Diffusion-Weighted Imaging Features of Breast Tumours and the Surrounding Stroma Reflect Intrinsic Heterogeneous Characteristics of Molecular Subtypes in Breast Cancer

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Word count: 6244 words

Abstract

Breast cancer heterogeneity is the main obstacle preventing the identification of breast cancer patients with poor prognosis and treatment responses, yet such heterogeneity has not been well characterized. The purpose of this retrospective study was to reveal heterogeneous patterns of the apparent diffusion coefficient (ADC) signals in tumours and the surrounding stroma to predict molecular subtypes in breast cancer. A dataset of 126 breast cancer patients who underwent preoperative diffusion-weighted imaging (DWI) on a 3.0-T image system was collected. Breast images were segmented into regions comprising the tumour and surrounding stromal shells in which features that reflect heterogeneous ADC signal distribution were extracted. In each region, imaging features were computed including the mean, minimum, variance, interquartile range (IQR), range, skewness, kurtosis and entropy of ADC values. Univariate and stepwise multivariate logistic regression modelling was performed to identify the MR imaging features that optimally discriminate luminal A, luminal B, HER2-enriched and basal-like molecular subtypes. The performance of the predictive models was evaluated using the area under the receiver operating characteristic curve (AUC). Univariate logistic regression analysis showed that the skewness in the tumour boundary achieved an AUC of 0.718 for discriminating between luminal A and non-luminal A tumours, while the interquartile range of the ADC value in the tumour boundary had an AUC of 0.703 for classifying the HER2-enriched subtype. Imaging features in the tumour boundary and the proximal peritumoural stroma corresponded to higher overall prediction performance than those in other regions. A multivariate logistic regression model

combining features in all the regions achieved an overall AUC of 0.800 for classifying the four tumour subtypes. These findings suggest that features in the tumour boundary and stroma around the tumour may be further assessed as potential predictors of molecular subtypes of breast cancer.

Kew Words

Diffusion-Weighted Imaging; Apparent Diffusion Coefficient; Breast cancer; Molecular subtype

Abbreviations used:

ADC	Apparent diffusion coefficient
AUCs	Areas under the curves
DWI	Diffusion-weighted magnetic resonance imaging
HER2	Human epidermal growth factor 2
IHC	Immunohistochemistry
ANOVA	Analysis of variance

Introduction

Breast cancer is a heterogeneous disease and can be differentiated into four subtypes, i.e., luminal A, luminal B, HER2-enriched and basal-like. Specific molecular subtypes have been shown to differ in clinical behaviours, providing a clinically useful basis for therapeutic selection (1). Compared with the luminal A subtype, patients with the luminal B subtype have higher proliferation and poorer prognosis (2). In the basal-like and HER2-enriched subtypes, patients are more sensitive to preoperative chemotherapy

and are more likely to obtain a pathologic complete response but worse prognosis than those with luminal cancers (3-7). The HER2-enriched and basal-like subtypes have been reported to exhibit more aggressive behaviours and lead to worse survival (8). Additionally, it has been reported that basal-like subtype patients are significantly associated with nodal involvement, an important determinant for prognosis in early-stage breast cancer (9). Therefore, accurate tumour characterization is important because it carries therapeutic and prognostic implications.

Tumour categorization is a nontrivial task due to the dramatic heterogeneity in cancer cells and the dynamic plasticity of the tumour microenvironment. The tumour and surrounding microenvironment are closely related and interact with extracellular signals, thereby inducing an altered stroma around the tumour (10). The altered tumour microenvironment can in turn contribute to increased tumour angiogenesis and microvessel density and a higher level of collagen deposition and fibre reorganization, leading to increased tissue stiffness (11). Radiomic analysis of the tumour and stroma can be performed using magnetic resonance imaging (MRI) to uncover quantitative features for association with genetic information such as breast cancer subtypes (12, 13).

Diffusion-weighted imaging (DWI) is an approach that may further improve the accuracy of tumour characterization by detecting the variability of the “Brownian motion” of water molecules in the breast tissue (14). DWI yields a quantitative parameter, defined as the apparent diffusion coefficient (ADC), which provides an estimation of biological tumour characteristics such as tissue cellularity, water content,

integrity of cell membranes and degree of vascularity (15). A decreased ADC value in malignant tumours may be caused by increased cellularity, larger nuclei with more abundant macromolecular proteins, and less extracellular space (14). The tumour ADC has been evaluated as a candidate biomarker for predicting responses to neoadjuvant chemotherapy and was also used for the differential diagnosis of benign and malignant breast masses (16-18). Moreover, the ADC has been utilized as an indicator of tumour aggressiveness, Ki-67 expression, tumour cellularity and the expression of prognosis biomarkers in breast cancer (19-23). Recent studies have reported a correlation between the ADC and the molecular markers of breast cancer (24-27). In addition to direct analysis of the entire tumour, DWI information of the breast tumour-stromal boundary and adjacent tissue surrounding the tumour has also been combined to associate DWI with pathological factors or to predict responses to treatment (28, 29).

In this study, we hypothesized that the ADC values of the tumour and surrounding peritumoural stroma may be distinct and reflect the heterogeneity of the tumour microenvironment in breast cancer. Therefore, the purpose of our study was to evaluate whether radiomic features obtained on DWI in tumours and adjacent stromal tissues show distinct characteristics and can be used as candidate biomarkers to predict the molecular subtypes of tumours in patients with breast cancer.

Materials and Methods

Subjects

This is a retrospective study approved by the Internal Research Review and Ethical Committee, and all patient information was anonymized before any data analysis was

conducted. The study initially collected 179 patients diagnosed with invasive breast cancer who underwent preoperative DCE-MRI and DWI between January and July 2011. Patients were excluded (n=21) due to the lack of pathological report or incomplete pathology data and no available MRI sequence. Patients who underwent breast cancer treatment (e.g., chemotherapy or radiation therapy, n=32) before MRI were also excluded. Finally, 126 patients who fit the selection criteria were included in our dataset for analysis.

Pathologic Assessment

The expression of ER, PR, HER2 and Ki-67 of each patient with invasive cancer was obtained from pathologic reports by a pathologist using streptavidin-peroxidase (SP) immunohistochemistry (IHC). A sample was scored as ER positive and/or PR positive when at least 1% of the tumour cell nuclei showed staining for ER or PR (30). HER2 status was assessed according to the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines for HER2 testing in breast cancer (31). An IHC HER2 score of 3+ was considered positive, a score of 0 or 1+ was considered negative, and a score of 2+ was further confirmed by fluorescence in situ hybridization (FISH). Tumours with Ki-67 levels greater than 14% were considered high-Ki-67 tumours, and the other tumours were considered low-Ki-67 tumours. Based on the receptor status, the molecular subtype classification was determined, and tumours were categorized as follows: luminal A: ER and/or PR positive, HER2 negative; luminal B: ER and/or PR positive, HER2 positive; HER2-enriched: ER and PR negative, HER2 positive; and basal-like: ER, PR, and HER2 negative. Luminal subtype with a

Ki-67 expression level above 14% was specifically assigned to luminal B (1).

MR Image Acquisition

All patients were scanned in the prone position with a Siemens Magnetom Verio 3.0 T MRI scanner (Siemens Medical Solutions, Erlangen, Germany) and a dedicated eight-channel double-breast array coil (Siemens Medical Systems). A precontrast series was first acquired, followed by six postcontrast series after intravenous injection of a contrast agent at a dose of 0.2 mmol per kilogram body weight and a saline flush of 20 ml at the same flow rate of 2 ml/s. All the precontrast and postcontrast image series were acquired with an axial T1-weighted 3D image scanning model. The imaging parameters were as follows: repetition time (TR)/echo time (TE), 4.5/1.6 ms; flip angle, 10°; acquisition matrix, 896 × 896; slice thickness, 0.8 mm in the MRI scan series; and spatial resolution, 0.38 × 0.38 × 0.8 mm.

DWI was performed before the precontrast MR scan using the following parameters: field of view (FOV): 104×320 mm; flip angle: 90°; repetition time (TR): 7000 ms; echo time (TE): 85 ms; slice thickness: 6 mm; spacing between slices: 6.6 mm; b values: 50 and 1000 sec/mm^2; acquisition matrix: 220×72; pixel bandwidth: 1196 Hz; in-plane resolution: 1.45×1.45 mm.

DW MR Imaging Analysis

DWI ADC maps were calculated for the whole breast on a pixel-by-pixel basis by the equation $ADC=-\ln[S_I/S_0]/(b_1-b_0)$, where S_0 and S_I are the signal intensities obtained by gradient factors b_0=50 s/mm^2 and b_1=1000 s/mm^2, respectively. Breast tumour regions of interest (ROIs) were drawn on DWI at b=50 by a radiologist with 20 years of

experience. To standardize the image analysis as much as possible, a representative ROI was manually drawn around the margin of the tumour on the DWI slice with the largest tumour diameter, and the ROI was then copied to the corresponding ADC map (32). Fibroglandular tissue was segmented using a fuzzy C-means clustering procedure on the b=50 image of the DWI, excluding the skin and fatty tissue. The ADC images were manually segmented into the inner tumour, tumour boundary, and the proximal, middle and distal peritumoural stroma shells (Figure 1b, c, d). Detailed descriptions for these regions are given below.

Feature extraction

In our study, we selected the stromal shell with a 4-pixel width (1.45 mm × 4 pixels=5.8 mm) based on the resolution of 1.45 × 1.45 mm on DWI. As shown in Figure 1a, we defined 6 regions for analysis: 1) S_1: the inner section of the tumour with a 2-pixel width; 2) S_2: the entire tumour; 3) S_3: the boundary region with a 2-pixel width inside and outside of the tumour; 4) S_4: the proximal stromal shell with a 4-pixel width outside the tumour; 5): S_5: the middle stromal shell with a 4-pixel width outside the S_4 region; and 6) S_6: the distal stromal shell with a 4-pixel width outside the S_5 region. To evaluate the tumour heterogeneity of the signal distribution, imaging features including the mean, minimum, variance, interquartile range (IQR), range, skewness, kurtosis and entropy of ADC values were calculated in these regions. The IQR is the difference between the 75th and 25th percentiles of ADC distribution. The skewness evaluates the asymmetry of the histogram about its mean, while kurtosis measures whether the ADC values are heavy-tailed or light-tailed relative to a normal distribution. Finally, the entropy

measures the randomness of the ADC distribution. We also calculated the ratios of all eight imaging features between each pair of regions (i.e., S_i and S_j, where $i < j \leqslant 6$). For example, the ratio of skewness between the inner tumour (S_1) and the tumour boundary (S_3) was calculated, which was termed S_1/S_3-skewness.

Statistical Analysis

Differences in the tumour characteristics were assessed using the χ^2 test or Fisher's exact test if the expected value in any cell of the table was less than five. Analysis of variance (ANOVA) was performed to evaluate the relationships between ADC values and histopathological variables. Univariate logistic-based classifier was utilized to evaluate the performance of the individual features to discriminate subtypes. Stepwise multinomial multivariate logistic regression modelling was performed to identify imaging features that optimally differentiate among the molecular subtypes. To evaluate the discriminative abilities of the models, receiver operating characteristic (ROC) analysis was performed, and the area under the ROC curve (AUC) was computed. The Kruskal–Wallis test was used to test the statistical significance of features selected for the four breast cancer molecular subtypes. All statistical tests were two-tailed, and significance was set at a P value < 0.05. In multiple comparison tests, P values were adjusted using the Bonferroni correction method. Statistical analyses were performed using MATLAB R2014a (MathWorks Inc, Natick, MA, USA).

Results

Patient clinicopathological characteristics

Table 1 summarizes the clinicopathological features among the breast cancer subtypes.

All patients were Han Chinese with a median age of 51 years (range: 27–71 years). These tumours were divided into 26 (20.6%) luminal A, 67 (53.2%) luminal B, 22 (17.5%) HER2-enriched and 11 (8.7%) basal-like tumours. In the ER/PR positive, HER2 negative cases, most (42 out of 68) were identified as luminal B tumour due to high Ki-67 expression. The other Luminal B cases were determined (n=25) by high HER2 expression in ER/PR positive tumours. Among all the subtypes, luminal A showed the lowest Ki-67 expression, while the other subtypes exhibited high Ki-67 expression. ANOVA revealed a significant association between molecular subtype and Ki-67 expression level ($p<0.001$) and maximum tumour diameter ($p=0.035$) (Table 1).

ADC of the tumour and the stromal shells

In the statistical analysis, one-way ANOVA conducted within each subgroup indicated no significant correlation between ADC values and Ki-67 expression status, histological type or menopausal status (Table 2). Further analyses were conducted to assess signal patterns of ADC values in the tumour and the surrounding stroma, which are shown in Table 3. The ADC values in various regions ascended as their distance from the tumour centre increased (Table 3 and Figure 2) for all the four subtypes. HER2-enriched tumours had the highest ADC value among all tumours and stromal shells. One-way ANOVA showed significant differences in the ADC values among the subtypes of breast cancer patients in all regions except for the proximal and distal peritumoural stromal shells. A follow-up post hoc correction for multiple comparisons was carried out to determine significant differences, as shown in Supplementary Table S1. The results revealed significant differences in the ADC values between luminal B

and HER2-enriched tumours in the inner tumour, the entire tumour and the tumour boundary with corrected p values <0.0001, 0.001 and 0.047, respectively.

Table 3 also shows the significant differences in the mean ADC values among various regions for luminal A, luminal B, HER2-enriched and basal-like subtypes, which corresponded to p values <0.0001, <0.0001, 0.0003 and 0.009, respectively. The Bonferroni-corrected p values for multiple comparisons of mean ADC values are shown in Supplementary Table S2. Interestingly, significant differences in the mean ADC values were found between regions with relatively larger distance (i.e., S_1 and S_5, S_1 and S_6, S_2 and S_5, S_2 and S_6) in luminal A, luminal B and HER2-enriched tumours, while additional significant differences in the mean ADCs were also observed between regions with smaller distances (i.e., S_2 and S_3, S_2 and S_4, S_3 and S_4, S_3 and S_5) in luminal B tumours. For the basal-like subtype, a significant difference in ADC values was observed only between the inner (S_1) and distal (S_6) peritumoural stromal shells (corrected p value=0.019).

Univariate analysis to discriminate among breast cancer subtypes

We evaluated the performance of individual imaging features for discriminating among breast cancer subtypes by univariate logistic regression analysis. Table 4 provides the best two individual statistical features and four ratio features for discrimination of the four molecular subtypes. The results suggested that a lower level of skewness of the ADC value in the tumour boundary is more likely to correspond to the luminal A subtype, with an AUC of 0.718, and a 95% confidence interval (CI) from 0.602 to 0.834 (Table 4 and Figure 3). Additionally, a higher value of ADC IQR in the tumour

boundary is associated with the HER2-enriched subtype, and the univariate logistic regression classifier yielded an AUC of 0.703 with a 95% CI from 0.563 to 0.844 (Table 4 and Figure 3). The highest performance among imaging features for discriminating basal-like tumours from other tumour subtypes was the maximum ADC value in the inner tumour region (AUC=0.687, 95% CI from 0.532 to 0.843).

Table 4 also presents five ratio features in the tumour and the surrounding stromal shells. The ratio of the IQR of the ADC between the entire tumour and the proximal stromal shell exhibited the highest performance for discriminating the luminal A and luminal B subtypes with AUCs of 0.716 and 0.698, respectively. The ratio of the minimum ADC value between the inner tumour and the middle stromal shell performed best to predict the HER2-enriched subtype at an AUC of 0.698 and a 95% CI from 0.606 to 0.790. Finally, the ratio of the variance of the ADC value between the proximal stromal shell and the middle stromal shell showed an AUC of 0.716, which was the individually best performing feature for predicting the basal-like subtype (Figure 3).

The Kruskal–Wallis test showed a statistically significant difference in the IQR in the entire tumour among the four molecular subtypes (corrected p=0.0081). Additionally, the ratio of the IQR between the tumour boundary and the proximal peritumoural stromal shell showed significant differences among the four subtypes with a corrected p=0.0081 (Figure 4). The detailed features that were significantly different among the breast subtypes with corrected p value less than 0.05 are listed in Supplementary Table S3.

Multinomial multivariate logistic regression analysis to classify molecular subtypes

For each region, multivariate stepwise logistic regression analysis was performed to produce the best subset of features to discriminate among the breast subtypes. Table 5 summarizes the performance of the model using features in the tumour and the surrounding shells for classification. For the tumour boundary, the multivariate logistic classifier using statistical features had an overall AUC of 0.677 for multiclass classification and AUCs of 0.729, 0.636, 0.756, and 0.645 for predicting the luminal A, luminal B, HER2-enriched and basal-like subtypes, respectively, which implies better predictive performance than that of other regions. The predictive model with combined statistical features in all the regions generated an overall AUC value of 0.774 (Table 5). The statistical features included in the predictive model were the IQR and skewness of ADC in the entire tumour, the skewness in the tumour boundary, the mean and kurtosis of ADC in the proximal stromal shell, and the entropy in the middle stromal shell.

In addition, the ratio features computed between two regions were also combined and exhibited an overall AUC of 0.763 (Table 5). The results indicated that the overall performance of the ratio features is comparable to the statistical features in these regions. The statistical features and ratio features were then combined in a stepwise multivariate analysis. The analysis identified seven statistical features and eight ratio features for multiclass classification of the four molecular subtypes and achieved an overall AUC of 0.800 with AUCs of 0.850, 0.766, 0.820, and 0.849 for predicting luminal A, luminal B, HER2-enriched and basal-like subtypes, respectively (Supplementary Table S4). Figure 5 shows the ROC curves representing the classification performances of applying the statistical features, ratio features and all features to classify the subtypes

of luminal A, luminal B, HER2-enriched and basal-like tumours, respectively.

Discussion

We evaluated the associations of imaging features in the tumour and the surrounding stroma with molecular subtypes in breast cancer patients. An ascending trend in the mean ADC values in stromal shells was found with increasing distance from the tumour, indicating improved water diffusion. Multivariate logistic regression classification indicated that the imaging features in the entire tumour, tumour boundary and proximal stromal shell contributed more to classification performance than those in the other regions.

Clinically, the four molecular subtypes and Ki-67 proliferation factor is generally determined by IHC (30). Ki-67 is a key biomarker for luminal B tumours in ER positive cases. In our dataset, most (42 out of 67) of ER positive, HRE2 negative cases are identified as luminal B subtype due to high Ki-67 expression. In addition, the luminal B tumours can be identified by HER2 high expression, while the major biological distinction between luminal A and B is the Ki-67 status (2). Our dataset showed more cases of luminal B determined by Ki-67 proliferation index than that of the HER2 status, which is consistent with the previous study (2).

Several studies have recently sought to identify DWI biomarkers that can predict molecular subtypes of breast cancer. These studies have reported that tumour ADC values are correlated with the subtypes of breast cancer, and HER2-enriched tumours have the highest ADC values among the four subtypes (20, 33). Our results are in line with those studies in that HER2-enriched tumours and stromal regions showed the

highest mean ADC values, while the luminal A subtype had the second highest values. A possible explanation is that HER2 overexpression accelerates cell growth and induces angiogenesis, leading to increased blood flow in tumours and increased ADC values (34). Studies have found associations between tumour ADC values and cancer aggressiveness biomarkers such as Ki-67 (23, 28). Because most luminal B samples had high Ki-67 expression in the present study, our result that the luminal B subtype had the lowest ADC value in the inner and entire tumour is partially consistent with the findings in the previous study.

Computer-extracted histogram-based imaging features have been shown to correlate with tumour heterogeneity (35). Mori et al. correlated the features of ADC histograms with Ki-67 expression (36). Kim et al. found that the 10th percentile, mean, median, 90th percentile, and maximum ADC features, but not the minimum ADC, were correlated with prognostic factors such as Ki-67 expression and breast cancer subtypes (20). Previous studies have also reported that the ADC range of the tumour boundary is associated with the prognostic factors of breast cancer (28, 29). However, these studies did not examine features such as skewness, kurtosis, and IQR of ADC values, which represent the heterogeneity of the signal distribution in separate regions. In our study, HER2-enriched tumours had the highest IQR in the tumour boundary among the breast cancer subtypes. We also observed a lower ADC skewness in the tumour boundary for the luminal A subtype than for the other subtypes, and this individual feature had the highest performance. The results are consistent with those of a similar study using the DCE-MRI technology in which lower skewness is more likely to indicate a luminal A

tumour (12). The results revealed that imaging features reflecting a heterogeneous distribution of ADC signals in the stroma around the tumour could be valuable for identifying subtypes in breast cancer.

Our study had several limitations. First, this study was retrospective in nature, and our patient numbers were relatively small, which could cause some selection bias. Second, we only focused on the diffusion characteristics of breast cancer and did not evaluate the features of DCE-MRI, which is valuable for discriminating among molecular subtypes in clinical practice (12, 13). Third, when quantifying DWI regions, we manually drew ROIs on the tumours and peritumoural stromal shells, which may not accurately reflect the characteristics of the breast stroma. Further prospective investigations are needed to validate the clinical usefulness of our work.

In conclusion, DWI parameters, including the ADC, were significantly associated with molecular subtypes in breast cancer. Classification analysis revealed that lower ADC skewness of the tumour boundary was the strongest predictor of luminal A, while the IQR of the ADC value in the tumour boundary was among the best predictors of HER2-enriched tumours. In multiclass classification experiments, the statistical features representing the heterogeneity of the tumour environment are of vital importance for classification. However, further work is needed before these quantitative MRI parameters can be used to non-invasively assess the intrinsic characteristics in breast cancer patients in clinical practice.

Acknowledgements

This work was supported in part by funding from the National Natural Science

Foundation of China (61401131, 61731008) and the Natural Science Foundation of Zhejiang Province of China (LZ15F010001).

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Tables

Table 1. Tumour characteristics

Characteristic	All 126	Luminal A (n=26) (%)	Luminal B (n=67) (%)	HER2-enriched (n=22) (%)	Basal-like (n=11) (%)	P value
Ki67[a]						<0.001
High (≥14%)	88	0 (0)	60 (48)	18 (14)	10 (8)	
Low (<14%)	38	26 (20)	7 (6)	4 (3)	1 (1)	
Histologic type[a]						0.854
Ductal	104	21 (17)	55 (44)	18 (14)	10 (8)	
Intraductal	9	2 (1.5)	5 (4)	2 (1.5)	0 (0)	
lobular	4	1 (0.8)	2 (1.5)	0 (0)	1 (0.8)	
N/A	9	2 (1.5)	5 (4)	2 (1.5)	0 (0)	
Menopausal status[a]						0.026
Premenopausal	8	5 (4)	2 (2)	0 (0)	1 (0.8)	
Postmenopausal	98	18 (14)	54 (43)	19 (15)	7 (5)	
N/A	20	3 (2)	11 (9)	3 (2)	3 (2)	
Age (y)[b]	51.66 (27-71)	53.2 (36-71)	50.64 (27-70)	52.45 (40-67)	52.73 (37-67)	0.628
Tumour volume (mm^3) [b]	15448 (180-96000)	9815 (180-49000)	15011 (560-72000)	32019 (1200-96000)	15254 (560-42000)	0.084
Maximum tumour diameter (mm)[b]	28.76 (10-80)	24.64 (12-70)	28.55 (10-70)	38.38 (12-80)	25.63 (11-40)	0.035

[a]Data were tested using the Fisher-exact test.

[b]Data were tested using one-way ANOVA.

Numbers in parentheses are range.

N/A, Not available

Table 2. Comparison of the ADC values according to histopathological features

Characteristic	Number	Mean ADC	P value[a]
Ki67			0.882
High (≥14%)	88	0.923±0.227	
Low (<14%)	34	0.939±0.204	
N/A	4	0.971±0.025	
Histologic type			0.302
Ductal	104	0.918±0.224	
Intraductal	9	1.061±0.186	
lobular	4	0.909±0.134	
N/A	9	0.929±0.164	
Menopausal status			0.059
Premenopausal	8	0.947±0.089	
Postmenopausal	98	0.906±0.192	
N/A	20	1.039±0.331	

[a]p values of one-way ANOVA showed differences in ADC values according to histopathological features.

N/A, not available

Table 3. Comparison of the mean ADC values in the tumour and the surrounding stromal shells

	Mean ADC value				
Proximity maps	Luminal A	Luminal B	HER2-enriched	Basal-like	p value[a]
S_1	0.942±0.181	0.858±0.160	1.067±0.324	0.879±0.209	0.0007
S_2	0.966±0.187	0.865±0.159	1.074±0.313	0.953±0.243	0.001
S_3	1.025±0.196	1.007±0.233	1.175±0.328	1.125±0.308	0.041
S_4	1.091±0.261	1.151±0.334	1.310±0.344	1.169±0.285	0.079
S_5	1.208±0.207	1.324±0.279	1.347±0.232	1.172±0.219	0.036
S_6	1.304±0.214	1.386±0.256	1.383±0.184	1.232±0.179	0.121
p value[b]	<0.0001	<0.0001	0.0003	0.009	

[a]One-way ANOVA for differences in the ADC values among the subtypes of breast cancer patients in each region.

[b]One-way ANOVA for differences in the ADC values among various regions for each molecular subtype.

Table 4. Univariate analysis of the best two individual statistical features and four ratio features in distinguishing molecular subtypes

Region	Luminal A		Luminal B		HER2-enriched		Basal-like	
	Feature	AUC	Feature	AUC	Feature	AUC	Feature	AUC
	Statistical feature							
S_1	max	0.586(0.449-0.723)	IQR	0.656(0.558-0.753)	min	0.687(0.553-0.821)	IQR	0.548(0.341-0.657)
	mean	0.529(0.387-0.642)	mean	0.633(0.534-0.732)	max	0.563(0.428-0.698)	mean	0.523(0.351-0.656)
S_2	skewness	0.649(0.512-0.787)	**IQR**	**0.688(0.585-0.778)**	min	0.688(0.552-0.825)	entropy	0.566(0.369-0.762)
	kurtosis	0.563(0.440-0.686)	min	0.643(0.543-0.743)	max	0.584(0.449-0.719)	max	0.539(0.357-0.721)
S_3	**skewness**	**0.718(0.602-0.834)**	skewness	0.640(0.542-0.737)	**IQR**	**0.703(0.563-0.844)**	range	0.591(0.411-0.772)
	max	0.628(0.503-0.755)	IQR	0.620(0.521-0.719)	min	0.673(0.531-0.815)	mean	0.538(0.357-0.719)
S_4	kurtosis	0.708(0.600-0.815)	range	0.615(0.516-0.715)	IQR	0.576(0.441-0.710)	max	0.579(0.407-0.751)
	mean	0.658(0.543-0.772)	max	0.619(0.520-0.718)	mean	0.573(0.442-0.704)	IQR	0.512(0.288-0.637)
S_5	mean	0.570(0.453-0.687)	max	0.597(0.498-0.697)	IQR	0.530(0.405-0.655)	**max**	**0.687(0.532-0.843)**
	skewness	0.531(0.418-0.645)	range	0.591(0.429-0.691)	mean	0.557(0.436-0.678)	mean	0.607(0.456-0.757)
S_6	IQR	0.575(0.459-0.691)	max	0.560(0.458-0.661)	variance	0.548(0.428-0.669)	max	0.676(0.552-0.802)
	mean	0.559(0.435-0.684)	mean	0.531(0.429-0.633)	kurtosis	0.533(0.406-0.661)	variance	0.648(0.486-0.811)
	Ratio feature							
	S_2/S_4-IQR	**0.716(0.625-0.811)**	**S_2/S_4-IQR**	**0.698(0.606-0.790)**	**S_1/S_5-min**	**0.698(0.565-0.832)**	**S_4/S_5-var**	**0.716(0.552-0.802)**
	S_3/S_4-IQR	0.708(0.608-0.807)	S_3/S_4-IQR	0.684(0.602-0.787)	S_3/S_5-min	0.677(0.534-0.819)	S_4/S_5-max	0.690(0.505-0.876)
	S_2/S_4-mean	0.675(0.568-0.781)	S_2/S_4-mean	0.685(0.592-0.778)	S_3/S_6-min	0.674(0.532-0.817)	S_3/S_6- max	0.684(0.516-0.851)
	S_1/S_4- IQR	0.665(0.560-0.769)	S_3/S_4-mean	0.671(0.576-0.766)	S_1/S_6-IQR	0.659(0.535-0.783)	S_4/S_5-range	0.681(0.502-0.864)
	S_3/S_4-mean	0.630(0.509-0.750)	S_1S_5-IQR	0.675(0.581-0.770)	S_3/S_4-min	0.653(0.522-0.785)	S_3/S_5-entropy	0.670(0.481-0.860)

Note: S_1: inner section of the tumour (-2 pixels); S_2: entire tumour; S_3: tumour boundary from -2 to 2 pixels; S_4: peritumour stromal shell of 4 pixels outside the tumour; S_5: middle stromal shell of 4 pixels outside S_4; S_6: distant stromal shell of 4 pixels outside S_5. The ratio feature, for example, S_2/S_4-IQR stands for the IQR between the S_2 and the S_4 regions.

IQR: Interquartile range.

A multivariate logistic regression analysis was performed. Numbers in parentheses are 95% CIs for AUC values.

The highest AUC value for the imaging feature in discriminating each subtype are shown in bold.

Table 5. Performance of multiclass classification in tumour and peritumoural stromal shells

Feature class	Overall	Luminal A	Luminal B	HER2-enriched	Basal-like
S_1	0.631	0.630 (0.516-0.744)	0.652 (0.555-0.749)	0.648 (0.575-0.821)	0.471 (0.282-0.659)
S_2	0.634	0.602 (0.476-0.728)	0.653 (0.553-0.752)	0.685 (0.553-0.817)	0.501 (0.338-0.661)
S_3	**0.677**	0.729 (0.619-0.838)	0.636 (0.538-0.743)	0.756 (0.631-0.882)	0.645 (0.476-0.813)
S_4	0.654	0.795 (0.704-0.886)	0.623 (0.502-0.706)	0.616 (0.494-0.738)	0.587 (0.397-0.730)
S_5	0.571	0.618 (0.585-0.798)	0.517 (0.413-0.618)	0.516 (0.387-0.643)	0.671 (0.517-0.825)
S_6	0.554	0.621 (0.502-737)	0.561 (0.460-0.663)	0.558 (0.442-0.674)	0.582 (0.412-0.753)
Statistical features	0.774	0.842 (0.767-0.917)	0.746 (0.657-0.834)	0.764 (0.649-0.879)	0.802 (0.692-0.911)
Ratio features	0.763	0.794 (0.706-0.883)	0.713 (0.622-0.804)	0.816 (0.713-0.920)	0.893 (0.786-0.998)
All features	**0.800**	0.850 (0.775-0.925)	0.766 (0.683-0.849)	0.820 (0.723-0.916)	0.849 (0759-0.939)

Multivariate logistic regression was performed. Numbers in parentheses are 95% CIs for AUC values.

Figure legends

Figure 1. Schematic illustration of the tumour and stromal shells and an example of a left breast of a patient. (a) Schematic illustration of the tumour and stromal shells. (b) DWI at b=50 s/mm^2 shows a mass with a high signal intensity (red circle). (c) Fibroglandular tissue was segmented in the image (b). (d) An ADC image of a breast masked by the fibroglandular map (c). (e) The inner tumour, tumour margin and proximal peritumoural stromal ADC map were segmented in the ADC image (d).

Figure 2. Boxplot of the mean ADC values in the tumour and peritumoural stromal shells for (a) luminal A, (b) luminal B, (c) HER2-enriched, and (d) basal-like tumour subtypes.

Figure 3. Distribution of individual features according to histopathological subtype. The features include (a) skewness of the ADC values in the tumour boundary, (b) ratio of the ADC IQR between the entire tumour and the proximal peritumoural stroma, (c) ADC IQR in the tumour boundary, and (d) the ratio of variances of the ADC values between the proximal and middle peritumoural stroma. The box extends from the 25th to 75th percentile. The line is the median feature value.

Figure 4. Distribution of individual features according to histopathological subtype. The features include (a) the interquartile of ADC values in the entire tumour and (b) the ratio of the interquartile of ADC values between the tumour boundary and the proximal peritumoural stromal shell. The box extends from the 25th to 75th percentile. The line is the median feature value.

Figure 5. ROC curves for the statistical features, ratio features and combined features in the (a) luminal A; (b) luminal B; (c) HER2-enriched and (d) basal-like tumour subtypes.