Gastric Cancer (2006) 9: 197–202
DOI 10.1007/s10120-006-0378-3

Original article

Assessment of lymph node micrometastasis in early gastric cancer in relation to sentinel nodes

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Abstract

Background. **The development of endoscopic resection and reduced surgical procedures has progressed in recent years. Lymph node micrometastases can be cited as one of the problems with reduced operations. In this study, we investigated clinicopathological findings and sentinel lymph nodes (SNs) for associations with micrometastases. We discuss the indications for endoscopic mucosal resection (EMR), reduced surgery, and sentinel node navigation surgery (SNNS) based on the results.**

Methods. **Immunostaining with anti-cytokeratin antibodies was used as the method of exploring for micrometastases. Comparisons and assessments were made in regard to the presence or absence of micrometastases and various clinicopathological factors.**

Results. **The relationship between the clinicopathological factors and micrometastases was investigated in 120 patients with pT1pN0 gastric cancer. Significant differences in depth of invasion (mucosal [m] versus submucosal [sm]) and histological type (differentiated versus undifferentiated) were observed in both univariate analysis and multivariate analysis. Micrometastases were observed in 32% of the sm cancers, and they were observed in group 2 lymph nodes (no. 7) in 8%. They tended to be more common in the undifferentiated type. The micrometastatic lymph nodes were restricted to blue nodes (BNs) and lymph nodes within the dye flow area of patent blue (used intraoperatively explore for SNs).**

Conclusion. **It is considered that the indications for current EMR and reduced surgery in early gastric cancer are valid from the standpoint of micrometastases. But if the SNNS that has been studied in recent years is introduced, the lymphatic basin dissection method seems valid only if the case is s-pN0 early cancer.**

Key words Lymph node micrometastasis · Reduced surgery · EMR · SNNS

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Received: December 15, 2005 / Accepted: April 11, 2006

Introduction

Systematic lymph node dissection, including normal lymph nodes, has been performed in conventional curative surgery for gastric cancer. The development of endoscopic resection and reduced surgical procedures (pylorus-preserving, vagus nerve-preserving, greater omentum-preserving, laparoscopic surgery) has progressed in recent years. Sentinel node navigation surgery (SNNS), the principle of which is the dissection of sentinel lymph nodes (SNs), has also been studied in recent years, and, if it were adopted clinically, it would be good news for patients [1–5]. However, because lymph node dissection is decreased with SNNS, there is apprehension that curativeness would be lost as a result of leaving behind metastatic lymph nodes. The basis for conventional reduced surgery is the result of exploration for lymph node metastases by routine H&E staining, and lymph node micrometastases can be cited as one of the problems with reduced operations. Although the significance of micrometastases is unclear at present, it seems impossible to ignore this point. In this study, we investigated clinicopathological findings and SNs for associations with micrometastases. We also discuss the indications for endoscopic mucosal resection (EMR), reduced surgery, and SNNS, based on the results.

Patients

This study was conducted in 120 patients with pT1pN0 disease among the 327 patients with gastric cancer diagnosed between January 2000 and August 2004 and treated surgically in our department. All lymph nodes submitted for pathological diagnosis (total number, 3505; mean, 29.1 ± 14.6) were explored for micrometastases. According to depth of invasion, there were 70 mucosal (m) cancer cases and 50 submucosal (sm)

cancer cases. The surgical procedures consisted of distal gastrectomy in 90 patients, proximal gastrectomy in 9 patients, total gastrectomy in 17 patients, partial resection in 1 patient, and laparoscopically assisted distal gastrectomy (LADG) in 3 patients. Lymph node dissection consisted of D1+α dissection in 66 patients, D1+β dissection in 17 patients, and D2 dissection in 37 patients. Patent blue was used intraoperatively to explore for SNs in the 76 most recent of the 120 patients, and blue nodes (BNs) were identified; of these 76 patients, 43 had m cancers, and 33 had sm cancers. According to sex, there were 83 males and 37 females, and the mean age of the patients as a whole was 64 years. Median follow-up time was 1250 days (range, 515 to 2180 days). The terminology used is in accordance with the Japanese Classification of Gastric Carcinoma (Japanese Research Society for Gastric Cancer).

Methods

Immunostaining

Immunostaining with anti-cytokeratin (CK) antibodies was used as the method of exploring for micrometastases. DAKO-AE1/AE3 CK antibodies (DAKO, Copenhagen, Denmark) were used. Three sections (about 3-μm thick), at approximately 50-μm intervals, were cut from each lymph node, and a dextran polymer method (Envision/HRP (DAKO) was used for staining. After the above procedure was performed, comparisons and assessments were made in regard to the presence or absence of micrometastases and various clinicopathological factors. The factors assessed were: depth of invasion, macroscopic type, histological type, greatest tumor diameter, lymphatic invasion, and venous invasion. The χ^2 test and multivariate analysis were adopted as the methods of statistical analysis, and the analyses were performed with StatView statistical software (StatView, San Jose, CA, USA). Micrometastases were judged based on the definition of isolated tumor cells (ITCs) as having a maximum diameter of 0.2 mm or less, as stipulated by the TNM classification. The ITCs consisted of single cells and clusters (Fig. 1).

Patent blue dyeing

BNs were identified by performing intraoperative endoscopy, with local submucosal injection of 0.2 ml of 2% patent blue at four sites around the tumor, and exploration for lymph nodes that had stained blue about 10 to 15 min later (dye method). The BNs were fixed with 10% formalin solution and explored for micrometastases by the immunostaining method described above.

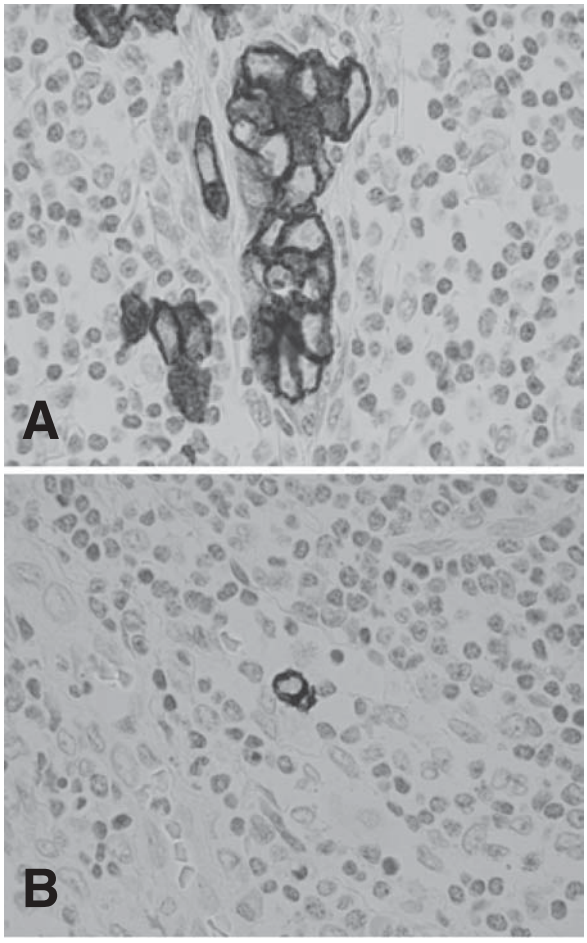

Fig. 1A,B. Lymph node micrometastases detected by immunohistochemistry. Lymph node micrometastases were clearly stained by AE1/AE3 antibody (**A** Cluster; **B** single cell. ×800)

Results

The relationship between the clinicopathological factors and micrometastases was investigated in 120 patients with pT1pN0 gastric cancer (Table 1). Micrometastases were detected in 27 patients (22.5%), and 37 metastatic lymph nodes (1.06%) were identified. Univariate analysis was performed in regard to clinicopathological risk factors. According to depth of invasion, micrometastases were detected in 11 (15.7%) of the 70 cases of m cancer and 16 (32%) of the 50 cases of sm cancer, and the difference was significant ($P = 0.035$). According to histological type, micrometastases were detected in 7 (10.9%) of the 64 cases of the differentiated type and 20 (35.7%) of the 56 cases of the undifferentiated type, and the difference was significant ($P = 0.003$). No significant differences were found for any of the other factors (macroscopic type, tumor diameter, lymphatic invasion, vascular invasion). Multivariate analysis revealed significant differences for depth of

invasion ($P = 0.048$) and histological type ($P = 0.003$), and they were concluded to be independent risk factors (Table 2).

EMR and reduced surgery

Cases of m cancer (in relation to EMR)

Micrometastases were found in 11 (15.7%) of the 70 patients with m cancer (Table 3), and in 16 of the 2026 nodes. Micrometastases were found in 10 (31.25%) of the 32 patients with the undifferentiated type and in 1 (2.63%) of the 38 with the differentiated type; in 8 (22.86%) of the 35 patients in whom tumor diameter was more than 2.0 cm, and in 3 (8.57%) of the 35 patients in whom it was 2 cm or less. No micrometastases were detected in the 19 patients with the differentiated type in whom the tumor diameter was 2.0 cm or less.

Cases of sm cancer (in relation to reduced surgery) among patients with group 2 lymph node micrometastases

Micrometastases were found in 16 (32%) of the 50 patients with sm cancer, and in 21 of the 1452 nodes. Micrometastases in group 2 lymph nodes were found in 4 (3.3%) of the 120 patients with pT1pN0 gastric cancer. All 4 (8%) were sm cancers, and 3 (75%) of the 4 were the undifferentiated type. There tended to be more micrometastases in the undifferentiated type of sm cancer (3 out of 24 cases, 12.5%; Table 4).

Findings in 76 patients explored for SNs

BNs were identified in all 76 patients explored. A total of 2272 lymph nodes were dissected (mean, 29.9 ± 13.9), and 492 had been stained (mean, 6.5 ± 4.7). Micrometastases were observed in 19 nodes in 15 (19.7%) of the 76 patients. Micrometastases were detected in the BNs alone in 11 of the 15 micrometastasis-positive patients, and micrometastases were detected in non-BNs in 4 patients, but these non-BNs were all within the dye flow area (Table 5). The BN identification rate was 100%. The diagnostic accuracy rate was 94.7%, and the sensitivity (micrometastasis-positive diagnosis rate) was 73.3% (Table 6).

Table 1. Relationship between clinicopathological findings and lymph node micrometastasis (MM) in 120 patients with pT1pN0 gastric cancer (univariate analysis)

	MM (–)	MM (+)	*P* value
Depth			0.035
m	59	11	
sm	34	16	
Tumor diameter			0.146
≤2.0 cm	48	9	
>2.0 cm	45	18	
Macroscopic type			0.332
Flat and elevated	43	9	
Depressed	50	18	
Histological type			0.0025
Differentiated type	57	7	
Undifferentiated type	36	20	
Ly			0.446
Negative	71	18	
Positive	22	9	
V			0.314
Negative	90	25	
Positive	3	2	

Table 2. Multivariate analysis of relationship between clinicopathological factors and lymph node micrometastasis in 120 patients with pT1pN0 gastric cancer

	P value	Relative risk ratio
Depth	0.0481	2.557
Tumor diameter	0.1991	1.867
Histological type	0.0028	4.487

Table 3. Clinicopathological findings in relation to lymph node micrometastasis in 70 patients with pT1(m)pN0 gastric cancer

	MM (–)	MM (+)	*P* value
Tumor diameter			0.1875
≤2.0 cm	32	3	
>2.0 cm	27	8	
Histological type			0.0017
Differentiated type	37	1	
Undifferentiated type	22	10	

Table 4. Micrometastasis (n2) in sm cancer

	Depth	Macroscopic	Tumor diameter (mm)	Histological	Ly	V	MM LN no.	Number of LNs with MM
Case 1	sm 2	IIc	10	tub2	0	0	7	1
Case 2	sm 1	IIa + IIc	15	sig	1	0	3, 7	2
Case 3	sm 2	IIc	12	sig	0	0	3, 7	2
Case 4	sm 2	IIc	12	por1	2	1	3, 7	2

Table 5. Detection of micrometastasis in node-negative gastric cancer

	Depth	Macroscopic	Location	Stained channel	MM LN no.	Number of LNs with MM
Case 1	m	IIa + IIc	M	RGEA	4d	1
Case 2	m	IIc	U	LGA, PGA	3[a]	2
Case 3	m	IIc	M	LGA, LGEA	3[a]	1
Case 4	m	IIc	L	RGEA, RGA	6	1
Case 5	sm 1	IIa + IIc	M	LGA, RGEA	3, 7	2
Case 6	sm 1	IIa	L	LGA, RGEA	3[a]	1
Case 7	sm 1	IIc	L	RGEA	6	1
Case 8	sm 2	IIc	M	LGA	7	2
Case 9	sm 2	IIc	M	LGA, RGEA	3[a], 6	1
Case 10	sm 2	IIc + III	L	RGEA	4d	2
Case 11	sm 2	I	M	LGA, RGEA	4d	1
Case 12	sm 2	IIc	M	LGA, RGEA	3, 7	1
Case 13	sm 2	IIc	M	LGA, RGEA, LGEA	3	1
Case 14	sm 2	IIc	M	RGEA	4d	1
Case 15	sm 2	IIa + IIc	M	LGA, RGEA	4d	1

RGA, right gastric artery; LGA, left gastric artery; PGA, posterior gastric artery; RGEA, right gastroepiploic artery; LGEA, left gastroepiploic artery; BN, blue node

[a] Cases with micrometastasis found in non–stained (non–BN) nodes. Micrometastases were found only in the non–BN nodes in cases 2, 3, 6, and 9, but they were all within the stained lymphatic channels

Table 6. Results of blue node detection at our department (January 2000–August 2004)

Number of of patients	76 (Male, 55; female, 21)	
Total number of resected lymph nodes	2272 (mean ± 2 SD, 29.9 ± 13.9)	
Blue dye-stained lymph nodes	492 (mean ± 2 SD, 6.5 ± 4.7)	
Detection rate of blue nodes	76/76	100.0%
Accuracy	72/76	94.7%
Sensitivity	11/15	73.3%

Discussion

The TNM classification stipulates that micrometastases are metastases that measure 2 mm or under but more than 0.2 mm. They are usually detected by immunohistochemical tests or by molecular biology tests, and single cells and small cell clusters under 0.2 mm in size, which can even be confirmed by H&E staining, are defined as ITCs [6]. Kobayashi et al. [7] created micrometastasis models in mice by intravenously and percutaneously transplanting them with Lewis lung carcinoma cells that had been transfected with the *lacZ* gene. They [7] reported that the results showed that single cells circulating in the blood vessels had died and degenerated within 2 to 3 days after transplantation, but cells that survived had formed micrometastases in the lungs [8]. Nakajo et al. [9] found an association between decreased E-cadherin expression and the presence of micrometastases. However, other genes are also thought to be related to metastasis, and analyses various methods, including the microarray method, are awaited [10].

Micrometastasis and its clinical significance has not yet been fully elucidated, and there have been reports of both associations with recurrence and outcome [9,11–14] and of no associations with recurrence and outcome [15–19]. There has also been a report of a 5-year survival rate of 100% in patients with micrometastasis-negative sm cancer as opposed to 82% those who were positive [11] and of a 50% survival time of 6.6 years in patients with T1 recurrent disease among patients with micrometastases-negative sm cancers, versus 3.3 years in those who were positive [12]. There have been no cases of recurrence yet in our own study, and the clinical significance of micrometastasis is unclear. It now appears impossible to ignore the existence of micrometastases.

In the present study we explored for micrometastases by CK staining in patients with m and sm cancers, according to depth of invasion. Micrometastases were newly detected in 22.5% of the n0 cases, and this rate is fairly consistent with the rates reported by other investigators (8% to 36%) [9,13,14,17–19] (Table 7).

Table 7. Reports of lymph node micrometastasis in gastric cancer detected by immunostaining

Author	Year	Antibody	No. of patients	Stage	Positivity (%)	Prognosis
Harrison [13]	2000	CAM5.2	25	T1–T4N0	36	(+)
Nakajo [9]	2001	AE1/AE3	67	T1–2N0	21	(+)
Cai [11]	2000	CAM5.2	162	T1	27	(+)
Stachura [17]	1998	CK18	40	T1	8	(–)
Saragoni [18]	2000	CK	139	T1N0	17	(–)
Choi [19]	2002	CK8	88	T1 (SM)	32	(–)

Various methods of exploring for micrometastases are available, including immunohistochemical staining for CKs (detection rate, 8% to 36%) [9,13,14,17–19], and reverse transcription-polymerase chain reaction (RT-PCR) using carcinoembryonic antigen (CEA) mRNA (detection rate, 30% to 61%) [20–22]. The immunostaining method is still superior in terms of convenience, and increasing the number of sections used for routine staining and immunostaining has been found to increase the detection rate. Increasing the number of sections excised from the lymph nodes to three in the present study seems to have made it possible to obtain results with detection sensitivity similar to that of RT-PCR. At this point in time, the three-section method would also seem adequate for immunostaining. Karsten et al. [23] recently reported an immunostaining method that can be performed in the brief span of 10 min, and research is currently under way to make the RT-PCR method, the real-time RT-PCR method [24], the RT-Loop-mediated isothermal amplification (LAMP) method [25], and the Transcription-Reverse Transcription Concerted (TRC) method [26], which are more sensitive than immunostaining, convenient, and to shorten the testing time.

Next, we will discuss the risk of reduced surgery from the standpoint of micrometastases. Micrometastases were observed in 22.5% of the patients with pT1pN0 cancer in the present study. Significant differences according to depth of invasion (m versus sm) and histological type (differentiated versus undifferentiated) were observed in both the univariate analysis and the multivariate analysis, and micrometastasis tended to be more common in those with a tumor diameter of more than 2.0 cm. Such micrometastatic foci may persist when EMR is performed without lymph node dissection. Thus, m cancer, differentiated type, and tumor diameter of 2.0 cm or less seem to be valid as current indications for EMR, from the standpoint of micrometastases as well [27]. D1+α dissection is said to be indicated for pT1(m)N0 outside the indications for EMR. Micrometastases were observed in 32% of the sm cancers in our study, and they were observed in group 2 lymph nodes (no. 7) in 8%. They tended to be more common in the undifferentiated type. From the standpoint of micrometastases, D1+α dissection is valid if the tumor is the differentiated type in a patient with pT1(sm)N0 cancer. D1+β dissection is more desirable in the undifferentiated type. This is almost completely consistent with current guidelines, and when the risk of micrometastases is taken into consideration, at least a D1+β dissection is desirable, given in the current stage of our knowledge. If the SNNS that has been studied in recent years is introduced, it will become possible to reduce surgery even more [28–32]. The micrometastatic lymph nodes in the present study were restricted to the BNs and lymph nodes within the dye flow area. Based on this finding as well, the lymphatic basin dissection method, in which lymph node dissection is performed within the dye flow area, seems valid only if the patient has s-pN0 early cancer. Early achievement of improvements in the accuracy of intraoperative micrometastasis detection methods and in convenience is awaited in order to make SNNS a reality.

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