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ORIGINAL ARTICLE

High false-negative proportion of intraoperative histological examination as a serious problem for clinical application of sentinel node biopsy for early gastric cancer: final results of the Japan Clinical Oncology Group multicenter trial JCOG0302

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Abstract
Background To evaluate the feasibility and accuracy of diagnosis using sentinel node (SN) biopsy in T1 gastric cancer, a multicenter trial was conducted by the Japan Clinical Oncology Group (JCOG).
Methods Sentinel node biopsy with indocyanine green (ICG) was performed in patients with T1 gastric cancer. Green-stained nodes (GNs), representing SNs, were removed first, and gastrectomy with lymphadenectomy was then performed. GNs in one plane (with the largest dimension) were histologically examined intraoperatively by frozen section with hematoxylin and eosin (H&E) stain. All harvested lymph nodes (GNs and non-GNs) were histologically examined by paraffin section after surgery. The primary endpoint was to determine the proportion of false negatives, which was defined as the number of patients with negative GNs by frozen section divided by those with positive GNs and/or positive non-GNs by paraffin section. The sample size was set at 1,550, based on the expected and threshold value as 5 and 10 % in the proportion of false negatives.
Results Accrual was suspended when 440 patients were enrolled because the proportion of false negatives was high. In the primary analysis, the proportion of false negatives was 46 % (13/28) after a learning period with 5 patients for each institution. Seven of 13 patients had nodal metastases outside the lymphatic basin. False negatives remained at 14 % (4/28) even by examining additional sections of GNs by paraffin section.
Conclusions The proportion of false negatives was much higher than expected. Intraoperative histological

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examination using only one plane is not an appropriate method for clinical application of SN biopsy in gastric cancer surgery.

Keywords Sentinel node · Gastric cancer · Indocyanine green · Multicenter clinical trial

Introduction

Early gastric cancer is almost completely curable, although nodal metastasis is found in approximately 20 % pathologically. Because nodal disease cannot be identified before or during surgery, the standard treatment is gastrectomy with nodal dissection, which has been uniformly selected for early gastric cancer. Theoretically, standard surgery is unnecessary for patients without nodal metastases.

The sentinel node (SN) technique has been used in the management of various cancers to avoid unnecessary lymphadenectomy [1–3]. The technique is based on the concept that the tumor-bearing status of the SN, which is defined as a lymph node (LN) that directly drains a specific cancer, reflects the tumor status of the remaining nodes.

Regarding gastric cancer surgery, two Japanese studies were reported in the early 2000s [4, 5]. Thereafter, several studies reported the validity of the SN concept for gastric cancer [6, 7]. To apply the SN concept and partial gastrectomy without radical lymphadenectomy to patients with T1 gastric cancer, the proportion of false negatives should be sufficiently low. Because nodal metastasis was only 20 % in T1 disease, a large number of patients was necessary to confirm the SN concept. However, most reports were from single-institutional studies with a small sample size and inadequate endpoint. Moreover, the SN technique varied according to the surgeon.

As a result of these limitations, the Japan Clinical Oncology Group (JCOG) conducted a multicenter clinical trial, JCOG0302 (GCSSG-SNB, UMIN-CTR ID: C000000059), to evaluate the feasibility and accuracy of diagnosis using SN biopsy in T1 gastric cancer.

Patients and methods

Patients

Patients had to fulfill the following preoperative eligibility criteria: T1 gastric cancer without indication for endoscopic resection, i.e., clinically T1a (cT1a) undifferentiated adenocarcinoma, cT1a differentiated adenocarcinoma with ulceration or with maximal diameter more than 2 cm, or cT1b adenocarcinoma; no existence of multiple foci; maximum diameter of 4 cm or less; distance from esophagogastric (EG) junction or pyloric ring of 2 cm or more; age 20–80 years; no prior treatment for gastric cancer; no prior surgery for gastric or duodenal ulcer. All patients provided written informed consent before surgery. Patients also had to fulfill the following intraoperative eligibility criteria: before dye injection; open surgery (laparoscopic surgery was excluded); cT1; palpable tumor or clips (four clips recommended) that were marked endoscopically for tumor location before surgery; no apparent lymph node metastasis; no severe adhesion around the stomach.

Sentinel node biopsy

Patients were enrolled intraoperatively before injection of indocyanine green (ICG) by means of a telephone call to the JCOG Data Center (Fig. 1). Then, 4–5 ml (25 mg/5 ml) ICG (Diagnogreen; Dai-Ichi Sankyo, Tokyo, Japan) dye was injected around the primary tumor using a fine needle (26-gauge) from the serosal surface of the stomach. Five minutes after dye injection, all LNs that stained green (GN), representing SNs, were excised one by one before lymphadenectomy. Each GN was then histologically examined intraoperatively in one plane (with the largest dimension) by frozen section with H&E staining. The protocol stated that harvesting of GNs must be finished within 30 min.

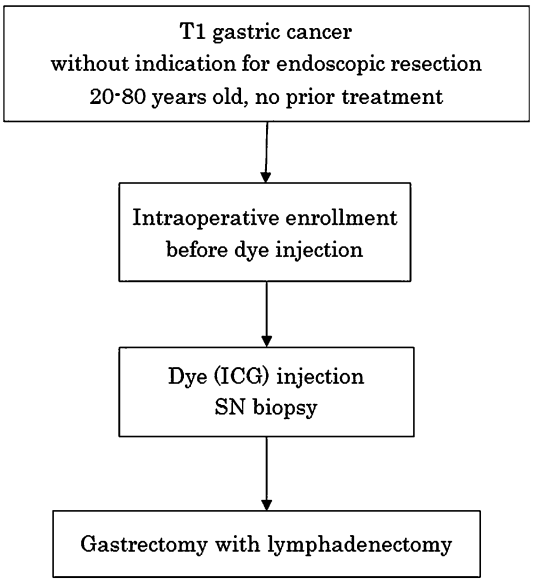

Fig. 1 Trial scheme of the JCOG0302 trial. After confirmation of eligibility criteria by the surgeon, patients were enrolled intraoperatively by means of a telephone call to the JCOG Data Center. Gastrectomy with lymphadenectomy was performed after sentinel node (*SN*) biopsy. *JCOG* Japan Clinical Oncology Group, *ICG* indocyanine green

Gastrectomy with lymphadenectomy was performed after SN biopsy according to the gastric cancer treatment guidelines edited by the Japanese Gastric Cancer Association [8]. After completing surgery, LNs were obtained as non-GNs from the resected stomach.

The initial five patients enrolled in each institution (but not per surgeon) were defined as patients during the learning period and all measurements referring to accuracy were calculated, excluding the learning period patients. Surgeons observed the video performed by the study principal investigator at starting the trial.

The GNs and non-GNs were fixed in formalin solution and embedded in paraffin for histological examination with H&E staining. Both GNs and non-GNs were diagnosed by one plane (with the largest dimension).

Adverse events were recorded according to the National Cancer Institute-Common Toxicity Criteria version 2.0 and the JCOG Surgical Morbidity Criteria [9].

This study was designed as a multicenter prospective clinical trial. The study protocol was approved by the JCOG Clinical Trial Review Committee and the institutional review boards of all participating institutions.

Study design and statistical analyses

The primary endpoint was to determine the proportion of false negatives, which was calculated by the number of patients with negative GNs by frozen section divided by those with positive GNs and/or non-GNs by paraffin section. The secondary endpoints were to calculate the proportion to detect GNs, the proportion of false positives, postoperative complications, the incidence of anaphylaxis reaction, and relapse-free survival of patients with nodal metastases. False positives were defined as patients with positive GNs by frozen section but negative GNs and non-GNs by paraffin section (Table 1). In cases in which GNs were diagnosed as positive in an intraoperative frozen section but negative in a paraffin section, this was regarded as a false positive and was not included in pathologically positive nodes.

The planned sample size was set at 1,550 based on the assumption that the expected and threshold proportion of false negatives was 5 and 10 % with one-sided alpha of 10 % and at least 90 % power. The proportion of nodal metastasis and the proportion to detect GNs were estimated as 20 and 95 %, respectively. If the proportion of false negatives was less than 10 %, the threshold, the risk to perform partial gastrectomy without lymphadenectomy erroneously for those with nodal metastasis, was supposed to be less than 2 %, which was considered sufficiently low.

The planned duration of accrual was 3.5 years with 5 years of follow-up. The protocol stated that accrual was suspended if 12 or more patients were diagnosed as false-negative at a semiannual monitoring.

Table 1 Definition of endpoints according to the JCOG0302 protocol

	Confirmed diagnosis of GNs and/or non-GNs by paraffin section	
	Positive	Negative
Intraoperative frozen section diagnosis of GNs		
Positive	A	B
Negative	C	D
GN undetectable	E	F

Proportion of false negatives = C/(A + C)

Proportion of false positives = B/(B + D)

Proportion to detect GNs = (A + B + C + D)/(A + B + C + D + E + F). Confirmed diagnosis of GN did not include intraoperative histological examinations of GNs by frozen section. Patients with only intraoperative histological positive GNs using frozen section were defined as false-positive and were not included in pathologically positive nodal metastases

Relapse-free survival was calculated from the date of enrollment to the date of relapse or the date of death from any cause. If patients remained alive without recurrence, they were regarded as censored cases at the date when no relapse was confirmed. Relapse-free survival was estimated by the Kaplan–Meier method.

All statistical analyses were conducted with SAS software (version 9.2; SAS Institute, Cary, NC, USA).

Results

The trial started in May 2004. However, accrual was suspended in September 2005 when 440 patients had been enrolled because false negatives were found in 13 patients by analysis excluding the learning period. According to the recommendation by the Data and Safety Monitoring Committee of JCOG, we decided to terminate the trial in November 2008. At that time, enrollment in each hospital ranged from 2 to 37 patients, with a median of 13 patients. The number of patients was more than 30 in 4 institutions, 21–30 in 4, 11–20 in 11, and 10 or less in 8.

Background factors, perioperative outcomes, and pathological findings are shown in Table 2. After the learning period, the number of GNs ranged from 0 to 19, with a median of 4 nodes (Fig. 2), and time to harvest all GNs after ICG injection ranged from 7 to 30 min, with a median of 18 min. Within the learning period, the time required for harvesting was less than 5 min in one patient and more than 30 min in another. Although the protocol specified that more than 4 ml ICG should be injected around a tumor, the result was that sufficient ICG was successfully

Table 2 Distribution of background, surgical, and pathological factors

	Learning period		Total ($n = 440$)
	Within ($n = 127$)	After ($n = 313$)	
Background factors			
Age (years)			
Median	63	61	62
Range	33–79	26–80	26–80
Sex			
Male	80 (63.0 %)	205 (65.5 %)	285 (64.8 %)
Female	47 (37.0 %)	108 (34.5 %)	155 (35.2 %)
Marking clips			
Yes	107 (84.3 %)	259 (82.7 %)	366 (83.2 %)
No	20 (15.7 %)	54 (17.3 %)	74 (16.8 %)
Surgical findings			
Operation time (min)			
Median	200	202	200
Range	107−410	113–410	107–410
Estimated blood loss (ml)			
Median	230	200	205
Range	21–1,010	10–1,360	10–1,360
Type of gastrectomy			
Proximal	8 (6.3 %)	13 (4.2 %)	21 (4.8 %)
Pylorus-preserving	29 (22.8 %)	82 (26.2 %)	111 (25.2 %)
Distal	87 (68.5 %)	205 (65.5 %)	292 (66.4 %)
Total	3 (2.4 %)	13 (4.2 %)	16 (3.6 %)
Tumor location (portion)			
Upper	11 (8.7 %)	18 (5.8 %)	29 (6.6 %)
Middle	75 (59.1 %)	174 (55.6 %)	249 (56.6 %)
Lower	41 (32.3 %)	121 (38.7 %)	162 (36.8 %)
Time required for harvest (min)			
Median	15	18	17
Range	3–31	7–30	3–31
Number of GNs			
Median	4	4	4
Range	0–12	0–19	0–19
Coloring around tumor			
Circumferential	89 (70.1 %)	240 (76.7 %)	329 (74.8 %)
Non-circumferential	38 (29.9 %)	73 (23.3 %)	111 (25.2 %)
Pathological findings			
Number of tumor foci			
Single	121 (95.3 %)	308 (98.4 %)	429 (97.5 %)
Two or more	6 (4.7 %)	5 (1.6 %)	11 (2.5 %)
Tumor histology			
Papillary	2 (1.6 %)	6 (1.9 %)	8 (1.8 %)
Well differentiated	26 (20.5 %)	75 (24.0 %)	101 (23.0 %)
Moderately differentiated	41 (32.3 %)	76 (24.3 %)	117 (26.6 %)
Poorly differentiated	27 (21.3 %)	73 (23.3 %)	100 (22.7 %)
Signet ring cell	29 (22.8 %)	82 (26.2 %)	111 (25.2 %)
Mucinous	2 (1.6 %)	1 (0.3 %)	3 (0.7 %)

Table 2 continued

	Learning period		Total ($n = 440$)
	Within ($n = 127$)	After ($n = 313$)	
Tumor diameter (cm)			
Median	2.4	2.3	2.3
Range	0.5–8.6	0–18	0–18
Depth of tumor invasion			
T1a	74 (58.3 %)	174 (55.6 %)	248 (56.4 %)
T1b	46 (36.2 %)	121 (38.7 %)	167 (38.0 %)
T2	5 (3.9 %)	12 (3.8 %)	17 (3.9 %)
T3	2 (1.6 %)	6 (1.9 %)	8 (1.8 %)
Number of dissected LNs			
Median	37	37	37
Range	4–90	1–137	1–137
Residual tumor			
R0	126 (99.2 %)	312 (99.7 %)	438 (99.5 %)
R1	1 (0.8 %)	1 (0.3 %)	2 (0.5 %)

Depth of tumor invasion and residual tumor were classified based on the *UICC TNM Classification of Malignant Tumors*, 7th edition

Learning period with five patients for each institution was adopted in the JCOG0302 trial

injected in all patients. However, circumferential coloring was not observed in 23.3 % (73/313) after the learning period. There were no remarkable differences in operation time or estimated blood loss between the period of learning and after learning. Pathological tumor diameter ranged from 0 to 18 mm, with a median of 2.3 mm overall. The number of resected LNs ranged from 1 to 137, with a median of 37 nodes after the learning period. Two patients underwent R1 resection, one because of positive peritoneal lavage cytology and the other was pathologically positive for proximal margin.

Two patients were judged as ineligible after the learning period because the presence of palpable tumor or clips was not confirmed before registration in one patient and registration was completed before all eligibility criteria were confirmed in the other (Fig. 3). No GN was detected in 7 of 311 patients enrolled after the learning period. Nodal metastasis, diagnosed by paraffin section, was found in 28 of these 311 patients.

The proportion of false negatives after the learning period, which was the primary endpoint, was 46.4 % (13/28; 80 % CI, 33.1–60.1 %, 95 % CI, 27.5–66.1 %) (Tables 3, 4). Seven of 13 false-negative patients had nodal metastases outside the lymphatic basin.

The proportion to detect GNs was 97.8 % (304/311; 95 % CI, 95.4–99.1 %). The proportion of false positives was 0.7 % (2/276; 95 % CI, 0.1–2.6 %). No patient had an adverse event caused by the ICG injection or grade 4 postoperative complications. Five-year relapse-free survival of patients with nodal metastases ($n = 44$) was 90.9 % (95 % CI, 77.6–96.5 %). Five patients developed a relapse after R0 resection: sites of recurrence were bone in three patients, peritoneum in one, and LN in one.

To further clarify the reason why the proportion of false negatives was unexpectedly high, additional exploratory analyses were performed. First, we examined the number of patients in whom GNs were negative by frozen section but positive by paraffin section. Seven of 13 false-negative patients were such cases, and thus the remaining number of false negatives was 6. Next, we examined the patients in whom GNs were negative in both frozen and single-plane paraffin section but were positive by examining multiple sections of the GNs left for final diagnosis. All the node-positive patients with negative GNs in both frozen and paraffin sections were encountered in the first 19 patients in each institution. Tumor deposits were found in the GNs of 2 of these 6 patients, although the remaining GNs were not entirely whole in some cases and sometimes comprised only half a side. Thus, these additional analyses of GNs decreased the proportion of false negatives to 14.3 % (4/28; 95 % CI, 4.0–32.7 %).

Discussion

The SN concept for gastric cancer surgery was first suggested by Japanese studies at the beginning of the 21st century. Kitagawa et al. [4] reported their preliminary data on the use of an intraoperative radiation technique with a gamma probe. Hiratsuka et al. [5] reported that SN biopsy using ICG can be performed with a high detection probability, and that SN status can predict LN status with a high degree of accuracy. Given the daily clinical setting, the JCOG0302 trial was basically designed in accordance with the study by Hiratsuka et al. [5], i.e., the ICG dye-guided method for open surgery followed by histopathological

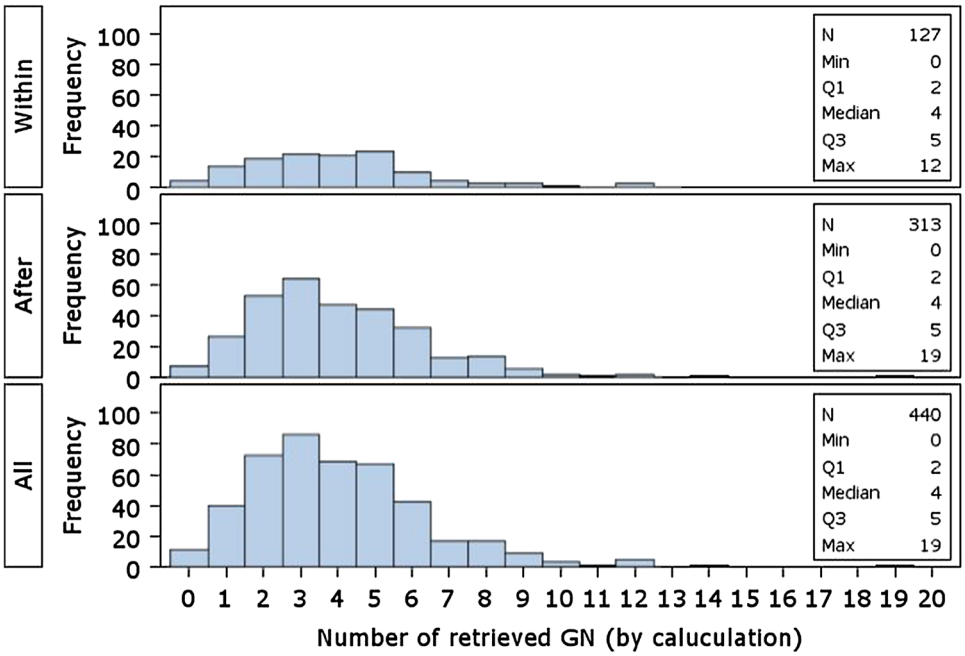

Fig. 2 Number of retrieved green nodes (GNs) in intraoperative frozen section diagnosis. Number of GNs from each patient ranged from 0 to 19 with a median of 4 nodes (0 to 12, with a median of 4 nodes within the learning period and 0 to 19, with a median of 4 nodes after the learning period, respectively). *GN* green node

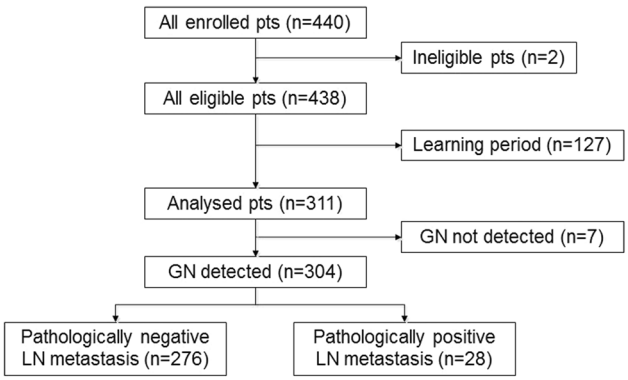

Fig. 3 Algorithm for analyses of the JCOG0302 trial. The proportion of false negatives as the primary endpoint was defined as the number of patients with intraoperatively negative GNs divided by 28, with pathologically positive nodal metastases in paraffin section. *LN* lymph node, *pts* patients

Table 3 Results of 311 eligible patients after learning period with 5 patients for each institution

	Confirmed diagnosis of GN and non-GN by paraffin section	
	Positive	Negative
Intraoperative GN-positive	15	2
Intraoperative GN-negative	13	274
GNs undetectable	3	4

examination with H&E staining. The dye-guided method is safe, convenient, inexpensive, and widely available in general hospitals, whereas the gamma probe-guided method has issues of legal restriction and the high cost of radioactive substances [10, 11]. ICG is a popular, approved, diagnostic reagent [12], and allergic reactions to ICG are fewer than those to blue dyes such as isosulfan blue (Lymphazurin) [13].

The present study evaluated the applicability of SN biopsy for T1 gastric cancer and demonstrated that the proportion of false negatives was too high to apply the current SN technique. The reasons for this are as follows.

The first critical issue is the histological examination of only one slice of GNs by frozen section, which might depend on the quality of section processing and the ability of pathologists in each institution. In the multicenter trial setting, only one plane of the largest dimension of the frozen section was adopted in the JCOG0302 trial for convenience in spite of the fact that multiple planes were adopted for detection of metastases in the study by Hiratsuka et al. [5]. The proportion of false negatives in this study was much higher than those of other studies; this is primarily because this is the only study in which GNs proven to have metastasis by final paraffin sections were not regarded as positive nodes if a frozen section failed to detect metastasis. The main purpose of the other studies was to evaluate the sensitivity of the SN concept using the final diagnosis of paraffin sections. Our primary interest was to test the intraoperative applicability of the SN concept in an ordinary clinical setting without any special tools or special staining methods such as immunohistochemistry, because there is a short timeframe during laparotomy in which to make the decision to proceed with gastrectomy. However, additional exploratory study clearly revealed that

Table 4 False negatives were found in 13 patients by analysis excluding the learning period

No.	Number of cases in each institute	Tumor location (portion)	Pathological tumor diameter (cm)	Number of GNs	Station number of GNs	Station number of metastatic nodes
1	7	Lower	7.0	3	4d, 6	4d
2	9	Upper	18.0	3	4d	1, 3, 4sb, 4d, 7
3	13	Middle	4.2	12	3, 4d 6	4d
4	11	Middle	3.1	3	3, 11p	3
5	12	Lower	2.8	5	4d	4d
6	7	Lower	2.5	3	3, 5, 7	3
7	9	Middle	3.6	5	3, 4sb	8a
8	16	Lower	3.2	5	3, 7	4d, 7
9	11	Lower	4.6	1	8a	1, 3
10	11	Lower	5.4	3	3, 7	3
11	19	Middle	3.2	6	3, 7	3, 6
12	23	Middle	3.0	5	4sb, 7	4sb, 4d, 6
13	32	Lower	3.5	6	6	3, 6

Station numbers: No. 1, right paracardial LN; No. 3, LN along the lesser curvature; No. 4sb, LN along the left gastroepiploic vessels; No. 4d, LN along the right gastroepiploic vessels; No. 5, suprapyloric LN; No. 6, infrapyloric LN; No. 7, LN along the left gastric artery; No. 8a, LN along the common hepatic artery (anterosuperior group); No. 11p, LN along the proximal splenic artery

9 of 13 patients with false negatives had metastases in GNs when the GNs were histologically examined in one slice or in serial slices by paraffin sections.

The second issue is the learning period. The planned learning period of only 5 patients in each institution (but not per surgeon) is presumed to be insufficient. A reasonable learning period is considered to be approximately 30 patients at present, as concluded from the survey conducted the Japanese Society for Sentinel Node Navigation Surgery (SNNS). Lee et al. [14] reported a learning period of 26 patients. Of 27 participating institutions in the JCOG0302 trial, only 4 institutions enrolled more than 30 patients. All the 6 node-positive patients with negative GNs in both frozen and paraffin sections were encountered in the first 19 patients in each institution. Nowadays, it is well known that surgeon inexperience was associated with detection failure [15–17]. Unfortunately, we could not find the appropriate learning period in the present trial setting because of limited sample size resulting from termination midway through the trial. It could be a critical point in the present study.

Lee et al. [18] reported that a small number of SNs ($\leq$3 nodes) was associated with false negativity. The median number of GNs in the JCOG0302 trial was 4 nodes per patients. It seems to be moderate because it is impractical for intraoperative histological diagnosis to harvest numerous SNs, which is in conflict with the SN concept, i.e., surgery without lymph node dissection.

Several investigators have argued that lymphatic basin dissection, which is a regional lymphadenectomy of one to two of five basins of the stomach, is better for harvesting SNs than node pickup biopsy adopted in the JCOG0302 trial because metastatic nodes would remain confined to the basins even in the case of false-negative SN technique [7]. However, 7 of 13 patients had nodal metastases outside the lymphatic basin in the present study, although an insufficient learning period might have affected the outcome, i.e., poor identification of lymphatic flow resulted in detection failure of GNs.

The JCOG0302 trial revealed the unreliability of frozen section examination using just one plane and highlighted the impact of the learning curve. Recently, Wang et al. [19] evaluated the diagnostic value of SN biopsy for gastric cancer in the systematic review. They concluded that further studies are needed to confirm the best procedure and standard criteria although the SN concept is technically feasible. A recent report suggested that intraoperative diagnosis using SN biopsy with ICG could be acceptable but with some additional requirements, such as multiple planes for detection, combination use of imprint cytology, and open surgery by experienced surgeons [20]. In terms of limitations of the ICG dye method, such as loss of visibility in dense fat and rapid transit, some novel ICG-based techniques such as infrared electronic endoscopy (IREE) [21, 22] and ICG fluorescence imaging [23–25] have been reported as convenient and reliable detection methods. Moreover, a prospective multicenter clinical trial of a novel semi-automated molecular-based rapid diagnostic method for LN metastases using one-step nucleic acid amplification (OSNA) indicated that the method is feasible for intraoperative detection of LN metastases in patients with gastric cancer (Kumagai et al., submitted). Such new technology could overcome the difficulties of clinical application of the SN technique [26].

Conclusions

The proportion of false negatives in the present study was unacceptably high. SN biopsy with ICG and intraoperative histological examination of a single plane is not recommended for clinical use in patients with early gastric cancer. Further improvement in the procedure should be explored to apply the SN concept to gastric cancer surgery. The JCOG0302 multicenter trial never denied the SN concept itself.

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Conflict of interest The authors declare no conflict of interest.

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