Heterogeneity of enhancement kinetics in dynamic contrast-enhanced MRI and implication of distant metastasis in invasive breast cancer

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\textbf{AIM:} To investigate the heterogeneity of enhancement kinetics for breast tumour in order to demonstrate the predictive power of dynamic contrast-enhanced (DCE) magnetic resonance imaging (MRI) features for distant metastasis (DM) in invasive breast cancer.

\textbf{MATERIALS AND METHODS:} Time–signal intensity curve (TIC) patterns from 128 patients with invasive breast cancer were analysed by a pixel-based DCE–MRI analysis. This MRI technique enabled pixels with varying TIC patterns (persistent, plateau, washout and non-enhancement) to be categorised semi-automatically and the percentage of different TIC patterns in each breast tumour to be calculated. The percentage of TIC patterns was compared between the DM and non-DM groups. DM-free survival was estimated using Kaplan–Meier survival analysis.

\textbf{RESULTS:} This study demonstrated a larger percentage of persistent TIC and non-enhancement TIC was associated with DM in invasive breast cancer. The cut-off values of persistent TIC and non-enhancement TIC were 22.5% and 2.5%. Combining TIC patterns and traditional predictors (tumour size and axillary lymph node status) can improve the prediction efficiency. The multivariable model yielded an area under the receiver operating characteristic curve (AUC) of 0.87 with 0.70 sensitivity and 0.87 specificity in leave-one-out cross-validation (LOOCV). These predictors showed significant differences in DM-free survival by Kaplan–Meier analysis.

\textbf{CONCLUSION:} This study shows that breast tumours with higher heterogeneity are more likely to metastasise, and pixel-based TIC analysis has utility in predicting distant metastasis of invasive breast cancer.

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Introduction

Distant metastasis (DM) is the main cause of death for breast cancer patients. Breast cancer metastasis can occur years after apparently successful treatment, emphasising the importance of efficient clinical management of the disease. Thus, it is important that the clinician has as much prognostic information as possible to monitor possible relapse and develop individualised treatment. From previous studies, the traditional predictive factors associated with DM included younger age, large tumour size, the presence of axillary nodal metastasis, negative oestrogen receptor (ER) expression and human epidermal growth factor receptor 2 (HER2) overexpression; however, the established prognostic markers do not accurately predict the risk of DM in breast cancer, so new prognostic markers are urgently needed to identify patients who are at high risk of DM.

Dynamic contrast-enhanced (DCE) magnetic resonance imaging (MRI) features can indirectly reflect the tumour proliferation rate and growth of the tumour by providing morphological and kinetic features. Preoperative DCE-MRI of primary breast tumours has the potential to aid prognostic evaluation. Several studies have confirmed the prognostic value of DCE-MRI parameters to predict the occurrence of metastasis for breast cancer, and some of these studies have also performed survival analysis.

Breast cancer is highly heterogeneous both genetically and histopathologically, and intratumoural heterogeneity has been considered to be a factor related to poor prognosis, owing to resistance to therapy. It is necessary to explore risk factors associated with prognosis of breast cancer from the perspective of heterogeneity. Region of interest (ROI)-based analysis cannot take into account intratumoural heterogeneity; therefore, pixel-by-pixel analysis may be a useful method to provide detailed information about the tumour response in different areas.

The present study sought to investigate the kinetic features associated with distant metastasis in invasive breast cancer by using pixel-based DCE-MRI analysis. This MRI technique enabled pixels with varying time–signal intensity curves (TIC) patterns to be categorised semi-automatically and the percentage of different TIC patterns in each breast tumour to be calculated. The aims of this study were to correlate TIC patterns with the occurrence of distant metastasis in invasive breast cancer and to investigate whether TIC patterns were associated with DM-free survival.

Methods and materials

Patients

The present retrospective study was approved by the ethical committee of hospital, and informed consent for all patients was obtained prior to the MRI examinations. The institution's database was searched for consecutive patients who underwent preoperative MRI between August 2011 and October 2016, and were newly diagnosed with invasive breast cancers, resulting in 2,531 being identified. Among them, the following patients were excluded: those who had received needle biopsy (n=429) or neoadjuvant chemotherapy (n=136) prior to MRI; those with distant metastasis before operation (n=14); those with synchronous or metachronous contralateral cancer (n=30). In this study, distant metastasis (DM) referred only to distant organ metastasis and did not include patients with ipsilateral or contralateral breast recurrence, local chest wall recurrence, and isolated regional lymph node metastasis. Of the 1,922 patients, 64 patients with DM were included in DM group. Due to the large quantity difference between patients with DM and without DM, a random number table method was used to select 64 patients on a one-to-one ratio as the non-DM group.

Each primary breast tumour was diagnosed by using core-needle biopsy analysis. Of these, 125 patients had invasive ductal cancer and three patients had mixed breast cancer. DM was confirmed by histopathological analysis in 13 patients and by imaging follow-up in 51 patients. Of the 64 patients with DM, 47 patients with single-organ metastasis and 17 patients with multiple-organ metastasis, bone (n=40) was the most frequent metastatic site followed by the lung (n=20), liver (n=16), and brain (n=7). Follow-up time for each patient was calculated in months from the date of the surgery to the date of last visit. The median follow-up time of patients in the non-DM group was 48 months (range, 25–88 months). DM-free survival was defined as the interval between the date of breast cancer operation and the date of DM. The median time to DM-free survival was 27 months (range, 1–64 months).

MRI technique

Sagittal DCE-MRIs were performed by using 1.5 or 3 T MRI machines (Signa, GE Medical Systems, Milwaukee, WI, USA) in the prone position. All examinations contained T1-weighted unenhanced and five or eight contrast-enhanced sequences. The five contrast-enhanced series were acquired with intervals of 90 seconds, and the eight contrast-enhanced series were acquired with intervals of 60 seconds. Gadolinium-based contrast material (Meglumine Gadopentatate, Magnevist, Bayer Healthcare, Germany) was injected intravenously at a dose of 0.2 ml/kg and at an injection rate of 2 ml/s.

Pixel-based TIC analysis

ROIs were delineated manually on a single section containing the largest cross-section of tumour regions and then tumour regions were extracted using ImageJ software (http://imagej.nih.gov/ij/). For the most invasive cancers,
the first 2 minutes after the injection of contrast medium is the period when the contrast between tumour and background is at its peak, so the ROI was firstly placed manually on the first contrast-enhanced phase of DCE sequence, and then was copied to the unenhanced phase and remaining contrast-enhanced phases. The TIC analysis was performed using MatlabR2018a (MathWorks, Natick, MA, USA) on pixel-by-pixel analysis. For three patients with multifocal carcinoma and four patients with multicentric carcinoma, this study was confined to the lesion with the highest tumour stage or the largest lesions with the same tumour stage.

DCE sequences have T time points, the signal intensity at each time point is $S(0), S(1), ..., S(T-1)$, where $S(t)$ is the signal intensity at time point $t (t=0,1,...,T-1)$. Denoting $S^*$ as the peak of the signal intensity in the first 120 seconds after contrast medium injection, $S(T_{peak})$ as the peak of the signal intensity in the whole enhancement process. Different types of TICs are automatically classified on the basis of maximum enhancement ratio ($E_{R_{max}}$) and washout ratio ($W_R$), these TIC parameters were calculated by the following equations:

$$E_{R_{max}}(\%) = \frac{S(T_{peak}) - S(0)}{S(0) \times 100\%} \quad W_R(\%) = \frac{S^* - S(T_{120})}{S^* - S(0)} \times 100\%.$$ 

TICs are categorised into four type patterns (Fig 1), they are Type 1 (persistent), Type 2 (plateau), Type 3 (washout) and Type 4 (non-enhancement). Let $P_i$ represent the $i$th pixel in the segmented tumour area, the following criteria were used to group the pixels:

$$\begin{align*}
P_i \in \text{Type 1} & \text{ if } E_{R_{max}} > 10\% \text{ and } W_R < -10\% \\
P_i \in \text{Type 2} & \text{ if } E_{R_{max}} > 10\% \text{ and } -10\% \leq W_R \leq 10\% \\
P_i \in \text{Type 3} & \text{ if } E_{R_{max}} > 10\% \text{ and } W_R > 10\% \\
P_i \in \text{Type 4} & \text{ if } E_{R_{max}} \leq 10\%
\end{align*}$$

(1)

Then a TIC map was created as the pixel’s membership determined by equation 1 for each pixel in the tumour area. A pixel was marked as blue, green, red, or grey if it was a member of Type 1, Type 2, Type 3 or Type 4, respectively (Figs 2 and 3).

**Statistical analysis**

Comparisons between DM group and non-DM group were made with the Mann–Whitney U test, chi-square test, or Fisher’s exact test. Multivariate logistic regression analysis was performed to identify the DCE-MRI features and clinicopathological features that could be used as important predictive indicators for DM risk in patients with breast cancer. The results were evaluated by using odds ratios and 95% confidence intervals (95% CI). Receiver operating characteristic (ROC) curve analysis and the area under the
ROC curve (AUC) were used to evaluate the performance of TIC patterns and clinicopathological features for DM prediction. The performance of the multivariable model was assessed by leave-one-out cross-validation (LOOCV) in terms of AUC, sensitivity and specificity. The features found to differ significantly across the DM group and non-DM group were further estimated with Kaplan–Meier survival plots. The log-rank test was used to analyse the difference in survival.

All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS, version 22; Chicago, IL, USA); p-values <0.05 were considered significant.

Results

Patient characteristics

Patient characteristics are listed in Table 1 for the DM group and non-DM group. The significantly greater tumour sizes were found in DM group than non-DM group (30.4 ± 14.1 versus 20.9 ± 7.4 mm, p<0.001). The DM group also had a larger proportion of positive axillary lymph nodes than the non-DM group (61% versus 22%, p<0.001). There were no significant differences in age, disease extent, tumour type, molecular subtyping, ER status, and HER2 status between the DM group and non-DM group (Table 1).

Pixel-based TIC analysis

There was no significant difference in the percentage of Type 2 (plateau) TIC between the DM group and the non-DM group (p=0.884); however, the percentage of Type 1 (persistent) TIC patterns and Type 4 (non-enhancement) TIC patterns was significantly larger in the DM group than in the non-DM group (30.5% versus 10%, p<0.001 and 10% versus 2.4%, p<0.001, respectively). In contrast, the percentage of Type 3 (washout) TIC patterns was significantly smaller in the DM group than in the non-DM group (43% versus 70%, p<0.001; Table 2).

Multivariate logistic regression analysis

A Mann–Whitney U-test demonstrated that the percentage of Type 1, 3, or 4 TIC patterns, tumour size, and axillary lymph node status significantly correlated with the distant organ metastasis in breast cancer. Hence, these five variables were selected for multivariate logistic regression.
analysis. Multivariate logistic regression analysis demonstrated that the percentage of Type 1 TIC patterns (OR = 1.087, 95% confidence interval [CI]: 1.015, 1.164; \( p = 0.017 \)), the percentage of Type 4 TIC patterns (OR = 1.054, 95% CI: 1.004, 1.107; \( p = 0.034 \)), tumour size (OR = 1.073, 95% CI: 1.009, 1.140; \( p = 0.025 \)), and axillary lymph node status (OR = 4.135, 95% CI: 1.533, 11.152; \( p = 0.005 \)) were significantly important for independently predicting DM (Table 3).

### Diagnostic abilities analysis

The best single feature performance was the percentage of Type 1 TIC (AUC = 0.79), followed by tumour size (AUC = 0.75), axillary lymph node status (AUC = 0.69), and the percentage of Type 4 TIC (AUC = 0.66). Combining the percentage of Type 1 TIC and Type 4 TIC had an AUC of 0.81, higher than that of combining tumour size and axillary lymph node status (AUC = 0.79), demonstrating the prediction efficiency of pixel-based TIC patterns were superior to clinicopathological features. In LOOCV, AUC was 0.87 when using all four features to predict the DM risk in breast cancer, this multivariable model was demonstrated a sensitivity of 0.70 and a specificity of 0.87 (Table 4).

### Survival analysis

The cut-off values were investigated using the Youden index. The cut-off values of the percentage of tumour area with Type 1 TIC and Type 4 TIC were 22.5% and 2.5%, and the cut-off value of tumour size was 21.1 mm. Kaplan–Meier analysis and log-rank comparisons revealed that higher percentage of Type 1 TIC (≥22.5%): HR = 3.58, 95% CI: 2.51, 7.73; \( p < 0.001 \), higher percentage of Type 4 TIC (≥2.5%): HR = 2.36, 95% CI: 1.55, 5.05; \( p = 0.004 \), larger tumour size (≥21.1mm: HR = 3.49, 95% CI: 1.99, 5.60; \( p < 0.001 \)) and positive axillary lymph node (HR = 2.80, 95% CI: 1.85, 5.57; \( p < 0.001 \)) were significantly associated with worse DM-free survival (Fig 4).

### Discussion

The present study demonstrated that TIC patterns were effective predictors of DM of invasive breast cancer. The results of the present study indicated that a higher percentage of Type 1 (persistent) TIC, higher percentage of Type 4 (non-enhancement) TIC, larger tumour size, and positive axillary lymph nodes were more common in the DM group and were associated with worse DM-free survival. Compared with the other three features, the percentage of Type 1 TIC had the highest AUC. A combination of all four features can obtain high AUC, and this multivariable model also had favourable sensitivity and specificity in LOOCV.

Many previous studies showed that the TIC can identify benign and malignant breast lesions effectively. Researchers suggest malignant lesions always have strong contrast enhancement and washout, so they more often show Type 2 (plateau) TIC and Type 3 (washout) TIC, and the latter is highly predictive of malignant lesions. In contrast, Type 1 (persistent) TIC corresponds to steadily increasing contrast enhancement over time and is more often found in benign lesions. It was an interesting finding that a higher percentage of Type 1 TIC was associated with DM in invasive breast cancer; however, the present study did not identify the mechanism. Type 1 TIC might be caused by the presence of a fibrotic focus (FF), which is a major histological prognostic factor for breast cancer. The proliferative activity of the fibroblasts forming the FF plays an important role in DM, and tumours with an FF are associated with poorer short- and long-term survival. Intratumoural hypoxia of tumours with an FF is an important driving force of angiogenesis. Hypoxia can stimulate the production of vascular endothelial growth factor (VEGF) directly through hypoxia inducible factor-1 (HIF-1)-induced transcription and indirectly through the accumulation of tumour associated macrophages (TAM) in hypoxic regions. VEGF induces microvascular permeability, then it attracts an influx of fibroblasts, inflammatory cells, and endothelial cells, which are conducive to initiating tumour

### Table 2
Pixel-based TIC patterns analysis.

<table>
<thead>
<tr>
<th>TIC patterns</th>
<th>Percentage (%)</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DM group (n=64)</td>
<td>Non-DM group (n=64)</td>
</tr>
<tr>
<td>Type 1</td>
<td>30.5±22.6</td>
<td>10.0±10.1</td>
</tr>
<tr>
<td>Type 2</td>
<td>18.3±10.7</td>
<td>17.6±15.3</td>
</tr>
<tr>
<td>Type 3</td>
<td>43.0±25.7</td>
<td>70.0±21.8</td>
</tr>
<tr>
<td>Type 4</td>
<td>10.0±17.2</td>
<td>2.4±7.1</td>
</tr>
</tbody>
</table>

TIC, time—signal intensity curve.

### Table 3
Multivariate logistic regression analysis of different features for the prediction of distant metastasis.

<table>
<thead>
<tr>
<th>Features</th>
<th>Odds ratio (95% CI)</th>
<th>( p )-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIC patterns</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 1</td>
<td>1.087 (1.015, 1.164)</td>
<td>0.017</td>
</tr>
<tr>
<td>Type 2</td>
<td>1.009 (0.968, 1.052)</td>
<td>0.669</td>
</tr>
<tr>
<td>Type 3</td>
<td>1.054 (1.004, 1.107)</td>
<td>0.034</td>
</tr>
<tr>
<td>Tumour size</td>
<td>1.073 (1.009, 1.140)</td>
<td>0.025</td>
</tr>
<tr>
<td>Axillary lymph node status</td>
<td>4.135 (1.533, 11.152)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

CI, confidential interval; TIC, time—signal intensity curve.

### Table 4
ROC curve analysis showing AUC, sensitivity and specificity for features associated with DM.

<table>
<thead>
<tr>
<th>Features</th>
<th>AUC</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of Type 1 TIC</td>
<td>0.79</td>
<td>0.63</td>
<td>0.91</td>
</tr>
<tr>
<td>Percentage of Type 4 TIC</td>
<td>0.66</td>
<td>0.47</td>
<td>0.83</td>
</tr>
<tr>
<td>Tumour size</td>
<td>0.75</td>
<td>0.81</td>
<td>0.63</td>
</tr>
<tr>
<td>Axillary lymph node status</td>
<td>0.69</td>
<td>0.60</td>
<td>0.78</td>
</tr>
<tr>
<td>Percentage of Type 1 TIC + percentage of Type 4 TIC</td>
<td>0.81</td>
<td>0.71</td>
<td>0.82</td>
</tr>
<tr>
<td>Tumour size + axillary lymph node status</td>
<td>0.79</td>
<td>0.65</td>
<td>0.75</td>
</tr>
<tr>
<td>Combining all four features</td>
<td>0.87</td>
<td>0.70</td>
<td>0.87</td>
</tr>
</tbody>
</table>

AUC, area under the receiver operating characteristic (ROC) curve, TIC, time—signal intensity curve.
angiogenesis. These changes lead to tumour progression and invasion.

This study demonstrated that the percentage of Type 4 TIC was also associated with DM in breast cancer. The non-enhanced areas were caused by the presence of necrosis, which was an important histopathological feature of the primary tumour to predict DM. The rapid tumour growth rate beyond the nutritional capabilities of their blood supply, which is a possible reason of tumour necrosis. Necrosis is a form of cell death caused by factors external to the cell, such as hypoxia, as mentioned above, hypoxia is often associated with rapidly growing and aggressive forms of breast cancer.

In the present study, breast tumours of the non-DM group consisted mainly of Type 3 TIC (70%); however, Type 1 TIC and Type 4 TIC constituted a larger proportion of the DM group than the non-DM group, and the percentage of Type 1 TIC (30.5%) and Type 4 TIC (10%) was close to percentage of Type 3 TIC (43%) in DM group. Diversified types of TICs in the DM group were observed. Therefore, the present study demonstrated that the DM group had higher intratumoural heterogeneity than the non-DM group. Many sections of the tumour exhibited differences in TICs, which may have contributed to the development of DM.

Figure 4 Kaplan–Meier curves showing DM-free survival rate in 128 patients with invasive breast cancer. (a) The blue line represents DM-free survival in patients with percentage of Type 1 < 22.5% (n = 82; the green line represents DM-free survival in patients with percentage of Type 1 ≥ 22.5% (n = 46; p < 0.001). (b) The blue line represents DM-free survival in patients with percentage of Type 4 < 2.5% (n = 87; the green line represents DM-free survival in patients with percentage of Type 4 ≥ 2.5% (n = 41; p = 0.004). (c) The blue line represents DM-free survival in patients with tumour size < 21.1 mm (n = 57); the green line represents DM-free survival in patients with tumour size ≥ 21.1 mm (n = 71; p = 0.001). (d) The blue line represents DM-free survival in patients with negative axillary lymph node (n = 76; the green line represents DM-free survival in patients with positive axillary lymph node (n = 52; p < 0.001).
studies have suggested that tumours with high heterogeneity have poorer prognosis than those without high heterogeneity, so the present results are accordant with previous studies. 30,31 Tumour size and the axillary lymph node status are generally accepted as the most powerful clinical indicator for breast cancer prognosis. 32–34 Consistent with previous studies, the present study demonstrated that tumour size and axillary lymph node status are independent prognostic indicators, although other clinicopathological features including age, tumour type, disease extent, molecular subtyping, ER status, and HER2 status were not significant. In the present study, tumour size ≥21.1 mm and positive axillary lymph nodes were associated with worse DM-free survival.

There are several limitations to the present study. First, this study was limited by its retrospective nature, which meant that the imaging parameters, such as magnet strength and contrast medium administration protocol, could not be controlled. Second, the study cohort included a small number of patients from a single institution. Multi-institutional prospective studies would help to verify these results. Third, the analysis is based on a single section that was manually selected and subject to selection bias. In addition, the selection of the lesion in three patients with multifocal carcinoma and four patients with multicentric carcinoma may again be subjected to selection bias. Fourth, the histological type of all patients with DM was invasive ductal cancer. Therefore, the results might only be appropriate for patients with invasive ductal cancer, and the heterogeneity of other tumour types should be explored in further studies. Despite these limitations, it is encouraging to see strong associations of the DCE-MRI features with DM in invasive breast cancer.

In conclusion, this study demonstrated that TIC patterns were associated with DM through pixel-by-pixel analysis. Combining TIC patterns, tumour size, and axillary lymph node status can improve prediction efficiency. DCE-MRI features can be used as prognostic factors to predict DM risk in breast cancer, enabling monitoring for possible relapse in individual patients.

Conflicts of interest
The authors declare no conflict of interest.

Acknowledgements
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