DCE-MRI for early evaluation of therapeutic response in esophageal cancer after concurrent chemoradiotherapy and its values in predicting HIF-1α expression

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Abstract
To examine the feasibility of quantitative dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) in the early assessment of the therapeutic response to concurrent chemoradiotherapy (CRT) in esophageal cancer (EC) patients and to determine its value in predicting HIF-1α expression. EC patients underwent DCE-MRI 1 week pre-CRT and 3 weeks post-CRT (3w-CRT). According to tumor regression post-treatment, patients were divided into sensitive group (SG) and resistant group (RG). HIF-1α expression was assessed by immunohistochemistry (IHC). Quantitative parameters (ktrans, kep, and ve) were compared between the SG and RG groups, as well as between the HIF-1α(+) and HIF-1α(-/) groups. Receiver operating characteristic (ROC) curve analysis was performed to detect the best predictor of the above parameters in the therapeutic response and in predicting HIF-1α expression. Totally 34 and 5 patients were included in the SG and RG, respectively. Pre-ktrans and pre-kep were decreased significantly in the SG at 3w-CRT (p < .01), whereas only pre-kep was decreased in the RG (p = .037). Pre-ktrans was higher in the SG compared with the RG (p < .01). Meanwhile, absolute Δktrans (post-ktrans – pre-ktrans) was reduced more substantially in the SG compared with the RG. Δktrans also had the highest area under the curve (AUC = 0.929) in distinguishing SG from RG. Based on IHC, 13 and 11 patients were HIF-1α(+) and HIF-1α(-/), respectively. At 3w-CRT, post-ktrans was markedly lower than pre-ktrans in the HIF-1α(+) group (p < .01); however, both ktrans and kep in the HIF-1α(-/) group were dramatically reduced than pre-treatment values (both p < .01). Pre-ktrans was significantly higher in the HIF-1α (-) group compared with the HIF-1α(+) group (p = .002) and constituted an excellent predictor of therapeutic response and HIF-1α expression.

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1 | INTRODUCTION

It is estimated that there are 570,000 new diagnoses of esophageal cancer (EC) and 510,000 deaths per year, representing the sixth most common cause of cancer-related deaths worldwide. In China, it is also a leading cause of death, and the majority of cases are diagnosed at advanced stages and not eligible for surgery. At present, a definitive CRT has been given priority as the standard treatment for inoperable EC, and good response could increase patient survival. However, owing to individual differences and tumor heterogeneity, not all patients could benefit from the CRT approach. The treatment effect depends heavily on the response to CRT, and it is crucial to predict the response as early as possible to avoid side effects for timely adjustment of treatment strategies.

The main traditional imaging methods for evaluating the treatment response of EC patients administered CRT include esophagography and computed tomography (CT), which analyze pathological changes of the esophagus only for morphology, lagging behind biometric changes. Although fluorodeoxyglucose positron emission tomography/computed tomography (FDG-PET/CT) could reflect metabolic changes and is helpful in evaluating the treatment response to CRT in EC patients, radiation exposure and high cost are fatal disadvantages, especially for long-term follow-up.

Magnetic resonance imaging (MRI) has been widely used in tumor detection and treatment evaluation thanks to excellent soft tissue resolution and nonionizing radiation. Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) is an advanced MRI; beside the advantages of traditional MRI, it measures the properties of tumor microvascular structure and permeability, and evaluates the functional tissue information with quantitative parameters. In DCE-MRI examination, quantitative parameters such as ktrans (volume transfer constant in min⁻¹), kep (rate constant in min⁻¹), and ve (volume fraction of the extravascular extracellular space, which is dimensionless) could be obtained from pharmacokinetic models. Current studies have reported the potential role of DCE-MRI parameters in assessing the treatment response in different tumors as well as its value in predicting tumor-related biomarkers.

Regarding the prediction of tumor-related biomarkers, based on the advantages of DEC-MRI in determining vascular permeability, hypoxia-inducible factor-1-alpha (HIF-1α) was chosen as a research indicator in this study. As a core transcription factor under hypoxia in the microenvironment, HIF-1α is involved in mediating biological behaviors such as apoptosis, proliferation, and migration. Current reports have further suggested that hypoxic cells could not only increase the resistance of tumor cells to CRT, but also render the tumor more invasive and prone to metastasis. In addition, higher HIF-1α expression is associated with lower treatment response and reduced survival. Therefore, the prediction of HIF-1α expression would also contribute to early efficacy assessment in EC patients. At present, data reported about tumor hypoxia are typically dependent on the pathological method, which is an invasive and undesirable approach for some patients. Hence, it would be beneficial for the patients to predict HIF-1α expression by DCE-MRI in a noninvasive manner.

Based on the above analysis, this study aimed at detecting the role of DCE-MRI in detecting early treatment response in EC patients administered CRT. In addition, the possible associations of DCE-MRI parameters with HIF-1α expression were assessed, to offer a novel and noninvasive approach for prediction. Moreover, the relationships among DCE-MRI parameters, therapeutic response, and HIF-1α expression were explored.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

The Institutional Review Board (IRB) of our hospital approved this study, and written informed consent was obtained from each participant. All experiments were performed in accordance with the ethical standards of the World Medical Association (Declaration of Helsinki).

2.2 | Patients

Inclusion criteria were (a) EC confirmed by endoscopic biopsy; (b) scheduled definitive concurrent CRT, without preoperative tumor-related treatment; (c) no contraindications for DCE-MRI; and (d) good quality of images, suggesting that motion artifacts were low enough to allow data analysis. Exclusion criteria were (a) previous tumor-related treatment, (b) contraindications for MRI, (c) unsuitability for CRT (n = 2), and (d) poor quality of images (n = 3). Eligible patients were enrolled from February 2018 to October 2020.

2.3 | MRI examination

All patients underwent 3.0 T DCE-MRI examination (Ingenia 3.0 T; Philips Medical Systems, Best, the Netherlands) at two time-points: pre-CRT (1 week before CRT) and 3w-CRT (3 weeks after CRT).
Examination was performed in the supine position. The MRI scanning sequences included conventional T1-weighted imaging (T1WI), small field of view (FOV) and high-resolution (HR) T2-weighted imaging (T2WI), diffusion weighted imaging (DWI), and DCE imaging. Small FOV and HR T2WI scanning parameters were repetition time (TR), 2000 ms; echo time (TE), 90 ms; slice thickness, 4 mm; matrix, 232 × 232; FOV, 140 mm × 140 mm; and navigator use for respiratory triggering. DWI adopted echo-planar imaging to scan cross sections with the following parameters: TR, 250 ms; TE, 64 ms; slice thickness, 4 mm; matrix, 64 × 64; b values of 0 and 800 s/mm², respectively). The apparent diffusion coefficients (ADC) diagram was reconstructed finally. DCE scanning comprised two parts. Before contrast injection, transverse volume sequences were obtained at two flip angles (α = 5° and 15°, respectively) to calculate T1 mapping. Then, DCE images were obtained with an axial dynamic T1-weighted 3D-fast field echo (TFE) with the following parameters: TR, 4 ms; TE, 2 ms; slice thickness, 4 mm; matrix, 172 × 172; FOV, 240 mm × 240 mm. A bolus dose of MRI contrast (Magnevist, Bayer) was injected at 0.1 mmol/kg of body weight at a rate of 2.5 ml/s with an automatic syringe pump, followed by saline flush.

2.4 | MR image analysis

The dynamic data were processed with the Omni-Kinetics post-processing software (GE Healthcare), which could fit T1-weighted DCE MRI data to the Tofts linear model, and quantitative kinetic parameters (ktrans, kep and ve) were calculated. Two radiologists with 8 and 11 years of experience in digestive radiology were blinded to the treatment results and independently performed data analysis. In this study, we artificially divided esophageal lesions into upper, middle, and lower segments, and three regions of interest (ROIs) were manually outlined randomly in each segment (Figure 1). Areas of necrotic tissue, hemorrhage, calcification, and blood vessels were avoided while setting the ROIs. Finally, the averages of the three segments (totally nine ROIs) for various parameters were obtained in every patient. On T2WI, the thickened esophageal wall had a relatively higher signal intensity compared with normal esophageal tissue. The small FOV and HR T2WI could make the signal intensity contrast more obvious; in addition, DWI/ADC could further help determine the exact boundaries of the esophageal lesions for delineating ROIs in CE-T1WI (Figure 2).

2.5 | Treatment protocol and therapeutic response evaluation

A total of 39 EC patients underwent CRT. According to the patient’s specific condition, the prescribed radiotherapy dose was 60–66 Gy (1.8–2.2 Gy/fraction, 5 fractions/week; totally 30–33 fractions). Chemotherapy was performed concurrently with radiation therapy with paclitaxel liposome at 90 mg/m² plus nedaplatin at 40 mg/m² for 4 weeks, and no more than 6 weeks. A month after CRT, based on combined esophageal barium swallow, chest enhancement CT and the Response Evaluation Criteria in Solid Tumors (RECIST) guideline version 1.1, patients were divided into the sensitive (SG) and resistant (RG) groups; in parallel, HIF-1α(+) and HIF-1α(−) groups were determined by immunohistochemistry (IHC).

2.6 | Immunohistochemical analysis of HIF-1α

HIF-1α was assessed in paraffin-embedded tissue samples sectioned at 5 μm. Briefly, all sections were deparaffinized, and antigen retrieval was performed under high pressure for 2 min. Nonspecific binding was blocked with serum for 15 min at 37°C. The sections were stained with primary monoclonal rabbit anti-human HIF-1α antibody (Abcam, Cambridge, UK) in a humidified chamber for 60 min at 37°C. The specimens were next stained with goat anti-rabbit secondary antibodies, in a humidified container for 30 min at 37°C. HIF-1α expression was visualized with 3, 3-diaminobenzidine (DAB) followed by counterstaining with hematoxylin. HIF-1α expression was determined by assessing the percentage of tumor cells with cytoplasmic staining, and staining intensity was evaluated with the following classification system: 0, no staining; I, staining in less than 10% of tumor cells; II, staining in 10%–50% of cells; III, staining in over 50% of cells. 0 and I were considered a negative (−) pattern, whereas II and III were positive (+) patterns.

2.7 | Statistical analysis

All statistical analyses were performed with the SPSS 23.0 statistical software (SPSS Inc., Chicago, IL). Categorical data were compared by the
Fisher’s exact test and Kruskal–Wallis test. The Shapiro–Wilk test was performed to determine whether quantitative parameters had a normal distribution. Normally distributed data were compared by the Student’s t test; the Mann–Whitney U test was adopted for non-normally distributed data. Paired comparisons were performed by the Wilcoxon test. The diagnostic performances of these parameters in predicting treatment response or HIF-1α expression were tested by receiver operating characteristic (ROC) curve analysis. The maximal Youden index (Youden index = sensitivity + specificity - 1) was calculated to obtain a reasonable threshold. \( p < .05 \) was considered statistically significant.

3 | RESULTS

3.1 | DCE-MRI-derived parameters for early evaluation of patient response to CRT in EC

3.1.1 | Clinical characteristics in the SG and RG groups

Demographic data of all patients are presented in Table 1. Totally 39 patients (22 males and 17 females; mean age, 66.77 years; age range, 46–82 years) were enrolled in the current study. According to RECIST guideline version 1.1, the CR and PR groups were defined as the SG, and the SD and PD groups as the RG. There were 18 males and 16 females (mean age, 66.65 ± 7.32 years) in the SG, and 4 males and 1 female (mean age, 67.6 ± 5.13 years) in the RG. No statistically significant differences were detected between the SG and RG in mean age (\( p = .782 \)), gender (\( p = .363 \)), pathological type (\( p = .701 \)), clinical T-stage (\( p = .819 \)), and N-stage (\( p = .397 \)), whereas tumor location showed a significant difference (\( p = .016 \)) between the SG and RG.

3.1.2 | Parameters in SG and RG at the pre-CRT and 3w-CRT time-points

As shown in Table 2, pre-\( k_{\text{trans}} \) and pre-\( k_{\text{ep}} \) were decreased significantly in the SG after 3w-CRT (\( p < .01 \)), whereas only pre-\( k_{\text{ep}} \) was significantly reduced in the RG (\( p = .037 \)). Pre-\( k_{\text{trans}} \) was also decreased in the RG at 3w-CRT, although it showed no statistically significant difference (\( p = .225 \)). Although ve was increased in the RG and decreased in the SG after 3w-CRT, no statistical significance was detected (\( p = .319 \) and .48, respectively).

3.1.3 | Changes in parameters between the SG and RG at the pre-CRT and 3w-CRT time-points

As shown in Table 3, pre-\( k_{\text{trans}} \) was higher in the SG compared with the RG (\( p < .01 \)), and absolute \( \Delta k_{\text{trans}} \) was reduced more substantially
in the SG compared with the RG. No statistically significant differences were detected between the SG and RG in post-ktrans ($p = .473$), pre-kep ($p = .579$), post-kep ($p = .225$), $\Delta$kep ($p = .685$), pre-ve ($p = .475$), post-ve ($p = .914$), and $\Delta$ve ($p = .38$). According to ROC analysis, $\Delta$ktrans was the best parameter for early distinction of SG from RG; at a threshold of 0.4416, its sensitivity was 97.1%, with a specificity of 80.0% (AUC = 0.929; Figure 3).

**TABLE 1** Demographic data in the SG and RG

|                      | SG (n=34) | RG (n=5) | p-value |
|----------------------|-----------|----------|---------|
| Mean age             | 66.65 ± 7.32 | 67.6 ± 5.13 | .782    |
| Gender (n%)          |           |          | .363    |
| Male                 | 18 (52.9) | 4 (80)   |         |
| Female               | 16 (47.1) | 1 (20)   |         |
| Location (n%)        |           |          | .016*   |
| Cervical and upper   | 3 (8.8)   | 0 (0)    |         |
| Cervical             | 2 (5.9)   | 0 (0)    |         |
| Upper                | 11 (32.4) | 1 (20)   |         |
| Middle and upper     | 8 (23.5)  | 0 (0)    |         |
| Middle               | 9 (26.5)  | 1 (20)   |         |
| Lower and middle     | 1 (2.9)   | 1 (20)   |         |
| Lower                | 0 (0)     | 2 (40)   |         |
| Clinical T-stage (n%)|           |          | .819    |
| II                   | 2 (5.9)   | 0 (0)    |         |
| III                  | 6 (17.6)  | 1 (20)   |         |
| IV                   | 26 (76.5) | 4 (80)   |         |
| Clinical N-stage (n%)|           |          | .397    |
| N0                   | 1 (2.9)   | 0 (0)    |         |
| N1                   | 15 (44.1) | 0 (0)    |         |
| N2                   | 6 (17.6)  | 4 (80)   |         |
| N3                   | 12 (35.3) | 1 (20)   |         |

Abbreviations: SCC, squamous cell carcinoma; SG, sensitive group; RG, resistant group.

* $p < .05$.

**TABLE 2** Comparisons of parameters in SG and RG at the pre-CRT and 3w-CRT time-points

|                      | SG (n=34) | 3w-CRT | p      |
|----------------------|-----------|--------|--------|
| $k_{trans}$          | 0.47 ± 0.09 | 0.27 ± 0.09 | <.01** |
| $kep$                | 3.02 ± 0.91 | 2.17 ± 0.74 | <.01** |
| $ve$                 | 1.86 ± 0.92 | 1.72 ± 0.84 | .48    |

|                      | RG (n=5)  | 3w-CRT | p      |
|----------------------|-----------|--------|--------|
| $k_{trans}$          | 0.27 ± 0.04 | 0.23 ± 0.08 | .225   |
| $kep$                | 2.78 ± 1.06 | 1.72 ± 0.95 | .037*  |
| $ve$                 | 1.46 ± 0.56 | 1.75 ± 0.78 | .319   |

Abbreviations: 3w-CRT, after 3 weeks of CRT; $k_{trans}$, volume transfer constant in min$^{-1}$; $kep$, reverse trans-vascular transfer rate constant in min$^{-1}$; Pre-CRT, 1 week before CRT; RG, resistant group; SG, sensitive group; ve, extravascular extracellular volume fraction.

* $p < .05$, **$p < .01$. 

**TABLE 3** Comparisons of parameters between the SG and RG at the pre-CRT and 3w-CRT time-points

|                      | SG (n=34) | 3w-CRT | p      |
|----------------------|-----------|--------|--------|
| $k_{trans}$          | 0.47 ± 0.09 | 0.27 ± 0.04 | .01*   |
| $kep$                | 0.27 ± 0.09 | 0.23 ± 0.08 | .473   |
| $ve$                 | 3.02 ± 0.91 | 2.78 ± 1.06 | .579   |
| $kep$                | 2.17 ± 0.74 | 1.72 ± 0.95 | .226   |
| $ve$                 | 1.86 ± 0.92 | 1.46 ± 0.56 | .475   |
| $ve$                 | 1.72 ± 0.84 | 1.75 ± 0.78 | .914   |

Abbreviations: Post-X, X acquired after 3 weeks of CRT; Pre-X, X acquired 1 week before CRT; RG, resistant group; SG, sensitive group; $\Delta$X, changes in X.

* $p < .05$, **$p < .01$. 

**FIGURE 3** Receiver operating curve (ROC) curve for $\Delta$ktrans. $\Delta$ktrans was the best parameter in early distinction of SG from RG with 97.1% sensitivity, 80.0% specificity, and an AUC of 0.929.
3.2 | DCE-MRI-derived parameters for predicting HIF-1α(−) and HIF-1α(+) EC patients

3.2.1 | Clinical characteristics of HIF-1α(−) and HIF-1α(+) EC patients

The clinical data of the HIF-1α(−) and HIF-1α(+) groups in EC patients are presented in Table 4. Totally 24 patients (12 males and 12 females; mean age, 66.33 years; age range, 46–82 years) were enrolled in this study. According to IHC analysis, the patients were divided into the HIF-1α(−) and HIF-1α(+) groups, respectively. There were 5 males and 8 females (mean age, 64.0 ± 7.0 years) in the HIF-1α(−) group, and 7 males and 4 females (mean age, 69.09 ± 6.98 years) in the HIF-1α(+) group. No statistically significant differences were detected between the two groups in mean age (p = .11), gender (p = .219), pathological type (p = .358), location (p = .096), clinical T-stage (p = .649), and N-stage (p = .665).

### Table 4
Clinical characteristics of HIF-1α(−) and HIF-1α(+) EC patients

| Gender (n%) | HIF-1α(−) | HIF-1α(+) | p-value |
|------------|-----------|-----------|---------|
| Male       | 5 (38.5)  | 7 (63.6)  | .219    |
| Female     | 8 (61.5)  | 4 (36.4)  |         |
| Pathological type (n%) | HIF-1α(−) | HIF-1α(+) | .358    |
| SCC        | 12 (92.31)| 11 (100)  |         |
| Adenocarcinoma | 1 (7.69) | 0 (0)     |         |

| Location (n%) | HIF-1α(−) | HIF-1α(+) | p-value |
|---------------|-----------|-----------|---------|
| Cervical and upper | 3 (23.1) | 0 (0)     | .096    |
| Cervical      | 1 (7.7)   | 0 (0)     |         |
| Upper         | 3 (23.1)  | 3 (27.3)  |         |
| Middle and upper | 2 (15.4) | 3 (27.3)  |         |
| Middle        | 4 (30.8)  | 3 (27.3)  |         |
| Lower and middle | 0 (0)   | 1 (9.1)   |         |
| Lower         | 0 (0)     | 1 (9.1)   |         |

| Clinical T-stage (n%) | HIF-1α(−) | HIF-1α(+) | .649    |
|-----------------------|-----------|-----------|---------|
| III                   | 2 (15.4)  | 1 (9.1)   |         |
| IV                    | 11 (84.6) | 10 (90.9) |         |

| Clinical N-stage (n%) | HIF-1α(−) | HIF-1α(+) | .665    |
|-----------------------|-----------|-----------|---------|
| N1                    | 6 (46.2)  | 4 (36.4)  |         |
| N2                    | 3 (23.1)  | 3 (27.3)  |         |
| N3                    | 4 (30.8)  | 4 (36.4)  |         |

Abbreviation: SCC, squamous cell carcinoma.

### Table 5
Comparisons of parameters in the HIF-1α(−) and HIF-1α(+) groups at the pre-CRT and 3w-CRT time-points

| Parameters | HIF-1α(−) (n = 13) | HIF-1α(+) (n = 11) | p  |
|------------|---------------------|---------------------|----|
| Pre-CRT    | 0.48 ± 0.1          | 0.37 ± 0.07         | .002**|
| Post-CRT   | 0.27 ± 0.1          | 0.23 ± 0.06         | .207  |
| Pre-kep    | 3.24 ± 0.89         | 2.89 ± 0.82         | .25   |
| Post-kep   | 2.16 ± 0.73         | 2.23 ± 0.87         | .848  |
| Pre-ve     | 1.5 ± 0.4           | 1.6 ± 0.74          | .448  |
| Post-ve    | 1.65 ± 0.71         | 1.48 ± 0.88         | .264  |

### Table 6
Comparisons of parameters between the HIF-1α(−) and HIF-1α(+) groups at pre-CRT and 3w-CRT

| Parameters | HIF-1α(−) (n = 13) | HIF-1α(+) (n = 11) | p  |
|------------|---------------------|---------------------|----|
| Pre-ktrans | 0.48 ± 0.1          | 0.37 ± 0.07         | .002**|
| Post-ktrans| 0.27 ± 0.1          | 0.23 ± 0.06         | .207  |
| ΔK trans   | −0.21 ± 0.12        | −0.14 ± 0.08        | .098  |
| Pre-Kep    | 3.24 ± 0.89         | 2.89 ± 0.82         | .25   |
| Post-Kep   | 2.16 ± 0.73         | 2.23 ± 0.87         | .848  |
| ΔKep       | −1.07 ± 0.63        | −0.8 ± 1.05         | .434  |
| Pre-Ve     | 1.5 ± 0.4           | 1.6 ± 0.74          | .448  |
| Post-Ve    | 1.65 ± 0.71         | 1.48 ± 0.88         | .264  |
| ΔVe        | 0.15 ± 0.68         | −0.36 ± 1.14        | .185  |

Abbreviations: ΔX, changes in X; Post-X, X acquired after 3 weeks CRT; Pre-X, X acquired 1 week before CRT. **p < .01.

### 3.2.2 | Comparisons of parameters in the HIF-1α(−) and HIF-1α(+) groups at pre-CRT and 3w-CRT

As shown in Table 5, both pre-ktrans and pre-kep were decreased significantly in the HIF-1α(−) group compared with the 3w-CRT (p < .01). Pre-ktrans was reduced significantly in the HIF-1α(+) group at 3w-CRT (p < .01). Although pre-kep was decreased in the HIF-1α(+) group at 3w-CRT, statistical significance was not reached (p = .066). Meanwhile, ve was increased in the HIF-1α(−) group and decreased in the HIF-1α(+) group at 3w-CRT, but with no significant differences (p = .432 and .508, respectively).

### 3.2.3 | Comparisons of parameters between the HIF-1α(−) and HIF-1α(+) groups at pre-CRT and 3w-CRT

As shown in Table 6, only pre-ktrans was markedly higher in the HIF-1α(−) group compared with the HIF-1α(+) group (p = .002). The
remaining parameters, including post-ktrans, Δktrans, pre-kep, post-kep, Δkep, pre-ve, post-ve and Δve, showed no statistically significant differences between the two groups (p = .207, .098, .25, .848, .434, .448, .264, and .185, respectively). Moreover, pre-ktrans was an excellent parameter in predicting HIF-1α expression in EC patients according to ROC analysis. At a threshold of 0.4358, pre-ktrans showed a sensitivity as high as 100%, and a specificity of 76.9%, with an AUC of 0.881 (Figure 4).

4 | DISCUSSION

Early prediction of CRT efficacy could bring many benefits to advanced EC patients, such as avoiding unnecessary side effects (esophageal fistula, esophagitis, pericarditis, pulmonary fibrosis, et al.) and timely adjusting to a proper strategy.27,28 At present, DCE-MRI not only can visually assess a given ROI with multi-modal imaging, but also quantitatively reflects the characteristics of tumor microcirculation/metabolism with parameters based on the blood supply situation of lesions.6,13 In this study, we fully exploited these advantages of DCE-MRI, which was applied to evaluate early treatment response to CRT and to predict the expression of HIF-1α, a useful tumor marker for efficacy assessment.

ROI selection in this study has never been reported before. We artificially divided the esophageal lesions into three equal segments, each containing three small ROIs. This was more advantageous than the classical method of choosing only one maximum ROI or three random ROIs in the maximum cross section of the tumor.29,30 Because of tumor heterogeneity, selection of a total of nine ROIs at the upper, middle, and lower levels could better reflect the tumor characteristics. Meanwhile, retention of data for the ROIs in the three different segments will lay a foundation for exploring differences in the recurrence of foci originated from the upper, middle, or lower levels at the later stage.

In this study, two patients were not suitable for continuous CRT due to complications (fistula), and three were excluded for poor image quality. Therefore, totally 39 patients were enrolled, and the SG and RG had 34 and 5 cases, respectively. The three excluded patients with poor images all had lower esophageal lesions, and the RG had only five individuals, which may explain the distribution difference of esophageal lesions between the RG and SG. A total of 24 individuals were enrolled in the HIF-1α prediction study. The lower number (15 cases) was mainly due to unavailable IHC data in patients who underwent gastroscopy and pathology in local hospitals before receiving further treatment in our hospital. According to the study aims, enrolled patients were divided into the SG and RG on the one hand, and HIF-1α(−) and HIF-1α(+) groups on the other hand. DCE-MRI examination was performed before CRT, and reexamination was conducted 3 weeks after treatment. Tumor reduction is typically observed at 3 weeks or more after the initiation of CRT.31,32 Thus, a 3-week reexamination time-point was set in order to acquire a more rapid evaluation or treatment response prediction.

Evaluating the early response to CRT, we found that pre-ktrans in the SG was higher than that of the RG (p < .01) and pre-ktrans was decreased significantly in the SG at 3w-CRT (p < .01). To obtain adequate nutrients for growing and metastasizing, malignant neoplasms have developed both structurally and functionally new vessels, which are leaky with a hazard pattern of interconnections.33 This results in higher blood flow and endothelial permeability in the tumor, thereby increasing k-trans. Therefore, we considered the relationship between high pre-ktrans and sensitive treatment may be associated with higher blood flow and endothelial permeability, which improves accessibility to chemotherapy and sensitivity to radiation.15,34 In addition, we found that absolute Δktrans was reduced more substantially in the SG compared with the RG. In addition, it was considered the best parameter in distinguishing SG from RG. Δktrans is believed to represent a relative reduction of vessel endothelial permeability due to fibrosis, and the relative variation is often thought to be more representative and stable.

In HIF-1α prediction research, we compared parameters between the HIF-1α(−) and HIF-1α(+) groups at pre-CRT and 3w-CRT. The data showed that pre-ktrans was significantly higher in the HIF-1α(−) compared with the HIF-1α(+), and its sensitivity in distinguishing SG from RG could reach 100% (specificity of 76.1%; AUC 0.881). These findings suggest that pre-ktrans derived from DCE-MRI could be an excellent and promising imaging biomarker for predicting the expression the HIF-1α. Moreover, higher pre-ktrans in the HIF-1α(−) may reflect a better treatment response compared with the HIF-1α(+), according to the above early response evaluation and previous studies.35

Inevitably, there were several limitations in this study. First, the sample size was relatively small, and a larger sample is necessary in further studies. Secondly, we used biopsy samples to evaluate the expression of HIF-1α in EC. Considering tumor heterogeneity, the

FIGURE 4 ROC curve for pre-ktrans. The pre-ktrans value was a promising parameter in predicting the expression of HIF-1α in EC patients, with 100% sensitivity, 76.9% specificity, and an AUC of 0.881.
The assessment of tumor response to CRT and HIF-1α cancer biomarkers and MRI parameters are warranted. Thus, more investigations of markers. Thirdly, other immunohistochemical biomarkers are related to study the associations of DCE-MRI parameters with tumor molecular 

Therefore, the assessment of the entire tumor is required to further results may not represent the biomarker expression of the entire tumor. In conclusion, DCE-MRI could be a promising tool for early assessment of tumor response to CRT and HIF-1α expression prediction in advanced EC patients. Δktrans was the best parameter in early distinction of SG from RG, and pre-ktrans represented an excellent parameter in predicting HIF-1α expression. Also, the prediction of HIF-1α expression could also contribute to efficacy evaluation. The core conclusions of this study are shown in Figure 5.

CONFLICT OF INTEREST
The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS
(I) Conception and design: Wenrong Shen, Zhengyang Zhou; (II) Administrative support: Wenrong Shen, Zhengyang Zhou; (III) Provision of study materials or patients: Xiaodong Xie, Lingling Gu, Zhen Guo, Hua Tao, Yiqin Zhou; (IV) Collection and assembly of data: Xiaodong Xie, Lingling Gu, Zhen Guo; (V) Data analysis and interpretation: Xiaodong Xie, Lingling Gu; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

ETHICS STATEMENT
Ethical approval for this investigation was obtained from the Ethics Committee of Nanjing Medical University affiliated Cancer Hospital (Nanjing, Jiangsu, China).

INFORMED CONSENT
All patients consent to participate, and all data were consent for publishing.

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