

RESEARCH ARTICLE

Expression and Significance of ER, PR, VEGF, CA15-3, CA125 and CEA in Judging the Prognosis of Breast Cancer

Su-Jie Zhang¹, Yi Hu^{1*}, Hai-Li Qian², Shun-Chang Jiao¹, Zhe-Feng Liu¹, Hai-Tao Tao¹, Lu Han¹

Abstract

Objective: To explore the expression and significance of estrogen receptor (ER), progesterone receptor (PR), vascular endothelial growth factor (VEGF), CA15-3, CA125 and carcinoma embryonic antigen (CEA) expression in judging the prognosis of breast cancer. **Materials and Methods:** Sixty-five patients with breast cancer undergoing operations in the general surgery department were considered as the observation group, and 50 healthy outpatients of our hospital as the control group. Cubital venous blood was drawn in the morning from fasting patients in the two groups and chemiluminescence immunoassays were used to detect the levels of CA15-3, CA125 and CEA in serum. The follow-up duration was from 4 months to 2 years, and change in levels of the indicators was detected by dynamically drawing blood. After surgery, cancer tissue samples of patients in observation group remained on file (the non-recurrent patients were biopsied). Immunohistochemistry was applied to determine the expression of ER, PR and VEGF in tissue. **Results:** The effective rate of 12 patients with negative ER and PR expression was 33.3% in the observation group, being associated with prognosis to varying extents. Serum CA15-3, CA125 and CEA in the observation group were all significantly higher than in control group ($p < 0.01$). With increase in pathological staging, levels of serum CA15-3, CA125 and CEA gradually increased ($p < 0.01$). Levels in patients with lymph node metastasis were markedly higher than in those without ($p < 0.01$). In addition, values with distal lymph node metastasis were notably higher than with adjacent lymph node metastasis ($p < 0.01$). The postoperative follow-up results revealed that positive VEGF and levels of serum VEGF, CA15-3, CA125 and CEA in recurrence group were obviously higher than in non-recurrence group ($p < 0.01$). **Conclusions:** Joint detection of ER and PR expression as well as levels of serum VEGF, CA15-3, CA125 and CEA is meaningful and can guide the diagnosis and treatment for breast cancer.

Keywords: Estrogen receptor - progesterone receptor - vascular endothelial growth factor - tumor markers - breast cancer

Asian Pacific J Cancer Prev, 14 (6), 3937-3940

Introduction

Breast cancer is one of the commonly-encountered malignant tumors, and its morbidity is on the rise year by year. Hence, joint detection of tumor markers is of great importance in the early discovery and treatment of patients as well as the judgment on progression and prognosis of disease. The previous studies revealed that the prognosis of patients with breast cancer who had both negative estrogen receptor (ER) and progesterone receptor (PR) was very poor, suggesting that detection of ER and PR expression plays a key role in judging the prognosis of disease (Patel et al., 2013; Petekkaya et al., 2013). Vascular endothelial growth factor (VEGF) capable of promoting angiogenesis exerts an important effect in the process of genesis, development, metastasis and recurrence of various tumors. CA15-3, one of the antigens related to breast cancer, can be used to monitor the recurrence and metastasis of breast cancer (Bolayirli et al., 2013; Tarhan

et al., 2013). Serological indicators CEA and CA125 are also associated with the genesis and development of breast cancer. In the study, the significance of jointly detecting indicators above in judging the prognosis of breast cancer is explored.

Materials and Methods

General data

Sixty-five female patients with breast cancer conducted operation in general surgery department of our hospital from Apr., 2009 to Jun., 2011 were selected as observation group. They were 25-59 years old, and the mean age was (45.6±6.2) years old. Their body weight was 44-59 kg, and the mean was (53.6±5.7) kg. According to pathological staging standards set by committee of National Cancer Institute and Union for International Cancer Control, the staging was performed, in which phase I included 6 cases, phase II 28 cases, phase III 25 cases and phase IV

¹Department of Oncology, Chinese PLA General Hospital, ²National Key Lab of Molecular Oncology, Cancer Institute & Hospital, CAMS, Beijing, China *For correspondence: huyi0401@yahoo.com.cn

6 cases. All patients were not treated with radiotherapy, chemotherapy and endocrinotherapy before operation. The examination results demonstrated that metastasis occurred in 35 out of 65 patients (in which adjacent and distal lymph node metastasis were 20 and 15 cases, respectively), and metastasis was not found in 30 cases. Meanwhile, 50 healthy female people by physical examination in the outpatient of our hospital were selected as control group. They were 26-60 years old, and the mean age was (47.3±4.9) years old. Their body weight was 46-60 kg, and the mean was (54.6±5.4) kg. There were no significant differences regarding the general data like the gender, age and body weight, indicating a comparability between two groups ($p>0.05$).

Methods

The cubital venous blood was drawn in the morning from fasting patients in two groups. After centrifugation at the speed of 1 500 r/min, the supernate was collected and preserved in the freezer at -20°C. Chemiluminescence immunoassay was used to detect the levels of CA15-3, CA125 and CEA in serum. The follow-up duration was from 4 months to 2 years, and the levels of indicators above were detected by dynamically drawing blood. After operation, cancer tissue samples of patients in observation group were on file in formation of wax stones (the non-recurrent patients were punctured to take the examination of biopsy tissue), and were given conventional dewaxing, hydration and antigen retrieval. The immunohistochemistry staining technique was applied to mark the expression of ER, PR and VEGF in tissue and follow-up results.

Evaluation on therapeutic effects

According to the revised edition (edition 1.1) of Response Evaluation Criteria in Solid Tumors (RECIST) in 2009, the therapeutic effect was divided into stable disease (SD) and progressive disease (PD), and the effective rate was calculated based on the SD patients.

Statistical data analysis

SPSS 12.0 statistical software was used to detect the data, and the results were expressed with ($\bar{x}\pm s$). Measurement data between groups was compared with t test, while enumeration data with χ^2 test. $p<0.05$ was represented differences had statistical significance.

Table 1. Comparison of Serum CA15-3, CA125 and CEA Levels in Two Groups ($\bar{x}\pm s$)

Groups	Number of cases	CA15-3/U·mL ⁻¹	CA125/U·mL ⁻¹	CEA/ng·mL ⁻¹
Control group	50	6.02±0.65	9.44±1.24	1.20±0.14
Observation group	65	37.65±4.66**	34.47±3.52**	9.57±1.36**

Compared with control group, ** $p<0.01$

Table 2. Expression of VEGF, CA15-3, CA125 and CEA in Different Pathological Stagings of Patients ($\bar{x}\pm s$)

Indicators	Phase I (n=6)	Phase II (n=28)	Phase III (n=25)	Phase IV (n=6)
Positive VEGF [n(%)]	4(66.67)	24(85.71)	21(84.00)	5(83.33)
CA15-3/U·mL ⁻¹	6.94±0.71	9.14±1.06**	14.36±1.55***	114.69±12.78***△△
CA125/U·mL ⁻¹	10.41±1.06	12.23±1.47**	15.36±1.97***	89.56±8.47***△△
CEA/ng·mL ⁻¹	1.69±0.21	2.72±0.20**	6.89±0.74***	27.56±2.30***△△

Compared with phase I, ** $p<0.01$; compared with phase II, *** $p<0.01$; compared with phase III, △△ $p<0.01$

Results

ER and PR immunohistochemistry staining results

ER and PR expression was negative in 12 patients in observation group, accounting for 18.46%, whereas their expression was positive in 24 patients, occupying 36.92%. ER expression was positive in 9 patients (13.85%), and PR expression was positive in 20 patients (30.77%). The follow-up results for all patients demonstrated that the effective rate of 12 patients with negative ER and PR expression was 33.33% (4/12), in which PD and SD were 8 and 4 cases, respectively. Among the rest, the effective rate was 71.70% (38/53), in which PD and SD were 15 and 38, respectively. It could be concluded that the expression of breast cancer receptors is associated with the prognosis of patients to a certain extent.

Comparison of serum CA15-3, CA125 and CEA levels

The levels of serum CA15-3, CA125 and CEA in observation group were all significantly higher than in control group, and the statistical significance was remarkably presented ($p<0.01$) (Table 1).

Expression of VEGF, CA15-3, CA125 and CEA in different pathological stagings of patients in observation group

In the study, VEGF expression in different pathological stagings of patients in observation group and the levels of serum CA15-3, CA125 and CEA were analyzed. The results revealed that with pathological staging increasing, the levels of serum CA15-3, CA125 and CEA in observation group gradually increased, and the statistical significance was remarkably presented by comparison ($p<0.01$). However, there was no significant difference regarding VEGF expression in different pathological stagings of patients ($p>0.05$) (Table 2).

Relationship between expression of VEGF, CA15-3, CA125 and CEA and lymph node metastasis in observation group

In the study, VEGF expression in the patients with and without lymph node metastasis and levels of serum CA15-3, CA125 and CEA were compared in observation group. Research results revealed the levels of serum CA15-3, CA125 and CEA in the patients with lymph node metastasis were markedly higher than those without lymph node metastasis, and the difference had statistical significance ($p<0.01$). Besides, the levels of serum CA15-3, CA125 and CEA in the patients with distal lymph node metastasis were notably higher than those with adjacent lymph node metastasis ($p<0.01$), whereas the significant difference was not presented concerning the positive VEGF expression rate between the patients with and without lymph node metastasis ($p>0.05$) (Table 3).

Follow-up results

During the follow-up period from 4 months to 2 years, there were 20 recurrent patients (recurrence group) and 45 non-recurrent patients (non-recurrence group). The positive VEGF rate and levels of serum VEGF, CA15-3, CA125 and CEA in recurrence group were obviously higher than in non-recurrence group ($p<0.01$) (Table 4).

Discussion

In recent years, the incidence of breast cancer is significantly on the rise in the East and West, hence, how to treat breast cancer, effectively assess the therapeutic effect, correctively evaluate the prognosis and find the postoperative recurrence of patients with breast cancer in an early stage have been paid attention by more and more scholars at home and abroad (Jiang et al., 2010; Yan et al., 2010; Gao et al., 2011; Huang et al., 2011; Deng et al., 2013; Sun et al., 2013).

ER protein is a dimer, composed of two hormones with molecular weight being 65 000 and sedimentation coefficient being 4S in combination with protein molecules (Bhattacharjee et al., 2012; Godfrey et al., 2013). In addition, ER is also an independent prognostic factor of breast cancer with the condition similar to axillary lymph nodes and the same size. As the end product of estrogen, its synthesis has to depend on the launch of estrogen, illustrating that it is indeed active. However, as a special protein capable of regulating the growth and differentiation of breast cancer, its detection with PR can be applied to judge the prognosis of disease and endocrinotherapy in clinic. Relevant literatures reported that positive ER expression was obviously higher than the negative in postoperative 5-year survival rate of breast cancer, and both recurrence and distal metastatic rate were lower, whereas this advantage was unobvious over 5 years (Dede et al., 2013; Rosato et al., 2013; Wang et al., 2013). There are also some relevant studies regarding PR in breast cancer (Joensuu et al., 2013; Lee et al., 2013). The breast cancer with both negative ER and PR, characterized by strong invasiveness, high exacerbation, great probability of distal metastasis and

poor prognosis, is a key factor to affect the long-term prognosis, especially for the prognosis of breast cancer (Omran et al., 2012; Prakhya et al., 2012; Sandoval et al., 2013). In the study, the results revealed that the effective rate of 12 patients with negative ER and PR expression in observation group was 33.33%, in which PD and SD were 8 and 4 cases, respectively. Among the rest, the effective rate was 71.70%, in which PD and SD were 15 and 38, respectively. It could be concluded that the expression of breast cancer receptors is associated with the prognosis of patients to a certain extent.

One of the major causes resulting in the patients with breast cancer death is postoperative tumor recurrence and metastasis. More effective clinical predictors have been explored in medical field for many years, for comprehensively and accurately understanding the probability of postoperative recurrence and metastasis of breast cancer (Godfrey et al., 2013; Vermeulen et al., 2013; Zubeda et al., 2013). The tumor-related indicators in all individuals cannot be detected systemically and comprehensively due to limited economics and techniques, thereby, joint detection of specific tumor markers is of great importance (Wang et al., 2012; Aktas et al., 2013; Lawicki et al., 2013).

In the process of tumor genesis and development, tumor regenerative capillaries capable of providing nutrients for tumor cells and favorable conditions for distal metastasis are the precondition to induce the local growth, infiltration and distal metastasis of malignant tumors, hence, how to inhibit tumor angiogenesis is a new research hotspot at present (Thielemann et al., 2013). VEGF, one of the key factors to promote tumor angiogenesis and with the strongest function and highest specificity, can not only promote the proliferation of endothelial cells, but also regulate and participate in angiogenesis. Due to an intimate association with genesis, development, metastasis and infiltration of breast cancer, it is an important indicator to judge the metastasis and infiltration of breast cancer in clinic (Luo et al., 2013). CA15-3, a variant of mammary epithelial surface glycoprotein and an antigen related to breast cancer, can be considered as the indicator to detect recurrence and metastasis of breast cancer. Studies have confirmed that as a sort of glycoprotein, CA125 can be identified by monoclonal antibody OC125, and has a certain correlation with the genesis and development of breast cancer, whereas CEA as a sort of broad-spectrum tumor markers is applied to detect the postoperative recurrence rate of various epithelial tumors and judge the prognosis (Lawicki et al., 2013). In the study, the clinical value of jointly detecting CA15-3, VEGF, CA125 and CEA on the breast cancer was primarily investigated, and achieved a better application effect. The results in the study revealed that VEGF was positive in the patients with early breast cancer, in which the positive rate at phase I came up to 66.67%. However, CEA, CA125 and CA15-3 also

Table 3. Relationship between Expression of VEGF, CA15-3, CA125 and CEA and Lymph Node Metastasis ($\bar{x}\pm s$)

Indicators	No lymph node metastasis (n=30)	Adjacent lymph node metastasis (n=20)	Distal lymph node metastasis (n=15)
Positive VEGF [n(%)]	21(70.00)	18(90.00)	15(100.00)
CA15-3/U·mL ⁻¹	7.45±0.84	20.23±2.74**	128.09±12.47***
CA125/U·mL ⁻¹	11.14±1.23	32.23±3.07**	85.41±8.52***
CEA/ng·mL ⁻¹	1.74±0.20	6.25±0.63**	36.20±4.72***

Compared with the patients without lymph node metastasis, ** $p<0.01$; compared with the patients with adjacent lymph node metastasis, ** $p<0.01$

Table 4. Expression of VEGF, CA15-3, CA125 and CEA in Follow-up Patients ($\bar{x}\pm s$)

Groups	Number of cases	Positive VEGF [n(%)]	CA15-3/U·mL ⁻¹	CA125/U·mL ⁻¹	CEA/ng·mL ⁻¹
Non-recurrence group	45	0(0.00)	12.47±1.35	11.66±1.78	16.34±2.14
Recurrence group	20	20(100.00)**	64.74±7.36**	43.87±5.20**	41.69±3.98**

Compared with non-recurrence group, ** $p<0.01$

had a certain clinical significance in the early diagnosis of tumors, and their early levels were remarkably higher than in control group. Meanwhile, the positive VEGF rate in the patients with lymph node metastasis was also conspicuously higher than those without lymph node metastasis. During a follow-up period, each indicator in recurrent patients was all higher than non-recurrent ones notably. These results suggest that joint detection of the indicators above can judge the prognosis better, which is of great importance to monitor recurrence and metastasis. Besides, joint detection of CA15-3, VEGF, CA125 and CEA can improve their own sensitivity without decreasing diagnostic accuracy and specificity of breast cancer, but the effect is still limited.

To sum up, joint detection of VEGF, ER, PR, CEA, CA125 and CA15-3 can complement mutually in the diagnosis, treatment and prognosis of breast cancer, and can be regarded as a crucial reference indicator. VEGF, a commonly-encountered effective tumor marker, is conducive to judging the patients with early breast cancer. The expression of CA15-3, CA125 and CEA has an important application value in the aspects of assessing lymph node metastasis, recurrence monitoring and clinical staging of tumors in clinic.

Acknowledgements

This study is supported by the Science Foundation of Jie-Ping Wu.

References

- Aktas B, Kasimir-Bauer S, Lehmann N, et al (2013). Validity of bone marker measurements for monitoring response to bisphosphonate therapy with zoledronic acid in metastatic breast cancer. *Oncol Rep*, **30**, 441-7.
- Bhattacharjee KG, Bhattacharyya M, Halder UC, et al (2012). The "Cross Talk" between the receptors of insulin, estrogen and progesterone in neutrophils in the synthesis of maspin through nitric oxide in breast cancer. *Int J Biomed Sci*, **8**, 129-39.
- Bolayirli M, Papila C, Korkmaz GG, et al (2013). Serum thymidine kinase 1 activity in solid tumor (breast and colorectal cancer) patients treated with adjuvant chemotherapy. *J Clin Lab Anal*, **27**, 220-6.
- Dede DS, Gumuskaya B, Guler G, et al (2013). Evaluation of changes of biologic markers ER, PR, HER 2 and Ki-67 in breast cancer with administration of neoadjuvant dose-dense doxorubicin, cyclophosphamide followed by paclitaxel. *J BUON*, **18**, 57-63.
- Deng QQ, Huang XE, Ye LH, et al (2013). Phase II trial of Loubol (Lobaplatin) and pemetrexed for patients with metastatic breast cancer not responding to anthracycline or taxanes. *Asian Pac J Cancer Prev*, **14**, 413-7.
- Gao LL, Huang XE, Zhang Q, et al (2011). 14.A Cisplatin and vinorelbine (NP) regimen as a postoperative adjuvant chemotherapy for completely resected breast cancers in China: final results of a phase II clinical trial. *Asian Pac J Cancer Prev*, **12**, 77-80.
- Godfrey AC, Xu Z, Weinberg CR, et al (2013). Serum microRNA expression as an early marker for breast cancer risk in prospectively collected samples from the Sister Study cohort. *Breast Cancer Res*, **15**, R42.
- Huang XE, Li CG, Li Y, et al (2011). Weekly TP regimen as a postoperative adjuvant chemotherapy for completely resected breast cancer in China: final result of a phase II trial. *Asian Pac J Cancer Prev*, **12**, 2797-2800.
- Jiang Y, Huang XE, Yan PW, et al (2010). Validation of treatment efficacy of a computer-assisted program for breast cancer patients receiving postoperative adjuvant chemotherapy. *Asian Pac J Cancer Prev*, **11**, 1059-62.
- Joensuu K, Leidenius M, Kero M, et al (2013). ER, PR, HER2, Ki-67 and CK5 in early and late relapsing breast cancer-reduced CK5 expression in metastases. *Breast Cancer (Auckl)*, **7**, 23-34.
- Lawicki S, Będkowska GE, Szmitkowski M (2013). VEGF, M-CSF and CA 15-3 as a new tumor marker panel in breast malignancies: a multivariate analysis with ROC curve. *Growth Factors*, **31**, 98-105.
- Lee M, Lee CS, Tan PH (2013). Hormone receptor expression in breast cancer: postanalytical issues. *J Clin Pathol*, **66**, 478-84.
- Luo T, Chen L, He P, et al (2013). Vascular endothelial growth factor (VEGF) gene polymorphisms and breast cancer risk in a chinese population. *Asian Pac J Cancer Prev*, **14**, 2433-7.
- Omran OM (2012). The prognostic value of breast cancer resistance protein (BCRB/ABCG2) expression in breast carcinomas. *J Environ Pathol Toxicol Oncol*, **31**, 367-76.
- Patel T, Gupta A, Shah M (2013). Pathological predictive factors for tumor response in locally advanced breast carcinomas treated with anthracyclin-based neoadjuvant chemotherapy. *J Cancer Res Ther*, **9**, 245-9.
- Petekkaya I, Sahin U, Gezgen G, et al (2013). Association of breast cancer subtypes and body mass index. *Tumori*, **99**, 129-33.
- Prakhya S, Prakash G (2012). Analysis of BRCA1 gene rearrangements in breast carcinomas by RT-PCR and ER, PR, HER2/NEU status by IHC and HPE correlation. *J Clin Diagn Res*, **6**, 1663-8.
- Rosato V, Bertuccio P, Bosetti C, et al (2013). Nutritional factors, physical activity, and breast cancer by hormonal receptor status. *Breast*, [Epub ahead of print].
- Sandoval C, Rahal R, Forte T, et al (2013). Indicator measures er/pr and her2 testing among women with invasive breast cancer. *Curr Oncol*, **20**, 62-3.
- Sun MQ, Meng AF, Huang XE, et al (2013). Comparison of psychological influence on breast cancer patients between breast-conserving surgery and modified radical mastectomy. *Asian Pac J Cancer Prev*, **14**, 149-52.
- Tarhan MO, Gonel A, Kucukzeybek Y, et al (2013). Prognostic significance of circulating tumor cells and serum CA15-3 levels in metastatic breast cancer, single center experience, preliminary results. *Asian Pac J Cancer Prev*, **14**, 1725-9.
- Thielemann A, Baszczuk A, Kopczyński Z, et al (2013). Clinical usefulness of assessing VEGF and soluble receptors sVEGFR-1 and sVEGFR-2 in women with breast cancer. *Ann Agric Environ Med*, **20**, 293-7.
- Vermeulen JF, van der Wall E, Witkamp AJ, et al (2013). Analysis of expression of membrane-bound tumor markers in ductal carcinoma in situ of the breast: paving the way for molecular imaging. *Cell Oncol (Dordr)*, [Epub ahead of print].
- Wang J, He Q, Shao YG, et al (2013). Chemokines fluctuate in the progression of primary breast cancer. *Eur Rev Med Pharmacol Sci*, **17**, 596-608.
- Wang KH, Kao AP, Lin TC, et al (2012). Promotion of epithelial-mesenchymal transition and tumor growth by 17 β -estradiol in an ER(+)/HER2(+) cell line derived from human breast epithelial stem cells. *Biotechnol Appl Biochem*, **59**, 262-7.
- Yan PW, Huang XE, Jiang Y, et al (2010). A clinical comparison on safety and efficacy of Paclitaxel/Epirubicin (NE) with Fluorouracil/Epirubicin/Cyclophosphamide (FEC) as postoperative adjuvant chemotherapy in breast cancer. *Asian Pac J Cancer Prev*, **11**, 1115-8.
- Zubeda S, Kaipa PR, Shaik NA, et al (2013). Her-2/neu status: a neglected marker of prognostication and management of breast cancer patients in India. *Asian Pac J Cancer Prev*, **14**, 2231-5.