DETECTION AND SIGNIFICANCE OF LYMPH NODE MICRO-METASTASES IN PATIENTS WITH NODE-NEGATIVE GASTRIC CARCINOMA

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ABSTRACT

Objective: To study micrometastases in lymph nodes from patients with node-negative gastric carcinoma by routine histologic examination and discuss their prognostic significance and the relationship between micrometastases and each of the clinicopathologic factors. Methods: A total of 1245 perigastric lymph nodes from 105 patients with node-negative gastric carcinoma was immunohistochemically detected using a monoclonal antibody against low molecular weight cytokeratin AE_1. The characteristics of the micrometastases, their related factors and effect on patients' survival after surgery were analysed and tested with statistical methods. Results: Micrometastases were observed in 81 lymph nodes (6.5%) of 31 patients (29.5%). The incidence of lymph node micrometastases was significantly higher in the diffuse type (41.5%) than in the intestinal type gastric carcinoma (17.6%, $P<0.01$, x^2 test). In addition, the presence of micrometastases was closely correlated with the size and invasion depth of the primary tumor, but had no relation to patient's age, sex and the location of primary tumor. The patients with micrometastases had significantly worse prognosis shown by Log-rank test. Their five-year survival rate after surgery was 61.29%; for those without micrometastases the rate was 82.43%, $P=0.0116$. When the number of patient's lymph nodes with micrometastases was three or more, the five-year survival rate of these patients was much lower (41.67%, $P=0.0012$). Conclusion: The detection of lymph node micrometastases is necessary to more accurately determine the prognosis and clinical staging of patients with node-negative gastric carcinoma by routine histologic examination. The presence of micrometastases may be regarded as one of the clues in adjuvant therapy of those patients.

Key words: Gastric carcinoma, Micrometastases, Lymph node, Immunohistochemistry

Lymph node micrometastases refer to minute cancer metastases in lymph nodes whose diameter is less than 2 mm,[1] and they are difficult to be observed with routine histologic examination. In early years serial sectioning was frequently used in detecting lymph node micrometastases. Since the 80's immunohistochemical technique has been commonly employed, and recently reverse transcriptases-polymerase chain reaction is also applied in order to detect micrometastases.[2-8] Although all techniques mentioned above could partly increase the detection rate of lymph node micrometastases, the significance of micrometastases is still uncertain. So far, the researches of micrometastases are mostly on breast cancer and those on gastric carcinoma are very few. However, the mortality of gastric carcinoma is the highest in all malignances in China.[9] In this study micrometastases of node-negative gastric carcinomas with routine histologic examination were detected, and the prognostic significance and correlated factors of micrometastases were discussed.

MATERIALS AND METHODS

Patients and Specimens

One hundred and five patients with gastric carcinoma who had undergone gastric resection between 1985 and 1993 at Beijing Cancer Hospital and been followed up over 60 months were selected for this study. All patients were node negative by routine histolgic examination. Their detailed clinicopathologic data were given in Table

Received December 10,1999; accepted January 19, 2000
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1. Two 4 μm-serial sections were prepared from formalin-fixed and paraffin-embedded blocks containing a total of 1245 perigastric lymph nodes from 105 patients (mean 11.9/each case). One was stained using the haematoxylin-eosin method, and the other using immunohistochemical technique.

Immunohistochemistry

After being dewaxed and rehydrated to distilled water through xylene and descending graded alcohols, the tissue sections were immersed in methanol containing 0.3% hydrogen peroxide for 20 minutes in order to inhibit endogenous peroxidase activity. Then the sections were digested using 0.4% pepsin in 0.1 M HCL for 15 minutes at 37°C. Before the sections were incubated with the primary antibody AE_1 (Zymed, San Francisco, California, USA), a monoclonal mouse antibody against low molecular weight cytokeratin (1:50 dilution) overnight at 4°C, non-immuno-goat-serum was dropped on the sections for 30 minutes. On the second day, the sections were sequentially incubated with biotinylated-goat-antimouse antibody (Zymed) and streptavidin-biotin-horseradish peroxidase complex (Zymed) for 30 minutes separately at room temperature. Then color reaction was developed using 3, 3'-diaminobenzidine solution for 5 minutes. Finally the sections were counterstained with haematoxylin and mounted. Tissue sections of gastric carcinoma were simultaneously stained as positive control, and those with the primary antibody omitted as negative control.

Statistical Analyses

Chi-square test was used to test the relationship between micrometastases and each of the clinico-pathologic factors. Survival date analysis of the patients with gastric carcinoma after surgery was performed using Log-rank test.

RESULTS

The strong positive reaction was diffuse in the cytoplasm of gastric carcinoma cells, especially in its periphery, for AE_1 immunohistochemical staining (Figure 1). Micrometastases were observed in 81 lymph nodes (6.5%) of 31 cases (29.5%) from 1245 lymph nodes of 105 patients. In the 31 cases with micrometastases there were 12 cases (38.7%) with three or more lymph nodes involved (range: 1–8). Micrometastases were scattered in the lymph sinus, mostly in the marginal sinus. They were presented in the forms of single or more discrete carcinoma cells or/and a small cluster of carcinoma cells consisting of two to three or more cells (Figure 2). Cross reaction was found in macrophages and plasmocytes of some nodes, even in some smooth muscle cells of the gastric wall. This manifestation had been also observed by other researchers.[2] The cross reaction could be excluded from positive staining according to the morphologic characteristics, place in lymph node and AE_1 immunostaining features of carcinoma cells, in conjunction with the adjacent haematoxylin-eosin staining section.

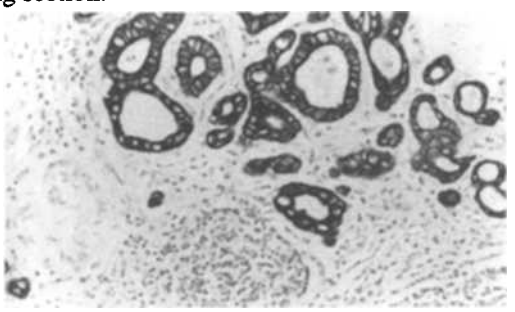

Fig. 1. Gastric carcinoma cells, positive for AE_1 immunostaining (200 ×)

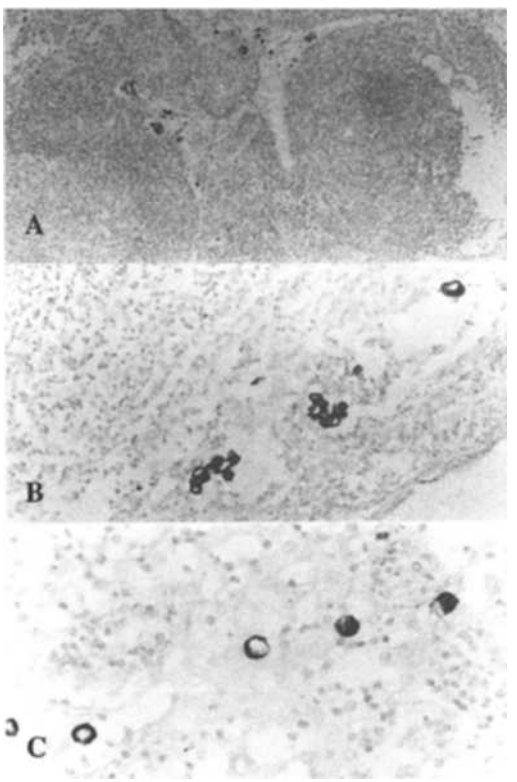

Fig. 2. Lymph node micrometastases, positive for AE_1 immunostaining a.100× b.200× c.400×

The details of micrometastases found in different sex, age, and different size, location, invasion depth and Lauren type of the primary tumors are shown in Table 1. The incidence of lymph node micrometastases was significantly higher in the diffuse type (41.5%) than in

the intestinal type gastric carcinoma (17.6%). The larger the size of the primary tumor, the more the chances of micrometastases. In addition, the number of micrometastasis-positive cases increased with the invasion depth of the primary lesion in the gastric wall. These results indicated that the presence of micrometastases was closely correlated with the histologic type of gastric carcinoma, the size and invasion depth of the primary tumor, but had no relation to patient's age, sex and the location of the primary tumor.

Table 1. Clinicopathologic details and the results of immunohistochemical detection of micrometastases in 105 patients

Clinicopathologic parameter		Total (n=105)	Positive (n=31)	Negative (n=74)	*P*
Sex	Male	74	24	50	NS*
	Female	31	7	24	
Age	≤45 yrs	20	7	13	NS*
	>45 yrs	85	24	61	
Lauren type**	Intestinal	51	9	42	<0.01
	Diffuse	53	22	31	
Invasion depth**	M-PM***	60	9	51	<0.001
	Subserosa	36	16	20	
	Extraserosa	8	5	3	
Tumor location	Upper	4	0	4	NS*
	Middle	15	5	10	
	Lower	86	26	60	
Tumor size**	≤2 cm	29	3	26	<0.01
	>2 cm	75	28	47	

* Not significant by x^2 test; ** There was one case without a known category, and it was excluded from statistical analyses; *** M-mucosa, PM-proper muscle layer

The patients with micrometastases had significantly worse prognosis shown by Log-rank test. Their five-year survival rate after surgery was 61.29%; for those without micrometastases the rate was 82.43%, P=0.0116 (Figure 3). When the number of patients' lymph nodes with micrometastases was three or more, the five-year survival rate of these patients was much lower (41.67%, P=0.0012).

DISCUSSION

The results of this study demonstrated that micrometastases were found in 29.5% cases with node-negative gastric carcinoma by routine histologic examination. Trojani et al.[4] reported that five monoclonal antibodies against epithelial cell antigen of cytokeratin, EMA etc. displayed equal sensitivity in revealing lymph node micrometastases. Ishida et al.[2] found CAM5.2 against cytokeratin was the most sensitive antibody in comparison with anti-EMA and anti-CEA. However, the detection of micrometastases using the antibody BC_2 against epithelial mucin core protein was superior to that using the antibody MNF116 against cytokeratin was also reported.[5] They interpreted that was due to the complex nature of the mucin protein itself and the influence of glycosylation on the reactivity of antibody reactive with mucin core protein; the reactivity of different antibodies with cancer-associated mucin can vary considerably, so that BC_2 was superior to MNF116 in their study. Meanwhile, if tissue sections were appropriately digested using trypsin, the anti-keratin antibody showed carcinoma cells very well. We employed two monoclonal antibodies against epithelial cell—AE_1 and anti-EMA in the preliminary experiments, and both results were entirely consistent in revealing either the primary gastric carcinoma tissue or their metastases in lymph nodes. Therefore we chose only AE_1 in this study.

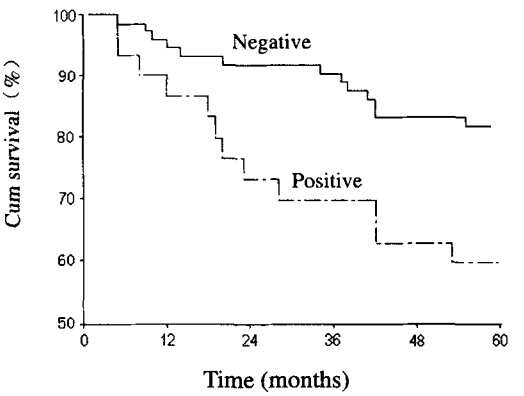

Fig. 3. The survival curves for lymph node micrometastases-positive and negative patients

Lymph node metastases are an important prognostic factor of gastric carcinoma. Extensive lymph node dissection during surgery was beneficial to the patients with node-negative gastric carcinoma by routine histologic examination.[10] The phenomenon suggested that there might be micrometastases in dissected lymph nodes of these patients that were difficult to be discovered by conventional haematoxylin-eosin staining. Moreover, these micrometastases would have adverse effects on the patients' prognosis. The current study indicated the five-year survival rate of the patients with micrometastases was much lower than those without micrometastases, i.e. the patients with micrometastases had significantly worse prognosis as previously reported.[2–5, 7] However, Gaelea et al. doubted this point of view; their results suggested there was no difference in overall survival, in recurrence-free survival, or in frequency of or time of presentation of recurrences between micrometastases-positive and micrometastases-negative patients.[6] This might result from a different case-choice, detection technique and decision standard of micrometastases. The presence of micrometastases in lymph nodes was an independent indicator of poor prognosis of breast cancer patients,[5–7] while micrometastases were also an indispensable factor in determining the prognosis of gastric carcinoma patients.[2] The survival time of micrometastases-positive patients with gastric carcinoma was significantly shorter than that of micrometastases-negative patients.[3] These findings in conjunction with our results suggested the detection of lymph node micrometastases is necessary to more accurately determine the prognosis and clinical staging of patients with node-negative gastric carcinoma by routine histologic examination. The presence of micrometastases may be regarded as one of the clues in adjuvant therapy of those patients.

Lymph node micrometastases were found more frequently in the diffuse type gastric carcinomas. The result is consistent with Ishida et al.[2] and may be associated with lower cell to cell adhesion, more easily dropping from the primary tumor and greater invasion ability of the cells of diffuse type gastric carcinoma. Our present results indicated the presence of micrometastases was not related to patients' age, sex and the primary tumor location, similar to the results of Maehara et al. in early gastric carcinoma.[3] The difference between the two studies is whether the size and invasion depth of the primary tumor could influence the incidence of micrometastases. The former was yes and the latter no. Early gastric carcinoma is generally small in size and only invades mucosa or submucosa, so their micrometastases have no relation to the tumor size and invasion depth. In addition, Ishida et al. also reported the number of micrometastases-positive cases increased with the invasion depth of the primary tumor in the gastric wall.[2]

After a few carcinoma cells migrate to lymph nodes, there may be three outcomes of the tumor cells: (1) proliferation to form micrometastases, (2) removal by the body's immune system, (3) withdrawal from cell cycle temporarily, getting into it and proliferating again when the environment is appropriate. Therefore the prognosis of these patients with lymph node micrometastases may not be bad. Further studies are necessary about how to help the human body to discern and wipe out a few carcinoma cells that migrated to lymph nodes.

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