

Associations Between Conventional Breast MRI Signal Characteristics and Immunohistochemical Biomarkers in Breast Cancer: A Retrospective Exploratory Study

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Abstract

BACKGROUND/AIMS: This study investigates whether quantitative magnetic resonance imaging (MRI)-derived signal measurements are associated with immunohistochemical biomarkers in breast cancer, extending beyond morphological assessment to signal-based imaging characteristics.

MATERIALS AND METHODS: The study retrospectively included 63 patients with 66 breast cancer lesions, selected from an initial cohort of 415 patients based on specific inclusion criteria. MRI scans were performed using a standardized protocol, and quantitative MRI signal measurements were extracted and analysed. Histopathological data-including estrogen receptor (ER), progesterone receptor, human epidermal growth factor receptor 2 status, and Ki-67 proliferation index-were also evaluated. Statistical analyses, including correlation and regression tests, were conducted to assess associations between MRI features and immunohistochemical biomarkers.

RESULTS: Weak associations were observed between pre-contrast T1 signal intensity (SI) and ER expression, and between T2 SI and the Ki-67 proliferation index (Spearman's $\rho=-0.17$ and 0.25 , respectively); neither remained statistically significant after Bonferroni correction for multiple comparisons nor after adjustment for within-patient lesion clustering. A more robust positive association was observed between the initial enhancement ratio (IER) and Ki-67 ($\rho=0.39$, 95% confidence interval: $0.16-0.57$, $p=0.001$), which remained statistically significant after Bonferroni correction (corrected $p=0.016$) and in both a single-lesion-per-patient sensitivity analysis and a cluster-robust regression.

CONCLUSION: The association between IER and Ki-67 remained statistically significant after correction for multiple comparisons and after accounting for within-patient lesion clustering, suggesting that it may represent a comparatively robust exploratory imaging correlate of tumor proliferation. Associations involving T1 SI and T2 SI were weak and did not survive correction. These findings are preliminary and hypothesis-generating, underscoring the need for further studies with larger datasets to clarify the clinical relevance of quantitative MRI analysis in breast cancer evaluation.

Keywords: Magnetic resonance imaging, breast cancer, signal intensity, initial enhancement ratio, Ki-67 index

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INTRODUCTION

Breast cancer is the most prevalent malignancy among women and the second leading cause of cancer-related death globally.^{1,2} These striking statistics have made breast cancer one of the most extensively studied topics in modern medicine. This increased attention has led to widespread screening programs, enabling earlier detection, improving treatment protocols, and enhancing survival outcomes.

Understanding the molecular subtypes of breast cancer plays a critical role in facilitating personalized treatment strategies.^{3,4} Gene expression studies have identified several distinct breast cancer subtypes that differ markedly in prognosis and in the therapeutic targets they express; however, these are not applied in daily clinical practice.⁵ Medical oncologists initiate treatment based on the predicted which are largely focused on estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and Ki-67 proliferation levels. There are significant discordances between molecular subtypes and immunohistochemical studies.⁶ However, assessment is costly, necessitating highly specialized equipment and significant technical expertise.

Medical imaging plays a pivotal role in breast cancer screening, diagnosis, treatment planning, and follow-up. Beyond the binary classification of lesions as benign or malignant, imaging parameters obtained through breast magnetic resonance imaging (MRI) may also yield insights into the histopathological subtypes of tumors.

Numerous studies have examined the association between molecular subtypes and morphological imaging features such as lesion shape, margins, spiculation, and location.⁷⁻¹² However, the number of studies examining the relationship between signal properties of MRI and immunohistochemical biomarkers of breast cancer lesions remains limited. In this study, we aim to assess the potential relationship between immunohistochemical markers and their corresponding quantitative MRI-derived parameters.

MATERIALS AND METHODS

Patient Population

This study was approved in University of Health Sciences Non-Interventional Ethics Committee (approval no: 18/84, date: 05.04.2018). All procedures involving human participants were conducted in accordance with the ethical standards of the institutional research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Written informed consent was obtained from all participants for the storage of medical data in the hospital database and its use for research purposes. A retrospective search of the local hospital's medical imaging archive was conducted to identify breast MRI scans performed between January 2014 and April 2018. Imaging data from 415 patients were initially identified. Of these, 91 patients had a histopathological diagnosis of breast cancer. Patients were excluded if they had poor image quality, had a history of neoadjuvant therapy, or lacked detailed histopathological reports. Additionally, cases of *in situ* carcinoma were excluded because these lesions were unsuitable for optimal region of interest (ROI) measurement and immunohistochemical biomarkers were unavailable in their pathology reports. In patients with multiple lesions, all histopathologically confirmed malignant lesions were included in the analysis. After applying exclusion criteria, 63 patients

with a total of 66 breast cancer lesions were included in the final analysis. A study flow diagram, prepared according to STROBE reporting guidelines for observational studies, summarizes the patient and lesion selection process (Figure 1). Exclusion criteria at the n=91 to n=63 stage (poor image quality, neoadjuvant treatment history, incomplete histopathology documentation) were applied concurrently in a single retrospective eligibility screen rather than sequentially. Some excluded patients met more than one criterion; per-criterion counts were not retained and are therefore not reported. Only the total number of exclusions at this stage (n=28) is available.

Imaging Protocol

All breast MRI scans were performed using a standardized protocol, with patients in the prone position, utilizing a 7-channel bilateral breast coil on a 3.0 Tesla superconducting MRI system (Achieva 3.0T X-series, Philips Medical Systems B.V., Eindhoven, The Netherlands). The pre-contrast sequences included axial Turbo Spin Echo T1-weighted (T1w), short tau inversion recovery (STIR), and T2-weighted (T2w) images. Detailed imaging parameters are provided in Table 1. A gadolinium-based contrast agent was administered at a dose of 0.1 mmol/kg, and dynamic contrast-enhanced images were acquired at one-minute intervals for a total of 6 minutes post-injection. MRI data were processed on a dedicated workstation using DynaCad version 2.1.6.81957. All MRI measurements were independently performed by two radiologists with 4 and 10 years' experience in breast imaging, respectively. Interobserver reliability, assessed using the intraclass correlation coefficient (ICC). Using a two-way random-effects, absolute-agreement, single-measures [ICC (2,1)=0.88], indicating good agreement between the two readers; a 95% confidence interval (CI) was not computed for this coefficient. Both radiologists were blinded to each other's results and to the histopathological findings at the time of measurement. Given this

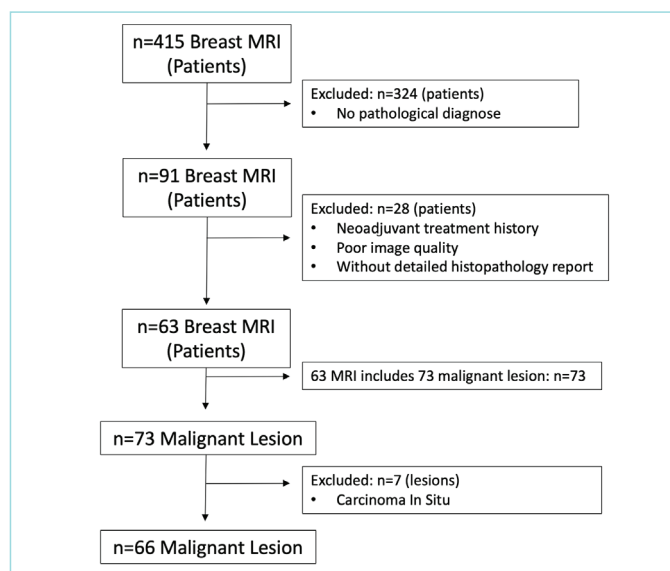


Figure 1. Study flow diagram (prepared according to STROBE reporting guidelines for observational studies). Because eligibility was assessed in a single concurrent screening pass rather than sequentially, per-criterion exclusion counts within this step were not retained and are not reported; only the total number excluded (n=28) is shown.

MRI: Magnetic resonance imaging, STROBE: Strengthening the Reporting of Observational Studies in Epidemiology.

confirmed agreement, the mean of the two radiologists' independent measurements for each lesion was used as the input for subsequent statistical analyses.

Breast MR images and corresponding histopathology reports were reviewed concurrently. Lesions confirmed as malignant by histopathology were identified on MRI, and mean signal intensities were calculated on each sequence using elliptical ROIs manually placed on the single axial slice that demonstrated the largest cross-sectional area of each lesion, encompassing at least two-thirds of that area. Areas of necrosis or hemorrhage were excluded from the ROIs to avoid signal intensity (SI) alterations unrelated to tumor tissue (Figure 2). Signal intensities were measured on unenhanced T1w and T2w sequences and compared with histopathological parameters. Additionally, the initial enhancement ratio (IER) was calculated as the percentage increase in T1 SI between the pre-contrast image and the first post-contrast time point, using the same ROI (Figure 3) to minimize measurement bias. $IER (\%) = [(SI \text{ post-contrast, first minute} - SI \text{ pre-contrast}) / SI \text{ pre-contrast}] \times 100$.

Histopathologic Assessment

Histopathological data were retrieved from patients' medical records and evaluated retrospectively. As this was a retrospective study, histopathological evaluations were performed by the pathologists on duty at the time of each biopsy, in accordance with the institution's routine clinical workflow. All reports were generated using standardized immunohistochemical staining protocols applied consistently across the study period. The following features were assessed: tumor histological subtype, grade, ER, PR, HER2 status, and Ki-67 proliferation index. Molecular subtypes were categorized as Luminal A, Luminal B, HER2-enriched, and Basal-like. Molecular classification was based on a combination of tumor grade, ER, PR, HER2 status, and Ki-67 index. HER2 status was extracted from each patient's final composite pathology report rather than from the immunohistochemical score alone; immunohistochemically equivocal (2+) cases were reflex-tested with fluorescence *in situ* hybridization (FISH) as part of routine clinical practice during the study period, consistent with ASCO/CAP guidelines, and the FISH result was incorporated into the final reported HER2 status. HER2 was therefore analyzed as a binary variable (positive vs. negative).

Table 1. Detailed imaging parameters of the MRI sequences			
	Pre-contrast T1	T2 STIR	Dynamic 3D T1
Slice thickness	3 mm	3 mm	0.76/0.76/2 mm
Gap	0 mm	0 mm	0 mm
Matrix	340×277	340×259	472×472
FOV (AP × RL × FH)	340×352×150	340×340×150	360×360×150
TR	495 ms	10927 ms (TI=230 ms)	5.1 ms
TE	10 ms	60 ms	2.6 ms
NEX	1	1	1
Time (min:sec)	3.21	4.22	1.07
Total time (min:sec)	-	-	7.52

MRI: Magnetic resonance imaging, STIR: Short tau inversion recovery, FOV: Field of view, AP: Anteroposterior, RL: Right-left, FH: Foot-head, TR: Repetition time, TE: Echo time, TI: Inversion time, NEX: Number of excitations.

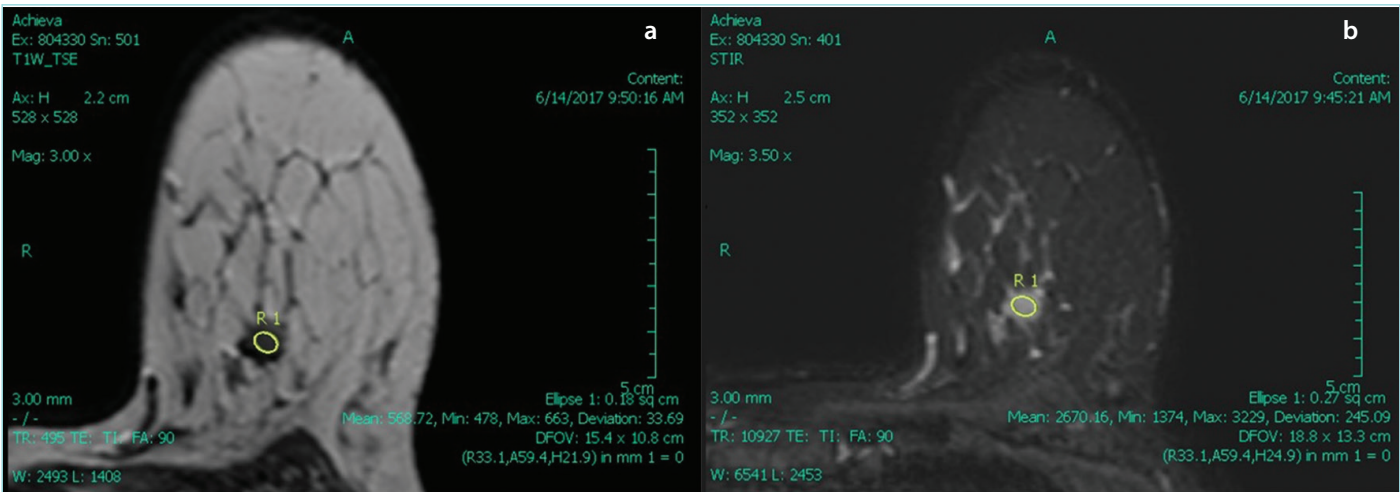


Figure 2. Mean signal calculations by using an ROI on T1w axial (a) and T2w axial (b) images (mean T1 SI on a=568.72, and mean T2 SI on b=2670.16).

ROI: Region of interest, T1w: T1-weighted, T2w: T2-weighted, SI: Signal intensity.

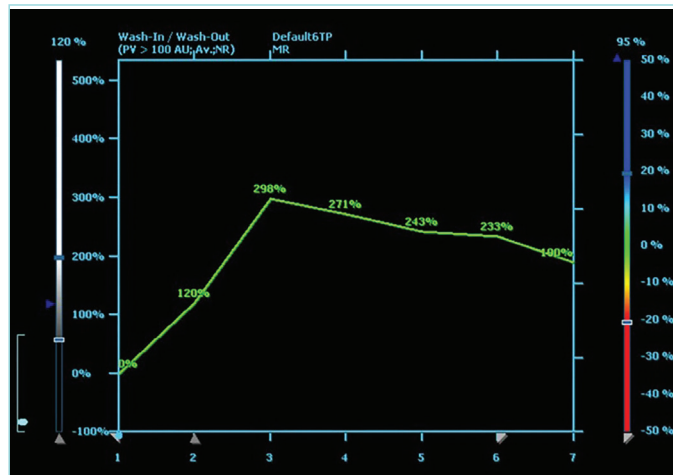


Figure 3. Calculation of initial enhancement ratio by using the time-intensity curve at the dedicated workstation. Time point 1 corresponds to the unenhanced T1 signal intensity, and time point 2 shows the first-minute signal change after contrast material injection. In this example, IER was 120%.

IER: Initial enhancement ratio.

Molecular subtypes were assigned using standard literature-based criteria: ER and PR positivity were defined as $\geq 1\%$ positively stained tumor nuclei by immunohistochemistry, consistent with ASCO/CAP reporting standards; a Ki-67 cut-off of 20% was used to distinguish low- from high-proliferation tumors, consistent with the St Gallen International Expert Consensus framework; HER2 positivity was determined according to ASCO/CAP guideline criteria, as described above; and histological grade was incorporated as a supportive criterion, consistent with the St Gallen consensus framework. These thresholds were applied solely for the descriptive molecular subtype classification; the primary correlation analyses (Table 2) used the continuous values of ER, PR, and Ki-67 directly, without dichotomization. Given the resulting skewed subtype distribution in this cohort-including a single basal-like lesion and no HER2-enriched lesions-comparisons across molecular subtypes were not adequately powered for formal statistical testing and

were presented as descriptive and exploratory findings only, rather than as confirmatory subgroup analyses.

Statistical Analysis

The Shapiro-Wilk test was applied to evaluate the normality of the distributions of all imaging (T1 SI, T2 SI, IER) and biomarker (ER, PR, Ki-67) variables. Only patient age was normally distributed ($p=0.559$); all other variables significantly deviated from normality (all $p<0.05$). Spearman's rank correlation coefficient was therefore used as the primary measure of association for all twelve pre-specified MRI parameter-biomarker combinations (T1 SI, T2 SI, and IER, each against ER, PR, HER2, and Ki-67) continuous; this approach treated HER2 as a two-level ordinal variable for this purpose, consistent with the ordinal/non-parametric treatment applied to the other markers. Because some patients contributed more than one lesion to the dataset, within-patient dependence was addressed using (1) a sensitivity analysis restricted to a single index lesion per patient, and (2) cluster-robust regression with standard errors (SEs) clustered at the patient level. Multiple linear regression was used to assess the combined association of T2 SI and IER with Ki-67, including collinearity diagnostics (variance inflation factor) and assessment of residuals. All statistical analyses were performed using STATA version 14 (StataCorp, College Station, TX, USA), with supplementary Spearman and cluster-robust analyses conducted in Python (SciPy/statsmodels). A p-value <0.05 was considered statistically significant. To account for the twelve bivariate correlation tests performed, a Bonferroni-corrected significance threshold of $\alpha=0.0042$ ($0.05/12$) was applied.

As the multiple regression residuals showed some deviation from normality, Spearman's rank correlation was retained as the primary analysis throughout, with linear regression presented as a secondary, exploratory analysis. A bootstrap resampling analysis (10,000 resamples, patient-level clustering) was additionally performed on the primary IER-Ki-67 correlation as a non-parametric robustness check.

RESULTS

A total of 63 patients with 66 malignant breast lesions were included in a retrospective analysis. The mean age of the patients was 49.5 ± 9 years

Table 2. Summary of bivariate correlations between MRI signal parameters and immunohistochemical biomarkers

MRI parameter	IHC marker	n	Spearman ρ	95% CI	p (uncorrected)	p (Bonferroni $\times 12$)
T1 SI	ER	66	-0.168	-0.39 to 0.08	0.177	1.000
T1 SI	PR	66	-0.138	-0.37 to 0.11	0.270	1.000
T1 SI	HER2	66	0.078	-0.17 to 0.31	0.535	1.000
T1 SI	Ki-67	66	0.124	-0.12 to 0.36	0.321	1.000
T2 SI	ER	66	-0.063	-0.30 to 0.18	0.618	1.000
T2 SI	PR	66	0.097	-0.15 to 0.33	0.436	1.000
T2 SI	HER2	66	-0.012	-0.25 to 0.23	0.924	1.000
T2 SI	Ki-67	66	0.250	0.01 to 0.46	0.043	0.513
IER	ER	66	-0.182	-0.41 to 0.06	0.143	1.000
IER	PR	66	-0.045	-0.28 to 0.20	0.719	1.000
IER	HER2	66	0.132	-0.11 to 0.36	0.290	1.000
IER	Ki-67	66	0.386	0.16 to 0.57	0.001	0.016

Spearman's rank correlation was used given non-normal distributions (Shapiro-Wilk testing). All twelve pre-specified MRI parameter-biomarker combinations were tested; the Bonferroni-corrected significance threshold was $\alpha=0.0042$ ($0.05/12$). Only the IER-Ki-67 association retained significance after correction.

MRI: Magnetic resonance imaging, SI: Signal intensity, IHC: Immunohistochemistry, CI: Confidence interval, IER: Initial enhancement ratio, ER: Estrogen receptor, PR: Progesterone receptor, HER2: Human epidermal growth factor receptor 2.

(range: 31-71 years). The majority of the lesions (68.4%) were classified as invasive ductal carcinoma. Based on immunohistochemical analysis, the molecular subtypes of the lesions were distributed as follows: Luminal A (n=18, 27.3%); Luminal B (n=47, 71.2%); and Basal-like (n=1, 1.5%). No lesions were categorized as HER2-enriched. Statistical analysis revealed no significant associations between quantitative MRI signal measurements and the molecular subtypes ($p>0.05$).

Pre-contrast T1 SI values ranged from 250 to 824, with a median of 375 SI and a mean of 391.8 ± 111.5 SI. T2w STIR SI values ranged from 303 to 3273 SI, with a median of 975 SI and a mean of 1035.2 ± 490.5 SI. IER values ranged from 10% to 220%, with a median of 91% and a mean of $101.4 \pm 60.7\%$.

Among the histopathological findings, Ki-67 values ranged from 2% to 80%, with a median value of 15% and a mean of 19.7 ± 15.2 . ER expression varied between 0% and 100%, with a median of 90% and a mean of 74.2 ± 31.3 . PR values also ranged from 0% to 100%, with a median of 40% and a mean of 44 ± 35.5 . The associations between MRI parameters and the expression levels of ER, PR, and HER2 were evaluated individually. A weak negative correlation between pre-contrast SI values and ER expression was observed (Spearman's $\rho = -0.17$, 95% CI: -0.39 to 0.08, $p = 0.177$), but it did not reach statistical significance (Figure 4a).

T2w STIR SI values showed no significant correlation with ER, PR, or HER2 expression levels (all Spearman's ρ , uncorrected $p>0.05$; Table 2). A weak positive correlation was observed between T2 SI values and the Ki-67 proliferation index ($\rho = 0.25$, 95% CI: 0.01 to 0.46, $p = 0.043$), but it did not remain significant after Bonferroni correction for multiple comparisons (corrected $p = 0.513$) nor in cluster-robust regression ($p = 0.105$) (Figure 4b).

Analysis of IER values revealed no significant associations between IER and ER, PR, or HER2 status (all Spearman's ρ , uncorrected $p>0.05$; Table 2). In contrast, a moderate positive correlation was found between IER and Ki-67 levels ($\rho = 0.39$, 95% CI: 0.16 to 0.57, $p = 0.001$), which remained statistically significant after Bonferroni correction for multiple comparisons (corrected $p = 0.016$), in a single-lesion-per-patient sensitivity analysis ($\rho = 0.43$, $p < 0.001$), and in cluster-robust regression ($p = 0.028$) (Figure 4c).

Multiple linear regression of Ki-67 on T2 SI and IER (n=66) showed that IER was independently associated with Ki-67 in the model ($B = 0.077$, $SE = 0.030$, 95% CI: 0.017-0.136, $p = 0.012$), whereas T2 SI was not independently associated ($B = 0.006$, $SE = 0.004$, 95% CI: -0.001-0.013, $p = 0.117$). The overall model was statistically significant [$F(2,63) = 5.54$, $p = 0.006$; $R^2 = 0.150$, adjusted $R^2 = 0.123$], with minimal collinearity between predictors (variance inflation factor = 1.04 for both). When SEs were adjusted for within-patient clustering, IER remained significant (cluster-robust $p = 0.028$), whereas T2 SI did not ($p = 0.178$). Residuals showed a deviation from normality (Shapiro-Wilk $p < 0.001$), consistent with the underlying skewed distribution of Ki-67. Results were nonetheless consistent across parametric and non-parametric approaches.

As an additional non-parametric sensitivity check, a bootstrap resampling analysis (10,000 resamples) was performed to evaluate the Spearman correlation between IER and Ki-67. A patient-level cluster bootstrap, resampling patients with replacement to account for within-patient lesion dependence, yielded a 95% CI of 0.12 to 0.62; a simple case-resampling bootstrap yielded a 95% CI of 0.13 to 0.60. Both intervals excluded zero and were consistent with the Fisher z-transformation-based CI reported above, further supporting the robustness of this association.

A complete summary of twelve bivariate correlations between MRI signal parameters and immunohistochemical biomarkers, including exact coefficients, 95% CIs, and both uncorrected and Bonferroni-corrected p-values, is presented in Table 2. After Bonferroni correction for multiple comparisons ($\alpha = 0.0042$), only the association between IER and Ki-67 retained statistical significance; the T1 SI-ER and T2 SI-Ki-67 associations did not retain statistical significance and should be interpreted as exploratory.

DISCUSSION

Recent studies in breast radiology, as well as globally accepted guidelines (e.g., BI-RADS), aim to assess the malignancy risk of detected lesions.¹³ Advances in imaging technology and computational analysis have allowed us to establish diverse associations with the pathological features of tumors beyond the differentiation of benign or malignant lesions.

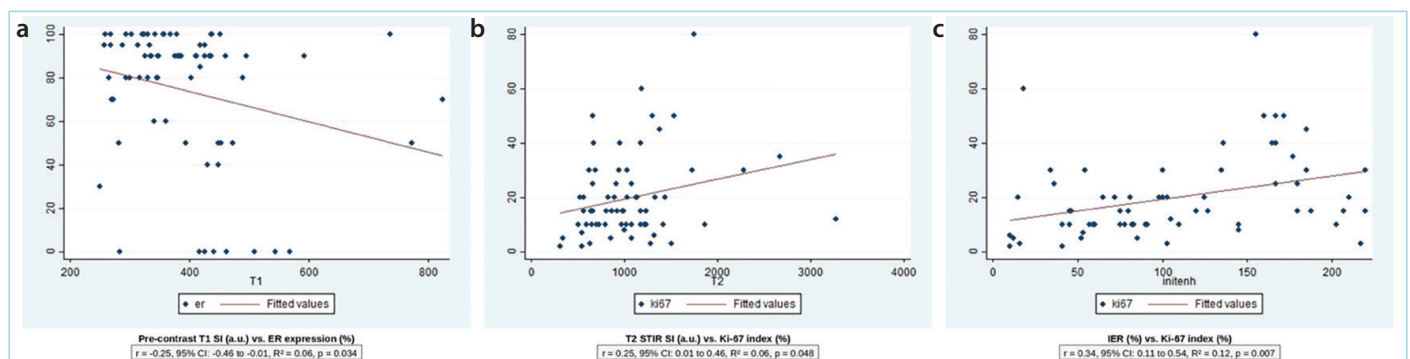


Figure 4. Bivariate correlations between MRI signal parameters and immunohistochemical biomarkers (Spearman's rank correlation). (a) Correlation between pre-contrast T1 SI values and ER expression (n=66, $\rho = -0.17$, 95% CI: -0.39 to 0.08, $p = 0.177$). (b) Correlation between T2 SI values and Ki-67 index (n=66, $\rho = 0.25$, 95% CI: 0.01 to 0.46, $p = 0.043$). (c) Correlation between initial enhancement ratio and Ki-67 index (n=66, $\rho = 0.39$, 95% CI: 0.16 to 0.57, $p = 0.001$).

IER: Initial enhancement ratio, ER: Estrogen receptor, MRI: Magnetic resonance imaging, SI: Signal intensity, STIR: Short tau inversion recovery, CI: Confidence interval.

Several recent studies have explored the role of MRI features in identifying breast cancer molecular subtypes.^{10-12,14-17} For instance, multiparametric MRI has demonstrated significant differences among subtypes, particularly between triple-negative and hormone receptor-positive tumors.¹⁸ Moreover, dynamic contrast-enhanced MRI radiomics, especially intratumoral and peritumoral features derived from late-phase images, have been shown to contribute substantially to subtype classification,¹⁹ while multiparametric approaches overall outperform semantic models in predicting molecular subtypes.²⁰ In addition, morphological characteristics such as mass shape and margin features have also been reported to correlate with specific subtypes, including round contours in triple-negative tumors and spiculated margins in hormone receptor-positive cancers.²¹ However, in our study, we did not observe significant associations of enhancement patterns or SI values (T1 SI, T2 SI, or IER) with molecular subtypes. This discrepancy may be related to differences in imaging protocols, sample size, or in the use of conventional imaging parameters rather than advanced radiomic or multiparametric approaches. Our findings suggest that while MRI has potential in molecular subtype assessment, classical enhancement and SI measurements alone may be insufficient for reliable subtype differentiation in routine clinical practice. This lack of association should also be interpreted in light of the skewed molecular subtype distribution in our cohort, which may limit the generalizability of this particular finding and is discussed further in the study limitations section.

We also examined the association between pre-contrast T1 SI values and ER expression (Spearman's $\rho = -0.17$, $p = 0.177$), which did not reach statistical significance after correction for multiple comparisons (see Table 2). While post-contrast T1 signal variability has been described in the literature, the relevance of pre-contrast T1 SI to tumor biology remains underexplored.²² To our knowledge, no previous study has specifically investigated the T1 SI of fibroglandular tissue or breast lesions on pre-contrast T_w images. In our cohort, lower T1 SI values were associated with higher ER expression. Previous studies have demonstrated lower ADC values in ER-positive tumors, reflecting higher cellularity.^{23,24} Given that this association did not remain statistically significant after Bonferroni correction for multiple comparisons or adjustment for within-patient lesion clustering (Spearman's $\rho = -0.17$, $p = 0.177$; see statistical analysis and Table 2), the association should be interpreted as a non-significant, exploratory observation rather than evidence of biomarker capability and the above hypothesis-generating interpretation should therefore be considered preliminary.

In the current study, we also examined the T2 STIR signal intensities of malignant lesions. While early studies such as Kuhl et al.²⁵ reported no statistically significant differentiation between benign and malignant lesions based on T2 STIR imaging, more recent evidence offers a different perspective. Notably, Kim et al.²⁶ found that intratumoral T2 hyperintensity correlated positively with Ki-67 in ER-positive/HER2-negative invasive ductal carcinoma, while ADC values did not show significant associations. Dietzel et al.²⁷ emphasized that T2w hyperintense signal is associated with higher proliferation rates and histologic grade, suggesting its potential as a non-contrast imaging biomarker. In our study, we similarly found that higher lesion SI values on T2 STIR were positively associated with the Ki-67 proliferation index. This observation is in line with the findings of Kim et al.,²⁶ who reported that intratumoral T2 hyperintensity correlated with high Ki-67 expression in ER-positive/HER2-negative breast cancers, and with the

work of Dietzel et al.,²⁷ who demonstrated that T2 hyperintensity and other semantic features were linked to increased proliferative activity and higher histologic grade. Taken together, these results should be interpreted cautiously, as the association did not survive correction for multiple comparisons or patient-level clustering (Table 2).

Recent studies have highlighted the role of dynamic contrast-enhanced MRI kinetics in reflecting tumor proliferative activity. Szepe et al.¹⁸ demonstrated that multiparametric MRI features, including kinetic parameters, show significant associations with molecular subtypes and proliferation indices. Similarly, Huang et al.¹⁹ reported that radiomic features derived from late-phase DCE-MRI, particularly intratumoral and peritumoral characteristics, contributed substantially to Ki-67 prediction, while Huang et al.²⁰ confirmed the superiority of multiparametric MRI over semantic models in this context. In our study, IER showed a statistically significant association with Ki-67 (Spearman's $\rho = 0.39$, $p = 0.001$). This association remained significant after Bonferroni correction for multiple comparisons (corrected $p = 0.016$), in a single-lesion-per-patient sensitivity analysis, and in cluster-robust regression accounting for within-patient lesion dependence. Taken together, these findings suggest that enhancement kinetics may serve as a non-invasive imaging biomarker for tumor proliferation, providing complementary prognostic information. However, MRI-derived parameters, including IER, cannot currently replace tissue-based immunohistochemical assessment and should be regarded as complementary exploratory signals that require prospective validation.

Weak, uncorrected relationships were observed between T1 SI and ER, and between T2 SI and Ki-67; however, neither remained significant after correction for multiple comparisons or adjustment for within-patient lesion clustering. Both should be interpreted as exploratory. In contrast, the relationship between IER and Ki-67 remained statistically significant in Spearman correlation with Bonferroni correction, in a single-lesion-per-patient sensitivity analysis, and in cluster-robust regression, representing a comparatively robust finding. An additional strength of our study was that IER could be calculated using dedicated software with minimal operator dependence, providing a quantitative and reproducible parameter. The objective nature of IER may support its role as an exploratory imaging correlate, though it cannot currently replace tissue-based immunohistochemical assessment. Overall, the MRI parameters evaluated in this study represent only a small component of a broader research field. However, our findings provide preliminary insights into the histopathological characteristics of malignant breast lesions beyond the classical benign-malignant differentiation and warrant further investigation.

Study Limitations

First, although the signal measurements in our study were performed at the dedicated workstation, they might not reflect the whole lesion due to operator-dependent ROI placement, which may not encompass the entire lesion. Second, all patients with malignant histopathology results were evaluated as a single group. Although the vast majority (68.49%), breast cancer subtypes and histopathological biomarkers may affect the MRI findings we described. Furthermore, the molecular subtype distribution in our cohort was heavily skewed toward Luminal A and Luminal B tumors, with only a single basal-like lesion and no HER2-enriched lesions; findings regarding molecular subtype should therefore not be generalized to underrepresented or absent subtypes. Third, the relatively small sample size (63 patients and 66 lesions)

limits the statistical power and generalizability of our findings. Future multicenter studies with larger cohorts are warranted. Additionally, because histopathological assessments were performed by multiple pathologists during routine clinical care, interobserver variability could not be controlled. A further limitation is the use of absolute, non-normalized SI values rather than SI ratios referenced to a stable tissue (e.g., pectoralis muscle or contralateral fibroglandular tissue). Absolute SI is influenced by scanner hardware, receiver gain, coil sensitivity, and acquisition settings, which may limit direct comparability across examinations. Although all scans were acquired on a single scanner using an identical, standardized protocol, prospective studies should incorporate reference-tissue normalization to improve reproducibility. Finally, although Spearman's rank correlation was used as the primary method following Shapiro-Wilk normality testing (see statistical analysis), residual non-normality in the multiple regression model (Shapiro-Wilk $p < 0.001$ for residuals) indicates that parametric regression estimates should be interpreted with some caution. However, results were consistent with the non-parametric Spearman correlation and cluster-robust analyses. Diagnostic threshold determination (e.g., via ROC analysis to identify a clinically actionable Ki-67 cut-off) requires a study specifically designed and powered for this purpose and is beyond the scope of the present exploratory analysis. This represents an important direction for future prospective, adequately powered studies.

CONCLUSION

In summary, the association between the IER and Ki-67 remains statistically significant after Bonferroni correction for multiple comparisons and after accounting for within-patient lesion clustering, and represents the most robust finding of this study. Associations between pre-contrast T1 SI and ER, and between T2 SI and Ki-67, were weak and did not survive correction. These findings should be regarded as preliminary and hypothesis-generating rather than as established imaging biomarkers, using normalized SI measures before any consideration of clinical implementation. Moreover, the increasing interest in digitalization, artificial intelligence, and quantitative data assessment indicates that radiomic features may play an important role in breast cancer evaluation in the near future.

MAIN POINTS

- A weak positive correlation was observed between T2 short tau inversion recovery signal and tumor Ki-67 expression levels, which did not remain significant after correction for multiple comparisons or for patient-level clustering.
- A correlation between initial enhancement ratio (IER) and tumor Ki-67 values was identified and remained statistically significant after Bonferroni correction and in sensitivity analyses accounting for within-patient lesion clustering. This finding suggests that IER may be a comparatively robust exploratory imaging correlate of Ki-67 expression, which provides important information about tumor prognosis and mitotic activity.
- Future studies involving larger datasets and analyzing additional magnetic resonance parameters could improve understanding of associations with immunohistochemical markers.

ETHICS

Ethics Committee Approval: This study was approved in University of Health Sciences Non-Interventional Ethics Committee (approval no: 18/84, date: 05.04.2018).

Informed Consent: Written informed consent was obtained from all participants for the storage of medical data in the hospital database and its use for research purposes.

Footnotes

Authorship Contributions

Concept: M.T., Design: H.T.S., Data Collection and/or Processing: U.B., Analysis and/or Interpretation: U.B., S.A., M.T., Literature Search: U.B., S.A., H.T.S., Writing: U.B., H.T.S.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

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Declaration Regarding the Use of AI and AI-Assisted Technologies:

During the preparation of this manuscript, the authors used Claude (Anthropic) and ChatGPT (OpenAI) for sentence-level editing and grammatical refinement. All AI-generated suggestions were carefully reviewed, critically evaluated, and edited as necessary by the authors to ensure accuracy and contextual appropriateness. The authors take full responsibility for the publication's content. The use of AI tools primarily affected the linguistic presentation and grammatical structure of the manuscript; all scientific content, data interpretation, and conclusions reflect solely the authors' own work.

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