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Original article

The prognostic significance of isolated tumor cells in the lymph nodes of gastric cancer patients

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Abstract

Background. **The clinical significance of isolated tumor cells (ITC) detected immunohistochemically in the lymph nodes of gastric cancer patients is controversial. The aim of this study was to examine the prognostic impact of ITC in patients with gastric cancer.**

Methods. **The data of a total of 402 patients with pathological T2N0 and T2N1 gastric cancer who underwent gastrectomy with D2 lymph node dissection between 1984 and 1990 at four participant hospitals were analyzed. All resected lymph nodes were reexamined by serial sectioning with hematoxylin & eosin (H&E) staining, and evaluated by immunohistochemistry using antibody against cytokeratin (AE1/3). The prevalence and prognostic significance of ITC were investigated.**

Results. **ITC were detected in 187 of the 402 (47%) patients. A multivariate analysis identified the nodal status, histological type, and tumor size as significant factors predictive of the presence/absence of ITC. The 5-year and 10-year overall survival rates of patients with vs those without ITC were 84.4% (95% confidence interval [CI], 79.1–89.0) and 70.4% (95% CI, 64.1–76.7) vs 83.9% (95% CI, 78.6–89.2) and 72.0% (95% CI, 65.4–78.5), respectively. The hazard ratio for death in patients with ITC as compared with those without ITC was 0.90 (95% CI, 0.64–1.26; $P = 0.53$).**

Conclusions. **The presence of ITC in the lymph nodes does not affect the prognosis of patients with gastric cancer who have undergone gastrectomy with D2 lymph node dissection.**

Key words ITC · Lymph node metastases · Gastric cancer · Immunohistochemistry · Lymph node dissection

Introduction

The major prognostic factors in patients with gastric carcinoma are the depth of the primary tumor and the presence/absence of lymph node, peritoneal, and distant metastases. Complete tumor removal is deemed to be the only potentially curative treatment in patients with gastric cancer. Locally advanced gastric cancer frequently recurs after a curative operation, and even early gastric cancer relapses occasionally [1]. In patients with recurrent disease, it is considered that such disease arises, presumably from residual tumor cells, in the form of occult micrometastases, left behind at the time of apparently curative surgery.

Recent advances in immunohistochemistry (IHC) and molecular biological techniques [2] allow the identification of discrete and occult tumor cells in the lymph nodes [3], peripheral [4] blood, and bone marrow [5–7] of patients with malignant diseases that remain undetected during routine pathological examination. After some debate regarding the terminology for occult tumor cells, micrometastases (MM) are now defined as deposits of tumor cells measuring 2 mm or less but larger than 0.2 mm, while the term "isolated tumor cells (ITC)" refers to single tumor cells or clusters of tumor cells measuring 0.2 mm or less [8, 9]. The prevalence and prognostic significance of ITC are still controversial.

The aim of this study was to analyze whether the presence of ITC in the lymph nodes of gastric cancer patients treated by curative resection portends a worse prognosis.

Methods

Patients

A total of 402 patients with pathological T2N0M0 or T2N1M0 gastric cancer (T2, tumor invades the muscularis propria or subserosa, N0, no lymph node metastases; N1, with perigastric lymph node metastases; M0, no distant metastases) who underwent gastrectomy with

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D2 lymph node dissection between January 1984 and December 1990 at any of the four participant hospitals in this study in Japan were included in this study. One hundred seventy-seven patients were treated at the National Cancer Center Hospital, Tokyo; 130 at Aichi Cancer Center Hospital, Nagoya; 67 at Teikyo University Hospital, Tokyo; and 28 at Kagoshima University Hospital, Kagoshima.

The patients in this study were basically consecutive, except for a few whose follow-up or material blocks were not available. All patients underwent partial or total gastrectomy with systematic lymphadenectomy, including complete dissection of perigastric lymph nodes and the second-tier lymph nodes along the common hepatic, proper hepatic, celiac, and splenic arteries.

Pathology and immunohistochemistry

All specimens containing the primary tumors were histologically classified according to the Japanese classification of gastric carcinoma [10] and the World Health Organization tumor classification system [11], and the following data were recorded at each hospital: tumor size, histological type, depth of invasion, and presence/absence of vascular or lymphatic invasion. Lymph nodes were examined in one cross-section obtained through the center of each lymph node.

Two consecutive sections measuring 4 μm in thickness were newly cut from 15 899 lymph nodes of the 402 patients for H&E staining and IHC. The median number of lymph nodes examined per patient was 32.5 (range, 6–124). The diagnosis in 32 patients who had been diagnosed as node-negative was revised to node-positive at this review, based on the examination of sections stained with H&E (T2N0 to T2N1).

IHC was performed using AE1/AE3 (Boehringer Mannheim, Indianapolis, IN, USA), a monoclonal antibody reactive with a broad spectrum of human cytokeratins. The procedure has been reported in detail previously [12]. Lymph nodes stained by IHC were evaluated by the pathologists at each hospital and revised by T.S., without any knowledge of any clinical information about the patients. ITC were defined as single tumor cells or clusters of tumor cells measuring 0.2 mm or less; they could not be detected by routine H&E staining and were detected by cytokeratin-specific IHC. When ITC were detected in a lymph node without overt metastases, this case was regarded as ITC-positive. When both single cells and clusters were observed in a lymph node, the ITC were classified as the cluster type.

Statistical analysis

Statistical analysis was carried out using the SPSS software, version 11.5 (SPSS, Chicago, IL, USA). The clinicopathological features of the studied cases were compared by a χ^2 test or Student's *t*-test. Multivariate analysis was conducted using a logistic regression model and Cox's proportional hazard model. The Kaplan-Meier method was used for drawing the survival curves, and the log-rank test was used for evaluating the statistical significance of the differences between the survival curves.

Results

Frequency and location of ITC

ITC were identified in 187 of the total of 402 patients (46.5%), in 81 of the 221 T2N0 patients (36.7%), and in 106 of the 181 T2N1 patients (58.6%). The median number of lymph nodes containing ITC was 2 (range, 1–25) per patient.

Among the 81 T2N0 patients detected as having ITC, 59 had the ITC in the perigastric lymph nodes, 19 in the second-tier lymph nodes, and 3 in distant lymph nodes including paraaortic lymph nodes. Among the 106 T2N1 patients detected as having ITC, 76 had the ITC in the perigastric nodes, 28 in the second-tier nodes, and 2 in distant nodes. Seventy-five patients had the single-cell type of ITC, while 112 had the cluster type.

Relationship between the presence of ITC and clinicopathological factors

The correlations between the presence of ITC and clinicopathological factors are shown in Table 1. Tumor size, histological type (differentiated or undifferentiated), lymphatic invasion, tumor depth (mp, muscularis propria; ss, subserosa) and nodal status (N0 or N1) were identified by univariate analysis as significant factors predictive of the presence/absence of ITC in the lymph nodes. Among these factors, the histological type, size of the tumor (<50 or ≥50 mm), and nodal status were identified as significant factors by multivariate analysis based on a logistic regression model (Table 2). Tumors of the undifferentiated type, large-sized tumors, and originally node-positive tumors were more likely to be associated with ITC in the lymph nodes; the odds ratios were 1.78 (95% CI, 1.18–2.69; $P = 0.006$), 1.67 (95% CI, 1.05–2.66; $P = 0.029$), and 2.11 (95% CI, 1.36–3.26; $P = 0.001$), respectively.

Relationship between the presence of ITC and the prognosis of patients

The median follow-up period of the surviving patients was 127 months (range, 3–215 months). Disease recurrence was observed in 32 patients. Of these, 18 patients

Table 1. Relationships between ITC and clinicopathological factors

	Positive	Negative	*P* value
Age (years)	59.0 ± 11.9	61.4 ± 11.9	0.04
Tumor diameter (mm)	50.6 ± 25.2	40.5 ± 19.0	<0.001
Sex			
Male	124	162	0.03
Female	63	53	
Histology			
Diff.	84	130	0.001
Undiff.	103	85	
V			
–	152	180	0.30
+	35	35	
Ly			
–	68	101	0.02
+	119	114	
Depth			
mp	105	147	0.008
ss	82	68	
Nodal status			
N0	81	140	<0.001
N1	106	75	

ITC, isolated tumor cells; Diff, differentiated; Undiff, undifferentiated; V, vascular invasion; Ly, lymphatic invasion; mp, muscularis propria; ss, subserosa; –, negative; +, positive

Table 2. Multivariate analysis to determine the relationship between the presence of ITC and clinicopathological factors

	OR	95% CI	*P* value
Histology (diff./undiff.)	1.780	1.179–2.687	0.006
Depth (mp/ss)	1.271	0.814–1.986	0.292
Tumor diameter (>50 / ≤50 mm)	1.673	1.053–2.658	0.029
Nodal status (N0/N1)	2.111	1.366–3.262	0.001
Lymphatic invasion (–/+)	1.146	0.734–1.791	0.549

OR, odds ratio; CI, confidence interval

had ITC, while 14 did not. The incidence of recurrence was not related to the presence of ITC ($P = 0.26$).

The 5-year and 10-year overall survival rates of the patients with and without ITC were 84.4% (95% CI, 79.1–89.0) and 70.4% (95% CI, 64.1–76.7); and 83.9% (95% CI, 78.6–89.2) and 72.0% (95% CI, 65.4–78.5), respectively. The hazard ratio for death in the patients with ITC as compared to those without ITC was 0.90 (95% CI, 0.64–1.26; $P = 0.53$).

There were no significant differences in the survival curves between patients with and without ITC ($P = 0.53$ by log-rank test; Fig. 1). The type of ITC (single cell, cluster), and the number of lymph nodes with ITC did not affect the prognosis. The effects of the clinicopathological factors on the prognosis of the patients are shown in Table 3. Age (<60 / ≥60 years), histological type (differentiated type/undifferentiated), depth of invasion (mp/ss), and nodal status (N0/N1) were identified as significant prognostic factors by univariate analysis. Multivariate analysis using representative factors from

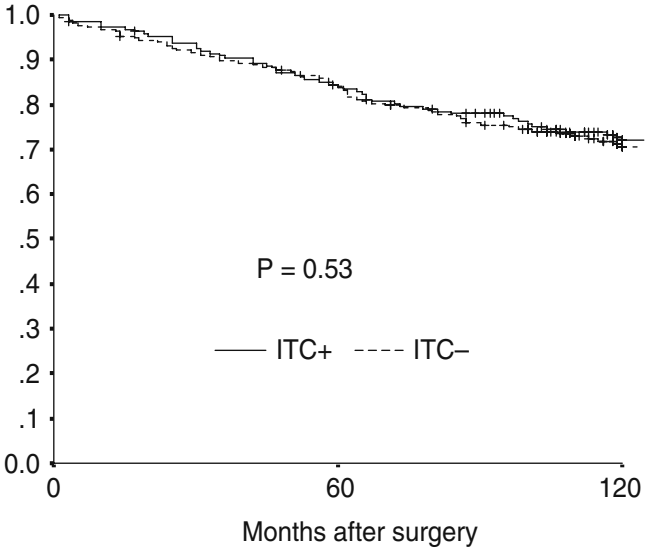

Fig. 1. Overall survival of pT2N0 and pT2N1 gastric cancer patients. There was no significant difference in survival between patients with and without isolated tumor cells (*ITC*)

Table 3. Univariate analysis to determine the clinicopathological factors related to overall survival

	HR	95% CI	*P* value
Age (<60/≥60 years)	3.539	2.375–5.273	<0.001
Histology (diff./undiff.)	0.531	0.371–0.760	0.001
Depth (mp/ss)	1.414	1.005–1.989	0.047
Tumor diameter (<50/≥50 mm)	1.035	0.716–1.495	0.855
ITC (–/+)	0.897	0.638–1.262	0.534
Nodal status (N0/ N1)	1.763	1.251–2.485	0.001
Sex (male/female)	0.762	0.515–1.130	0.176
Lymphatic invasion (–/+)	1.503	1.052–2.148	0.025
Vascular invasion (–/+)	1.413	0.936–2.113	0.100

HR, hazard ratio

Table 4. Multivariate analysis to identify the clinicopathological factors determining the overall survival

	HR	95% CI	*P* value
Age (>60 / ≤60 years)	3.189	2.113–4.814	<0.001
Histology (diff./undiff.)	0.693	0.476–1.008	0.055
Depth (mp/ss)	1.178	0.821–1.689	0.374
Nodal status (N0/N1)	1.800	1.229–2.638	0.003
Ly (–/+)	1.127	0.765–1.661	0.546
ITC (–/+)	0.888	0.621–1.268	0.513

Table 3 showed that age and nodal status were the most significant prognostic factors (Table 4). The presence of ITC did not have any impact on the prognosis of the patients.

Discussion

The results of this study suggest that the presence of ITC in lymph nodes does not imply systemic involvement by the disease, and has no influence on the prognosis of gastric cancer patients who have undergone gastrectomy and systematic lymph node dissection.

There is increasing interest in the presence and prognostic relevance of occult tumor cells in various malignant diseases [13]. In breast cancer and melanoma, lymph nodes involved with tumor metastases are no longer the objects of drastic dissection, but are an indicator of patients with a poor prognosis needing intensive adjuvant therapy. Lymph nodes containing occult tumor cells have been considered similarly in some other reports as well [14, 15], and this concept has been adopted even in the sentinel nodes theory [16, 17]. In contrast, metastatic lymph nodes are targets for local control in gastric cancer patients [18], although extended lymph node dissection is still controversial [10]. The clinical significance of the detection of occult tumor cells in the lymph nodes of gastric cancer patients is an important subject for more intensive study, because it may provide some directions regarding the extent of lymph node dissection [19] and the necessity for adjuvant therapy after curative surgery.

The biology of ITC in the lymph nodes has not been fully elucidated, but several conclusions can be drawn from a recent analysis [20]. ITC may be present in many lymph nodes that are originally diagnosed as tumor-negative by H&E staining, as previously reported. In the present study, such an occurrence was significantly more frequent in T2N1 than in T2N0 gastric cancer patients. In addition, ITC were detected in second-tier lymph nodes more frequently in N1 patients than in N0 patients (29 of 181 patients vs 19 of 221 patients, $P = 0.02$). This behavior of ITC is consistent with the concept that lymph node metastases proceed from the perigastric area to the next area in order. ITC were found even in distant lymph nodes, including paraaortic lymph nodes, as reported before [21]. This shows that ITC can also reach lymph node stations far away from the primary tumor, but further discussion may have to be limited because only a proportion of patients in the present study underwent super-extended lymph node dissection including the paraaortic area. In this study, the frequency of ITC in lymph nodes was higher in patients with larger and more undifferentiated tumors. It is conceivable that tumor cells can escape more easily from such tumors.

Many authors have reported on the clinical significance of occult cancer cells in the lymph nodes of gastric cancer patients. Morgagni et al. [22] and Choi et al. [23] reported a negative impact on the prognosis for early gastric cancer, while others have refuted such a suggestion [24–26] by the analysis of patients including those with early and advanced gastric cancer. The studies including a majority of patients with early gastric cancer have the problem of too low an incidence of disease-specific death to allow reasonable prognostic evaluation. If many cases of locally advanced gastric cancer invading the serosal surface of the stomach were to be included, the prognostic significance of occult tumor cells in the lymph node would be confounded by the

high frequency of peritoneal recurrence. For this reason, we previously carried out a study similar to the present one in patients with T2N0 disease [27]; in the present study, T2N1 gastric cancer patients with perigastric lymph node metastases were examined in addition for the purpose of including a larger number of patients. T2N2 patients with lymph node metastases in the second-tier lymph nodes were not included, because such patients might include those with paraaortic lymph node metastases which cause potential stage migration.

Previous discussions about occult tumor cells in the lymph nodes have always included diagnostic problems. Occult tumor cells detected by IHC have been divided into ITC and MM, as mentioned above. Some of the reported MMs may actually be small metastases, associated with a worse prognosis, which could probably have been detected by routine H&E staining by well-experienced pathologists. Many lymph node metastases diagnosed at our institution measure less than 2 mm in greatest dimension [28]. Furthermore, decisions reached among pathologists for resolving this delicate problem are quite mandatory, as shown by some studies reporting difficult reproducibility of the diagnosis of occult tumor cells [29, 30].

In the present study, the presence of ITC was not found to be an independent factor for worse prognosis, as assessed by both multivariate analysis of prognostic factors and survival analysis. Although one of the purposes of an investigation of ITC might be to find a target for additional therapy after curative surgery, based on the results of the present study, it will not be necessary hereafter to take into account the presence of ITC in the lymph nodes of gastric cancer patients. In Japan, pT2N1 gastric cancer patients are already candidates for adjuvant chemotherapy, based on the results of a clinical trial [31]. In the West, node-positive gastric cancer patients are included as candidates for postoperative chemoradiotherapy, based on the results of the INT0116 trial [32].

The viability and clinical significance of the presence of ITC in lymph nodes is better discussed separately. Some reports [6, 33] suggest that ITC do not progress to become metastatic lesions, and will probably die or be eliminated by the host immune response, even if they have reached distant sites, but the potential tumorigenicity of single cells in the lymph nodes has also been reported [20, 34]. Therefore, what is the malignant potential of ITC? Evaluation of the viability of a small number of tumor cells and the discrimination of actual malignant tumor cells are probably subjects of great interest and importance that need to be studied [34, 35]; however, further basic investigation will be needed before there are clinical applications. We still cannot provide a clear answer to the essential question of whether or not a lymph node containing ITC should be dissected. The similar outcomes in patients with and without ITC in our study may have occurred because all of the participants in this study underwent standard D2 lymph node dissection, but these similar outcomes could also be interpreted to suggest that ITC in the lymph nodes may be basically ignorable without dissection. However, these essential issues are so far from being clearly resolved that any discussion about the indications for limited surgery or the necessity for extended lymph node dissection based on the prevalence of ITC in the lymph nodes is futile.

In conclusion, the results of this study, an analysis of a large group of patients with limited disease, may provide some suggestions regarding the clinical impact of ITC in the lymph nodes of gastric cancer patients.

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