Chinese Journal of Cancer Research 9(2):146 — 149, 1997

DETECTION OF MICROMETASTASES IN AXILLARY LYMPH NODES OF NODE-NEGATIVE BREAST CANCER PATIENTS AND ITS CLINICAL SIGNIFICANCE

Wu Jindong 吴进冬　Yu Zihao* 余子豪　Li Guomin 李国民　Zhang Hongtu* 张宏图

Cancer Center. Academy of Military Medical Science, Beijing 100039

** Cancer Hospital, Chinese academy of medical Sciences. Beijing, 100021*

Monoclonal antibodies against cytokeratin (AE1/AE3) were applied as a probe, using APAAP immunohistochemistry technique to detect 635 lymph nodes from 45 breast cancer patients with negative lymph nodes. Micrometastases were identified in 14 lymph nodes of 9 cases (20%). A significant difference was found between cytokeratin staining positive group and cytokeratin staining negative group in disease-free and over-all Kaplan-Meier survival curves. The detection of micrometastases had more clinical value for T_1 and T_2 patients. One of 2 T_1 cytokeratin positive cases relapsed while only 1 of 19 T_1 negative cases relapsed within 5 years; three of 5 T_2 cytokeratin positive cases relapsed while 1 of 17 negative cases did. The presence of micrometastases had the same value in predicting local recurrence and distant metastases.

Key words: Breast neoplasm, Keratin, Lymphatic metastasis, Immunohistochemistry

The breast cancer patients with axillary node-negative (ANN) have better prognosis, but 15-20% of them have a recurrence within 10 years after primary treatment. The presence of axillary nodal metastases is the most important prognostic factor in primary operable breast cancer patients. So it was guessed that in operation there were lymph node micrometastases missed on routine staining in these relapsed patients with ANN breast cancer. In this study, monoclonal antibodies against cytokeratin (CK) were applied as a probe, using APAAP immunohistochemistry technique to detect 635 lymph nodes from 45 ANN patients with detailed clinical information. By analyzing the relationship between micrometastases and prognosis, we discuss detection of micro-metastases and its clinical value.

Accepted Jan 28, 1997

MATERIALS AND METHODS

Patients' Information and Sample Examined

A series of 48 consecutive patients operated in cancer Hospital of Chinese Academy of Medical Sciences for breast cancer (N_0 M_0) between 1980 and 1983 were selected for study. All of patients were treated by a radical operation or a modified radical operation. Three cases were excluded because of inadequate material. Finally, 45 cases were included in this study and all of them were female. The mean age of the patients was 48 years at operation with a range from 24 to 67.

21 patients had T_1 stage and the average diameter of the primary tumor was 1.5 cm; 22 patients had T_2 stage with 3.4 cm average diameter; two cases had T_3

stage and the diameters were 5.5 cm and 6.0 cm.

Seven cases were postoperatively irradiated and one received single drug of 5-Fu of chemotherapy.

Histological type: 20 patients had carcinoma simplex, 14 had invasive ductal carcinoma (IDC), 5 had medullary carcinoma, 3 had mucinous carcinoma, and 3 had other types.

635 lymph nodes of 45 patients were tested and the mean number in each case was 14 lymph nodes with a range from 4 to 23. The 4 μm thick section of each lymph node was floated onto slide before Immunohistochemical staining.

Methods of Staining

Main reagents

Monoclonal antibodies against cytokeratin was AE1/AE3 produced by ZYMED, USA and APAAP kit was produced by Beijing Basic Medical Research Institute, China.

Staining procedures

First, the 4 μm sections of lymph nodes were floated onto slides and allowed to dry overnight at 55 ℃ . Then the sections were deparaffinized in dimethylbenzene, and dehydrated in descending grades of ethanol and distilled water. Next, sections were digested with 0.1% trypsin at 37 ℃ for 20 min. After a wash in tap water the sections were in cubated with normal bovine serum at a dilution of 10% at room temperature for 20 min. AE1/AE3 at a dilution of 1/50 were applied over night as first antibodies in a humid chamber at 4 ℃ . After washing in PBS (phosphate-buffered saline), a polyvalent rabbit anti-mouse immunoglobin (secondary antibodies) and preformed complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase antibodies (third antibodies) were used. Finally, the sections were developed with fast red developer solution for 3-5 min., coverstained in hematoxylin for 30s, and overglass was applied using glycerol gelatin.

Result judgment

The CK antigen was present in cytoplasm, and the labeled cells were developed to be rose red while the negative cells showed no color. In normal condition there should not be present CK labeled cells in lymph nodes. If there were CK labeled cells. It must be come from other subtype tissue. Combing the shape of the CK labeled cells, we decided that if these cells were micrometastases (CK positive).

Follow-up Materials and Statistical Analysis

After finishing the examination of the micrometastases in lymph nodes, we got the follow-up materials of the 45 patients and the statistical analysis were completed with the help of the statistical department of Academy of Military Medical Science. The disease-free and overall Kaplan-Meier survival curves were studied between the CK positive and the CK negative groups. The results were tested by several statistical methods and it was considered to be the significant value if P value was smaller then 0.05.

RESULTS

Result of the Patients' follow-up

The average time of follow-up from surgery to death or end of study was 87 months with range from 21 to 160 months and the follow-up time of the survival cases was over 5 years, the nine of 45 cases had local recurrence and distal metastases and 7 of 9 had died from the cancer (Table 1).

Result of the Micrometastases' Test

The micrometastases were detected in 14 lymph nodes (14/635) from 9 cases of 45 ANN patients. In positive lymph nodes the micrometastases had been shown three types: the small cell clusters, the disseminated cells and the single cell. The majority of positive cases (8/9) had only 1 or 2 positive lymph nodes. The positive staining of micrometastases was more frequent with increasing of the original lesion (T stage). The detection rates of micrometastases were separately 9.5% in the group with T_1 stage (2/21), 22.7% in the group with T_2 stage (5/22) and 2/2 with T_3 stage (Table 2).

Relationship between Result of Micrometastases and Prognosis

Five from 9 cases with micrometastases have developed local recurrence and distant metastases (2

chest wall recurrence, 1 supraclavicular node metastases, 2 distant metastases) while only 4 from 36 cases without micrometastases have developed local recurrence and distant metastases (1 chest wall recurrence, 1 supraclavicular node metastases, 2 distant metastases). A significant difference had been shown between cytokeratin staining positive group and cytokeratin staining negative group in Kaplan-Meier curves. Five-year recurrence rates of two groups with CK+ and CK - were 44.4% (4/9) and 5.6% (2/36, P <0.005) and 10-year recurrence rates 55.6% and 12.8% (P<0.01).

Table 1. The 9 failure patients' data and CK status

T stage	Tumor size (cm)	CK status	The site of recurrence	metastases distant	The time of recurrence (Mo)
T1	1 × 1	–	chest wall		9
T1	1.5 × 1.5	+	chest wall		20
T1	2 × 2	–		bone, pleura	139
T2	3 × 2	–		lung	24
T2	4 × 4	+	chest wall		24
T2	3 × 4	+		lung	72
T2	2.5 × 2.5	–	supraclavicular node		75
T2	2 × 2.5	+	supraclavicular node		27
T2	3 × 2	+		liver, bone	15

Table 2. The 9 CK+ patients' data

T stage	Tumor size (cm)	Pathological classification	Lymphnodes examined	CK+	Survival (Mo) (free of tumor)
T1	2 × 2	scirrhous cancer	18	1	82
T3	5 × 5.5	squamous carcinoma	11	1	88
T2	4 × 4	IDC	18	1	123
T2	4 × 4	carcinoma simples	18	1	24
T1	1.5 × 1.5	carcinoma simples	11	1	20
T2	3 × 4	carcinoma simples	6	2	72
T3	5 × 6	medullary carcinoma	21	2	118
T2	2.5 × 2	carcinoma simples	15	3	27
T2	3 × 2	IDC	18	2	15

DISCUSSION

The researchers have been trying and hoping to find the biological behavior of the breast neoplasm through studying the original lesion in order to expect the metastasis ability of the cancer cell and predict ANN patients prognosis. It is more value to detect the micrometastases in lymph nodes of ANN patients than to study the original lesion in order to estimate indirectly the metastases ability. Pickren[1], Fisher[2] and Wilkinson[3] had demonstrated that the detection of the different slides from the ANN cases' lymph nodes by serious sections could increase 14% to 24% of the metastasis detected rate in lymph nodes. It was impossible to use it as the routine way in clinical practice because its work was too much. The detection of the micrometastases in lymph nodes from the ANN patients by applying AE1/AE3 as a probe and using immunohistochemistry technique differs from the detection by serious sections in that it labeled the epithelial content coming from the outside and identified the micrometastases undetected by the routine microscopy. Trojani, a French researcher, reexamined primary HE slides of lymph nodes from

150 ANN patients and made sure that there was no metastases. 14% of these cases had been found with micrometastase by immunohistochemistry. The HE primary had not been tested by immunohistochemistry in our study, but the retested slides were as near as possible to the levels of the primary HE slides which had been reexamined by the routine way and had no metastases. Through observing the CK positive samples, we had found that the micrometastases were in either very small cell cluster or a single cell type. In spite of many tumor cells in a few lymph nodes, they disseminated in the lymph nodes and did not break the normal structure of the lymph nodes. As we known, there are the same color between the normal cells and the tumor cells in the HE slides, so the metastases must be identified according on its tissue structure and its cell shape. Observing the HE slides is a simple and efficient way to detect the metastases in the lymph nodes for the big enough site of metastases, but it is not sensitive enough for the above micrometastases.

The micrometastases rate was 20% (9/45) among 14-29% reported by the other researchers.[4,5] The CK+ rates increased with the increasing of T stage (T_1 9.5%, T_2 22.7%, T_3 2/2). This phenomena correspond to the law that the more frequent the metastases are in the lymph nodes, the bigger the original lesions. Whether there was relationship between the prognosis and the micrometastases is more important question for us to study. According to the other research[5], the CK positive rate (labeled by AE1/AE3) of 61 ANN patients is 29% and 3 of 4 distant metastases' cases were detected out micrometastasis in lymph nodes.

The value of the detection of micrometastases had been shown from several aspect below in our study: 1) It is possible to spread out the way, because the monoclonal antibody AE1/AE3 had been reliable and the immunohistochemistry technique APAAP had been mature; 2) Five of 9 positive cases had developed local relapse and/or distant metastases while only 4 of 36 negative cases had; 3) The disease-free survival and the overall survival of the ANN patients without nodal micrometastasis overmatch those with nodal micrometastases mucy more. The 5-year recurrence rates (5.6%, 44.4%) and 10-year rates (12.8%, 55.6%) between them had significant differences.

In conclusion, it is a effective approach to detect the micrometastases in lymph nodes of the ANN breast cancer patients by the way of immunohistochemistry technique APAAP and using AE1/AE3 as the probe. The detected micrometastases is correlated with the prognosis of the patients. The technique is worthy to recommend. It is necessary to study on large samples for our samples were not enough.

REFERENCES

1. Pickren JW. Significance of occult metastases: a study of breast cancer. Cancer 1961; 14: 1266.
2. Fisher ER, Swamicoss S, Lee CI, et al. Detection and significance of occult axillary node metastases in patients with invasive breast cancer. Cancer 1987; 42: 2025.
3. Wilkinson EJ, Hause LL, Kuzma JF. Occult axillary lymph node metastases in patients with invasive breast carcinoma. Lab Invest 1981; 44: 83.
4. Torjani M, Mascarel I, Bonichon F, et al. Micrometastases to axillary nodes from carcinoma of breast: detection by immunohistochemistry and prognostic significance. Br J Cancer 1987; 55: 303.
5. Chen Zhaolun, wen Duanren, Coulson WF, et al. Occult metastases in the axillary lymph nodes of patients with breast cancer node negative by clinical and histologic examination and conventional histology. Disease Markers 1991; 9: 239.