Original Article

Location of Sentinel Lymph Node in Gastric Cancer: A Modified, Painless And Noninvasive Approach

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ABSTRACT

Objective: The presence of lymph node metastases is an important factor in the prognosis of gastric cancer patient. Therefore, the precise identification of sentinel lymph nodes (SLN) in these patients is critical. In this work, we investigated the feasibility and preciseness by injection of ^{99m}Tc-sulfur colloid (SC) 2 hours before operation after general anesthesia, instead of one day before surgery.

Methods: Thirty-one patients of gastric cancer diagnosed as T1–T3 were enrolled in this study. During operation, a SLN was defined as those containing 10 times more radioactivity than surrounding tissue with a hand-held gamma probe and removed. All the patients underwent radical gastrectomy with extended lymphadenectomy. All resected nodes were examined postoperatively by routine H&E stain and those negative SLNs were examined with further cytokeratin immunohistochemistical staining.

Results: The incidence of metastasis was significantly higher in SLNs than in non-SLNs (x^2=67.48, P<0.001). The overall sensitivity, specificity and accuracy of the SLN status in the diagnosis of the lymph node status of the patient were 86.36%, 100% and 96.77%, respectively. The positive predictive value and negative predictive value of SLN biopsy were 100%, and 75.0%, respectively. SLNs were used to diagnose the lymph node status with 100% accuracy in the T1 group. In the T2 and T3 groups, the sensitivity, specificity, and diagnostic accuracy were 92.3%, 100%, and 94.44%, 60.0%, 100%, and 66.66%, respectively. Most of the SLNs were located in the #1, #2, #3, #4, #5, and #6, except in 3 patients (9.68%).With cytokeratin immunohistochemical staining, lymphatic pathologic staging in l patient was upstaged.

Conclusion: SLN biopsy with the above approach is a feasible and accurate diagnostic procedure for detecting lymph node metastasis in patients with gastric cancer, which is painless, noninvasive, easily accepted by patients and suitable for extensive clinical applications.

Key words: Gastric cancer; Sentinel lymph node location; Radiocolloid; Injection time

INTRODUCTION

Gastrectomy with extend lymphadenectomy is considered potentially curative, but the overall prognosis for patients with gastric cancer remains poor, with 5-year survival of 23.0% in the US compared with 39.0% in China[1, 2]. An important prognostic indicator for gastric cancers is the presence or absence of tumor in the nodal drainage basin[2, 3]. Accordingly, the precise staging of gastric malignancies relies on the meticulous detection of lymph node metastases[4].

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Sentinel lymph nodes (SLNs), the first draining lymph node on the direct lymphatic pathway from the primary tumour site[5], should be theoretically the first site of lymph node metastases. In 1992, Morton et al.[5] reported pioneeringly that Sentinel lymph nodes was successfully detected by dye injection in a cutaneous melanoma. Since then, sentinel lymph node mapping and biopsy has entered medical practice with astonishing rapidity. This technique has been applied to breast[6], thyroid[7] and colorectal cancer[8]. Recently, some studies have showed that the SLN concept can be applied in gastric cancer[9–11].

However, currently, radiocolloid has to be endoscopically injected one day before surgery in gastric cancer. This technique is painful, invasive and not accepted easily by patients and their families. Limitations of the current radiocolloid methodology for localizing SLNs have spurred studies to improve this technology. In an attempt to create a better method for localizing SLNs with radiocolloid in gastric cancer patients, we developed a new injection time of 2 h before operation after general anesthesia. The purpose of this study was to evaluate its clinical feasibility and sensitivity in the detection of lymph nodal metastasis in gastric cancer patients.

MATERIALS AND METHODS

Patients

From January 2006 through March 2007, thirty-one patients (21 males and 10 females) with histologically confirmed primary gastric adenocarcinoma and without clinically diagnosed lymph node and distant metastasis were prospectively enrolled in this study. Their preoperative stages by imaging studies, computed tomography (CT), and abdominal ultrasonography were T1, T2 or T3 without lymph node (N0) or distant metastasis (M0). All patients were scheduled to undergo gastrectomy with extended lymphadenectomy, including the sampling of paraaortic lymph nodes, at Department of Surgical Oncology, the First Hospital of Xiamen, Fujian Medical University, Xiamen. None of the patients had received preoperative treatment. All patients enrolled in this study gave written informed consent, the procedure was conducted with the permission of the hospital's ethics committee.

Detection of Sentinel Lymph Nodes and Surgical Procedure

After general anesthesia, two hours prior to operation, we injected one depot of ^{99m}Tc-radiolabeled sulphur colloid solution (SC) containing 37 MBq of tracer in 2 ml saline (SC, Syncor Star Medicinal Technology Co. Ltd., Beijing, China) into the submucosa at 4 sites around the primary tumor using gastroscopy. Followed by the opening of the abdominal wall, radioactivity was monitored using a hand-held gamma probe (NEO2000TM Gamma Detection System; Neoprobe Corporation, USA) as soon as possible and without significant manipulation of stomach or greater omentum. SLN defined by a level of radioactivity 10 times higher than background[10, 11] was removed. Excised SLNs were immediately made to frozen sections for histologic examination, and then routine radical gastrectomy with extended lymphadenectomy was performed in all patients.

Histologic Evaluation

Routine histopathological examinations of SLNs were performed using hematoxylin and eosin (H&E). Immunohistochemical (IHC) studies for cytokeratin AE1/AE3 (Maixim Biology Corparation, Fuzhou, Fujian, China) were performed when SLN nodes were interpreted histologically as negative for metastases at frozen biopsy.

Statistical Analysis

In the calculation of the diagnostic accuracy, sensitivity, specificity, positive predictive value and negative predictive value for sentinel node biopsy, we used the definitions given by Gretschel[11].

The incidence of metastases detected in sentinel nodes and non-sentinel nodes was compared by the x^2 test. P values <0.05 were considered statistically significant.

RESULTS

Patient Characteristics

The clinicopathologic characteristics of all the 31 patients enrolled in the study are summarized in Table 1.

Sentinel Node Identification and Lymph Node Status

The average number of dissected lymph nodes

per patient was 17.6 (range, 10–29). The number of SLNs identified on the basis of radioactivity ratio criteria was 2.8 (range, 1–5) per patient. In the 31 patients identified with SLNs, metastatic SLNs were found in 19; the remaining 12 were free of metastases in SLNs. Among 12 patients in whom SLNs were negative for metastasis, all non-SLNs were also negative except for 3 patients. Those 3 patients were false negative (25.0%, 3 of 12) by SLN biopsy. Among 19 patients with positive SLNs, 9 were positive and 10 negative in non-SLNs. In those 10 patients, the SLN was the only site of lymph node metastasis. The positive predictive value and negative predictive value of SLN to predict regional lymph node status were 100% (19 of 19) and 75.0% (9 of 12), respectively. Sensitivity and specificity of the SLN biopsy were 86.36% (19 of 22) and 100% (9 of 9), respectively (Table 2).

In the patients with T1 gastric cancers, SLNs were used to diagnose the lymph node status with 100% (4/4) accuracy, and there were no false-negatives (Table 3). In the patients with T2 gastric cancer, the sensitivity, specificity, and diagnostic accuracy were 92.3% (12/13), 100% (5/5), and 94.44% (17/18), respectively. In the patient with T3 gastric cancer, the sensitivity, specificity, and diagnostic accuracy were 60.0% (3/5), 100% (1/1), and 66.66% (4/6), respectively.

All of the SLNs were located in the perigastric lymph nodes (station I), such as LN $^{\#}$1, $^{\#}$2, $^{\#}$3, $^{\#}$4, $^{\#}$5, and $^{\#}$6, except in 3 patients (9.68%, 3 of 31) where, though not metastatic in pathology, they were skipped over the perigastric area and identified along left gastric artery (LN $^{\#}$7, station II, Table 4).

Table 1. Patient characteristics

Clinical parameters	Number of cases (n=31)
Median age (y)	58 (range, 34–69)
Sex ratio (M:F)	21 : 10
Histologic type [n (%)]	
Well differentiated	3 (9.68)
Moderately differentiated	20 (64.52)
Poorly differentiated	5 (16.13)
Signet ring cell	4 (12.90)
Anatomic site [n (%)]	
Upper third	10 (32.26)
Middle third	5 (16.13)
Lower third	17 (54.83)
Depth of invasion [n (%)]	
T1	7 (22.58)
T2	19 (61.29)
T3	6 (19.35)
Retrieved LN number [mean (range)]	17.6 (10–29)
SLN number [mean (range)]	2.8 (1–5)

SLN: Sentinel lymph node

Table 2. Nodal status according to sentinel lymph node

Sentinel lymph node (SLN)	Nodal status (n)	
	Negative (n=9)	Positive (n=22)
Negative SLN (n=12)	9	3
Positive SLN (n=19)	0	19

Sentinel Node Distribution

The relationship between the location of a gastric carcinoma and the sentinel node distribution is shown in Table 5. Most of SLNs were in the region along the lesser curvature ($^{\#}$3), followed in frequency by those along the right gastroepiploic artery ($^{\#}$4d). For gastric carcinoma

of the upper third of the stomach, SLNs often were distributed along the lesser curvature (#3) and among the right paracardial lymph nodes (#1). For gastric carcinoma of the lower third, distribution included the suprapyloric (#5) and infrapyloric lymph nodes (#6). They were not distributed among the lymph nodes along the left gastric artery (#7) and the CHA (#8a) in this series.

Table 3. Sentinel lymph node and non-sentinel lymph node status in patients with lymph node metastasis

Depth of invasion	Patients with lymph node metastasis	Metastasis in SLN n (%)	Metastasis in non-SLN n (%)
T1 (n=7)	4	4 (100%)	0
T2 (n=18)	13	12 (92.3%)	1 (7.69%)
T3 (n=6)	5	3 (60.0%)	2 (40.0%)
Total (n=31)	22	19 (86.36%)	3 (13.63%)

Table 4. Results for 3 patients with negative SLNs for lymph node metastasis

Case	Histologic type	Depth of invasion	Metastasis in SLNs/non-SLNs	Metastatic site	SLNs site
1	PD	T2	0/1	#7 LN	#3 LN
2	PD	T3	0/3	#7 LN	#3 LN
3	SRC	T3	0/2	#7 LN	#3 LN

PD: poorly differentiated; SRC: signet ring cell

Table 5. Distribution of SLNs according to the location of the neoplasm

	Upper third n=9, (%)	Middle third n=5, (%)	Lower third n=17, (%)	Total n=31, (%)
Right paracardial (#1)	3 (33.33%)	0	0	3 (9.68%)
Left paracardial (#2)	1 (11.11%)	0	0	1 (3.22%)
Along the lesser curvature (#3)	4 (44.44%)	3 (60.0%)	5 (29.41%)	12 (38.71%)
Along the SGA & LGEA (#4s)	1 (11.11%)	1 (20.0%)	0	2 (6.45%)
Along the RGEA (#4d)	0	1 (20.0%)	7 (41.17%)	8 (25.8%)
Suprapyloric (#5)	0	0	3 (17.64%)	3 (9.68%)
Infrapyloric (#6)	0	0	2 (11.76%)	2 (6.45%)
Along the LGA (#7)	0	0	0	0
Along the CHA (#8a)	0	0	0	0

SGA: short gastric artery; LGEA: left gastroepiploic artery; RGEA: right gastroepiploic artery; LGA: left gastric artery; CHA: Common hepatic artery.

Pathologic Examination

In 19 patients, SLNs were positive for metastasis by frozen biopsy, which was also confirmed by permanent H&E staining. Among 12 patients in whom SLNs were negative for metastasis on frozen biopsy, 11 were also negative by IHC for cytokeratin AE1/AE3, 1 patient was positive by IHC for cytokeratin AE1/AE3 (Figure 1). Three micrometastases of SLNs in the patient were found on IHC for cytokeratin AE1/AE3. Therefore, the accuracy of the SLN frozen biopsy was 96.77% (30/31).

The total number of dissected sentinel nodes and non-sentinel nodes were 78 and 468. The incidence of metastases detected in sentinel nodes and non-sentinel nodes were 65.38% (51/78) and 22.72% (97/468), respectively. There was a significant difference between the incidence of metastases of the two phases (x^2=67.48, P<0.001).

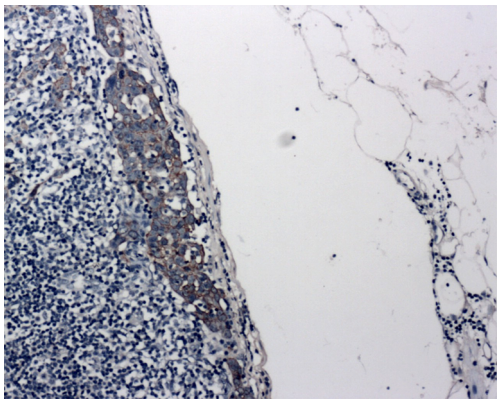

Figure 1. Micrometastases in SLNs of gastric cancer were found on IHC for cytokeratin AE1/AE3.

DISCUSSION

The role of lymphadenectomy for gastric carcinoma remains controversial. Accurate determination of the presence or absence of lymph node metastases provides important information for the treatment of gastric cancer. However, preoperative diagnostic techniques, including computed tomography (CT) and ultrasonography, do not provide an accurate prediction of metastasis in the regional lymph node. So, gastrectomy with extended lymphadenectomy has been considered as a standard surgical approach for early gastric cancer. In fact, the frequency of lymph node metastasis is less than 5% in patients with mucosal gastric cancer and 16% in patients with submucosal gastric cancer[12]. Therefore, 84% to 95% of patients with early gastric cancer could avoid lymphadenectomy and preserve a large volume of the stomach. It has been reported that extended lymphadenectomy does not improve the prognosis of lymph node negative patients, in addition, the morbidities accompanying extended lymph-adenectomy, such as bleeding, leakage, pancreatitis, subdiaphragmatic abscess, lymphorrhea, and chylous ascites cannot be trivialized[13]. Therefore, determination of the extent of lymph node dissection required on the basis of actual node involvement in patients with gastric cancer is important as less extensive dissection may reduce postoperative morbidity and mortality rates[14].

After the sentinel lymph node (SLN) biopsy procedure for patients with primary cutaneous melanomas was first introduced in the early 1992s[5], the concept of SLN characterization is of great interest to many surgical oncologists because it may be a guideline to the determination of the extent of cancer surgery. Many reports support the hypothesis that the absence of metastasis in SLNs correlates with absence of metastasis downstream of the lymph nodes[6−8].

Currently, there are 2 main methods (dye-guided and radio-guided) for detecting sentinel lymph nodes. In gastric cancers, Hayashi et al.[15] reported 2 possible difficulties exist in performing the dye-guided technique. First, because the pericardial area or prepyloric area is rich in fat, skeletonization around vessels is often required to detect positive staining nodes. Second, identification and removal of SLNs must be performed quickly because the dye flows into non-SLNs, and staining of primary lymph nodes or lymph vessels diminishes in 2 or 3 hours. In radio-guided method, radiocolloid has to be endoscopically injected one day before surgery and the patient must be performed endoscopy again. So, the technique is painful, invasive and not accepted easily by patients and their families. Additionally, unlike breast cancer and malignant melanoma, a preoperative lymphoscintigraphy in cases of SLN biopsy for gastric cancer may be not available[10]. For these reasons, we used ^{99m}Tc-sulphur colloid, with relatively larger particles, as a radioactive tracer instead of blue dye, and engaged in improving this technology and developing a new injection time of 2 hours before operation after general anesthesia.

In this study, SLN mapping and biopsy in patients with gastric cancer using ^{99m}Tc-sulphur colloid were proved satisfactory. The feasibility of the SLN biopsy was demonstrated in gastric cancer by an acceptable success rate of 96.88 %, and the mean number of SLNs in patients was 2.8 (range, 1−5); these results seem to be comparable with previously reported[14] in patients with gastric cancer. SLN positivity accurately predicted metastasis in the regional lymph nodes of gastric cancer patient (positive predictive value, 100%). This suggested that the use of the SLN technique may be warranted to select patients with metastatically involved lymph nodes who would be candidates for lymph node dissection. SLN negativity accurately predicted the absence of metastasis in all regional lymph nodes (negative predictive value, 75.0%). The overall sensitivity, specificity and accuracy were 86.36%, 100% and 96.77%, respectively. These results were in line with the literature reports[9, 10, 14].

The skip metastasis in gastric cancer has been considered an obstacle to the utilization of the SLN concept. There are different critical points: first of all, the complexity of the lymphatic network makes

SLN detection in the stomach more complicated than in other areas. Gastric lymphatic channels are multidirectional and form complex networks. Additionally, there can be a large number of sentinel lymph nodes. However, The incidence of skip metastasis in gastric cancer was found to be 0% to 10% in other retrospective studies[10, 16]. In our study, the skip metastasis occurred in 9.68 % (3/31; Table 4). Santoro et al.[17] claimed that, despite its complexity, the lymphatic flow occurred in an orderly, regular, repetitive, and predictable manner. Kim et al.[10] concluded that skip metastasis in gastric cancer was not an obstacle to the use of SLN detection, as SLN biopsy made it possible to locate and identify such metastases.

In our own experience, the SLN detection rate was 100%, and the accuracy was 100% in early gastric cancer (T1). However, the SLN detection rates in the T2 and T3 gastric cancers were 92.3% and 60.0%, respectively. The 3 patients with false-negative results had advanced tumors (T2, T3). The aspect is the block of lymphatic drainage caused by the tumor. As a matter of fact, the lymphatic flow can be altered if the main lymphatic routes are invaded and blocked by the tumor, leading to a lower success rate of SLN detection and to an increase in false negatives[18, 19]. This phenomenon occurs more frequently in the more advanced stages because of the greater extent of lymph node involvement. Hiratsuka M et al.[20] reported that the SLN biopsy was applicable and safe for T1 gastric cancer patients but not for T2 cases. These findings suggested that patients with early-stage gastric cancer might be better-suited for SLN mapping. Even though patients tend to have a lower incidence of early gastric cancer, the sentinel lymph node mapping technique is still useful to identify the lymphatic basin that should be dissected for T2 and T3 tumors.

Micrometastases in regional lymph nodes that are undetectable by single sectioning and hematoxylin and eosin staining are gaining importance in prognosis and potential survival in breast, melanoma, and other cancers[21, 22]. Various studies have shown that multiple sectioning and immunohistochemical staining increase the detection of metastatic lymph nodes in patients who are node-negative on hematoxylin and eosin staining[16, 22]. However, routine multiple sections and immunohistochemical staining of each node is too costly and time consuming. Therefore, in our study, immunohistochemical staining for cytokeratin AE1/AE3 was performed when SLNs were histologically interpreted as negative for metastases at frozen biopsy. In the field of gastric cancer, a considerable proportion of N0 patients have been upgraded to N (+) by multistep sectioning[23] or/and immunohistochemical staining of regional lymph nodes[24]. In the present study, one case of N0 patients was upgraded to N (+) by immunohistochemical staining of regional lymph nodes.

SLN biopsy for gastric cancer using the intraoperative injection ^{99m}Tc-SC and gamma-detecting is a feasible and accurate novel diagnostic procedure for detecting lymph node metastasis in patients with gastric cancer, especially in clinical T1 or T2, which is painless noninvasive, easily accepted and suitable for extensive clinical applications. This new technique may be of benefit to surgeons in planning the extent of lymph node dissection in gastric cancer.

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