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Appendix E1

Methods

US and Near-infrared Data Acquisition

Four optical wavelengths (740 nm, 780 nm, 808 nm, and 830 nm) were used to sequentially deliver the light to the breast tissue, and 10 parallel photomultiplier detectors were used to acquire data from each wavelength. The entire near-infrared data acquisition interval was less than 5 seconds. For each patient, US images and optical measurements were acquired simultaneously before biopsy procedures at multiple locations, including the lesion region and a normal region of the contralateral breast in the same quadrant as the lesion. The optical data acquired from the healthy contralateral breast were used as reference to calculate the background optical absorption and to reduce scattering coefficients that were used in image reconstruction of lesions. The chest-wall depths at both lesion and reference sites were matched. Mammograms and US images before the near-infrared scan were reviewed by attending radiologists and all contralateral measurements were performed at healthy areas based on the available information.

US Image Grading

By using US BI-RADS lexicon, target lesions were categorized as the following: 4A, low suspicion for malignancy (2%–10% likelihood of malignancy); 4B, moderate suspicion for malignancy (10%–50% likelihood of malignancy); 4C, high suspicion for malignancy (50%–95% likelihood of malignancy); and 5, highly suggestive of malignancy (≥95% likelihood of malignancy).

All lesions were presented to each radiologist separately in three different reading sessions over a 1-month period. Twelve percent of the lesions were randomly selected beforehand to be presented twice to each reader in different reading sessions. For lesions with different readings, the higher score was used as the final score. A binary decision of low and moderate suspicion (4A and 4B) and high suspicion (4C and 5) was used to compute sensitivity, specificity, positive predictive value, and negative predictive value for each reader. The binary decision provided a means to evaluate radiologists' diagnostic confidence of identifying malignant versus intermediate lesions with low and moderate suspicion.

Near-infrared Imaging

The near-infrared reconstruction used US localization of lesions and segmented the imaging volume into a region of interest and background nonlesion regions. Because the spatial resolution of diffused light is poorer than that of US, the region of interest is chosen to be at least two to three times larger than that seen by using US in x and y dimensions. In addition, because the depth localization of diffused light is poor, a tighter region of interest in the depth dimension is set by using coregistered US. For patient with more than one lesion, if adjacent lesions were located close to each other within the same field of view of an US image, the image reconstruction used a larger region of interest to reconstruct these lesions. If different lesions

were located further apart than the US and diffuse optical tomography probe size (9 cm) at a different quadrant or different breast, the image reconstruction reconstructed these lesions separately, with is the same procedure used for a single lesion.

Pathologic Assessment of Benign Lesions

The two pathologists classified benign lesions as proliferative and nonproliferative on the basis of biopsy reports. The proliferative lesions included lobular carcinoma in situ, atypia, adenosis variants, moderate-to-florid degrees of hyperplasia, sclerosing papilloma, complex or radial sclerosing lesions, and fibroadenoma with complex proliferative features (1). The nonproliferative lesions were fibrocystic changes and included mild hyperplasia, fibroadenoma without complex features, fat necrosis and/or other inflammatory and reactive changes, complex cysts (2), intramammary lymph nodes, lipoma, and healthy or physiologic breast tissue. Therefore, seven benign groups were classified as proliferative lesions, fibrocystic changes, fibroadenoma, fat necrosis and/or other inflammatory and reactive changes, complex cyst, lymph node, and breast tissue including one lipoma.

Results

US Imaging Evaluation

For reader 1, tHb levels of 10 out of 40 Tis–T1 carcinomas and one out of 19 T2–T4 carcinomas scored as BI-RADs 4B were above the 80 μmol/L threshold. While for reader 2, 11 out of 40 Tis–T1 carcinomas (10 scored as 4B, one scored as 4A) and one out of 19 T2–T4 carcinomas scored as 4B were above the threshold. Thus, the tHb improves the radiologists' diagnosis mainly on indeterminate BI-RADS 4B breast lesions. For the 12% lesions that were randomly selected to be shown twice to each reader in different reading sessions, the repeatability was 89% for reader 1 and 81% for reader 2. Small changes of 8%–9% were between 4B and 4C, and these could cause at most 2% changes on sensitivity, specificity, positive predictive value, and negative predictive value; 3%–9% were between 4A and 4B, which did not change these diagnostic values.

T2–T4 Malignant Lesion Features

These tumors either manifested high tHb levels ($n = 8$) by showing posterior shadowing or heterogeneous high peripheral hemoglobin contrast enhancement or lobulated distributions ($n = 9$). Eight of 10 T2–T4 tumors with tHb levels less than the threshold had either heterogeneous peripheral enhancement ($n = 5$) or lobulated distributions ($n = 3$) that were not seen in the benign large lesions (Table E4 [online]). Two had uniform tHb distributions indistinguishable from large benign lesions of a similar size. Reviews of hematoxylin-and-eosin slides obtained at excision showed that one was high-grade invasive ductal carcinoma with a well-circumscribed border, which may explain the lack of the peripheral enhancement. The second case consisted of three adjacent hypovascular and circumscribed T1 tumors, which appeared as a single large tumor mass shown by using US. These may account for the observed low vascularity because a large region of interest is used for imaging reconstruction.

Benign Lesions with tHb above Threshold

The fibroadenoma group had the highest number of lesions that exceeded the tHb threshold. The average age of this subgroup of patients was 39 years (age range, 18 to 55 years) and the average size of the fibroadenomas measured by using US was 1.7 cm. Hematoxylin-and-eosin reviews of the core biopsy samples showed that six were myxoid fibroadenomas and four were fibrotic or sclerotic fibroadenomas. Six lesions in the proliferative group exceed the threshold because of increased angiogenesis as lesions moved along the spectrum from nonproliferative to proliferative to malignant cancers. Four cases of fat necrosis and inflammatory changes showed a spectrum of acute and chronic inflammation, including hypervascular granulation tissue that could account for the increased tHb level. Two benign intramammary lymph nodes found close to the axilla were also above the threshold. One lymph node showed follicular hyperplasia with substantial blood vessel content and another lymph node had abundant amorphous debris and calcifications. One lesion in the group with fibrocystic change also showed a higher tHb level, and on histologic review this case was reassigned as mammary hamartoma. One complex cyst with blood-tinged fluid also showed high tHb content.

Discussion

Literature Data on Breast Lesion Deoxygenation

In a recent study that used diffuse optical spectroscopy, a significantly higher deoxygenation in invasive and ductal carcinoma in situ was found in larger breast tumors than in that of benign tumors, but no significant difference was seen in smaller tumors that were 1 cm or less (3). Diffuse optical spectroscopy is known to have much lower spatial resolution than US-guided diffuse optical tomography, which could account for the difference observed in smaller tumors.

References

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2. American College of Radiology. Breast Imaging Reporting and Data System (BI-RADS). 4th ed. Reston, Va: American College of Radiology, 2003.

3. Nioka S, Shnall M, Conant E, et al. Breast cancer detection of large size to DCIS by hypoxia and angiogenesis using NIRS. Adv Exp Med Biol 2013;789:211–219.

Table E1. Tumor Histopathological Characteristics of Two Groups of Malignant Breast Lesions

Tumor size	Tumor type	HER2	ER
Tis			
Tis (*n* = 3) 0.6 cm (*n* = 1) ∼1–2 cm (*n* = 1) Multifocal DCIS of three adjacent tumors: 2 cm, 1.5 cm, 0.5 cm (*n* = 1)	DCIS nuclear grade II-III with comedo (*n* = 1) DCIS nuclear grade eII without comedo (*n* = 2)		ER NA (*n* = 2)
T1			

T1b (*n* = 9) Mean size = 0.9 cm (0.8–1.0) T1c (*n* = 24) Mean size = 1.5 cm (1.1–2.0) T1 based on US size (*n* = 4) (0.6–1.3 cm in larger dimension)	IDC (*n* = 31) ILC (*n* = 2) Mixed IDC and ILC (*n* = 3) Medullary carcinoma (*n* = 1)	HER2 (*n* = 4) HER2– (*n* = 29) NAC (*n* = 4)	ER (*n* = 31) ER- (*n* = 2) NAC (*n* = 4)
T2–T4			
Gross measurements T2-T4 (*n* = 10) Mean: 3.5 cm (2.3–6.2cm) Three adjacent primary tumors Two T1b (0.8cm 1.0cm)and one T1c (2.0 cm) (*n* = 1) NAC: cT2-T4 (*n* = 8)	IDC (*n* = 11) ILC (*n* = 2) Mixed IDC and ILC (*n* = 6)	HER2 (*n* = 5) HER2- (*n* = 13) NA (*n* = 1)	ER (*n* = 14) ER- (*n* = 4) NA (*n* = 1)

Note.—DCIS = ductal carcinoma in situ, ER = estrogen receptor, HER2 = human epidermal growth factor receptor 2, IDC = invasive ductal carcinoma, ILC = invasive lobular carcinoma, NAC = neoadjuvant chemotherapy, NA = not available.

Table E2. Mean Maximum tHb, oxyHb, deoxyHb Values with Respect to Lesion Type

Group	Average Age (y)*	No. of Lesions	US Size (x, z*), cm Tumor Center Depth z, cm	tHb (unit: μmol/L)	oxyHb (unit: μmol/L)	deoxyHb (unit: μmol/L)
Tis-T1	60 (36–77)	40	0.8 ± 0.3 · 0.7 ± 0.3 (x: 0.2–1.8; z: 0.2–1.5) depth 1.6 (0.6–2.6)	89.3 ± 20.2	65.0 ± 20.8	33.5 ± 11.3
T2-T4	59 (34–94)	19	2.3 ± 1.0 · 1.9 ± 0.8 (x: 1.0–5.0; z: 0.7–4.1) depth 1.9 (1.2–2.7)	84.7 ± 32.8	57.1 ± 19.8	34.7 ± 18.9
Proliferative lesions	55 (27–82)	34	0.8 ± 0.5 · 0.6 ± 0.3 (x: 0.2–2.7; z: 0.2–1.5) Depth 1.5 (0.5–2.4)	61.9 ± 25.6	43.9 ± 18.3	27.5 ± 14.2
Fibroadenoma	40 (17–66)	75	1.4 ± 0.9 · 0.8 ± 0.4 (x: 0.2–4.7; z: 0.2–2.5) depth 1.5 (0.5–3.2)	58.6 ± 22.0	42.1 ± 17.0	23.9 ± 10.8
Fat necrosis and/or inflammatory and reactive changes	54 (26–84)	29	1.5 ± 1.2 · 1.1 ± 1.0 (x: 0.2–5.0; z: 0.2–5.5) depth 1.4 (0.5–2.8)	61.7 ± 27.2	41.3 ± 15.6	28.9 ± 16.7
Fibrocystic Changes	49 (19–77)	44	1.0 ± 0.6 · 0.6 ± 0.4 (x: 0.2–2.6; z: 0.2–2.3) depth1.5 (0.4–3.3)	46.5 ± 18.6	30.7 ± 14.9	26.2 ± 14.6
Complex Cysts	50 (29–81)	38	1.1 ± 0.9 · 0.6 ± 0.5 (x: 0.3–3.6; z: 0.2–1.8) depth = 1.6 (0.7–3.3)	47.8 ± 20.3	33.4 ± 17.1	24.4 ± 15.0
Breast tissue and other benign	52 (39–79)	7	1.3 ± 0.9 · 0.6 ± 0.3 (x: 0.6–3.0; z: 0.4–1.0) depth = 1.7 (1.0–2.4)	36.2 ± 17.4	31.9 ± 16.8	17.7 ± 13.4
Lymph nodes	45 (27–61)	6	1.2 ± 0.6 · 0.7 ± 0.3 (x: 0.4–2.1; z: 0.2–1.0) depth 1.9 (0.8–3.3)	43.9 ± 39.0	34.0 ± 28.3	14.8 ± 13.1
All benign lesions		233		54.1 ± 23.5	38.0 ± 17.4	25.2 ± 13.8
Control	49 (39–75)	12		40.2 ± 12.6	31.3 ± 11.1	20.0 ± 12.6

* Numbers in parentheses are ranges.

† z is the US depth direction.

Table E3. Two-Sample *t* Test Results of tHb, oxyHb, and deoxyHb for T2–T4 and Tis–T1 Carcinomas, and Seven Categories of Benign Lesions

Parameter	Proliferative Lesions	Fibroadenoma	Fat Necrosis/ Inflammatory Changes	Fibrocystic changes	Cyst	Breast Tissue and Other Benign	Lymph Nodes
T2–T4 carcinoma							
tHb	22.9 (5.1, 40.7)	26.1 (9.7, 42.6)	23.1 (4.7, 41.5)	38.2 (22.6, 54.2)	37.0 (20.1, 53.9)	48.6 (27.8,69.4)	40.8 (–0.9, 82.5)
P value	.013	.003	.016	<.001	<.001	< .001	.054
oxyHb	13.1 (1.9, 24.3)	15.0 (4.8, 25.2)	15.8 (4.8, 26.8)	26.4 (16.0, 36.8)	23.7 (12.8, 34.3)	25.2 (8.1, 42.2)	23.0 (–7.3,53.3)
P value	.023	.006	.006	<.001	<.001	.007	.113
deoxyHb	7.2(–3.0,17.3)	10.7(1.3,20.1)	5.7 (–5.1, 16.5)	8.4 (–1.5, 18.4)	10.2 (0.1, 20.4)	16.9 (2.7,31.1)	19.8 (4.8, 34.8)
P value	.161	.027	.289	.094	.049	.023	.014
Tis–T1							
tHb	27.4 (16.5, 38.2)	31.0 (22.9, 39.1)	28.2 (16.7, 39.7)	42.7 (34.3, 51.2)	42.1 (33.0, 51.2)	53.1 (36.6, 69.7)	45.3 (3.5, 87.1)
P value	<.001	<.001	<.001	<.001	<.001	<.001	.039
oxyHb	21.1 (12.0, 30.1)	23.2 (15.6, 30.9)	24.5 (15.9, 33.1)	32.7 (23.6, 40.8)	31.9 (23.4, 40.4)	33.1 (16.9, 49.4)	31.0 (0.1, 61.8)
P value	<.001	<.001	<.001	<.001	<.001	.001	.049
deoxyHb	6.0 (–0.1,12.0)	9.7 (5.4,14.0)	4.7 (–2.1, 11.5)	7.3 (1.6, 12.9)	9.0 (3.0,14.9)	15.7 (3.0, 28.4)	18.6 (4.9, 32.4)
P value	.053	<.001	.170	.012	.004	.022	.016

Note.—Units are μmol/L and data in parentheses are 95% confidence intervals.

Table E4. Carcinomas with tHb below Threshold

Category	Age of Patient (y)	Tumor size (cm)	Nottingham Score	Tumor Type	Maximum Value (μmol/L)			US Measurements (cm)	Depth (cm)	US Reviewed Score	
					tHb	oxyHb	deoxyHB			Reader No. 1	Reader No. 2
T1c	51	1.2	NS 2	IDC	55.3	26.2	51.9	0.9 × 0.6	1.3	5	4c
T1c	64	1.7	NS 2	IDC	60.9	42.5	25.2	0.9 × 0.5	1.3	5	4c
T1c	62	1.3	NS 1	IDC	36.4	35.2	13.4	0.9 × 0.6	1.8	5	4c
T1c	62	1.5	NS 1	IDC	77.2	52.3	27.1	1.0 × 0.8	1.5	5	4c
T1c	50	1.1	NS 1	mixed IDC & ILC	74.6	49.6	25.5	0.5 × 0.2	1.4	5	5
T1b	65	1.0	NS 1	mixed IDC &ILC	20.0	7.9	20.9	0.5 × 0.4	1.6	4b	4c
T2	76	3.5	NS 1	IDC	68.6 periphery	58.3	20.0	2.1 × 1.4	1.2	5	4c
3 adjacent primary tumors	47	0.8, 1.0, 2.0	NS 2	2T1b, 1T1c, mixed IDC&ILC	49.9 uniform	53.8	10.1	3.2 × 2.5*	1.5	5	4c
T2	49	3.6	NS 1	IDC	65.7 periphery	44.4	23.5	2.0 × 2.3	1.4	4b	4c
NAC	60	NAC	NS 2	IDC	59.5 lobulated	50.9	28.5	2.2 × 2.1	2.0	5	5
T2	38	2.3	NS 3	mixed IDC&ILC	66.1 periphery and heterogeneous	54.3	20.9	1.0 × 1.4	1.8	5	4c
T2	49	T2	NS 1	mixed IDC&LC	46.8 lobulated	34.6	18.2	1.9 × 1.3	2.7	5	4c
NAC	73	NAC	NS 3	IDC	64.8 periphery	42.6	27.1	3.1 × 1.9	2.2	4b	4c
NAC	69	NAC	NS 2	IDC	66.6 periphery	38.3	33.9	2.8 × 1.7*	2.2	4c	4c
NAC	38	NAC	NS 2	IDC	52.8 lobulated and heterogeneous	33.0	41.3	1.7 × 1.0*	1.3	5	4c
NAC	35	NAC	NS 3	IDC	60.4 uniform	35.7	25.1	2.5 × 1.7	1.5	4c	4c

Note.—For T2–T4 tumors, features of tHb distribution included periphery enhancement, periphery and heterogeneous, lobulated and/or heterogeneous, and uniform. IDC = invasive ductal carcinoma, LC = lobular carcinoma, max = maximum, NAC = neoadjuvant chemotherapy NS = Nottingham Score.

* Patients had palpable tumors with ill-defined and heterogeneous US images. Tumor size and depth were estimates.

Table E5. Benign Lesions with tHb above the Threshold

a.

Category	Mean Age	Core Biopsy Results	Max tHb (μmol/L)	Max OxyHb (μmol/L)	Max DeoxyHb (μmol/L)	US Measurements (size, x, z) in cm
Proliferative Lesions (*n* = 6)	49 (27–65)	proliferative changes with and without atypia, intraductal papillomas, sclerosing papillomas, sclerosing adenosis and epithelial complex fibroadenomas	104.6 ± 20.7	69.6 ± 5.8	41.2 ± 16.3	1.4 ± 0.8 × 0.8 ± 0.3 (x: 0.7–2.7; z: 0.5–1.4)
fibroadenoma (*n* = 10)	39 (18–55)	fibroadenoma	96.9 ± 12.5	67.5 ± 18.6	36.6 ± 7.7	1.7 ± 0.7 × 1.1 ± 0.4 (x: 0.7–3.3; z: 0.4–1.7)
Fat necrosis and inflammatory changes (*n* = 4)	62 (49–84)	fat necrosis, inflammatory changes, hemosiderin histiocytes	110.0 ± 20.5	66.9 ± 9.1	56.4 ± 14.7	2.4 ±1.9 × 2.0 ± 2.4 (x: 0.9, >5.0; z: 0.4–5.5)
Fibrocystic Changes (*n* = 1)	41	benign nonproliferative breast parenchyma with pseudoangiomatous hyperplasia of mammary stroma and columnar cell changes. (mammary hamartoma*)	81.8	62.9	30.2	1.4 × 1.0
Complex Cyst (*n* = 1)	45	Complex cyst (blood-tinged fluid)	80.6	57.7	28.6	1.7 × 0.5

b.

Category	Age (y)	Core Biopsy Result	Max tHb (μmol/L)	Max OxyHb (μmol/L)	Max DeoxyHb (μmol/L)	US Measurements (cm)
PBL	65	intraductal papilloma	97.4	66.1	40.8	2.7 × 1.4
PBL	47	sclerosing papilloma, and sclerosing adenosis,	90.6	63.2	34.5	0.7 × 0.7
PBL	54	sclerosing papilloma	113.4	71.6	45.8	0.7 × 0.5
PBL	53	stroma fibrosis and focal sclerosing adenosis	142.1	79.7	70.8	1.0 × 0.6
PBL	45	adenosis and miminal epithelial hyperplasia	99.9	70.5	30.3	1.0 × 1.0
PBL	27	epithelial complex fibroadenoma	84.5	66.9	24.9	1.8 × 0.8
FA	18	myxoid fibroadenoma	91.1	64.5	29.9	1.1 × 1.3
FA	55	fibrotic/sclerotic fibroadenoma	84.2	44.9	39.4	3.3 × 1.7
FA	40	myxoid fibroadenoma	103.7	85.7	28.8	1.6 × 1.0
FA	23	myxoid fibroadenoma	86.4	62.1	37.7	2.2 × 1.3
FA	50	myxoid fibroadenoma	88.3	58.5	33.6	0.7 × 0.4
FA	44	fibrotic fibroadenoma	119.9	94.6	29.0	1.7 × 0.8
FA	46	fibrotic fibroadenoma	109.6	60.1	51.3	1.7 × 1.7
FA	35	fibrotic fibroadenoma	103.6	61.6	47.7	1.5 × 1.2
FA	40	myxoid fibroadenoma	81.6	53.5	32.6	1.5 × 0.8
FA	40	myxoid fibroadenoma	101.1	69.0	36.4	1.2 × 1.0
FN/Inf/ Reactive changes	64	Fat necrosis, focal giant cell tissue reaction	80.6	56.3	36.2	2.6 × 1.4

FN/Inf/ Reactive changes	52	fat necrosis, stromal fibrosis	113.1	65.4	55.1	1.1 × 0.8
FN/Inf/ Reactive changes	49	fat necrosis with chronic inflammation	127.8	78.4	66.7	0.9 × 0.4
FN/Inf /Reactive changes	84	Adnexal structures with chronic inflammation and hemosiderin laden histiocytes	118.6	67.4	67.8	>5 × 5.5
FC*	41	benign nonproliferative breast parenchyma with pseudoangiomatous hyperplasia of mammary stroma and columnar cell changes. (mammary hamartomas*)	81.8	62.9	30.2	1.4 × 1.0
Complex cyst	45	Complex cyst (blood-tinged fluid)	80.6	57.7	40.2	1.7 × 0.5

Note.—FA = fibroadenoma, FC = fibrocystic changes, FN = fat necrosis, Inf = inflammatory, max = maximum, PBL = proliferative benign lesion.

* On histologic review, this case was diagnosed as pseudoangiomatous stromal hyperplasia. CD31 immunostain was performed on this case, which showed no concentration of true vascular profiles. However, pseudoangiomatous stromal hyperplasia can occur as a so-called pure lesion or in association with mammary hamartoma (a circumscribed benign breast lesion that is known to be associated in MR imaging contrast enhancement). When the US images were retrospectively reviewed, this particular case was shown to be a discrete lesion, so a revised histologic diagnosis of mammary hamartoma was assigned.