Clinicopathological value of Immunohistochemical Detection of Micrometastases in pT3N0M0 Gastric cancer

Ziwei Wang

Department of surgery, the First Affiliated Hospital, Chongqing University of Medical Sciences

Abstract **bjective** Exploring the significance of micrometastases of lymph nodes in cases of $pT_3N_0M_0$ gastric cancer. **Methods** We examined 2230 lymph nodes that had been removed from 95 patients with stage II gastric cancer ($pT_3N_0M_0$) with immunostaining of cytokeratin. **Results** In 38 of 95(40%) patients with gastric cancer, evidence of micrometastases was found in 290 of 2230(13%) lymph nodes. The incidence of micrometastases in undifferentiated carcinoma was higher than in differentiated carcinoma. An analysis of survival demonstrated the 5-year survival of patients with micrometastases in lymph nodes was significantly lower than that of patients without micrometastases (P<0.01). **Conclusions** The accuracy of predicting the prognosis of patients with $pT_3N_0M_0$ gastric cancer should be greatly enhanced if cytokeratin-specific immunostaining is performed in conjunction with routine histopathological examination of lymph nodes.

Key Words gastric cancer; micrometastases; cytokeratin; immunohistochemistry

Metastasis to lymph nodes is one of the most important predictive factors In judgment of the prognosis of patients with gastric cancer[1-3]. Recent Studies have demonstrated that patients with stage II gastric cancer have favorable prognosis by D_2 lymphadenectomy[4] . Recent advances in histochemical and molecular biological techniques allow identification of micrometastases of lymph nodes in cases of breast and colorectal cancers that are generally not detected with standard hematoxylin and eosin staining, and these patients had unfavorable prognosis[5-7]. However, in cases of gastric cancer, the value of micrometastases of lymph nodes remains argument. In this study, we tried to determine the existence of micrometastases in lymph nodes by immunostaining of cytokeratin and evaluate the clinicopathological significance of such immunostaining in improving the accuracy of diagnosis and the prediction of prognosis for patients with locally advanced gastric cancer.

MATERIAL AND METHODS

Clinical material

A total of 95 patients with stage II gastric cancer ($pT_3N_0M_0$) who had undergone surgery in our hospital from 1980 to 1995 were included in this study. All cases were followed up of 5-20 years. 27 patients had died of recurrence of the cancer by the end of 2000, Lymph node metastases or peritoneal dissemination were detected in 21 cases and hematogenic metastases were detected in 6 cases of these patients. Another 4 patients who died of postoperative complications within 30 days of surgery were excluded from the statistical analysis of survival of the patients.

A total of 2230 lymph modes were dissected from 95 patients of gastric cancer. All samples were fixed in formalin and embedded in paraffin. Two consecutive sections of 4μm in thickness were prepared from each block of resected lymph node. One section was stained with HE and another was subjected to cytokeratin-specific immunostaining, so that we could compare the results obtained from the two methods in adjacent sections of each lymph node. All 95 primary tumors and 15 lymph nodes in which metastases had been found by HE staining were used as positive controls. In addition, sections of 15 normal lymph nodes obtained from patients with benign diseases were used as negative controls.

Immunohistochemistry

Immunohistochemical staining was performed by the streptavidin-biotin (SAB) immunoperoxidase method with the murine monoclonal antibody CAM5.2. First, an experienced pathologist without the knowledge of sample groupings and previous diagnosis assessed the HE-stained slides for the presence of metastases. Then, immunostained slides were examined and the results were compared with

Ziwei Wang, Prof, 023-68814868,
A68718645@online.cq.cn

those obtained from HE-stained slides. Micrometastases in lymph nodes were recognized when tumor cells overlooked by ordinary HE-staining. were detected by cytokeratin-specific immunostaining.

Statistical analysis

Student´s t-test was used to examine the association between the number of lymph nodes with occult cancer cells and the clinicopathological characteristics of the primary tumor. The Kaplan-Meier method was used for construction of cumulative survival curves and the generalized Wilcoxon test was used for tests of the statistical significance of differences. Multivariate survival analysis was performed using Cox´s proportional hazards model. A $P<0.05$ were considered an indicator of statistical significance.

RESULTS

38 of 95(40) patients had immunohistochemically detectable micrometastases cells in their resected lymph nodes that had been overlooked after routine staining with HE. All 95 primary tumors were found to have positive immunoreactions consistently with the monoclonal antibody against cytokeratin, and all 15 sections of lymph nodes with metastases were detectable by conventional HE staining contained immunoreactive cancer cells. But no immunopositive cells were found in the 15 sections of normal lymph nodes.

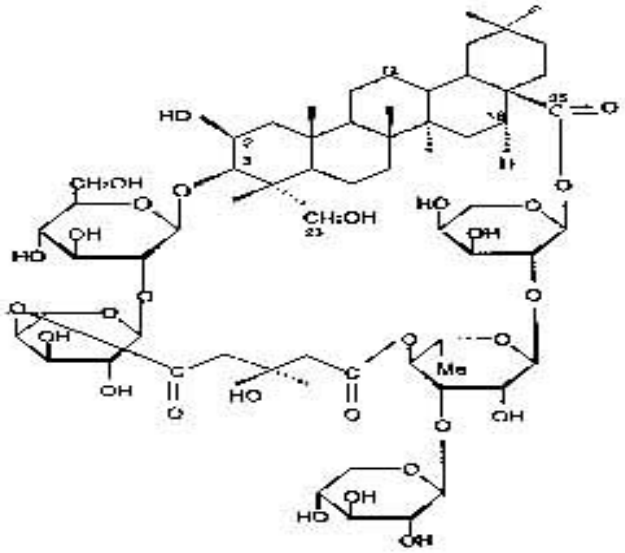

Fig.1 Kaplan-Meier postoperative survival curves for 91 patients with $pT_3N_0M_0$ gastric cancer.
a. The 5-year survival of 54 patients without micrometastases in lymph nodes(91%);
b. The 5-year survival of 37 patients with micrometastases in lymph nodes (52%).
Generalized Wilcoxon test: a vs. b, $P<0.01$.

Table 1. Number of lymph nodes with micrometastases and clinicopathological characteristics of the primary tumor

Variable	No. of patients (n=95)	No. of lymph nodes with micrometastases (mean±SD)	*P*
Gender			
Male	51	4.21±2.25	>0.05
Female	44	4.09±2.54	
Age(years)			
<60	45	4.11±3.12	>0.05
⩾60	50	4.30±2.88	
Maximum diameter(cm)			
⩾7	42	4.27±2.86	>0.05
>7	53	4.18±2.47	
Histology			
Differentiated	37	3.01±2.66	<0.05
Undifferentiated	58	5.82±4.25	
Growth pattern			
Infiltrative	54	5.99±4.32	<0.05
Expanding	41	2.81±2.24	
Lymphatic invasion			
Negative	46	4.17±3.22	>0.05
Positive	49	4.25±3.01	
Vascular invasion			
Negative	48	4.50±3.63	>0.05
Positive	47	4.08±3.13	

We found no significant relationship between the number of lymph nodes with micrometastases and other clinicopathological features of the primary tumor, such as patient´s age, sex, location of the tumor and lymphatic or vascular invasion. There was a statistically significant increase in the incidence of micrometastases in cases of poorly differentiated tumors as compared with well–differentiated tumors (P<0.05) and in cases of infiltrative tumor as compared with expanding tumor (P=0.03; Table 1). The 5–year survival rate of the 91 patients who did not die of postoperative complications was 70%. Survival analysis using the Kaplan –Meier method demonstrated the 5–year survival rate of the patients with micrometastases (52%) was significantly lower than the patients without micrometastases in lymph nodes (91%; P<0.05; Fig.1) The multivariate survival analysis shown the micrometastases in lymph nodes was one of the independent prognostic factors (Table 2).

Table 2. Multivariate analysis of independent prognostic factors in 91 patients with $pT_3N_0M_0$ gastric cancer

Variable	Relative risk	P
Gender	1.15	0.71
Age	0.98	0.86
Maximum diameter of the primary tumor	2.66	0.19
Histological type	3.24	0.05
Growth pattern	2.42	0.22
Lymphatic invasion	0.87	0.69
Vascular invasion	1.65	0.30
Micrometastases in lymph nodes	3.88	0.02

DISCUSSION

Recently, the focus of increasing interest was the relationship between the prognosis of patients with carcinomas and the micrometastases in lymph nodes. In cases of beast and colorectal cancers with micrometastases in lymph nodes, the 5–year survival rate was significantly lower. However, for the patients of gastric cancer with micrometastases in regional lymph nodes, it was argument about their significance in prediction of the prognosis of patients. The reason may be the difference of quantitative samples or the stage of tumor[8, 9].

Recently published studies have demonstrated the presence of isolated tumor cells or small clusters of such cells in lymph nodes of patients with early and advanced gastric cancer. These cells probably affected the prognosis of the patients. Our data shown that 38 of 95 (40%) patients who had been diagnosed as being free of lymph node metastases by routine HE staining had micrometastases in their regional lymph nodes by immunohistochemical staining. This result appears to explain why patients in whom no metastases have been found in lymph nodes with routine HE staining still tend to die of recurrence of the tumor.

The major risk factors for metastasis to lymph nodes are generally consisted of large size, growth pattern, histological type and the depth of invasion of the primary tumor[10]. Fukagawa[8] found that the infiltrative tumor is more tend to be micrometastases than the expanding tumor, but not significantly difference between them. In the present study, we also found that micrometastases were more frequent in lymph nodes of patients with infiltrative tumor and with undifferentiated tumors.

Many studies reported there were lymph nodes micrometastases in patients with gastric cancer, but their significance for prognosis were different each other. Some studies were retrospective analysis with small samples. Meanwhile, in cases of cancer with T_3 or T_4 stage, the pattern of recurrence was peritoneal implantation[2] . If there were advanced cancers in samples, the significance of micrometastases for prognosis might be interfered because of their peritoneal metastases. Therefore, we selected the cases of gastric cancer with $pT_3N_0M_0$ for analysis samples, in order to make more exact judgment about the significance of micrometastases in lymph nodes.

In this study, we found the 5–year survival rate of gastric cancer was significantly lower in patients with micrometastases in lymph nodes than in patients without micrometastases in lymph nodes (P< 0.05), and the micrometastases in lymph nodes was an independent prognostic factor by multivariate survival analysis. These findings are comparable with Cai´s results [11], which indicated that micrometastases in lymph nodes affected the prognosis of patients with $pT_3N_0M_0$ gastric cancer.

The failure to detect micrometastases in lymph nodes is clearly a drawback of conventional HE staining. Immunostaining with the cytokeratin–specific monoclonal antibody CAM 5.2 has been suggested to be the most useful method for detecting discrete or small clusters of tumor cells in lymph nodes[9] . In this study, we found that all primary

tumors and all lymph nodes with metastases that have been detected by HE staining were positive immunoreaction. By contrast, none of the normal lymph nodes removed from patients with benign diseases was immunostained. Among the resected lymph nodes with no evidence of metastasis by HE staining, 13% of them were found to contain micrometastases when only one section of each node was immunostained. The results indicate that immunostaining with cytokeratin –specific monoclonal antibody CMA 5.2 is an accurate and sensitive method for detecting micrometastases in lymph nodes. Such immunostaining would seem to be a useful supplement to standard HE staining especially in cases of patients with high–risk factors, such as infiltrative growth, poorly differentiated and deeply invasive tumors.

Our study shown that the immunohistochemical staining could detect micrometastases in lymph nodes of patients with $pT_3N_0M_0$ gastric cancer, which was usually overlooked by routine HE staining. Moreover, the patients with micrometastases in lymph nodes had significant poor prognosis than those without micrometastases in lymph nodes. It indicated that the immunohistochemical staining method can more exactly judge the prognosis of patients with $pT_3N_0M_0$ gastric cancer and the micrometastases in lymph nodes is one of independent prognostic factors.

REFERENCES

1. Roder JD, Bottcher K, Siewert JR, et al. Prognostic factors in gastric carcinoma: results of the German gastric carcinoma study 1992. Cancer (phila), 1993, 72(7): 2089–2097.
2. Katai H, Maruyama K, Sasako M, et al. Mode of recurrence after gastric cancer surgery. Digest Surg, 1994, 11: 99–103.
3. Moriguchi S, Maehara Y, Korenaga D, et al. Risk factors which predict pattern of recurrence after curative surgery for patients with advanced gastric cancer. Surg Oncol, 1992, 1: 341–346.
4. Siewert JR, Kestlmeier R, Busch R, et al. Benefits of D2 ymph node dissection for patients with gastric cancer and pN0 and pN1 lymph node metastases. Br J Surg, 1996, 83:1144–1147.
5. McGuckin MA, Cummings MC, Walsh MD, et al. Occult axillary node metastases in breast cancer: their detection and prognostic significance. Br J Cancer, 1996; 73:88–95.
6. Liefers GJ, Jansen AMC, van de Velde CJH, et al. Micrometastases and survival in stage II colorectal cancer. N Engl J Med, 1998, 339: 223–228.
7. Davidson BR, Sams VR, Styles J et al. Detection of occult nodal metastases in patients with colorectal carcinoma. Cancer (phila), 1990, 65: 967–970.
8. Fukagawa T, Sasako M, Mann GB, et al. Immunohistochemically detected micrometastases of the lymph nodes in patients with gastric carcinoma. Cancer, 2001, 92(4): 753–760.
9. Ishida K, Katsuyama T, Sugiyama A, et al. Immunohistochemical evaluation of lymph node micrometastases from gastric carcinoma. Cancer (phila), 1997, 79: 1069–1076.
10. Maehara Y, Orita H, Okuyama T, et al. Predictors of lymph node metastases in early gastric cancer. Br J Surg, 1992; 79: 245–247.
11. Cai JH, Ikeguchi M, Maeta M, et al. Clinicopathological value of immunohistochemical detection of occult involvement in pT_3N_0 gastric cancer. Gastric cancer, 1999, 2: 95–100.