Multi-slice spiral CT evaluation of breast cancer chemotherapy and correlation between CT results and breast cancer-specific gene 1

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Summary

Purpose: To investigate multi-slice spiral CT (MSCT) imaging with breast cancer chemotherapy and the correlation between MSCT and breast cancer-specific gene 1 (BCSG1).

Methods: 86 patients with breast cancer were enrolled from January 2016 to May 2017. All of them were treated with neoadjuvant chemotherapy, and underwent MSCT scan before and after treatment to evaluate the efficacy of chemotherapy. The expression of BCSG1 in tumor tissue was detected by immunohistochemistry and the correlation between CT results and BCSG1 was analyzed.

Results: MSCT evaluation of the efficacy of chemotherapy in breast cancer patients was consistent with pathological evaluation (p<0.05). MSCT in patients after chemotherapy was significantly better than before chemotherapy (p<0.05).

CT examination showed that tumor diameter and lymph node size were significantly reduced after chemotherapy (p<0.05). The positive rates of BCSG1 in patients with different TNM stages after chemotherapy were significantly decreased (p<0.05) and the CT perfusion value of BCSG1 in the low expression group was significantly higher than in the high expression group (p<0.05).

Conclusions: MSCT can accurately evaluate the effect of chemotherapy in breast cancer. The results of MSCT were closely related to the expression of BCSG1, which may provide a reference for predicting the effect of chemotherapy in breast cancer which could have important clinical significance.

Key words: BCSG1, breast cancer, CT, efficacy, ultrasound

Introduction

Breast cancer is one of the most common malignant tumors in women, and its incidence rate ranks first among female populations [1]. The clinical manifestations of breast cancer are mainly palpable lumps, enlargement of axillary lymph nodes, skin changes, etc [2]. There are many treatment methods of breast cancer, including surgery, chemotherapy, radiotherapy and biological target therapy, among which surgical treatment is the most commonly used. However, radical surgery will cause local deformity with serious psycho-

logical impact on the patients. Breast-conserving surgery minimizes such kinds of problems as the volume of surgical resection is lower, improving thus the patient quality of life [3,4]. Neoadjuvant chemotherapy administered before surgery or radiotherapy can effectively reduce the tumor volume and actively control the micrometastatic spread, thus expanding the scope of breast-conserving surgery [5]. BCSG1 is closely associated with the sex hormone-related tumors, such as breast cancer and endometrial cancer [6]. In this

study, MSCT examination was performed in breast cancer patients undergoing neoadjuvant chemotherapy, and the BCSG1 expression was assessed, so as to investigate the value of CT in evaluating the therapeutic effect of chemotherapy and analyze the correlation between CT results and BCSG1, thus providing a scientific basis for the chemotherapy of breast cancer.

Methods

General material

A total of 78 patients with breast cancer treated in our hospital from January 2016 to May 2017 were selected and studied. Inclusion criteria: 1) patients meeting the diagnostic criteria of breast cancer [7]; 2) patients having a preoperative CT examination; 3) patients without mental disorders; 4) patients or their family members that provided signed informed consent. Exclusion criteria: 1) pregnant patients or with moderate-severe anemia; 2) patients who were in menstrual period or with unexplained vaginal bleeding; 3) patients with history of other chemotherapies and surgical operations. Patients aged 25-58 years (42.83±7.53 on average). Body mass index was 22.63±1.31 kg/m^2. TNM staging showed 13 cases in stage I, 11 cases in stage II, 26 cases in stage III and 28 cases in stage IV.

Chemotherapy

All patients were treated with neoadjuvant chemotherapy. On the 1st day, 75 mg/m^2 doxorubicin hydrochloride (Zhejiang Hisun Pharmaceutical Co., Ltd., Approval No.: NMPN H33021980) was injected i.v.; on the 2nd and 8th days, 550 mg/m^2 cyclophosphamide (Zhejiang Hisun Pharmaceutical Co., Ltd., Approval No.: NMPN H32020857) was injected i.v.; on the 2nd and 7th days, 450 mg/m^2 5-fluorouracil (Shanghai Xudong Haipu Pharmaceutical Co., Ltd., Approval No.: NMPN H31020593) was also injected i.v. Patients were treated for 3 weeks as 1 treatment cycle for a total of 3-4 cycles.

CT examination

A 16-slice spiral CT machine (GE, USA) was used. In prone position, the breasts of patients prolapsed into the hollow foam frame. Plain scan was performed from the lower boundary of breast to the supraclavicular area, followed by perfusion scan after the tumor was found. Under the film mode, 50 mL non-ionic contrast agent containing iodine, iohexol (Guangzhou Schering Corporation, 300 mg/mL), was injected using high-pressure syringe (flow rate: 3.5 mL/s). Under quiet breathing, the examination was delayed for 5 s, and data were collected for 50 s.

BCSG1 detection

The expression of BCSG1 in tumor tissue samples was detected using immunohistochemistry. The goat anti-human BCSG1 monoclonal antibody was purchased by Santa, USA. The paraffin-embedded tissues were cut into 4 μm-thick sections using a microtome

(Leica, Germany), and baked in an incubator (Shanghai Medical Equipment Workshop, Shanghai, China) at 60°C overnight, followed by dewaxing via xylene. Then, the sections were placed into 95%, 85%, 75% and 65% ethanol for 10 min, respectively, soaked in distilled water for 5 min, added with 50 μL 3% H_2O_2 and incubated in the incubator (20°C) for 10 min. The activity of endogenous peroxidase was blocked, the sections were washed with phosphate buffered saline (PBS) for 3 times (5 min/time), and added with 50 μL primary antibody (1:100) at 4°C overnight. After that, the secondary antibody was added for incubation at 20°C for 10 min, followed by color development using the reagents in DBA kit (Wuhan Boster Biological Technology Co., Ltd. Wuhan, China), and observed under a microscope. Distilled water was used to terminate the color development, followed by re-staining with hematoxylin for 2 min and sealing with neutral gum.

Evaluation indexes

Determination of therapeutic effect [8]

1) Complete remission (CR): All lesions disappeared; 2) partial remission (PR): The tumor diameter was reduced by ≥ 30%; 3) non-remission (NR): Lesions had no change or were increased.

Imaging diagnosis was made by two senior ultrasound physicians using the double-blind method. The marginal and central area of the tumor were used as regions of interest (ROI), and their mean was taken as the blood perfusion value of breast tumor. The relevant indexes included blood flow (BF), blood volume (BV), mean transit time (MTT) and permeability surface area product (Ps).

The BCSG1 expression in breast cancer tissues was detected via immunohistochemistry. Five high-power fields (400×) were randomly selected in each section; the brown-yellow stained cells indicated positive staining, the percentage of positive cells was calculated, and the percentage point (PP) was scored: 1) 0 point: no positive cells; 2) 1 point: percentage of positive cells < 5%; 3) 2 points: 5% < percentage of positive cells ≤ 20%; 4) 3 points: percentage of positive cells > 20%. The staining intensity (SI) was also scored: 1) 0 point: no staining; 2) 1 point: pale yellow; 3) 2 points: brown yellow; 4) 3 points: dark brown. The immune response score (IRS) was calculated according to the formula: IRS = PP × SI; IRS > 4 points indicated high expression, while IRS < 4 points indicated low expression [9].

Statistics

Data were processed using the Statistical Package of Social Sciences (SPSS) 19.0 software (SPSS Inc., Chicago, IL, USA). Quantitative data were presented as mean ± standard deviation, and calculated using *t*-test. Numerical data were presented as cases and percents, and analyzed by chi-square test. Kappa value test was used for the consistency: Kappa value = 0.0-0.20: very low consistency; 0.21-0.40: general consistency; 0.41-0.60: moderate consistency; 0.61-0.80: high consistency; 0.81-1: almost exactly the same. p<0.05 suggested that the difference was statistically significant.

Results

Comparison of CT evaluation with pathological evaluation

Kappa test showed that the two evaluation methods were almost exactly the same (Kappa value = 0.832, p<0.001) (Table 1).

Comparisons of CT perfusion values before and after chemotherapy

The CT perfusion values of patients after chemotherapy were significantly superior to those before chemotherapy (p<0.05) (Table 2).

Tumor diameter and lymph nodes of patients before and after chemotherapy

Results of CT examination revealed that the tumor diameter and lymph node size after chemotherapy were significantly reduced compared with those before chemotherapy (p<0.05) (Table 3, Figure 1).

Comparisons of BCSG1 positive rates in patients with different TNM stages before and after chemotherapy

The BCSG1 positive rates in patients with different TNM stages after chemotherapy were significantly decreased (p<0.05) (Table 4).

Correlation analysis between CT perfusion values and BCSG1 after chemotherapy

CT perfusion values of low-expression BCSG1 group were significantly superior to those in high-expression BCSG1 group (p<0.05) (Table 5).

Table 1. Comparison of CT evaluation with pathological evaluation

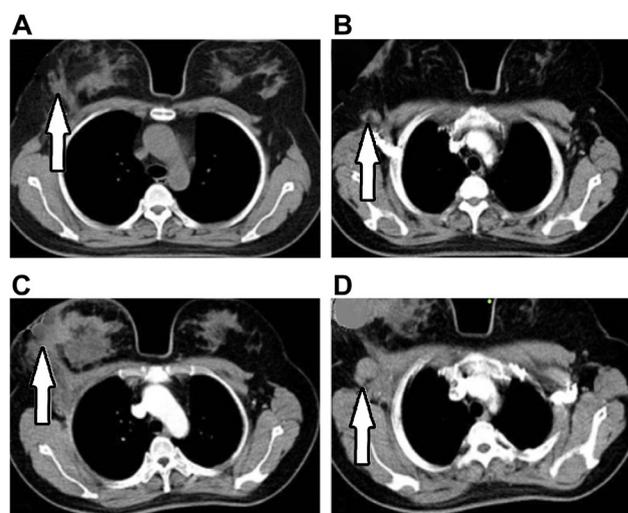| CT evaluation	Pathological evaluation			Total n
	CR n	PR n	NR n	
CR	34	2	1	37
PR	3	26	1	30
NR	1	0	10	11
Total	38	28	12	78

For abbreviations see text

Figure 1. A 51-year-old female patient with invasive breast cancer. **A,B:** The lesion and lymph nodes (arrows) before chemotherapy. **C,D:** The lesion and lymph node sizes are significantly reduced after chemotherapy (arrows).

Table 2. Comparisons of CT perfusion values before and after chemotherapy

Group	n	BF (mL.min^{-1}.100g^{-1})	BV (mL.100g^{-1})	MTT (s)	PS (mL.min^{-1}.100g^{-1})
Before chemotherapy	78	32.25±3.04	6.57±0.75	12.25±2.04	16.57±2.75
After chemotherapy	78	10.56±2.52	2.69±0.46	15.56±2.52	3.69±0.46
t		48.513	38.948	9.016	40.798
p		<0.001	<0.001	<0.001	<0.001

For abbreviations see text

Table 3. Comparisons of tumor diameter and lymph nodes of patients before and after chemotherapy by CT

Group	n	Tumor diameter (cm)	Lymph node size (cm)
Before chemotherapy	78	5.75±2.04	1.97±0.35
After chemotherapy	78	2.36±0.72	1.19±0.26
t		13.840	15.800
p		<0.001	<0.001

Table 4. Comparisons of BCSG1 positive rates in patients with different TNM stages before and after chemotherapy

Group	n	I n(%)	II n(%)	III n(%)	IV n(%)
Before chemotherapy	78	11 (14.10)	13 (16.67)	19 (24.36)	21 (26.92)
After chemotherapy	78	5 (6.41)	6 (7.69)	8 (10.26)	11 (14.10)
x^2					31.081
p					<0.001

Table 5. Comparisons of CT perfusion values in patients with different BCSG1 expressions

Group	n	BF (mL.min^{-1}.100g^{-1})	BV (mL. 100g^{-1})	MTT (s)	PS (mL.min^{-1}.100g^{-1})
Low-expression BCSG1 group	50	8.73±2.05	2.38±0.72	15.78±2.14	3.52±0.65
High-expression BCSG1 group	28	34.78±3.52	7.52±0.86	11.53±2.02	17.48±2.15
t		41.385	28.184	8.582	33.511
p		<0.001	<0.001	<0.001	<0.001

For abbreviations see text

Discussion

Among all malignancies breast cancer has the highest incidence rate in females, seriously impacting the women's physical and mental health. The data show [10] that the incidence rate of breast cancer in women in the United States is up or even more than 25%; this rate in women in China is not high, but its rate is becoming significantly higher than in developed countries in Europe and the United States. The incidence rate of breast cancer in urban areas is significantly higher than in rural areas, showing an obvious geographical difference distribution. The pathogenesis of breast cancer is not yet fully understood, but it is generally believed to involve many factors and steps, such as invasion and infiltration of basement membrane, mutation and proliferation of tumor cells, imbalance of protease secretion, and inactivation of tumor suppressor genes [11]. The early symptoms of breast cancer are not obvious, so they are easily ignored and undiagnosed. Therefore, early detection and early treatment initiation are important control strategies for this disease [12].

Neoadjuvant chemotherapy, also known as preoperative chemotherapy, can play a positive role in the comprehensive treatment of breast cancer, which is also an important part of the total treatment. Neoadjuvant chemotherapy can lower the viability of breast cancer cells, reduce the degree of intraoperative spread, effectively eliminate the micrometastatic lesions, and actively prevent the postoperative lymph node metastasis [13]. In this study, 78 patients received neoadjuvant chemotherapy, and the pathological evaluation showed that the CR rate was 48.72%, PR rate was 35.90%, and the effective remission rate was 84.62%, indicating that this treatment can act on tumor early, so as to ensure the smooth performance of subsequent surgery and promote the postoperative recovery of patients.

With the continuous development of imaging technology, its value in the diagnosis and treatment of cancer has far exceeded serology. CT examination is characterized by high scanning speed, clear images and less susceptibility to peripheral organs. In particular, with the rapid development of CT technique, MSCT is less influenced by motion artifacts, shorter scanning time and higher resolution, so it is more and more widely used in clinical practice [14]. Generally, conventional CT scan can accurately detect the breast cancer mass and assess the degree of infiltration and whether there is enlargement of lymph nodes or not [15]. In the 1990s, with the application of CT perfusion imaging technique, continuous dynamic scan could be performed for ROI after high-pressure i.v. bolus injection of contrast agent, so as to reflect the hemodynamics in tumor tissues; moreover, BF, BV, MTT, PS and other perfusion parameters can be calculated through the relevant professional software based on the time-density curve of blood flow characteristics, and quantitative analysis can be performed for lesions [16].

The results of this study showed that the therapeutic effect of chemotherapy on breast cancer evaluated by MSCT was almost the same as that by pathological evaluation in Kappa consistency test (Kappa value = 0.832, p<0.001). The CT perfusion values of patients after chemotherapy were significantly superior to those before chemotherapy, and tumor diameter and lymph node size were significantly reduced compared with those before chemotherapy (p<0.05), indicating that CT can accurately reflect the size, shape and site of tumors, and show the lesion shrinking more intuitively, which can accurately evaluate the efficacy of chemotherapy in breast cancer patients. This simple method with high evaluation speed is completely comparable to the pathological evaluation [17]. BF and BV before chemotherapy were significantly higher compared with those after chemotherapy, owing to the neovascular basement membrane lesions before chemotherapy allowing high vascular permeability, while vascular resistance is small and blood flow is large. In a more free description, when the contrast agent passes through these new tumor vessels, it will increase the contrast agent content and flow rate in the unit volume at the same time, thus increasing BF and BV. Besides, neoadjuvant chemotherapy can inhibit the proliferation of tumor cells and accelerate the tumor cell apoptosis, thereby reducing the mass [18].

BCSG1 is almost undetectable in benign breast tumors and normal breast tissues, but highly expressed only in malignant breast tumors, which is one of the reasons for its name [19]. BCSG1 can promote the activity and speed up the differentiation and proliferation of breast cancer cells. The results of this study showed that the positive rates of BCSG1 in patients with different TNM stages after chemotherapy were significantly decreased (p<0.05), and the CT perfusion values in the low-expression BCSG1 group were obviously superior compared with those in the high-expression BCSG1 group (p<0.05), indicating that BCSG1 is highly expressed in breast cancer tissues of patients with high pathological grading, because BCSG1 can enhance the motility of cancer cells, result in more aggressiveness, and inhibit tumor growth inhibitory factors, thereby promoting the cell proliferation. Changes in BCSG1 expression will lead to varying blood supply and metabolic status of tumors, and high expression will result in increased blood flow or perfusion, which can be displayed via CT perfusion imaging, so as to grasp the dynamic effect of chemotherapy and win the precious time for the best operation opportunity [20].

Conclusions

In conclusion, the MSCT evaluation for breast cancer patients receiving chemotherapy not only can help understand the changes in lesion morphology and lymph node metastasis before and after chemotherapy, but also detect the hemodynamic changes of tumors; its blood perfusion parameters are closely related to BCSG1 expression, which can provide a basis for determining the chemotherapeutic effect on breast cancer.

Conflict of interests

The authors declare no conflict of interests.

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