Original article

Risk factors for nodal micrometastasis of submucosal gastric carcinoma: assessment of indications for endoscopic treatment

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Abstract:

Background. Despite the widespread use of endoscopic mucosal resection (EMR) for intramucosal gastric carcinoma, there is no standardized therapy for those patients in whom the carcinoma is found, after EMR, to have invaded the submucosa.

Our aim in this study was to examine the relationship between the clinicopathological features of submucosal invasive carcinomas and their incidence of nodal micrometastasis, as detected by anti-human cytokeratin immunohistochemistry, in order to assess the curative potential of submucosal carcinoma by EMR.

Methods. Fifty surgically resected submucosal gastric carcinomas which would have satisfied the absolute indications for EMR, except for the criterion of submucosal invasion, were examined. The extent of submucosal invasion was determined by measuring its vertical and horizontal spread. Immunohistochemical analysis was performed with anti-human cytokeratin antibody (CAM5.2).

Results. Three of 50 cases (6.0%) were positive for nodal metastasis by routine H&E examination. Nodal micrometastases were detected in 11 of 47 cases (23.4%). Statistical analysis revealed that both the depth and the width of carcinoma in the submucosa were significantly larger in cases with than in those without micrometastasis ($P = 0.019$ and $P = 0.006$, respectively). The group with lymphatic invasion showed more frequent micrometastasis than the group without ($P = 0.014$). There were no micrometastases in submucosal carcinomas whose submucosal invasion was less than 200 μm vertically and less than 320 μm horizontally.

Conclusions. The present study indicates that differentiated gastric adenocarcinoma with minimal submucosal invasion (less than 200 μm vertically and less than 320 μm horizontally) and not accompanied by peptic ulcer or other risk factors, such as lymphatic invasion, can be considered as having high curative potential by EMR alone, without the necessity for further radical surgery.

Key words: gastric carcinoma, lymph nodes, micrometastasis, cytokeratin, endoscopic mucosal resection

Introduction

It has been reported that no lymph node metastasis occurs in gastric mucosal carcinomas that are: (1) differentiated adenocarcinomas; (2) 20 mm or less in size; and (3) not associated with peptic ulcer [1–4]. These three conditions are generally adopted as the indications for endoscopic mucosal resection (EMR) therapy. Such carcinomas are usually cured by EMR alone, without performance of the surgical operations generally associated with lymph node resection.

In consideration of the quality of life of patients, EMR has been the treatment of choice for mucosal gastric carcinomas. However, it is not easy to make an accurate diagnosis of the depth of cancer invasion (i.e., whether the cancer remains in the mucosa or has invaded to the submucosa) by endoscopic examination alone [5–7]. And it is not infrequent that lesions treated by EMR are revealed by pathological examination as having invaded the submucosa.

Lymph node metastasis is the major prognostic factor for patients with carcinomas. Since submucosal carcinomas have clear potential for lymph node metastasis, an additional surgical operation including lymph node resection has been recommended to patients who have undergone EMR for gastric submucosal carcinomas. However, it has been suggested in several reports [8–11] that gastric submucosal carcinomas could be cured by EMR alone in patients in whom the degree of cancer invasion is minute. Clearly, there is a need for a set of standardized indications for additional surgical operations on endoscopically resected submucosal gastric carcinomas.

Recent advances in immunohistochemical [12–14] and molecular biological [15,16] techniques have enabled us to identify the presence of a single or several small clusters of metastatic carcinoma cells that are undetectable by routine histopathological examination (so-called micrometastasis). The presence of micrometastasis is considered to be an independent prognostic factor for several human cancers, including those of the breast [13] and colorectum [14]. It has been shown that micrometastasis is a more sensitive marker than overt lymph node metastasis (as detected by routine histopathological examination) for the prediction of cancer recurrence [15,16].

Numerous studies have been published on the relationship between the histopathological characteristics of gastric submucosal carcinomas, and overt lymph node metastasis [1,3,4,8–10,17–20]. However, to our knowledge, no systematic study has been made of the correlation between their histopathological characteristics and the incidence of micrometastasis. The histopathological features of the primary lesion that correlate with micrometastasis could be the most useful prognostic markers for gastric submucosal carcinomas in patients who have undergone EMR, since information on the lymph nodes is not available in such patients.

In this study, we performed an immunohistochemical analysis, using anti-human cytokeratin antibody (CAM5.2), in order to detect micrometastases of gastric submucosal carcinomas. We also examined the relationship between the histopathological characteristics of the primary lesion — particularly the extent of cancer invasion — and micrometastasis. This study should provide significant information toward the establishment of a standardized therapy for patients with gastric submucosal carcinomas treated by EMR.

Subjects and methods

Patients

In this study, we examined 50 surgically resected primary gastric submucosal carcinomas. All were of differentiated type, 20 mm or less in size, and had not been associated with peptic ulcer (either open nor healed). Regional lymph nodes were resected simultaneously and 47 of the 50 patients, were diagnosed as negative for nodal metastasis by routine histopathological examination.

Histopathological investigation of the primary lesion

The resected stomachs were opened and fixed in 10% formalin, and the entire carcinomatous lesions were cut into step-wise sections and embedded in paraffin. Pathological diagnoses of the primary lesions were done with H&E staining according to the *General rules for*

Fig. 1. Measurement of extent of submucosal cancer invasion. *Vsm*, Maximum vertical distance of submucosal invasion; *Hsm*, maximum horizontal distance of submucosal invasion

the gastric cancer study in surgery and pathology of the Japanese Research Society for Gastric Cancer [21]. The extent of cancer invasion to the submucosa and the extent of venous and lymphatic permeation were assessed in greater detail by methods described below. The extent of cancer invasion to the submucosa was analyzed according to depth (extent of vertical invasion: *Vsm*) and width (extent of horizontal invasion: *Hsm*) (Fig. 1). The depth of the invasion was defined as the distance between the deepest point of the cancer invasion and the lower edge of the muscularis mucosae. The width was defined as the horizontal extent of the cancer in the submucosa. In order to confirm the deepest and the widest points of invasion of the carcinoma, 7–24 serial sections were cut for each paraffin block.

For the examination of venous and lymphatic permeation, two 3- μ m-thick sections and one 7- μ m-thick section were prepared. One of the two 3- μ m sections was used for H&E staining and the other was used for Victoria blue elastic fiber staining to visualize the elastic fibers of the venous wall. A 7- μ m-thick section was used for immunostaining with anti-human endothelial cell antibody CD31 (diluted 1:100; Dako, Glostrup, Denmark) to visualize the endothelial cells in the lymphatic channels.

Investigations of lymph nodes

Two to 57 lymph nodes (average, 22.0) were obtained from each primary cancer. To detect micrometastases of the lymph nodes, one 3- μ m-thick section and three consecutive 10- μ m-thick sections were prepared from all lymph nodes. A 3- μ m-thick section was stained with H&E, and 10- μ m-thick sections with anti-human cytokeratins 8 and 18 antibody (CAM5.2, diluted 1:3; Becton Dickinson, San Jose, CA, USA) to visualize the epithelial cells in the lymph nodes. Three 10- μ m-thick sections (30- μ m-thick in total) were used for the CAM5.2 staining, because our preliminary study²² showed that examination of nine 3- μ m-thick sections (27- μ m-thick in total) by CAM5.2 immunostaining was sufficient to achieve the maximum detection rate of micrometastasis.

Table 1. Relationship between nodal involvement and clinicopathologic factors of primary lesions by univariate analysis

	Nodal Micrometastasis		P value (χ^2 test)
	Positive/positive + negative (<i>n/n</i>)	Positive ratio (%)	
Gross type			
I	0/2	0%	NS
IIa	0/2	0%	
IIc	5/26	19.2%	
Mixed	6/17	35.3%	
Location of tumor			
Lower third	8/27	29.6%	NS
Middle third	3/16	18.8%	
Upper third	0/4	0%	
Lymphatic invasion			
Negative	4/36	11.1%	<0.05
Positive	7/11	63.6%	
Venous permeation			
Negative	9/39	23.1%	NS
Positive	2/8	25%	
Histologic classification			
Tub1	6/28	21.4%	NS
Tub2	5/19	26.3%	
Total	11/47	23.4%	

Histologic classifications represent the predominant histologic type of each tumor

NS, No significant; tub1, well differentiated type; tub2, moderately differentiated type

Immunohistochemistry

Immunohistochemistry for CD31 and CAM5.2 was performed by the conventional streptavidin-biotin (SAB) immunoperoxidase method, using commercially available SAB complex (Nichirei, Tokyo, Japan). Diaminobenzidine was used as the chromogen, and the counterstaining was done with hematoxylin for CD31 and methyl green for CAM5.2.

Results

Lymph node micrometastasis was detected by CAM5.2 immunostaining in 11 of 47 patients (23.4%) diagnosed as without lymph node metastasis by routine H&E examination. The results of univariate analysis of the relationship between the pathological findings and micrometastasis are summarized in Table 1. The presence of lymphatic invasion (detected by CD31 immunostaining) was the only pathological factor significantly correlated with the micrometastasis. A comparison of the extent of cancer invasion to the submucosa in patients with and without micrometastasis is shown in Table 2. Both the depth and width of submucosal invasion were significantly greater in patients with micrometastasis.

The pathological findings of the 11 patients with micrometastasis and the 3 patients with overt lymph

Table 2. Relationship between micrometastasis and extent of submucosal cancer invasion

Micrometastasis	Extent of submucosal invasion	
	Vsm	Hsm
Negative	827.9 $\pm$ 760.0*	2817.6 $\pm$ 3389.1**
Positive	2026.4 $\pm$ 1720.8*	6810.9 $\pm$ 4439.6**

* $P < 0.05$; ** $P < 0.01$, Mann-Whitney *U*-test

Data values are means $\pm$ SD

Vsm, Depth of submucosal invasion (μ m); Hsm, width of submucosal invasion (μ m); NS, not significant

node metastasis are shown in Tables 3 and 4, respectively. The minimal extent of cancer invasion in patients with micrometastasis was 200 μ m in depth and 0.32 mm (320 μ m) in width. These measurements were far greater in the patients with overt lymph node metastasis (more than 2440 μ m and 10.5 mm, respectively). Six of the 11 micrometastases consisted of only a single carcinoma cell (Fig. 2), while the others were detected as one to three clusters of several aggregated cancer cells each (Fig. 3). Nine of the 11 micrometastases were found in the subcapsular sinus of the lymph nodes and 2 were found in the medullary sinus. No correlation was found between the site of the micrometastasis and the aggregate or single status of cancer cells.

Figure 4 shows a scattergraph of the relation between the extent of submucosal invasion of the cancer and the

Fig. 2. Single cell metastasis consisting of an isolated cancer cell is clearly demonstrated (arrow) by immunostaining with CAM5.2. $\times 100$

Fig. 3. Immunostaining with CAM5.2 detected aggregated metastasis consisting of several cancer cells forming a cluster (arrow). $\times 100$

Table 3. Clinicopathological findings of the 11 patients with micrometastasis

Patient no.	Age (years)	Gross type	Maximum diameter (mm)	Location	Histologic type	Extent of sm invasion		Lymphatic invasion	Venous invasion	Number of metastatic cells
						Vertical; μm	Horizontal; μm			
1	55	IIC	7	M	Tub1 > tub2	200	320	—	—	Single cell
2	39	IIC	11	A	Tub2	540	3 050	+	—	Single cell
3	42	IIC	8	M	Tub1	600	1 050	—	—	Single cell
4	81	IIC + IIa	8	A	Tub1	900	6 800	+	—	About 10 cells $\times$ one cluster
5	60	IIC	7	A	Tub1	1050	6 400	+	—	About 10 cells $\times$ one cluster
6	66	IIa + IIC	18	A	Tub2	1350	2 800	—	+	About 10 cells $\times$ one cluster
7	47	IIC	17	M	Tub1 > tub2	2500	8 800	+	—	About 10 cells $\times$ two clusters
8	71	IIC + IIa	15	A	Tub2 > tub1	2650	10 500	+	—	Single cell
9	52	IIa + IIC	18	A	Tub1 > tub2	2800	11 000	—	—	Single cell
10	55	IIa + IIC	15	A	Tub2	3800	11 700	+	+	About 10 cells $\times$ three cluster
11	48	IIa + IIC	18	A	Tub2 > por	5900	12 500	+	—	Single cell

M, Middle third; A, lower third; por, poorly differentiated type; Sm, submucosal

Table 4. Clinicopathological findings of the three patients with overt lymph node metastasis

Patient no.	Age (years)	Gross type	Maximum diameter (mm)	Location	Histologic type	Extent of sm invasion		Lymphatic invasion	Venous invasion	Metastasis
						Vertical; μm	Horizontal; μm			
1	59	IIC	15	M	Tub2 > por	2440	10 500	+	+	Overt
2	47	IIC + IIa	18	M	Tub1 > Tub2, por	3800	7 800	+	+	Overt
3	52	IIa	20	A	Tub1 > tub2	5500	15 500	+	—	Overt

M, Middle third; A, lower third

Fig. 4. Relation between lymph node metastasis and extent of submucosal cancer invasion. *Vsm*, Maximum vertical distance of submucosal invasion; *Hsm*, maximum horizontal distance of submucosal invasion. *Circles*, No metastasis; *Squares*, overt metastasis; *gray triangles*, single-cell micrometastasis; *black triangles*, aggregated micrometastasis

lymph node metastasis status in all 50 patients. The minimal extent of cancer invasion in patients with aggregated micrometastasis was greater (900 μm in depth and 2800 μm in width) than that in patients with single-cell metastasis (200 μm in depth and 320 μm in width). Within the range of $Vsm < 900 \mu m$ and $Hsm < 2800 \mu m$, micrometastases detected in the lymph nodes were all single-cell metastases.

Discussion

Several studies have suggested the possibility of extending the indications for EMR to gastric submucosal carcinomas [8–11]. Nishida et al. [8] reported that well differentiated adenocarcinomas with shallow submucosal invasion (less than 400 μm vertically) in patients without any risk factors, such as lymphatic permeation and infiltrative growth, or association with peptic ulcer, may be considered appropriately indicated for endoscopic treatment. Fujisaki et al. [10] also suggested that the indications for EMR for gastric carcinoma could be extended to submucosal carcinomas whose invasion was 500 μm or less vertically and 1500 μm or less horizontally. These proposals stress the importance of the extent of cancer invasion to the submucosa. Note also that they were based on the findings that gastric submucosal carcinomas with such histopathological features showed no lymph node metastasis by routine H&E examination (so-called overt lymph node metastasis).

Since lymph node metastasis is the major prognostic factor in patients with carcinomas, the complete curative potential of EMR for gastric submucosal carcinoma should be evaluated in terms of whether the histopathological features of the primary lesion are those expected to have considerably low potential for lymph node metastasis. This is why the absolute indica-

tions for EMR for gastric cancer have been confined to intramucosal carcinomas.

Recently, immunohistochemical analysis using anti-human cytokeratin antibodies [12–14] enabled us to visualize the presence of a single carcinoma cell or a small cluster of carcinoma cells in lymph nodes (so-called micrometastases) that were not discernible by routine H&E staining. For submucosal gastric carcinomas, the presence of micrometastasis has been reported as 16% [23] to 21% [24] by cytokeratin immunohistochemistry. In the present study, micrometastasis was detected in 11 of 47 cases (23.4%). The presence of micrometastasis has been regarded as a more sensitive prognostic marker than the presence of overt lymph node metastasis (metastasis detected by routine H&E staining) in various human cancers, including gastric carcinoma [13,14,23–25]. It now seems necessary to re-evaluate the results of earlier studies [8–10] that suggested extending the indications for EMR to gastric submucosal carcinoma, because these studies were based on the correlation between the histopathological features of the primary lesion and overt lymph node metastasis alone. However, to date, no systematic studies have been conducted to investigate the relationship between the histopathological features of gastric submucosal carcinoma and micrometastasis. Focusing on the curative potential of EMR in terms of micrometastasis, we selected 50 surgically resected gastric submucosal carcinomas that would have satisfied the absolute indications for EMR, except for the criterion of submucosal invasion (differentiated-type carcinomas 20 mm or less in size and not associated with peptic ulcer). The present study showed that micrometastasis was significantly correlated with two histopathological factors: (1) lymphatic permeation of the carcinoma; and (2) the extent of cancer invasion to the submucosa. The correlation between lymphatic permeation and micrometastasis was not found in an earlier study [24]. This contradictory finding

of the present study can be explained by our more detailed examination for the presence of lymphatic permeation using CD31 immunohistochemistry. The extent of cancer invasion to the submucosa has also been regarded as a factor highly indicative of overt lymph nodes metastasis [8–10,17,26]. In this study, the average depths and widths of submucosal invasion were significantly higher in micrometastasis-positive cancers than in those without micrometastasis (Table 2). The minimum amount of cancer invasion in patients with micrometastasis was 200µm vertically and 320µm horizontally; the present results suggest that submucosal carcinomas without lymphatic permeation and with the extent of submucosal invasion less than these values may be curable by EMR alone, without additional radical surgery.

EMR-treated gastric carcinomas with submucosal invasion exceeding 200µm vertically or 320µm horizontally have the potential for micrometastasis. However, this does not necessarily mean that these cancers require additional radical surgery. There has been considerable controversy as to whether the size of micrometastasis has prognostic significance. In breast cancer, McGuckin et al. [13] stated that the presence of micrometastasis itself had prognostic value, while Nasser et al. [27] demonstrated that only micrometastases exceeding 0.2mm in size had prognostic value. Jeffers et al. [28] also suggested that micrometastases consisting of an isolated single cancer cell or several small clusters of cancer cells had no prognostic significance in colorectal cancer. In our study, six micrometastases consisted of a single carcinoma cell and five consisted of aggregations of cancer cells (Figs. 2 and 3 and Table 3), and cancers with submucosal invasion of less than 900µm vertically and 2800µm horizontally had no aggregated micrometastasis (Fig. 4). These cancers would also be expected to show complete curative potential by EMR alone, if in fact an isolated single micrometastasis has negligible prognostic value compared with that of an aggregated micrometastasis. These findings must therefore be further clarified by a comparative study of the survival or recurrence rate in patients bearing cancers with a single-cell metastasis and those with aggregated micrometastasis, and we are currently investigating this matter.

If the aggregation of micrometastasis alone has prognostic significance, the indications for EMR in patients with gastric carcinoma could be extended to carcinomas with submucosal invasion of up to 900µm vertically and 2800µm horizontally. These figures are greater than the earlier estimations for patients with overt lymph node metastasis, in whom the maximum cancer invasion without overt lymph node metastasis was 400 [8] to 500 [10] µm vertically and 1500µm [10] horizontally. We speculate that the earlier studies may have underestimated

the maximum extent of cancer invasion in patients without lymph node metastasis. Furthermore, in these studies it is unclear whether entire lesions were examined by stepwise sectioning [8], or by producing stepwise sections at 5- to 6-mm intervals [9,10]. Thus, it is not certain whether the deepest and/or widest portions of invasion were detected precisely by representative sections alone or by broad step-wise sectioning. In this study, we confirmed the deepest and the widest points of invasion by producing 7–24 serial sections for each paraffin block. This detailed procedure seems not unpracticed, since it has generally been recommended that EMR materials be examined histologically using stepwise sections made at 2–3-mm intervals.

Based on the present results, we are confident that those EMR-treated gastric carcinomas shown to be submucosal carcinomas could have complete curative potential by EMR alone when they show no lymphatic permeation and when the extent of submucosal invasion is less than 200µm vertically and 320µm horizontally. These cancers could even be expected to be free of micrometastasis. Furthermore, when the submucosal invasion is 200–900µm vertically and 320–2800µm horizontally, the requirement of additional radical surgery may also be avoided.

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