

Contrast Enhancement on Cone-Beam Breast-CT for Discrimination of Breast Cancer Immunohistochemical Subtypes^{1,2,3}



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Abstract

PURPOSE: To evaluate whether contrast enhancement on cone-beam breast-CT (CBBCT) could aid in discrimination of breast cancer subtypes and receptor status. **METHODS:** This study included female patients age >40 years with malignant breast lesions identified on contrast-enhanced CBBCT. Contrast enhancement of malignant breast lesions was standardized to breast fat tissue contrast enhancement. All breast lesions were approved via image-guided biopsy or surgery. Immunohistochemical staining was conducted for expression of estrogen (ER), progesterone (PR), human epidermal growth factor receptor-2 (HER2) and Ki-67 index. Contrast enhancement of breast lesions was correlated with immunohistochemical breast cancer subtypes (Luminal A, Luminal B, HER2 positive, triple negative), receptor status and Ki-67 expression. **RESULTS:** Highest contrast enhancement was seen for Luminal A lesions (93.6 HU) compared to Luminal B lesions (47.6 HU, $P = .002$), HER2 positive lesions (83.5 HU, $P = .359$) and triple negative lesions (45.3 HU, $P = .005$). Contrast enhancement of HER2 positive lesions was higher than Luminal B lesions ($P = .044$) and triple negative lesions ($P = .039$). No significant difference was evident between Luminal B and triple negative lesions ($P = .439$). Lesions with high Ki-67 index showed lower contrast enhancement than those with low Ki-67 index ($P = .0043$). ER, PR and HER2 positive lesions demonstrated higher contrast enhancement than their receptor negative counterparts, although differences did not reach statistical significance ($P = .1714$; $P = .3603$; $P = .2166$). **CONCLUSIONS:** Contrast enhancement of malignant breast lesions on CBBCT correlates with immunohistochemical subtype and proliferative potential. Thereby, CBBCT might aid in selecting individualized treatment strategies for breast cancer patients based on pre-operative imaging.

Translational Oncology (2017) 10, 904–910

Introduction

Breast cancer is the most common malignant disease in women, comprising approximately 16% of incident malignomas [1]. With recent diagnostic advances, breast cancer is no longer considered a single entity. Rather, breast cancer subtypes can be defined according to genetic array testing [2].

Immunohistochemistry provides a convenient clinical approximation to intrinsic breast cancer subtypes [3,4]. This approach defines breast cancer subtypes according to expression of estrogen and progesterone receptor (ER, PR), amplification of the human epidermal growth factor receptor-2 (HER2) and Ki-67 index as a marker for cell proliferation [4,5]. Breast cancer subtypes include Luminal A, Luminal B, HER2 positive and triple negative cancers [5]. These subtypes directly influence breast cancer prognosis and clinical behavior [6]. For example, Onitilo et al. have demonstrated that triple

negative breast cancer had worse overall survival and disease free survival compared to Luminal A type breast cancer [6]. On the level of breast cancer receptors and proliferation markers, HER2 receptor

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¹ Conflict of Interest: There is no conflict of interest.

² Funding statement: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

³ Acknowledgement: none.

Received 10 August 2017; Accepted 29 August 2017

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<http://dx.doi.org/10.1016/j.tranon.2017.08.010>

status and the Ki-67 index have been shown to correlate with cancer recurrence, overall survival and clinical stage [7–9].

Due to the impact on breast cancer prognosis and treatment, prior knowledge of immunohistochemical breast cancer subtypes and receptor status is crucial for an optimal, individualized treatment strategy of breast cancer patients. Several authors have evaluated imaging features to predict breast cancer molecular properties. On magnetic resonance imaging (MRI), triple negative breast cancer subtypes showed excess necrotic tissue and regularly presented as non-mass lesions [10,11].

Recent advances in breast imaging could further promote the discrimination of breast cancer subtypes. Cone-beam breast-CT (CBBCT) is a novel breast imaging technique, utilizing a dedicated flat-panel CT for acquisition of true breast 3D images [12,13]. Although it has been shown that lesion intensity on non-contrast (NC) CBBCT did not correlate with breast cancer receptor status or histopathological diagnosis, there is no literature on the added benefit of iodinated contrast media in this emerging imaging modality [14].

Therefore, the purpose of the present study was to evaluate whether contrast enhancement in dedicated CBBCT could aid in discrimination of immunohistochemical breast cancer subtypes and receptor status.

Material and Methods

The study was approved by the institutional review board and conducted in accordance to the Declaration of Helsinki. Written informed consent was obtained from all patients prior to inclusion. The study protocol is available from the corresponding author upon request.

This prospective study was conducted at a University affiliated breast imaging center in central Germany from December 2015 to March 2017. Inclusion criteria were malignant breast lesions (invasive breast cancer and ductal carcinoma in situ (DCIS)) identified by CBBCT and proven by image-guided breast biopsy, female gender and age over 40 years. Patients were excluded if enrolled in the German breast cancer screening-program, pregnant, presenting with renal insufficiency, or having a history of allergic reaction to iodinated contrast media.

CBBCT

CBBCT examinations were performed using a dedicated flat-panel breast-CT (Koning Breast CT, CBCT 1000; Koning Corporation, West Henrietta, NY, USA). CBBCT imaging was done in a standard manner [13,15]. After initial NC-CBBCT, 90 mL non-ionic contrast media (Iopromide, Ultravist® 300, Bayer-Schering, Berlin, Germany) were intravenously injected, followed by a 30 mL saline solution chaser. Contrast-enhanced (CE) CBBCT scans were obtained 2–3 minutes after contrast media (CM) administration.

Table 1. Definition of Immunohistochemical Breast Cancer Subtype According to Receptor Status and Ki-67 Index

Immunohistochemical Breast Cancer Subtypes	Receptor Status	Ki-67 Index	Reference
Luminal A	ER and/or PR positive; HER2 negative	< 0.14	[31,32]
Luminal B	ER and/or PR positive; HER2 negative	> 0.14	[4,5]
HER2-Subtyp	HER2 positive	any	[32,33]
triple negative	ER and PR negative and HER2 negative	any	[5,34]

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor-2.

Post-acquisition image processing and reconstructions were performed to achieve isotropic reconstructed volumes using a soft tissue filter and a voxel size of 0.273 mm³ (standard mode).

Image Analysis

CBBCT intensity was measured in Hounsfield Units (HU). Three representative rectangular regions of interest (ROIs) were positioned in the peripheral region of the suspicious breast lesions and fat tissue on CBBCT images in coronal view with a slice thickness of 2 mm. Corresponding image slices were used to measure lesion intensity on NC-CBBCT and CE-CBBCT as well as contrast enhancement on CE-CBBCT. To ensure stable estimates of lesion intensity, HU values were averaged over the three ROIs measured.

As proposed by Prionas et al., contrast enhancement of breast lesions was standardized to enhancement of fat tissue and defined as [16]:

$$enhancement \Delta HU = (HU_{lesion}^{post\ CM} - HU_{fat}^{post\ CM}) - (HU_{lesion}^{pre\ CM} - HU_{fat}^{pre\ CM})$$

Histopathological Analysis

All breast lesions were fixed in 5% formalin and processed into paraffin blocks for further histopathological examination. Immunohistochemical staining for expression of ER, PR, HER2 and Ki-67 was performed using a fully automated system (Dako Omnis; Dako, Glostrup, Denmark). Tissue sections were cut at 4 µm slice thickness, including the largest cut surface of the breast lesion. Staining was performed with primary antibodies against ER (EP1, Ready-to-use; Dako, Glostrup, Denmark), PR (PgR 1294; Ready-to-use, Dako, Glostrup, Denmark), HER2 (A0485, 1:400, Polyclonal Rabbit-Anti-Human c-erbB-2; Dako, Glostrup, Denmark), and Ki-67 (MIB-1, Ready-to-use; Dako, Glostrup, Denmark). Positivity-cutoff for ER and PR was 1% positive cells with nuclear staining [17].

HER2 staining was scored as: 0 (no staining/faint membrane staining); 1+ (faint membrane staining in >10% of tumor cells, incomplete membrane staining); 2+ (weak to moderate membrane staining in >10% of tumor cells), and 3+ (uniform, intense membrane staining in >30% of invasive tumor cells). Breast lesions with HER2 staining score of 3+ or 2+ and FISH-amplified were considered HER2-positive [18]. The Ki-67 index was evaluated as the percentage of positively staining cells among at least 1000 invasive cells in the scoring area without taking staining intensity into account [19].

All histopathological analyses were performed by a board certified pathologist with 15 years of experience in breasts pathology. Histological grades and biological features were evaluated based on invasive components.

Table 2. Histopathological Diagnosis of Included Breast Lesions

Histopathology	Number (Percent) n = 38 (100)	Grading	
		G2	G3
IDC	n = 27 (71.0)	n = 23	n = 4
NST + DCIS	n = 7 (18.4)	n = 6	n = 1
ILC	n = 1 (2.6)	n = 1	-
Intramammary metastasis	n = 1 (2.6)	-	n = 1
Mixed papillary/mucinous serous carcinoma	n = 1 (2.6)	n = 1	-
Metaplastic carcinoma	n = 1 (2.6)	-	n = 1

Abbreviations: IDC, invasive ductal carcinoma; NST, carcinoma of non-special type; DCIS, ductal carcinoma in situ; ILC, invasive lobular carcinoma.

Table 3. Immunohistochemical Breast Cancer Subtypes with Associated Enhancement

Immunohistochemical Breast Cancer Subtype	Number (Percent)	Mean Enhancement (\pm SD)
Luminal A	n = 9 (23.7)	93.6 (\pm 39.8) HU
Luminal B	n = 17 (44.7)	47.6 (\pm 18.1) HU
HER2 positive	n = 4 (10.5)	83.5 (\pm 49.8) HU
triple negative	n = 8 (21.0)	45.3 (\pm 23.0) HU

Abbreviations: SD, standard deviation; HER2, human epidermal growth factor receptor-2; HU, Hounsfield unit.

For this study, immunohistochemical breast cancer subtypes were defined as Luminal A, Luminal B, HER2 positive and triple negative as summarized in Table 1.

Statistical Analysis

Continuous variables are given as mean with standard deviation (\pm SD) as measure of dispersion, categorical variables as absolute number and percent. For analyses, the Ki-67 index was dichotomized according to its median value in “high” versus “low”.

The normality assumption of continuous variables was tested via the Shapiro-Wilk test. Non-parametric Wilcoxon signed-rank and Kruskal-Wallis tests were utilized for comparison of enhancement of non-normally distributed samples.

An alpha level of 0.05 was considered statistically significant. All provided p-values are two-sided. Due to the explorative design of this study, *P* values were unadjusted for multiple testing and should be interpreted accordingly. Statistical analyses were performed using R and RStudio (R Core Development Team, Vienna, Austria; RStudio Inc, Boston, MA, USA).

Results

Patients

A total of 23 patients (26 breasts) fulfilled the inclusion criteria. No patient withdrew study participation consent or was lost to follow-up. NC-CBBCT and CE-CBBCT were available for all patients. One mild contrast related adverse event with nausea was observed.

Mean patient age at inclusion was 59.6 years (\pm 10.6 years). Six patients (26%) were pre-menopausal, 17 patients post-menopausal (74%). Nine patients (39%) presented with clinically palpable breast lesions. Breast density was rated as ACR type c in 17 breasts (65.4%) and type d in 9 breasts (34.6%), according to the Breast imaging reporting and data system (BI-RADS) 5th edition [20].

Histopathology

A total of 38 malignant breast lesions were identified by CBBCT, proven by image-guided biopsy or surgery and histopathologically analyzed. Mean number of breast lesions per patients was 1.6 (range, 1–6 lesions). Histopathological diagnoses of all included breast lesions are summarized in Table 2.

Immunohistochemical analyses confirmed expression of ER on 30 breast lesions (78.9%) and PR on 29 lesions (76.3%). The HER2 receptor was expressed on 4 breast lesions (10.5%). The mean Ki-67 index among the breast lesions was 0.28 (SD \pm 0.22; range, 0.05–0.9).

Luminal subtype of breast lesions was grouped as: Luminal A in 9 lesions (23.7%), Luminal B in 17 lesions (44.7%), HER2 positive in 4 lesions (10.5%) and triple negative in 8 lesions (21%).

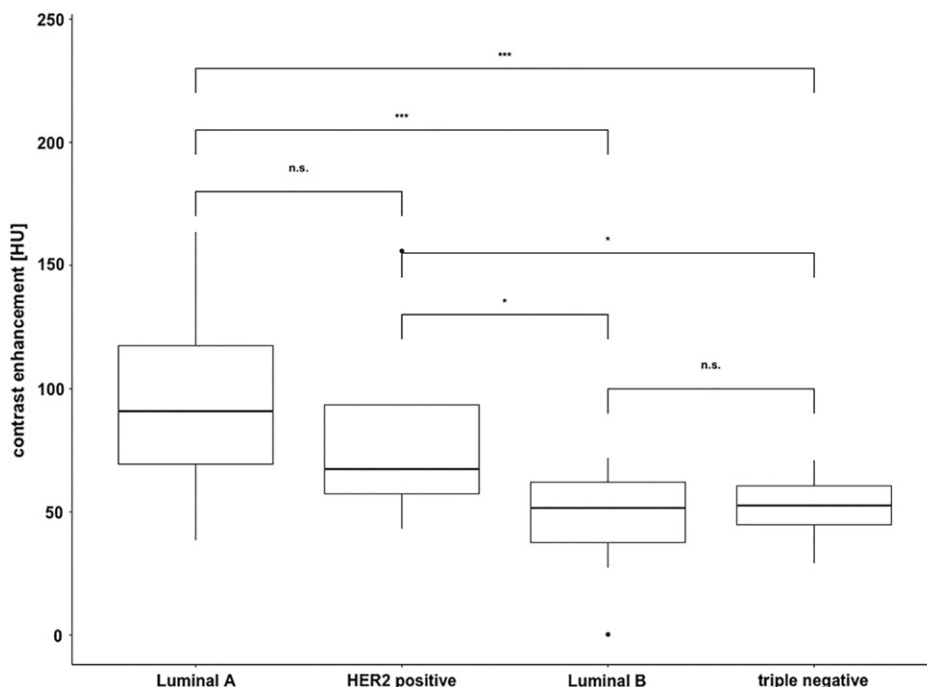


Figure 1. Boxplot chart of contrast enhancement among immunohistochemical breast cancer subtypes; boxes indicate interquartile range and horizontal bars median enhancement. * $P < .05$; *** $P < .01$; n.s. non-significant. Luminal A vs. triple negative $p = .005$; Luminal A vs. Luminal B $p = .002$; Luminal A vs. HER2 positive $p = .359$; HER2 positive vs. triple negative $p = .044$; HER2 positive vs. Luminal B $p = .039$; Luminal B vs. triple negative $P = .439$.

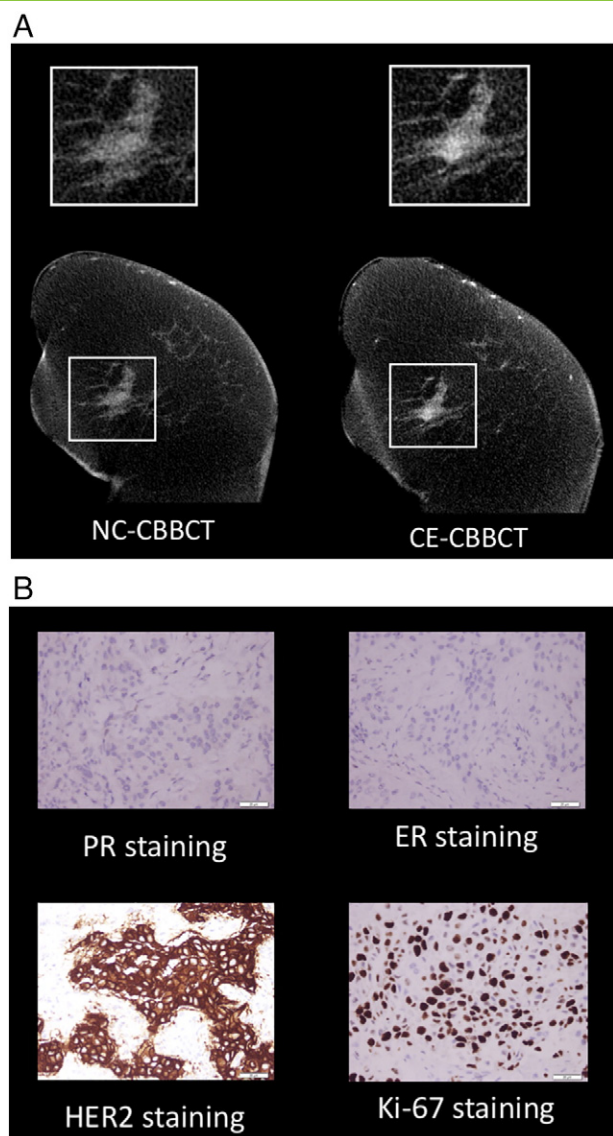


Figure 2. (A) Case of a postmenopausal 69-year-old woman presenting with right-sided breast mass adjacent to the thoracic wall. Contrast enhancement on CBBCT was 62 HU. (B) Immunohistochemical analyses revealed “HER2 positive” breast cancer subtype with a Ki-67 index of 0.8 and without expression of ER or PR. HER2 expression was positive (Dako-Score 3+).

Contrast Enhancement

Contrast enhancement was compared across immunohistochemical breast cancer subtypes and receptor status. Contrast enhancement differed significantly among immunohistochemical breast cancer subtypes (P value for overall difference: $P = .0093$). Luminal A lesions showed highest enhancement (93.6 HU) as compared to Luminal B lesions (47.6 HU, $P = .002$), HER2 positive lesions (83.5 HU, $P = .359$) and triple negative lesions (45.3 HU, $P = .005$). Enhancement of HER2 positive lesions was higher than that of Luminal B lesions ($P = .044$) and triple negative lesions ($P = .039$). No significant enhancement difference was evident for Luminal B and triple negative lesions ($P = .439$).

Contrast enhancement measures among luminal types are summarized in Table 3 and plotted in Figure 1. Representative cases of breast cancer subtypes with imaging features on NC-CBBCT

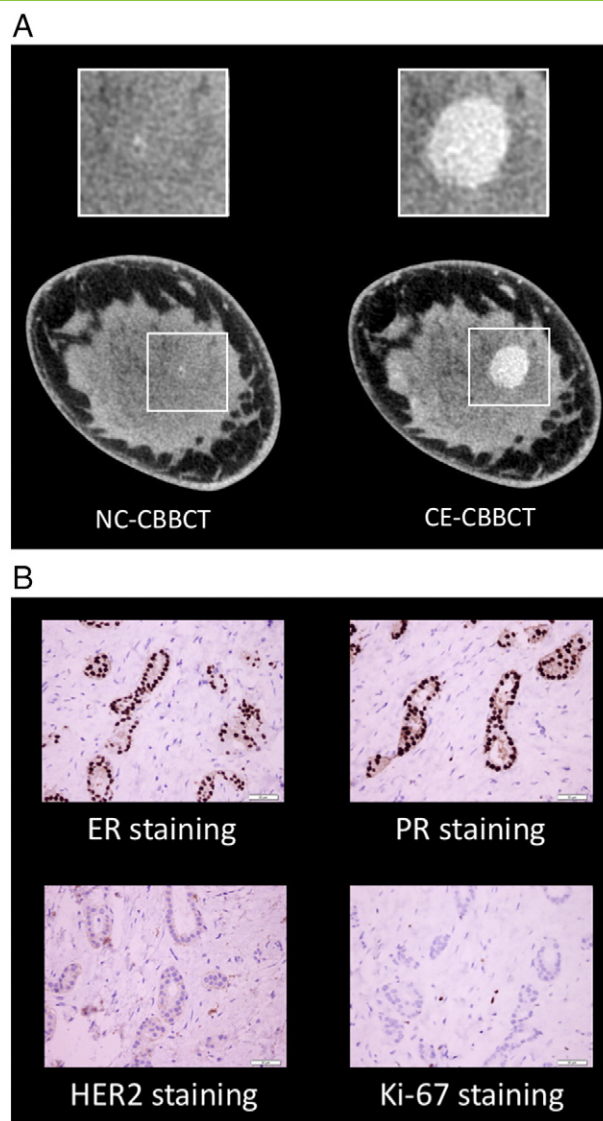


Figure 3. (A) Case of a premenopausal 43-year-old woman presenting with right-sided breast mass. Contrast enhancement on CBBCT was 113 HU. (B) Immunohistochemical analyses revealed “Luminal A” breast cancer subtype with a Ki-67 index of 0.1 and expression of ER and PR. HER2 expression was negative (Dako-Score 0).

and CE-CBBCT and associated immunohistochemical staining are provided in Figure 2 for HER2 positive subtype, Figure 3 for Luminal A, Figure 4 for Luminal B, and Figure 5 for triple negative subtype.

In separate analyses, ER-, PR-, and HER2-positive lesions demonstrated higher enhancement than their receptor negative counterparts as shown in Table 4. However, enhancement differences did not reach statistical significance ($P = .1714$; $P = .3603$; $P = .2166$).

After dichotomization at its median value (0.3), lesions with high Ki-67 index showed lower enhancement than those with low Ki-67 index ($P = .0043$).

Discussion

In our study on CBBCT, we showed that contrast enhancement correlated with breast cancer immunohistochemical breast cancer subtypes.

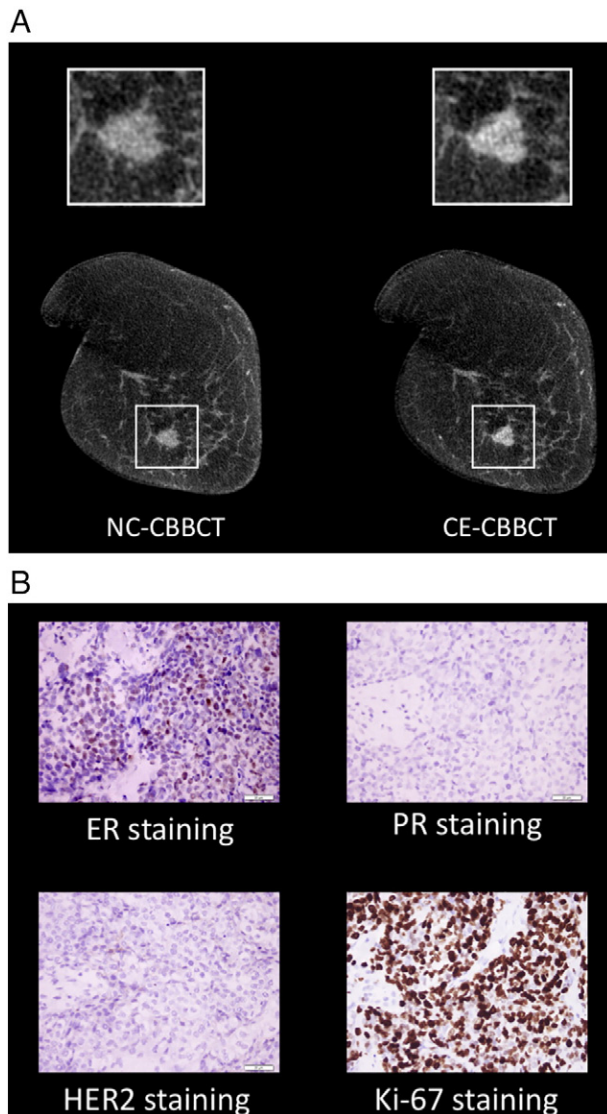


Figure 4. (A) Case of a postmenopausal 62-year-old woman presenting with right-sided breast mass. Contrast enhancement on CBBCT was 72 HU. (B) Immunohistochemical analyses revealed “Luminal B” breast cancer subtype with a Ki-67 index of 0.8, weak expression of ER and no expression of PR. HER2 expression was negative (Dako-Score 0).

CBBCT contrast enhancement was significantly correlated with breast cancer subtypes: highest contrast enhancement was evident for the Luminal A subtype, followed by the HER2-Subtyp, when compared to lesions of the Luminal B or triple negative receptor subtype.

In addition, separate analyses were conducted for breast cancer receptor status and proliferative potential. When evaluating the expression of individual receptors on breast cancer lesions, higher contrast enhancement was observed for ER, PR and HER2 positive breast cancer lesion when compared to their receptor negative counterparts. The missing statistical significance of these findings could be attributable to small and imbalanced sample sizes. Analyzing the proliferative potential, breast cancer lesions with low expression of the proliferative marker Ki-67 showed significantly higher contrast enhancement than those lesions with high Ki-67 index.

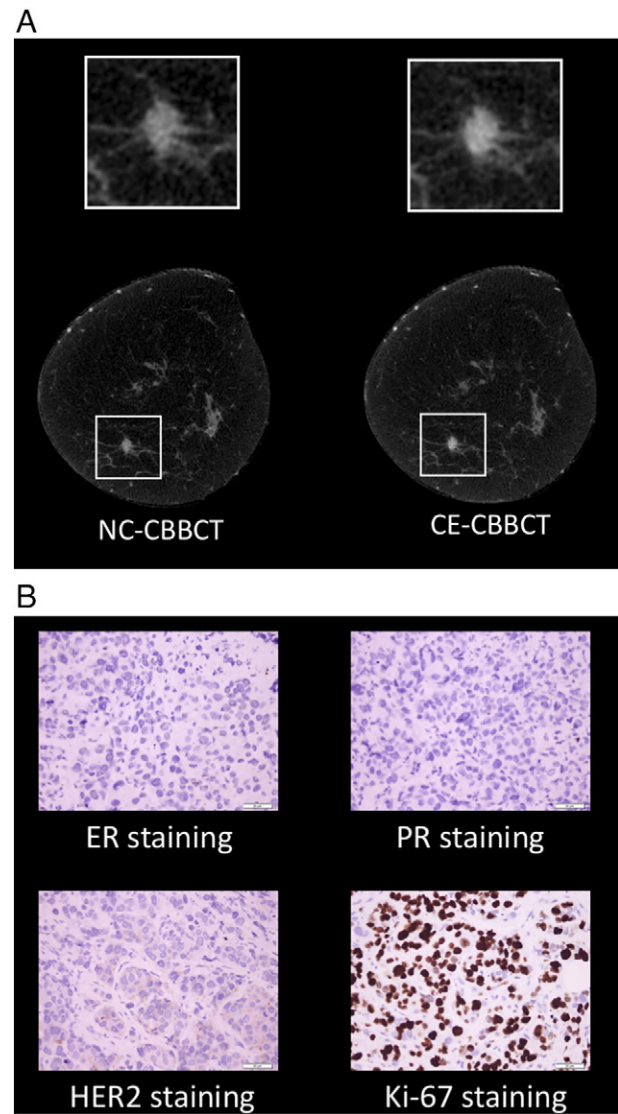


Figure 5. (A) Case of a postmenopausal 57-year-old woman presenting with right-sided breast mass. Contrast enhancement on CBBCT was 50 HU. (B) Immunohistochemical analyses revealed “triple negative” breast cancer subtype with a Ki-67 index of 0.8 and without expression of ER and PR. HER2 expression was negative (Dako-Score 0).

Our results might have immediate implications for therapeutic strategies and patient prognosis: in contrast to other breast cancer subtypes, chemotherapy showed reduced benefit for Luminal A breast cancer [21]. Further, there is expert consent on neoadjuvant cytotoxic treatment and administration of adjuvant monoclonal antibodies (Trastuzumab) for HER2 positive breast cancers [5]. Independent of any systemic adjuvant treatment, Blows et al. have shown distinct survival patterns depending on breast cancer subtype, with worst prognosis for those cancers with HER2 expression [9].

The effect of breast cancer subtype and receptor status on cellular behavior of malignant breast lesions might underlie our findings on distinct contrast enhancement patterns. Several authors reported central desmoplasia and cancerous necrosis in highly proliferative, Ki-67 expressing tumors which might explain the low contrast enhancement observed in Ki-67 intense breast lesions [22,23].

Table 4. Immunohistopathological Status with Associated Enhancement and *P* Values

Immunohistochemical Status	Number (Percent)	Mean Enhancement (\pm SD)	P-Value for Difference in Mean Enhancement
ER positive	n = 30 (78.9)	66.2 (\pm 36.7) HU	0.1714
ER negative	n = 8 (21.1)	45.3 (\pm 23.0) HU	Reference
PR positive	n = 29 (76.3)	65.8 (\pm 37.4) HU	0.3603
PR negative	n = 9 (23.7)	48.2 (\pm 23.3) HU	Reference
HER2 positive	n = 4 (10.5)	83.5 (\pm 49.8) HU	0.2166
HER2 negative	n = 34 (89.5)	59.2 (\pm 33.1) HU	Reference
Ki-67 > 0.3	n = 18 (47.4)	45.2 (\pm 21.5) HU	0.0043
Ki-67 < 0.3	n = 20 (52.6)	80.2 (\pm 38.5) HU	Reference

Abbreviations: SD, standard deviation; HU, Hounsfield unit; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor-2.

Tumor induced immune responses are another potential explanation for diverse contrast enhancement patterns. Della Rovere et al. demonstrated that breast cancer ER and PR receptor status correlated with inflammatory mast cell response [24]. Furthermore, the HER2 receptor has been shown to affect cancer angiogenesis [25–27]. Both mechanisms could in turn influence contrast enhancement patterns of breast cancer subtypes.

To the best of our knowledge, this study is the first to correlate CBBCT contrast enhancement with receptor status, proliferative properties and subtypes of breast cancer.

To date, there is a paucity of studies on the novel imaging technique of CBBCT [12,13,15]. Only one study evaluated image features of breast cancer subtypes and receptor status: Wienbeck et al. concluded that absolute density of lesions on CBBCT does not correlate with breast cancer receptor status or Ki-67 expression [14]. However, absolute lesion intensity on CBBCT might show high inter-individual variability depending on breast density, menopausal and menstrual status as well as age [28]. In our study, these limitations were addressed by analyses of contrast enhancement rather than absolute intensity and standardization of lesion enhancement to enhancement of fat tissue, thereby accounting for any fluctuations between image acquisitions. In addition, contrast enhancement as a dynamic parameter might better reflect intrinsic tissue properties than exclusive measurement of lesion intensity on CBBCT.

Other imaging studies evaluating breast cancer receptor status and proliferative properties focused on MRI but failed to cover analyses of breast cancer subtypes [29,30].

Several studies evaluated MRI contrast enhancement patterns: contrast enhancement curves with washout phenomena have been described for highly proliferative, Ki-67 rich breast cancer lesions [29]. Furthermore, MRI enhancement patterns correlated with expression of ER and PR on breast cancer cells [29].

Another MRI technique applied in breast cancer imaging is diffusion weighted imaging with calculation of apparent diffusion coefficients (ADC). Matsubayashi et al. reported low ADC values for breast cancer lesions with high Ki-67 expression as well as for ER and PR positive breast cancers [30].

A major limitation of our study is the comparably small sample size that might explain the high dispersion of enhancement measures. In addition, subgroup imbalances reduce statistical power for analyses of individual receptor status. Moreover, considering the single-center inclusion of only Caucasian women older than 40 years with ACR density type c or d breasts, the generalizability of our findings might be questioned.

Still, our study is the first to evaluate contrast enhancement for discrimination of breast cancer subtypes using the novel imaging

technique CBBCT. Further, lesion enhancement measures were averaged and standardized to fat tissue enhancement to ensure stable and generalizable estimates. Finally, commercially available and validated immunohistochemical methods were applied to assure reproducible results.

Conclusion

In summary, we have shown that contrast enhancement of histologically approved malignant breast lesions on CBBCT correlates with immunohistochemical breast cancer subtype, receptor status and proliferative potential. Highest enhancement was evident for Luminal A breast cancer lesions.

Large scale studies are indicated to confirm our findings in representative cohorts and to investigate whether therapeutic decision for breast cancer patients could validly be achieved based on contrast enhancement in a CBBCT.

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