBS scores and percent density and the areas of dense and nondense tissue among 104 premenopausal and 121 postmenopausal women. Mammographic density is the quantitative feature most often considered in studies of breast cancer risk (1-14); however, some studies suggest that the total area of dense tissue is a better marker of the total number of cells at risk of malignant transformation (16) and that etiologic studies should consider dense and nondense tissues separately (36). In the present study, different TiBS scores were associated with percent density and the dense area of the breast in premenopausal versus postmenopausal women, whereas their associations with nondense area were comparable in both groups. Among postmenopausal women, some TiBS scores were also correlated with age.

The reasons for the differences in the associations of the four t values with quantitative mammographic features, age and menopause, are 2-fold. First, each t score captures varying information about overall tissue scattering as well as absorption by water, lipid, deoxyhemoglobin, and oxyhemoglobin. Second, the proportion of stromal and epithelial tissue relative to adipose tissue is on average smaller in postmenopausal compared with premenopausal women due to a reduction in the area of radiologically dense tissue and an increase in the area of nondense tissue after menopause (37, 38). Consequently, the potential for cellular proliferation and the magnitude of the overall metabolism also varies in the breast tissue of each group (1, 11).

Principal component p_1 and associated $\overline{t_1}$ values provide information on the light-scattering properties of the tissue, such that decreasing values suggest increased light scattering and overall attenuation (20–23). Stromal and epithelial tissues have a larger scattering coefficient than fatty tissue due to increased cellular content and structural support tissues (that is, the collagen matrix; refs. 24, 25, 39, 40). A positive relationship between percent density and light scattering has been shown by others (23-30).

Among premenopausal and postmenopausal women, $\overline{t_1}$ was inversely associated with percent density and positively associated with BMI. Hence, in both groups, $\overline{t_1}$ captures comparable information to BMI about breast tissue composition. Consistent with previous studies (41), the association of BMI with percent density in the present study was largely through its positive association with the nondense component of the breast (Table 4). However, among postmenopausal women, $\overline{t_1}$ explained 15% of the variation in percent density beyond that explained by BMI, suggesting that $\overline{t_1}$ captures additional information about breast tissue physiology in this group regarding light scattering and attenuation by the dense component of the breast (Tables 3 and 4).

Principal components p_2 to p_4 and associated scores $\overline{t_2}$ to $\overline{t_4}$ provide information relating to the water, lipid, deoxyhemoglobin, and oxyhemoglobin content of the tissue (20-23). Although $\overline{t_3}$ was inversely associated with both percent density and dense area among all women, its contribution to both quantitative measures was larger in premenopausal compared with postmenopausal women (Tables 2-4). TiBS score $\overline{t_2}$ was also inversely associated with the dense area of the breast among premenopausal women only. Decreasing values of $\overline{t_3}$ suggest a higher water content, which correlates with higher-density tissue (32, 33), as well as greater contributions from deoxyhemoglobin (that is, lower oxygen saturation) due to increased cellular proliferation and metabolism in denser tissue (28, 31). Decreasing values of $\overline{t_2}$ indicate greater deoxyhemoglobin and oxyhemoglobin absorption (that is, total hemoglobin content) as a result of increased tissue vascularization in higher-density tissue (31).

Although water associated absorption correlates with fibroglandular tissue (32, 33), contributions from oxyhemoglobin are expected in less proliferating dense tissue (31). Increasing values of $\overline{t_4}$ suggest some contributions from water as well as oxyhemoglobin (23). Given the reduction in tissue proliferation and metabolism due to changes in hormonal exposure consequent with menopause (37, 38), the positive association of $\overline{t_4}$ with percent density and dense area in postmenopausal women is physiologically plausible.

The significant correlations between TiBS score $\overline{t_2}$ and $\overline{t_4}$ and age among postmenopausal women are also consistent with the observed radiologic changes associated with menopause and ageing (37, 38). Increasing values of $\overline{t_2}$ and decreasing values of $\overline{t_4}$ suggest greater lipid-associated absorption characteristic of lower-density postmenopausal breast tissue (37).

There are two potential limitations to the present study. The first is the timing of TiBS measurements relative to mammography. If timing of measurements had any effect on our results, we would expect to see stronger relationships between TiBS scores and mammographic features among women who had both measurements done closer together and weaker associations as the timing between measurements increased. However, when stratified according to timing between measurements, the observed associations between the TiBS scores and quantitative mammographic features were comparable.

A second potential issue is variation in the timing of measurements during the menstrual cycle among premenopausal women. All TiBS measurements were carried out during the first week of the luteal phase; however, menstrual cycle phase at mammography was not recorded. There is some suggestion of a small absolute increase in percent density and dense area in the luteal compared with the follicular phase, but the evidence is inconsistent (42-44). Although it is current procedure to recommend that mammograms be taken during the follicular phase of the cycle, we do not know whether this was a standard practice at the breast imaging center where our study was conducted. Although we do acknowledge this as a potential limitation, we do not feel that it is a major one, as we observed no significant variation in the TiBS scores as a function of menstrual cycle phase. Moreover, if both measurements were done during the same cycle phase, we would anticipate that the associations between TiBS scores and quantitative mammographic features would have been slightly stronger.

Conclusions

TiBS scores are associated with quantitative mammographic features in both premenopausal and postmenopausal women. After adjusting for BMI, TiBS scores explained an additional 30% to 40% of the variation in percent density and dense area, beyond the contribution of BMI, which also explained ~30% of the variation in percent density. Given this relationship, TiBS scores may prove useful as intermediate markers in studies of breast cancer etiology and prevention. Although TiBS reflects light scattering by cellular and structural components, similar to mammographic X-ray attenuation, it also captures additional information relating to the water, lipid, and hemoglobin content of the tissue, all of which are related to the amount of proliferating tissue in the breast. Hence, TiBS may be useful for assessing physiologic tissue differences related to breast cancer risk and/or measuring differences from cumulative exposure to risk factors known to modulate breast cancer risk, particularly in younger women where mammography is not an option. Work is currently under way to study directly the relationship between TiBS scores and breast cancer incidence by comparing the contralateral breast in women with positive mammograms and biopsy-confirmed breast cancer versus negative mammograms, similar to the method used by Boyd et al. (2) in showing the relationship between percent density and cancer risk. Other studies include examining differences in TiBS scores as a function of age and parity in younger women (18-40 years old) and determining if TiBS can monitor changes in breast tissue over time in the same woman using a first full-term pregnancy and breast-feeding as modifying factors.

TiBS has several advantages, including no exposure to ionizing radiation, no compression of the breast, and assessment based on numerical variables only; hence, no special medical training is required for measurement execution and interpretation. TiBS can be made widely available due to its reduced cost, which is an order of magnitude lower than mammography, and because of its smaller size and portability.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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We thank all participants in this study and Dr. Norman Boyd for help with the Cumulus reads.

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