Prognostic Significance of Axillary Lymph Node Micrometastases and Microvessel Count in Breast Cancer

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OBJECTIVE To investigate the influence of axillary lymph node micrometastases and the microvessel count on the prognosis of patients with breast cancer.

METHODS Forty-eight patients with breast cancer, who had no tumor cells in their regional lymph nodes based on conventional histopathologic examination, were re-examined with immunohistochemical LSAB techniques. H&E, anti-EMA, CK 19 and FVIII factor staining was used to identify tumor cells in both lymph nodes and tumor tissues and to count the microvessels. A total of 882 lymph nodes were examined.

RESULTS Immunostaining-positive tumor cells were found in 9.0 %(79/882) of the dissected lymph nodes. The positive rates were not significantly different between a surviving group and a deceased group ($P>0.05$). The microvessel count was significantly higher in group that had died ($P<0.001$).

CONCLUSION The lymph node micrometastases did not show any correlation with patients' survival, but the microvessel density had a negative correlation with the survival period in breast cancer patients who had negative axillary lymph nodes.

KEYWORDS: lymph node micrometastases, breast cancer, prognosis, microvessel count.

It is widely accepted that tumor cells disseminated from a primary breast tumor are first harbored in regional lymph nodes. They may remain quiescent there for a variable time but ultimately reach the systemic circulation. A more recent investigation confirms that most breast cancers are systemic diseases, but whether the axillary lymph node metastases or not is still a major prognostic factor. Therefore it is understandable that the lymph node status, especially the axillary lymph node status, can play an important role in evaluation of the clinical stage of breast carcinoma as well as therapy and outcome. In approximately 15% of patients in whom tumors were classified as lymph-node negative by standard histological examination, tumors recurred within 5 years after initial treatment.[1] The reliability of routine histological examination was about 70% . Examination of serial sections of axillary lymph nodes stained either with H&E or anti-cytokeratin and anti-EMA antibodies revealed undetected micrometastases in 9~30% of cases that were missed on routine examination.[1-10]

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MATERIALS AND METHODS

A total of 564 patients with breast cancer were operated on in 1993 at the Tianjin Cancer Hospital. Among them, 533 cases (98.0%) were followed-up over a 10-year period. No lymph node metastases were detected in 277 cases (49.1%) on routine histological examination, but 24 of them (8.7%) died of metastases within 5 years after initial treatment. The shortest survival period was 6 months. These 24 cases were designated as the deceased group. Another 24 cases who were alive were selected as the control group.

Materials

The median age of the 24 deceased patients was 47 years (range 29~70). Fourteen patients were premenopausal and 10 postmenopausal. One case was stage 0, 1 stage I, 21 stage II, and 1 stage III. Hormone receptors were measured in 14 cases. Three were positive for both estrogen (ER) and progesterone receptors (PR), 3 were negative for both ER and PR, 9 were positive for only with ER and 3 were positive for only PR. Histological examination showed that 13 were simple carcinomas (54.2%). Their median size was 3.2 cm (range 0~12 cm). The median survival time was 34 months.

Methods

A total of 882 lymph nodes from 48 cases were dissected and fixed in 102 paraffin blocks. These lymph nodes were re-examined at 5 μ m interval serial sections. One of every four sections was stained with H&E or EMA and CK19. Tumor vascularity was quantified using antibody to factor VIII in order to identify the number of microvessels per high-power field (at × 200). Results were analyzed with SSPS 10 software.

RESULTS

In 882 resected lymph nodes, a total of 79 micrometastatic nodes were detected, accounting for 9.0% . These positive nodes were found by the following staining: 7 were found by EMA, CK and H&E, 9 by EMA and CK, 43 by only EMA, and 14 by only CK.

H&E staining and outcome

Conversion of lymph nodes from negative into positive was observed in 13 cases out of the 882 (1.5%) nodes. Seven (53.8%) were in the deceased group and 6 (46.2%) were in the control group. There was no statistician difference between the two groups (P>0.05).

EMA staining outcome

The micrometastases were detected in 60 (7.0%) of the 882 nodes. Positive tumor cells showed a brown color in the cytoplasm and on the membrane, without coloring in cytoblast. Of the sixty-two nodes with detected micrometastases, 32 (54.5%) were in the deceased group and 30 (45.5%) in the control group. There was no significant difference between the two groups(P>0.05).

CK staining outcome

Thirty (3.4%) of the 882 nodes were detected to have micrometastases by CK staining. The positive tumor cells had a blue color in the cytoplasm. Of the 30 positive nodes, 16 (54.2%) were in the deceased group and 14 (45.8%) in the control group. There was no significant difference between the two groups(P>0.05).

Microvessel count outcome

In the deceased group, there were more than 80 microvessels, and the highest count in the control group was 72 per 200× field. These results indicated that when there was a high microvessel count in single field, the patient survival was shorter.

Linear Regression outcome

The survival time was treated as a dependent variable; the microvessel count was an independent variable. Overall variation explained by the model was 0.870, and variation explained by microvessels count was 0.756, which suggests that the survival time was decided by the microvessel count.

The results from ANOVA showed that the regression coefficient was-0.628, P=0.000, and that the microvessel count was statistically significant.

In the equation of the number of lymph nodes micrometastases qua independent and survival time qua dependent, R=0.179, R^2=0.032, it showed that the 3.2% of survival time was decided by the number of lymph nodes micrometastases. ANOVA analysis outcome: F=0.675, P=0.515, it showed that the regression equation was effectless. Regression coefficient was 3.917, P=0.266, it showed that the number of lymph nodes micrometastasis did not have statistical significance.

Correlation analysis outcome

The was a negative correlation between tumor's microvessel count and survival time. The coefficient of correlation which was -0.87, P=0.000, showed that between the tumor's microvessel count and the survival time there was a high correlation.

The results suggest that discriminant analysis was useful for clinical prognosis and determine the effects of factors associated with breast cancer. Therefore it could be used to predict pathogens or/and prognosis of disease.

This study utilized discriminance to analyze the effect of lymph node micrometastasis and tumor microvessel count on survival time. According to lymph node micrometastasis and tumor microvessel count, qua independent were used by processing discriminated analysis and setting up the whole model.

The outcome of ANOVA, P=0.000, showed that the discriminant equation was efficient.

The correlative coefficient derived from the discriminated analysis shows that tumor microvessel was statistically associated disease prognosis.

Discriminant analysis outcome: the discriminated correct rate of tumor microvessel count was 100% in the deceased group and was 95.5% in the control group. The overall discriminated correct rate was 97.7%.

Cox's multivariate analysis outcome: Cox regression equation χ^2=35.581, P=0.000, showed that the regression equation fitted in preferably. RR of a microvessel count was 7.635; which showed that the probability of death of the patients with a microvessels count ≥ 71 per 200 × field was 7.635 times greater than that the patients with a microvessel count ≤ 70 per 200 × field. Lymph nodes micrometastasis had no relation to survival time.

In general, the lymph node micrometastases did not show a relation to patients'survival in this study. The microvessel count of the tumors and the survival time of the breast cancer patients with negative axillary lymph nodes resulted in a negative correlation.

DISCUSSION

Axillary lymph node metastases are an important indicator in estimating prognosis of breast cancer patients.[1-11] The 5-year overall survival (OS) rate in patients with negative axillary lymph nodes was 91.3% (515/564) and but at 5 years after treatment 8.7% of the patients had died because of metastases. The 5-year OS in 85 cases of more than 10 lymph node metastasis was 35.2%.

In our study, the general accuracy of the lymph node status in predicting prognosis was 75.0% (56.5% in the deceased group and 80.5% in the survival group.); however the microvessel count of the tumors was more reliable than lymph nodes status.

Lymphatics are a major route by which tumor cells are transferred in breast cancer. Almost 50% of tumor cells that are transferred go through lymphatics. Our investigation showed that formation of lymphatic was very important in the tumor transfer process. The degree of lymphatic-canal formation had a positive correlation with lymph node metastasis. The wide space of the capillary lymphatic canal lining with a single layer of endothelial cells, their loose connection with each other, and lack of intact basal membranes, provides a route for the tumor cells to easily penetrate the wall of vessels. It has been reported that lymphangiogenesis and angiogenesis have different routes for signal transudation and lymphangiogenesis has a particular mechanism of regulation.[9]

Additional studies have shown that angiogenesis plays an important role in development, invasiveness, and metastasis of tumors.[9] In order to access the extent of tumor angiogenesis, microvessels in the tumor tissues are counted, a quantifying method, which reflects the intensity of angiogenesis. Tumors cannot grow larger than 2~3 mm^3 without neovascularization. When tumors grow, regulation of angiogenesis is disorganized, resulting in the abnormality of new capillary blood vessels, such as incomplete basal membranes, very thin vessel walls, easy permeation, and disorganization. The tumor cells easily penetrate the wall of vessels, and then the chance of tumor metastases is increased. Pervious investigators have examined a large number of histological cells' vascular endothelial growth factor and microvessel density in benign and malignant diseases and analyzed lymph node status, tumor size and histological grade. They found that lymph node status, the size of the tumor, histological grade and microvessel density were independent prognosis indicators.[12] But lymph angiogenesis and the mechanism of metastasis have been unclear.

The significance of occult axillary lymph node metastases or axillary lymph node micrometastases in patients with breast cancer is controversial.[1-9] Millis and Mscarel's study found that micrometastases were of no prognostic significance.[2-3] The results were the same with this study. Dowlathshahi et al. suggested that the effect of axillary lymph node micrometastases

on survival appeared to need a great cases and follow-up above 10-year.[1] This is a question to be resolved. Investigators agree that the prognosis for breast carcinoma patients is a very complex problem and involves consideration of many unknown factors. The use of multivariable analysis of the interaction of various findings should reduce errors of prognosis.

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