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Predictive value of neoadjuvant chemotherapy on breast cancer subtypes evaluated by DCE-MRI and MRI perfusion parameters

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Dynamic contrast enhanced MRI (DCE-MRI) is valuable for evaluating tumor angiogenesis. We aimed to predict the effects of neoadjuvant chemotherapy on breast cancer by DCE-MRI parameters. A total of 162 eligible patients were divided into response and non-response groups. K^{trans}, K_{ep} and V_e before, after two courses and at the end of neoadjuvant chemotherapy were compared. ER, PR, HER-2 and Ki-67 expressions in breast cancer tissues were detected by immunohistochemical staining. DCE-MRI parameters at different ER, PR and HER-2 expression levels were compared, and their correlations with Ki-67 expression were analyzed. K^{trans} and K_{ep} of response group were lower after two courses and at the end of neoadjuvant chemotherapy than those before neoadjuvant chemotherapy (P <0.05). K_{ep} of ER-negative group was higher than that of ER-positive group (P <0.05). K^{trans} and K_{ep} of PR-negative group were higher than those of PR-positive group (P <0.05). K_{ep} of HER-2-negative group exceeded that of HER-2-positive group (P <0.05). There were negative correlations between ER expression and K_{ep}, among PR expression, K^{trans} and K_{ep}, and between HER-2 expression and K_{ep} (P <0.05). Ki-67 expression was positively correlated with K^{trans} and K_{ep} (P <0.05). K^{trans} and K_{ep} of TNBC patients were higher and V_e was lower than those of other types. There were significant differences in K^{trans}, K_{ep} and V_e among Luminal A, Luminal B and TNBC types (P <0.05). DCE-MRI parameters reflect the biological behaviours of breast cancer, based on which the prognosis of patients can be assessed.

Keywords: Biomarker Ki-67, Estrogen receptors, HER-2 expression, Progesterone receptors

Breast cancer is one of the common malignant tumors in women worldwide, with the prevalence rate increasing annually[1,2]. Breast cancer can show complex heterogeneity due to different pathological types, histological grades and expressions of immunohistochemical molecules, which may affect its clinical biological behaviors, treatment methods and prognosis. Traditional prognostic factors, such as tumor size, pathological type, histological grade and lymph node metastasis, are the reference indices for clinical treatment. The expressions of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor-2 (HER-2) and Ki67 play key roles in the treatment and prognosis of breast cancer[3,4], of which ER, PR and HER2 are recommended as grade I evidence in the 8th version of the The American Joint Committee on Cancer breast cancer prognostic staging system. The proliferation rate of Luminal type A is low. The histological grade of Luminal type B is higher than that of Luminal type A, and most are accompanied by HER-2 gene amplification.

HER-2 overexpression breast cancer has a high degree of malignancy, a high rate of visceral metastasis and a low survival rate[5]. Triple negative breast cancer (TNBC) has strong invasiveness and unique biological behaviours, with poor prognosis[6]. For patients with locally advanced breast cancer (LABC), preoperative neoadjuvant chemotherapy combined with surgical treatment can effectively improve the survival rate, reduce primary lesions, promote the decrease of tumor grade and relieve micrometastasis[7,8]. In the past, molybdenum targets and ultrasonography were often used for early diagnosis and evaluation of the efficacy of neoadjuvant chemotherapy. Compared with the above-mentioned imaging methods, dynamic contrast enhanced MRI (DCE-MRI) is of greater value to the evaluation of tumor angiogenesis[9]. The quantitative dynamic enhanced MRI examination can calculate the quantitative enhancement parameters with physiological significance through the pharmacokinetic model, which can more accurately monitor the microvascular perfusion and infiltration in breast cancer lesions, conduct qualitative diagnosis of lesions, and evaluate the efficacy of neoadjuvant chemotherapy by detecting the vascular endothelial permeability and increased blood flow in tumor tissues[10].

Until now, most studies have mainly focused on the correlations between semi-quantitative parameters and biological prognostic factors. However, the quantitative enhancement parameters of MRI remain

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largely unknown. In this study, DCE-MRI quantitative parameters were used to assess the efficacy of neoadjuvant chemotherapy on breast cancer, and to analyze the correlation between these parameters and related biological prognostic factors.

Patients and Methods

Baseline clinical data

This study has been approved by the ethics committee of Affiliated Hangzhou First People's Hospital (approval No. AHFPH201701005). A total of 162 patients with breast cancer who were treated with neoadjuvant chemotherapy in Affiliated Hangzhou First People's Hospital from January 2017 to December 2019 were selected, with a total of 167 lesions. They were aged 40 to 66 years, (45.37 ± 6.23) on average ± SD. All patients have signed informed consents. Inclusion criteria: (i) Imaging examination revealed focal lesions without distant metastasis (The diameter of lesion was smaller than 5 cm, and the boundary between the lesion and its surrounding areas was unclear); (ii) Patients had invasive ductal carcinoma; and (iii) CET chemotherapy (cyclophosphamide, pharmorubicin, paclitaxel) was selected at the same dose.

Scan parameters

The patients received DCE-MRI by Discovery 750W 3.0T MRI scanner (GE Healthcare, USA) before and after chemotherapy. The axial 3D dynamic SRGR sequence was used. First, the patients were scanned at the flip angles of 5, 10 and 15 degrees to obtain T1 mapping. Afterwards, DCE-MRI was performed continuously for 30 time phases, with the total time of 7 min and 30 s.

Measurement of K^{trans} and K_{ep}

The raw data of multi-phase scanning were imported into Omni-Kinetics software for image post-processing. The extended Tofts pharmacokinetic model was employed to calculate the following quantitative parameters. (i) Volume transfer constant (K^{trans}): velocity constant of diffusion of intravascular contrast agent into the outside blood vessel; (ii) velocity constant (K_{ep}): velocity constant of infiltration of extravascular contrast agent into the blood vessel; and (iii) extracellular space volume ratio (V_e): (vascular + extracellular space)/total volume.

Evaluation of therapeutic effects

According to the Response Evaluation Criteria in Solid Tumors (RECIST), the changes in the maximum diameter of tumor before and after chemotherapy were measured and classified into effective or ineffective. Effective: Tumor body shrinks by over 30% after chemotherapy; ineffective: tumor body shrinks by less than 30% or new lesions appear. The changes in the longest diameter of lesion before, after two courses and at the end of chemotherapy were compared.

Immunohistochemical staining and analysis

Tumor tissues were subjected to immunohistochemical staining. The brownish yellow cells were found to be: (i) ER- and PR-positive: Positive staining was located in the nucleus, with the percentage of >10%; ER- and PR-negative: with the percentage of ≤10%; (ii) HER-2: Positive staining was located in the cell membrane, with low expression of HER-2: – and +, HER-2 overexpression: ++ and +++; and (iii) Ki-67: Positive staining was located in the nucleus, and the percentage of positive cells to 1,000 cells was recorded under high-magnification microscope, negative (–): <110%; weakly positive (+): 10%-50%; strong positive (++): >50%[11].

According to the molecular typing method proposed at the St. Gallen 2011 International Breast Cancer Conference[12], breast cancer can be classified into the following 4 types. (i) Luminal type A: ER- and/or PR-positive, HER-2-negative and low Ki-67 expression (<14%); (ii) Luminal type B: ER- and/or PR-positive, HER-2-negative and high Ki-67 expression (≥14%); ER- and/or PR-positive, HER-2-positive and any Ki-67 expression; (iii) HER-2 overexpression: ER- and PR-negative, HER-2-positive and any Ki-67 expression; and (iv) TNBC: ER-, PR- and HER-2-negative and any Ki-67 expression.

In the 167 lesions, there were 31 Luminal A, 100 Luminal B, 20 HER-2 overexpression and 16 TNBC types.

Statistical analysis

All data were statistically analyzed by SPSS22.0 software. Parameters were compared by analysis of variance, and therapeutic effects were evaluated with the 't' test. The DCE-MRI parameters at different expressions of each receptor were subjected to the Mann-Whitney U test. The correlations of DCE-MRI parameters with the positive expression rates of ER, PR, HER-2 and Ki-67 were studied by the Spearman's analysis. P <0.05 was considered statistically significant.

Results and Discussion

DCE-MRI parameters before and after neoadjuvant chemotherapy

In response group (n=104), after two cycles of neoadjuvant chemotherapy and at the end of

chemotherapy, K^{trans} and K_{ep} were lower than those before treatment (P <0.05), and V_e rose slightly after 2 cycles of neoadjuvant chemotherapy and declined at the end of chemotherapy in comparison with that before treatment (P >0.05) (Table 1 and Fig. 1).

No response group (n=58) showed a little raised K^{trans} after 2 cycles of neoadjuvant chemotherapy and slightly decreased K^{trans} at the end of chemotherapy compared with that before treatment (P >0.05). Besides, K_{ep} declined after 2 cycles of neoadjuvant chemotherapy and at the end of chemotherapy (P >0.05), while V_e was elevated after 2 cycles of neoadjuvant chemotherapy and at the end of chemotherapy (P >0.05).

DCE-MRI parameters of patients with different expressions of ER, PR and HER-2 before treatment

Before treatment, ER-negative group exhibited higher K_{ep} than ER-positive *group (P <0.05). K^{trans} and K_{ep} in PR-negative group were higher than those in PR-positive group (*P <0.05), whereas K_{ep} in HER-2-negative group was higher than that in HER-2-positive group (P <0.05) (Table 2).

Table 1 — DCE-MRI parameters before and after neoadjuvant chemotherapy

	Response group (n=104)			No response group (n=58)		
	Before	After 2 neoadjuvant chemotherapy cycles	After	Before	After 2 neoadjuvant chemotherapy cycles	After
K^{trans} (min^{-1})	1.40±0.43	0.94±0.42*	0.65±0.19*	1.26±0.42	1.48±0.35	1.08±0.36
K_{ep} (min^{-1})	1.85±0.39	1.22±0.47*	0.78±0.32*	1.60±0.36	1.49±0.44	1.42±0.42
V_e	0.76±0.18	0.81±0.13	0.77±0.23	0.84±0.38	0.90±0.27	0.85±0.28

[*Compared with before neoadjuvant chemotherapy within the same group, P <0.05]

Table 2 — DCE-MRI parameters of patients with different expressions of ER, PR and HER-2 before treatment

	ER		PR		HER-2	
	Negative (n=72)	Positive (n=90)	Negative (n=86)	Positive (n=76)	Negative (n=54)	Positive (n=108)
K^{trans} (min^{-1})	1.34±0.43	1.35±0.38	1.15±0.19	1.63±0.41*	1.36±0.32	1.33±0.46
K_{ep} (min^{-1})	1.89±0.34	1.57±0.44*	1.98±0.32	1.68±0.34*	1.95±0.41	1.70±0.41
V_e	0.79±0.19	0.80±0.27	0.79±0.23	0.81±0.32	0.78±0.22	0.81±0.24

[*Comparison within the same group, P <0.05]

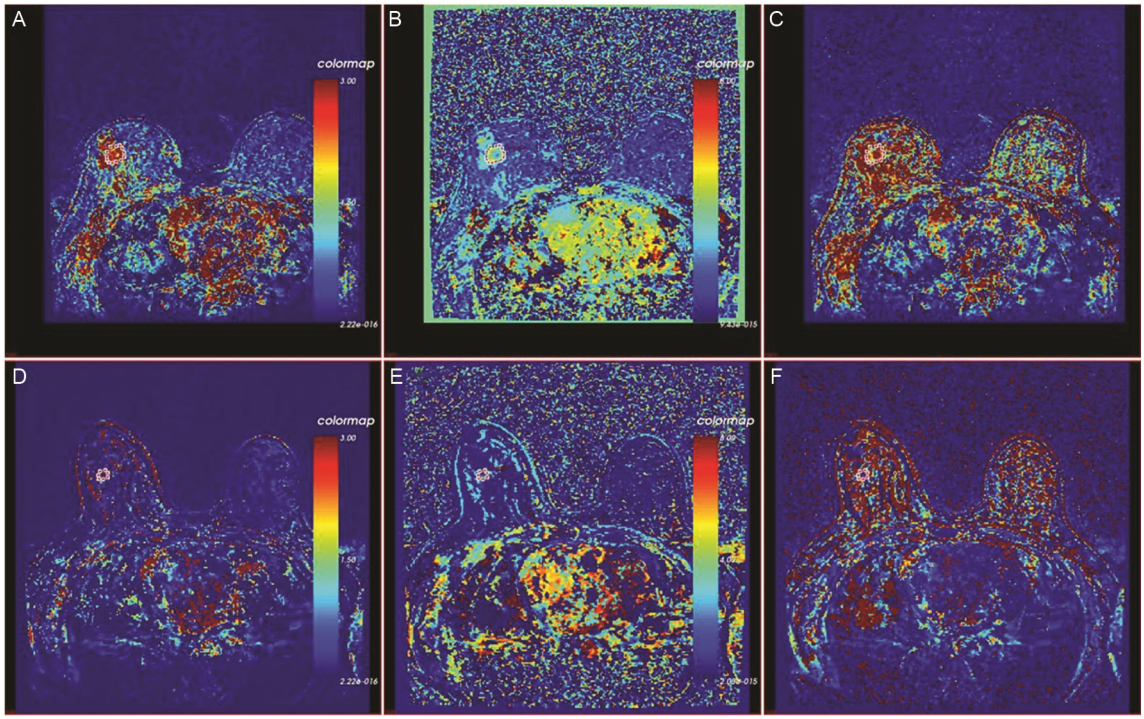

Fig. 1 — DCE-MRI images (K^{trans}, K_{ep} and V_e) of a 40-year-old female patient before and after neoadjuvant chemotherapy. (A-C) Before neoadjuvant chemotherapy, K^{trans}=2.0659 min^{-1}, K_{ep}=4.59003 min^{-1} and V_e=0.513918; and (D-F) after neoadjuvant chemotherapy, K^{trans}=1.34042 min^{-1}, K_{ep}=1.79043 min^{-1} and V_e=0.47946.

Table 3 — Correlations of ER, PR, HER-2 and Ki-67 expressions with DCE-MRI parameters before treatment

	K^{trans} (min^{-1})		K_{ep} (min^{-1})		V_e	
	r	*P*	r	*P*	r	*P*
ER	–0.321	0.564	–0.523	0.000	0.045	0.639
PR	–0.454	0.000	–0.572	0.000	–0.291	0.745
HER-2	–0.326	0.639	–0.632	0.000	0.189	0.843
Ki-67	0.656	0.000	0.734	0.000	–0.121	0.224

Correlations of ER, PR, HER-2 and Ki-67 expressions with DCE-MRI parameters before treatment

ER was significantly negatively correlated with K_{ep} (P <0.05), PR had significantly negative correlations with K^{trans} and K_{ep} (P <0.05), HER-2 was significantly negatively associated with K_{ep} (P <0.05), and Ki-67 was significantly correlated with K^{trans} and K_{ep} (P <0.05) (Table 3). (A plot would add more weightage of the article)

DCE-MRI parameters of patients with different molecular subtypes before treatment

TNBC patients had higher K^{trans} and K_{ep} and lower V_e than those with other types of breast cancer. Significant differences in K^{trans}, K_{ep} and V_e were found among different molecular subtypes of breast cancer (P <0.05). As shown in further pairwise comparisons, there were significant differences among luminal A breast cancer, luminal B and TNBC regarding K^{trans}, K_{ep} and V_e, whereas ADC differed among luminal B breast cancer, TNBC and HER-2 overexpressed breast cancer (P <0.05) (Table 4).

In recent years, the incidence rate of breast cancer has been gradually increasing. Neoadjuvant chemotherapy can create the chances of surgery for patients with locally advanced breast cancer, raise the rate of breast conservation for breast cancer patients with tumors of larger volume and improve the quality of life and survival of patients. Hence, such a treatment regimen has been mostly applied in clinic currently. The application of imaging techniques is the most common and important in evaluating the efficacy of breast neoadjuvant chemotherapy. MRI excels in accuracy and preciseness[13,14], while DCE-MRI examination is preferable since it can be used to dynamically observe both the morphological changes and the enhancement features of lesions and detect tumor tissue response after chemotherapy and hemodynamic changes[15].

Traditional breast MRI examination is relatively limited, as it can be performed only to observe the morphological features of lesions by dynamic contrast enhancement, diagnose lesions combining time-signal intensity curves, but cannot understand the perfusion

Table 4 — DCE-MRI parameters of patients with different molecular subtypes before treatment

Type	No. of lesions	K^{trans} (min^{-1})	K_{ep} (min^{-1})	V_e
Luminal A	31	0.86±0.22	1.19±0.44	0.68±0.21
Luminal B	100	0.96±0.27	1.87±0.43	0.81±0.22
HER-2 over-expression	20	1.16±0.33	1.44±0.41	0.89±0.23
TNBC	16	1.46±0.31	2.06±0.42	0.51±0.20
F		15.427	12.127	11.643
P		0.000	0.000	0.000

of tumor tissues[16]. However, quantitative DCE-MRI is able to show hemodynamic indicators by dynamically monitoring the *in vivo* absorption and metabolism of contrast agents, thereby precisely quantifying the blood perfusion of tumors. In the present study, both K^{trans} and K_{ep} declined in response group after 2 cycles of neoadjuvant chemotherapy and at the end of chemotherapy, with inconspicuous decreases and even slight rises in no response group. These results suggest that chemotherapy fails to decrease the local permeability of tumors and blood perfusion and has poor efficacy[17]. After 2 cycles of neoadjuvant chemotherapy, V_e rose a little, and it declined at the end of chemotherapy, probably because the edema around tumor tissues weakens the stability of V_e during chemotherapy. According to the results of this study, there were no statistically differences in V_e changes, but significant differences in K^{trans} and K_{ep} changes during the follow-up of every treatment cycle. Based on the analysis, the mechanism is that chemotherapy drugs impair the tumor vessels, decrease blood perfusion and the supply of blood oxygen, constantly destroy and cleave tumor cells and widen the space between extravascular cells, and the treatment with such chemotherapy drugs is efficacious. Therefore, DCE-MRI can non-invasively quantify the vascular distribution and permeability of tumor tissues and their enhancement features, in which K^{trans} and K_{ep} are able to quantitatively reflect the hemodynamic features of lesions, thereby enabling clinicians to objectively and accurately judge efficacy.

Different types of molecular biological indicators, such as ER, PR, HER-2 and Ki-67, are mostly applied to evaluate the prognosis of breast cancer and guide the clinical treatment[18-20]. The present study revealed that ER-negative group had higher K_{ep} than ER-positive group, and ER was significantly negatively correlated with K_{ep}. Moreover, the K^{trans} and K_{ep} rose in PR-negative group compared with those in PR-positive group, whereas PR was significantly

negatively associated with K^{trans} and K_{ep} ($P<0.05$). According to the analysis results, the mechanism is that capillary permeability is increased and there are more contrast agents reversely flowing from the extravascular-extracellular space to the lumen, when ER and PR expressions are negative. ER suppresses tumor angiogenesis by decreasing vascular endothelial growth factors[21]. Hence, ER-negative and PR-negative patients have obvious tumor angiogenesis and increases in the blood perfusion and hemodynamic indicators.

HER-2 is a proto-oncogene, and patients with HER-positive breast cancer usually manifest highly malignant and poorly differentiated tumor tissues, with unsatisfactory treatment effects and a poor prognosis[22]. This indicator is clinically used to guide clinical administration of drugs, such as targeted drug trastuzumab (herceptin)[23]. In the present study, the comparisons of K^{trans}, K_{ep} and V_e showed that K_{ep} in HER-2-negative group was higher than that in HER-2-positive group, and HER-2 was significantly negatively associated with K_{ep}. HER-2 induces tumors by amplifying HER-2 gene, overexpressing its protein product p185, activating tyrosine kinases, promoting cell proliferation and decreasing cell apoptosis[24].

Ki-67 antigen is associated with cell growth cycle and reflects the level of cell proliferation, and a higher expression level of Ki-67 antigen indicates a higher degree of malignancy and a higher rate of recurrence[25]. Kim *et al.* reported that breast cancer tissues with highly expressed Ki-67 had obviously higher K^{trans} and K_{ep} than those with lowly expressed Ki-67[19]. This study further corroborated that Ki-67 was significantly positively correlated with K^{trans} and K_{ep} in 162 cases of breast cancer tissue specimens. The mechanism may be that K^{trans} and K_{ep} reflect the differentiation and permeability of tumor vessels, and the higher the indicators are, the poorer the vascular differentiation is, and the higher the vascular permeability, blood flow and malignant degree are, which is consistent with the fact reflected by Ki-67 positively correlated with the above two indicators.

The treatment effects and survival outcomes substantially vary among different molecular subtypes of breast cancer, which is an important basis for clinical endocrine therapy. Hence, predicting the molecular subtypes of breast cancer *in vivo* plays an important guiding role in clinical treatment. Based on the findings in the present study, K^{trans} and K_{ep} in TNBC were higher than those in other subtypes of breast cancer, indicating that new capillaries in TNBC have a high density and more obvious microvascular perfusion. Wang *et al.* reported that vascular endothelial growth factors were more frequently expressed in TNBC, showing higher tumor invasiveness[26].

Conclusion

The present study has demonstrated that the quantitative DCE-MRI parameters K^{trans} and K_{ep} can be evaluated to identify the biological behaviours and assess prognosis of breast cancer, thus providing precise indicators for identification of clinical treatment schemes. Nevertheless, this study has limitations. The parameters herein were not compared with the routine prognostic markers for breast cancer, such as ER, PR (hormone receptors), MIB 1 antibody (proliferation status), c-erb-B2 (indicating oncogene activation), cathepsin D (promoting tumour metastasis) and nm 23 (indicating tumour suppressor potential). Additionally, the receiver operator characteristic curves were not plotted.

Conflict of interest

Authors declare no competing interests.

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