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Analysis of Sentinel Node Involvement in Gastric Cancer

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Background & Aims: Sentinel node navigation surgery (SNNS) is performed for patients with early gastric cancer. Because sentinel nodes (SNs) to gastric cancer exist but they have not been well-described, we attempted to validate the SN concept at the micrometastasis level. Methods: For 53 patients who underwent curative gastrectomy for T1/T2 (<4 cm) N0 gastric cancer, SNNS was performed with radioactive tin colloid and/or indocyanine green, and subsequent modified D1 lymphadenectomies were added. Whole formalin-fixed paraffin-embedded tissues of all resected lymph nodes from these patients were cut into 5-μm thick serial step sections at 85-μm intervals, and occult metastases were examined immunohistochemically. Results: Metastases were detected in 3 (1.5%) of 204 SNs and 3 (0.33%) of 901 non-SNs in pN0 cases and in 18 (46%) of 39 SNs and 3 (1.9%) of 158 non-SNs in pN1 cases. On a patient basis, metastases were detected in 4 (9%) of 46 pN0 patients, 2 (4%) each in SNs and non-SNs, and in 7 pN1 patients, of whom 7 and 4 had SN and non-SN metastases, respectively. The sensitivity, false-negative rate, and accuracy of SN identification by SNNS were 82%, 18%, and 96%, respectively, at the occult metastasis level. However, on the basis of the concept of the sentinel lymphatic station (SLS), which represents all lymphatic stations to which SNs belong, metastases were always limited to the lymph nodes in SLS in the 11 cases with metastases. Non-SN metastases occurred in 3 (60%) of 5 patients with SN metastases >2.0 mm in diameter but not in 4 patients with SN metastases ≤2.0 mm in diameter. Conclusions: The sentinel node concept held true at the occult metastasis level in 96% of patients with gastric cancer, and the accuracy of SNNS was elevated to 100% by introducing the concept of the sentinel lymphatic station. The size of SN metastasis was a predictive factor for metastasis beyond the sentinel node.

Advances in diagnostic technology have made it possible to detect gastric cancer at an early stage. Currently, stage I gastric cancer accounts for approximately 61% of all surgically resected cases in Japan.[1] Gastrectomy with lymph node dissection, usually D2 lymphadenectomy, is accepted in Japan as the standard surgical procedure for gastric cancer and contributes to improvement in patient outcome. Because lymph node metastasis occurs in only 10%–16% of patients with early gastric cancer, reduction or omission of regional lymph node dissection would be reasonable if it were possible to predict the extent of lymph node metastasis in each patient.[2–4]

The sentinel node navigation surgery (SNNS) is now widely applied as reduction surgery for various types of cancer, such as cancers of the breast, colorectal region, prostate, lung, female genital tract, and stomach.[5–10] A sentinel node (SN) is defined as the lymph node that is first to receive the flow of lymphatic fluid from the area containing the primary tumor in an organ. According to the SN hypothesis, lymph node dissection can be omitted when no metastases are detected in SNs. In breast cancers and skin melanomas, the SN hypothesis has been shown to be mostly applicable clinically, and the procedure of SNNS with radioisotope-guided and/or dyeing methods is now established. The accuracy of SN detection by these methods is reported to be 83%–100% in SNNS for breast cancer.[6,11–21] Some authors have also reported successful SNNS for gastric cancer, but it is still unclear whether the concept of the SN is valid, or whether the SN hypothesis holds true in surgery for gastric cancer.[22–25] To answer these questions, we performed a study to examine occult metastasis in serial step sections of whole formalin-fixed paraffin-embedded tissue blocks of all dissected SNs and non-SNs and compared the status of "true" metastasis between the SN and non-SN in 53 patients with early, T1/T2 (<4 cm) N0, gastric cancer.

Patients and Methods

Sentinel Lymph Node Navigation Surgery and Lymph Node Dissection

This study was reviewed and approved by the internal review board of the National Defense Medical College, Japan, and informed consent was obtained from all patients. The patients were diagnosed as having gastric carcinoma by endoscopic examination with histopathologic confirmation. By preoperative examinations, eg, barium radiography, endoscopy, ultrasonography, and computed tomography, we confirmed that the largest diameter of the tumor was less than 4 cm, and that lymph node metastasis or distant metastasis was not detectable. A total of 53 patients who underwent curative gastrectomy for primary gastric cancer received SNNS at the Department of Surgery I, National Defense Medical College Hospital, between October 2000–October 2003. The identification of SNs was carried out with radioactive tin colloid (Nihon Mediphysics, Tokyo, Japan) and/or indocyanine green (Daiichi Pharma-

Abbreviations used in this paper: SLS, sentinel lymphatic station; SN, sentinel node; SNNS, sentinel node navigation surgery.

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ceutical, Tokyo, Japan). Endoscopically, 2.0 mL of technetium-99m tin colloid (74 MBq/mL) was injected into the submucosal layer of the stomach at 4 sites around the tumor 21 hours before surgery. Just after laparotomy, 4 mL of 1.25% indocyanine green was delivered endoscopically into the same areas as the radiocolloid injection. A hand-held gamma-detector probe (Navigation; Tyco Healthcare Japan, Tokyo, Japan) was used to identify hot nodes and guide the surgery intraoperatively. An SN was defined as any hot node whose ex vivo radioactivity count was at least 10 times higher than the background count and/or any node for which green dye uptake could be identified visually.[26] All hot nodes and/or green nodes were subjected to intraoperative pathologic examination.

As shown in a previous publication, the double staining method with dye and radioisotope is the most reliable.[24] We performed the double staining method in principle. However, the method with dye alone or with technetium alone was performed when the radioactive reagent or an intraoperative endoscopist was not available.

For the patients in whom no metastasis to SNs was evident by frozen section examination, modified D1 lymphadenectomy was performed after SNNS. If an SN was found to include metastasis by frozen section examination, D2 lymphadenectomy was added. Because this is a retrospective study, we followed up the patients even if occult metastases were newly detected in permanent sections of SNs or non-SNs in the present study. For all SNs, intraoperative histopathologic diagnosis was performed routinely. Each SN was mounted in OCT compound (Sakura Finetek USA, Torrance, CA) and frozen immediately without being cut; 1 section was subjected to histologic diagnosis. After intraoperative diagnosis, the residual lymph node tissues were fixed in formalin and processed for routine permanent histologic diagnosis. After gastrectomy, all lymph nodes dissected were also fixed with formalin, embedded with paraffin, and processed for permanent histologic diagnosis. For each dissected lymph node, 1 section was prepared from a representative cut surface. By permanent histologic diagnosis, lymph node metastasis was absent (pN0) in 46 patients but was detected in 1-6 lymph nodes (pN1) in 7 patients. The locations of all SNs and non-SNs were classified to any of the lymphatic stations defined in the Japanese Classification of Gastric Carcinoma, 2nd English edition, by the Japanese Gastric Cancer Association (Figure 1).

The numbers of patients who underwent the combination method with dye and radioisotope together, dye method only, and radioisotope method only were 35, 2, and 16, respectively. Total gastrectomy, proximal gastrectomy, distal gastrectomy, and pylorus-preserving gastrectomy were performed on 4, 6, 29, and 14 patients, respectively. Patient characteristics are shown in Table 1. A total of 1302 lymph nodes, 24 per patient on average, were obtained. There were 243 SNs and 1059 non-SNs or 4.6 and 20.0 per patient on average, respectively.

Serial Step Sections

All tissue blocks of dissected lymph nodes were serially enumerated for identification and classified into those ≥2.0 mm and those <2.0 mm in diameter. Nine tissue microarray blocks were constructed by using 360 lymph nodes that were less than 2.0 mm in diameter. In addition, 942 lymph nodes with a diameter of 2 mm or larger were rearranged in 40 tissue blocks and re-embedded in paraffin. From all these tissue

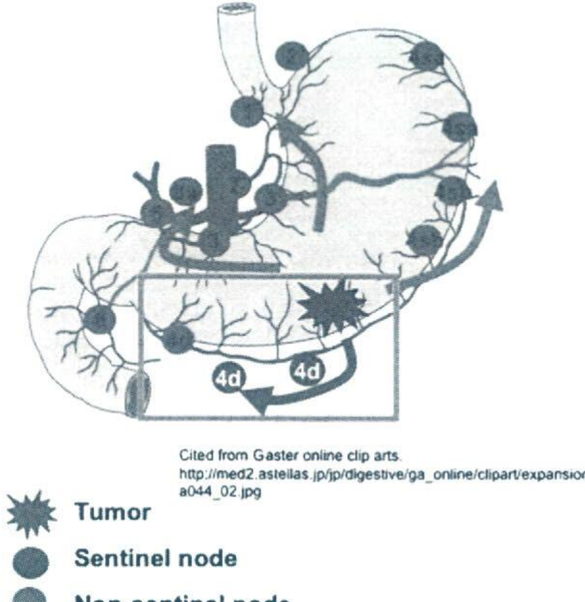

Figure 1. Concepts of the SN and SLS in the stomach. *Numbers* stand for lymphatic stations, which are defined in the Japanese Classification of Gastric Carcinoma, 2nd English edition. *Each number* corresponds to 1 lymphatic station. Perigastric lymphatic stations are classified as nos. 1–6. If 2 SNs are identified in lymphatic station no. 4d (*blue*), and no. 4d contains not only these SNs but also another non-SN (*red*), these 3 nodes are regarded as belonging to the SLS. *No. 1*, right paracardiac lymph nodes (*LNs*); *No. 2*, left paracardiac LNs; *No. 3*, LNs along the lesser curvature; *No. 4sa*, LNs along the short gastric vessels; *No. 4sb*, LNs along the left gastroepiploic vessels; *No. 4d*, LNs along the right gastroepiploic vessels; *No. 5*, suprapyloric LNs; *No. 6*, infrapyloric LNs; *No. 7*, LNs along the left gastric artery; *No. 8a*, LNs along the common hepatic artery.

blocks, triplet 5-μm thick serial sections were obtained every 85 μm until all the tissue in the block had been used. These sections were mounted on silane-coated glass slides (Muto Pure Chemicals, Tokyo, Japan). The total number of sections obtained was the triplets of 980 (mean, 19.6 slides per block). One of the triplet sections was stained with hematoxylin-eosin and subjected to histopathologic examination and the second to immunohistochemical study. The third sections were stocked as spare sections.

Immunohistochemistry. These sections were deparaffinized in xylene, rehydrated with ethanol, and reacted with 5% hydrogen peroxide for 10 minutes. Antigen retrieval of the sections was performed by exposure to microwave radiation in 10 mmol/L citrate buffer (pH 6.0) at 95°C for 5 minutes 3 times. The slides were then incubated with 10% normal goat serum (Nichirei, Tokyo, Japan) for 30 minutes, followed by anti-human cytokeratin antibodies (clone: AE1+AE3, dilution: 1/200; Dako, Glostrup, Denmark) at room temperature for 2 hours. The slides were then reacted with EnVision polymer reagent (Dako) at room temperature for 30 minutes. The slides were washed in phosphate-buffered saline with 0.1% Tween 20 (Sigma-Aldrich, Steinheim, Germany) 3 times and subsequently reacted with 3, 3′-diaminobenzidine tetrahydrochloride and hydrogen peroxide as a chromogen. Two observers (D.M., H.T.)

Table1. Clinicopathologic Characteristics of Study Population

Measure	Number
Patients	53
Dissected lymph nodes	1302
(Average ± SD)/patient	24.5 ± 5.3
SNs	243
(Average ± SD)/patient	4.6 ± 1.7
SLSs	88
(Average ± SD)/patient	1.7 ± 0.7
Age (y)	61.6 ± 8.4
Sex ratio (male:female)	38:15
Depth of tumor invasion	
pT1 (mucosa)	23
pT1 (submucosa)	26
pT2 (muscularis propria)	3
pT2 (subserosa)	1
Type of surgery	
Total gastrectomy	4
Proximal gastrectomy	6
Distal gastrectomy	29
Pylorus-preserving gastrectomy	14
Method for detection of SNs	
Dyeing only	16
Radioactive only	2
Dyeing and radioactive	35

SD, standard deviation.

independently judged the presence of tumor cells in hematoxylin-eosin-stained sections and immunohistochemistry sections. If 1 or more cancer cells were revealed by hematoxylin-eosin or if 1 or more cells were positive by immunohistochemistry, we judged the lymph node to be positive for occult metastasis. If there was a discrepancy in judgment between 2 observers, consensus was acquired by using a discussion microscope.

We also applied the new TNM classification, 6th edition[27] for the lymph node status of the 53 patients. Routinely defined pN0 and pN1 were reclassified into pN0, pN0 i+, pN1mi, and pN1 (>2 mm). The pN1 was classified into 2 subgroups according to the TNM classification of breast cancer: pN1 (>2 mm) and pN1mi were defined as statuses with metastatic foci >2 mm in diameter and with those >0.2 mm but ≤2 mm in diameter, respectively.[27] Tumor cell nests or single cells <0.2 mm in diameter were defined as isolated tumor cells or pN0 i+. By the present method, pN1 (>2 mm), pN1mi, and nests of pN0 i+ 90 mm or larger were always detectable. If a single tumor cell was speculated to approximately 10 mm in diameter, at least 9 or more tumor cells were estimated to be always identified in the lymph node by the present method.

Concept of the Sentinel Lymphatic Station

We validated the sentinel lymph node concept in gastric cancer from the viewpoint of both individual SNs and the sentinel lymphatic station (SLS). A lymphatic station denotes a group of lymph nodes that are localized in an identical area that is numbered in the Japanese Classification of Gastric Carcinoma, 2nd English edition (Figure 1). The SLS is defined here as the area of 1 or more lymphatic stations at which lymphatic flow arrives first from the primary lesion of gastric cancer. When SNs were identified, all lymphatic stations that contained the SNs were regarded as SLSs. In the present study, a total of 88 SLSs were detected (mean, 1.7 per patient).

Statistical Analysis

A "true metastasis" was defined as a metastasis that was detected by routine examination or occult metastasis detected by the present serial step section method. We compared the rate of metastases detected by routine examination with that of true metastases at the patient level and at the lymph node level. We also calculated sensitivity, specificity, positive and negative predictive values, and overall accuracy of SNNS with regard to true metastasis at the SN level and at the SLS level.

Mean values between groups were compared by t test. Fisher exact test or the Mann-Whitney U test was used to examine differences between groups. Differences at $P < .05$ were considered to be statistically significant. All data were analyzed with the Statistical Package for JMP version 5.1.1J software package (SAS Institute, Cary, NC).

Results

Detection of Occult Metastasis by Serial Section Examinations

The results of both hematoxylin-eosin and immunohistochemistry were always concordant, and the level of interobserver agreement for these results was 100%. Occult metastases were detected by serial section examination in 4 of 46 patients with pN0 (Table 2), 2 in SNs only, and 2 in non-SNs only. In these 4 pN0 patients, occult metastases were detected in 6 lymph nodes comprising 3 SNs and 3 non-SNs. In 7 patients with pN1, metastases were detected in 21 lymph nodes; 18 were detected by routine examination and 3 occult. The average size of nodes with metastases was 5.3 ± 3.8 mm (standard deviation) and 5.0 ± 3.3 mm in these 4 pN0 cases and 7 pN1 cases, respectively. However, the average sizes of the metastases in these 2 groups were 0.45 ± 0.33 mm and 3.3 ± 3.2 mm, respectively, and the difference was significant ($P = .0005$).

Table 2. Correlation Between Routine Diagnosis and Serial Step Section Diagnosis of SN and non-SN Metastasis

		No. of patients with lymph node metastases		
	Total	Serial step section diagnosis[a] (occult metastasis)[a]		Routine diagnosis[a]
pN0 (n = 46)				
SN	46	2 (4.4%)	(2)	0
Non-SN	46	2 (4.4%)	(2)	0
pN1 (n = 7)				
SN	7	7 (100%)	(1)	7
Non-SN	7	3 (43%)	(2)	1
Total	53	11		7

[a]Two pN0 cases with metastasis in SN deleted by serial step section diagnosis and 2 pN0 cases with metastasis in non-SN detected by serial step section diagnosis were always occult metastases and were exclusive together. Three pN1 cases with non-SN metastasis, 2 of which were detected by serial step section diagnosis and 1 of which was diagnosed routinely, also had SN metastasis.

Table 3. Demographics of Metastatic Lymph Nodes

Case no.	No. of SNs detected	SLS	Detection in routine pathologic diagnosis	Lymph node status	Location	Size of a lymph node (*mm*)	Size of metastasis (*mm*)
pN0							
1	3	No. 4d	–	SN	No. 4d	12	0.2
2	5	No. 3, 4d, 5	–	Non-SN	No. 4d	5	0.8
3	7	No. 6	–	SN	No. 6	2	0.9
			–	SN	No. 6	2	0.1
4	2	No. 1, 3	–	Non-SN	No. 3	7	0.2
			–	Non-SN	No. 3	4	0.5
pN1							
5	7	No. 3, 4d, 6, 8a	+	SN	No. 3	1.5	0.7
6	5	No. 4d	+	SN	No. 4d	6	0.1
			+	SN	No. 4d	5	3
			+	SN	No. 4d	4	2
7	9	No. 3, 4d	+	SN	No. 3	12	12
			+	SN	No. 3	10	10
			+	SN	No. 3	5	5
			+	SN	No. 3	2.5	2.5
			+	SN	No. 3	2	2
			+	SN	No. 3	1.5	1.5
			–	SN	No. 3	1.5	1.5
			+	Non-SN	No. 4d	3	3
8	3	No. 6	+	SN	No. 6	4	4
			+	SN	No. 6	2	0.5
9	2	No. 3, 8a	+	SN	No. 3	8	0.05
10	3	No. 3, 4sb	+	SN	No. 3	2.5	2.5
			–	Non-SN	No. 3	10	0.9
11	10	No. 3, 4d	+	SN	No. 4d	8	8
			+	SN	No. 4d	4	4
			+	SN	No. 4d	3	2
			–	Non-SN	No. 3	10	5

At the lymph node level, true metastases were detected in 21 (8.6%) of 243 SNs and 6 (0.57%) of 1059 non-SNs. The rate to metastasis to SNs was approximately 15 times as high as that to non-SNs. The rate of true metastasis to SN was high (46%, 18 of 39 SNs) in pN1 patients, but it was only 1.5% (3 of 204 SNs) in pN0 patients. The rate of true metastasis to non-SNs was also low, 1.9% (3 of 158 non-SNs) and 0.33% (3 of 901 non-SNs), in pN1 and pN0 patients, respectively.

Accuracy of Routine Pathologic Diagnosis

True metastases to SNs were detected in 9 patients, by routine pathologic diagnosis in 7 and occult metastases in 2. With regard to SNs, the false-negative rate, sensitivity, specificity, and overall accuracy of routine pathologic diagnosis against the status of true metastasis were 22% (2 of 9), 78% (7 of 9), 100% (44 of 44), and 96% (51 of 53). Likewise, true metastases to non-SNs were detected in 5 patients, 3 (43%) of 7 pN1 patients, and 2 (4%) of 46 pN0 patients. Only 1 of these 5 non-SN metastases was detected by routine diagnosis. With regard to all lymph nodes dissected, true metastases to SNs or to non-SNs were detected in 11 patients, by routine pathologic diagnosis in 7, and occult metastases in 4. The false-negative rate, sensitivity, specificity, and overall accuracy of routine pathologic diagnosis against the status of true metastasis were 36% (4 of 11), 64% (7 of 11), 100% (42 of 42), and 92% (49 of 53) of lymph nodes as a whole.

Validation of the Sentinel Node Concept on a Serial Step Section Basis

"Skip metastases," defined as metastases to non-SNs without presence of metastasis in any SNs, were detected in 2 (4%) of 53 patients. Therefore, the false-negative rate, sensitivity, specificity, and overall accuracy of SNNS at the occult metastasis level were 18% (2 of 11), 82% (9 of 11), 100% (42 of 42), and 96% (51 of 53). Thus, the sentinel node theory was applicable to 96% of the patients in this study.

Validation of the Sentinel Lymph Node Concept From the Viewpoint of Sentinel Lymphatic Station

The distribution of SLS was investigated in 11 patients with true metastases to SNs and/or non-SNs (Table 3). Two (cases 1 and 3) of 4 pN0 patients had occult metastases to SNs, including a metastasis to no. 4d in case 1 and 2 metastases to no. 6 in case 3. The other 2 pN0 patients (cases 2 and 4) had occult skip metastases to non-SNs: In case 2, a metastasis occurred in a no. 4d non-SN, and 5 SNs without metastases were located in no. 3 (2 nodes), no. 4d (2 nodes), or no. 5 (1 node). In case 4, metastases occurred in no. 3 non-SNs, and 2 SNs without metastases were located in no. 1 or no. 3. Therefore, in both cases 2 and 4, occult skip metastases always belonged to SLSs. In 7 pN1 patients, 21 nodes had metastases, 18 of which were detected routinely in SNs, whereas the other 3 were occult metastases to SNs or non-SNs (Table 3). All

Table 4. Patient and Tumor Characteristics According to SN and/or non-SN Status

	No. of cases	SN/non-SN status			
		With Metastasis(%)	[With occult Metastasis]	Without Metastasis	*P* value
Age (*y*)					
<60	23	5 (22)	[2]	18	.9623
≥60	30	6 (20)	[2]	24	
Histologic type					
Well-differentiated tubular	16	0 (0)	[0]	16	.0077[a]
Moderately differentiated tubular	17	7 (41)	[3]	10	
Poorly differentiated adenocarcinoma	18	3 (17)	[1]	15	
Undifferentiated carcinoma	2	1 (50)	[0]	1	
Depth of invasion					
T1 (mucosa)	23	1 (4)	[0]	22	.0253[b]
T2 (submucosa)	26	8 (31)	[4]	18	
T2 (muscularis propria or subserosa)	4	2 (50)	[0]	2	
Location of tumor					
Upper	8	1 (13)	[1]	7	.7561
Middle	25	5 (20)	[1]	20	
Lower	20	5 (25)	[2]	15	
Lymphatic vessel invasion					
Negative	32	1 (3)	[0]	31	<.0001
Positive	21	10 (48)	[4]	11	
Blood vessel invasion					
Negative	45	7 (16)	[3]	38	.0942
Positive	8	4 (50)	[1]	4	
Method for SN identification					
Dyeing only	16	4 (25)	[2]	12	.4740
Radioactive only	2	1 (50)	[1]	1	
Dyeing and radioactive	35	6 (17)	[1]	29	
Type of surgery					
Total gastrectomy	4	0 (0)	[0]	4	.0440
Proximal gastrectomy	6	1 (17)	[1]	5	
Distal gastrectomy	29	10 (35)	[3]	19	
Pylorus-preserving gastrectomy	14	0 (0)	[0]	14	

[a]Well-differentiated tubular adenocarcinoma vs moderately differentiated tubular adenocarcinoma, poorly differentiated adenocarcinoma, and undifferentiated carcinoma.
[b]T1 (mucosa) vs T1 (submucosa) and T2 (muscularis propria or subserosa).

metastases were included in SNs in 4 pN1 patients (cases 5, 6, 8, and 9), whereas metastases were detected in both SNs and non-SNs in the other 3 (cases 7, 10, and 11). In case 10, both a routinely detected SN metastasis and an occult non-SN metastasis were located in no. 3. In case 7, all 9 SNs were located in either no. 3 or 4d, and 7 routinely detected SN or non-SN metastases and another occult SN metastasis were also located in no. 3 or no. 4d. In case 11, all 10 SNs were located in either no. 3 or no. 4d, and 3 routinely detected SN metastases and 1 occult non-SN metastasis were also located in no. 4d, or in no. 3. Therefore, in 7 pT1 cases, metastatic lymph nodes were always included in SLSs, irrespective of whether the metastatic nodes were SN or non-SN, and non-SLSs examined did not have metastasis. Thus, false-negative rate, sensitivity, specificity, and accuracy of SNNS based on the SLS concept were 0%, 100%, 100%, and 100%, respectively.

According to the TNM classification 6th edition, 1 and 3 of 4 pN0 patients were classified as pN0 i+ and pN1mi, and 1, 1, and 5 of 7 pN1 patients were classified as pN0 i+, pN1mi, and pN1 (>2 mm), respectively. We investigated the risk factors of non-SN metastasis among 9 patients with SN metastases. Six patients without non-SN metastases had SN metastases 1.48 mm in diameter on average. In contrast, 3 patients with non-SN metastases had SN metastases 7.5 mm in diameter on average ($P = .0695$). Three (60%) of 5 patients with pN1 (>2 mm) to SNs also had metastases in non-SNs, whereas none of 4 patients with pN1mi or pN0 i+ to SNs had metastasis to non-SNs. Between the group of pN1 (> 2 mm) and the group of pN1mi or pN0 i+, the F value was 0.010, and the mean diameter of SN metastases differed significantly by *t* test ($P = .033$).

Clinicopathologic Correlation

In the 11 patients with true lymph node metastases, tumor invasion was deeper and the ratio of lymphatic vessel invasion was higher than in patients without metastases (Table 4). In the 4 pN0 patients with occult metastases, tumor invasion was limited to the mucosa or submucosal layer. In 7 pN1 patients, tumor invasion was limited to the mucosa or submucosal layer in 5 but reached the muscularis propria or deeper in 2.

In all of these 11 pN0 patients, the primary tumors were moderately or poorly differentiated adenocarcinoma, and no case was well-differentiated adenocarcinoma. There were no significant correlations of true lymph node metastasis with patient age, tumor size, tumor location, blood vessel invasion, or SN detection method. At present, none of the 53 patients has

shown relapse or metachronous metastasis (mean follow-up period, 36 ± 13 months).

Discussion

Intraoperative use of dye for lymph node mapping was described >50 years ago.[28,29] In the present study with currently used radioactive labeling and/or dyeing methods, it was possible to identify lymph nodes in which initial metastasis had developed in 96% (51 of 53) of the patients examined. The sensitivity and accuracy of SNNS for gastric cancer at the routine diagnostic level are reported to be 92% and 98% with radioactive labeling and 83%–89% and 96%–98% with dyeing, respectively.[22-25] At the occult metastasis level, the sensitivity and accuracy were also 82% and 96%, respectively, and in addition, the rate of metastasis to SNs was approximately 15 times higher than that to non-SNs. From these results, we were able to show that SNs did exist in most of the patients, thus providing histopathologic proof of the validity of SNNS for gastric cancer. Even with the present serial sectioning method, there were 2 patients with skip metastases, indicating that initial metastasis to lymph nodes cannot always be detected perfectly by the current SNNS procedure. To overcome this situation, we suggest that the SLS concept be applied to gastric cancer surgery. In the 11 patients with any type of lymph node metastasis, all the affected lymph nodes belonged to the SLS. Therefore, metastatic cancer cells from the primary site always reached lymph nodes in the SLS first. We believe that the SN theory based on the SLS concept is applicable to patients with gastric cancer. In 9 patients with SN metastasis, we investigated factors correlated with non-SN metastases. Non-SN metastasis was detected in 60% of the patients with pN1 (>2 mm), but it was not detected in the patients with pN1mi or pN0 i+. These results suggested that the size of SN metastasis based on the new TNM classification was useful for predicting non-SN metastasis. Therefore, it seems useful to prepare sections every 2 mm from SNs or lymph nodes in SLSs for intraoperative pathologic diagnosis when the diameter of these lymph nodes is >2 mm.

The present results validate the rationale of SNNS for early gastric cancer on the basis of the SLS concept. If there are no metastases in lymph nodes in SLSs, no further dissection would be necessary. Furthermore, the possibility of metastasis beyond the SLSs would appear low if there are no lymph nodes with metastases >2.0 mm in diameter in SLSs. It would be worth studying the validity of omitting further dissection of non-SLSs in this situation.

To detect all clinically significant metastases in SLSs intraoperatively, it would be necessary to extend pathologic examination to all lymph nodes belonging to SLSs. This would naturally increase the work burden for histopathology staff. Thus, whether it is possible to diagnose all lymph nodes in SLSs by using a sectioning interval of 2 mm in routine pathologic practice is problematic. To minimize the burden of intraoperative pathologic diagnosis, the application of novel techniques for detecting lymph node metastasis would be helpful. Reverse transcription polymerase chain reaction and other molecular diagnostic modalities have been developed and are emerging as quantitative and highly sensitive methods for rapid detection of metastasis.[30,31] The application of these molecular diagnostic tools will facilitate the spread of SNNS as a standard procedure for gastric cancer worldwide as well as improving the accuracy of intraoperative diagnosis.

In the present study, all 11 patients with 1 or more lymph node metastases underwent curative resection, and none of them has shown relapse yet. Skip metastasis foci to the 3 non-SNs in these 2 patients were 0.2, 0.5, and 0.8 mm in diameter and were not pN0 i+ but pN1mi. It is unclear whether these occult metastases would have caused cancer recurrence if they had not been dissected. Lee et al[32] reported that 5-year overall survival rates differed significantly between patient groups with gastric cancer with and without micrometastasis to regional lymph nodes (49% and 76%, respectively). Their results suggest that surgical resection of micrometastases is mandatory to improve cure rate of patients with early gastric cancer.

SNNS is usually performed for the patients with T1 or T2 gastric cancer without clinically detectable lymph node metastasis. It is shown and is the consensus that adjuvant chemotherapy is not necessary for the patients with pT1 or pT2 pN0 gastric cancer.[1] The utility of adjuvant chemotherapy to patients undergoing gastrectomy with D1 or D2 lymphadenectomy to gastric cancer has been shown by randomized clinical trials, but pT1 or pT2 pN0 cases were excluded from these studies.[1] Therefore, at present, there is no evidence for adjuvant chemotherapy to most of gastric cancers' SNNS procedures.

In conclusion, the accuracy of the current SNNS procedure for detecting SNs in patients with early gastric cancer was 96% at the occult metastasis level. By application of the SLS concept, the accuracy of SN detection would be improved further. The size of SN metastasis was a predictive factor of non-SN metastasis. SNNS is a valid and practically useful method for reduction surgery in patients with early gastric cancer.

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特集 センチネルリンパ節理論の消化器癌への応用

3 リンパ節転移の術中診断の精度とその向上策

上田 重人* 津田 均**

Key words：乳癌リンパ節

要旨

わが国における乳癌，消化器癌のセンチネルリンパ節ナビゲーション手術(SNNS)においては，センチネルリンパ節(SLN)転移の診断はおもに術中病理診断ないし細胞診で行われるが，約10～40%の頻度で転移見落とし，とくに微小転移巣(長径>0.2～2 mm)の見落としが起きる．微小転移見落としを防ぐためSLNを半割，3分割，あるいは2 mmごとの切片作製などこまかい検索を行う施設も増えてきた．しかし病理部門のマンパワーの面で限界があるのも事実である．微小転移検出の補助としてサイトケラチン(CK)免疫組織化学(IHC)法は有用であるが，IHC法のみで見つかりルーチン病理診断では検出困難な遊離腫瘍細胞(長径≦0.2 mm)の意義は，現時点では不明とされる．最近では分子生物学的手法によるSLNの術中転移診断法が開発されている．

はじめに

センチネルリンパ節(SLN)は癌原発巣周囲のリンパ流が最初に流れつくリンパ節と定義される．癌細胞が増殖・浸潤して原発巣から離れ，その周囲組織のリンパ管に侵入すると，これらの癌細胞はまずSLNに到達する．悪性黒色腫，乳癌などにおいて色素や放射性薬剤が腫瘍周囲から最初に流れ込むSLNは，確かに存在することが示されてきた[1),2)]．

乳癌のsentinel node navigation surgery (SNNS)では，SLNを同定して転移の有無を調べ，センチネル理論に基づいて転移がなければほかの腋窩リンパ節(非SLN)の郭清を省略し，転移があれば腋窩郭清を追加している．

わが国では，SLN生検と乳癌原発巣の手術を1回の手術枠内で行うことがほとんどであり，SLNの転移診断は通常，術中迅速病理診断または細胞診によって行われる．術中病理診断は臨床的にきわめて有用性の高い診断法であるが，ルーチンで行われる永久病理診断(ホルマリン固定パラフィン包埋組織ブロックから作製された切片で診断する．標本作製から結果が出るまで数日かかる)と比べると診断の質，精度が低くなる．

胃，食道，大腸など消化器癌においてもSNNSを行う施設が増えつつあり，センチネル理論が確立されつつある．

本稿ではSLN転移の術中病理診断の利点と問題点並びに，その解決法について考えてみたい．

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Ⅰ．術中病理診断の利点と問題点

この項のポイント
- 術中病理診断は，一期的にSLN生検と原発巣の手術を行うためには不可欠である．
- 術中病理診断は，転移診断の偽陰性がしばしば起こりうる問題点がある．

術中病理診断の際には，通常，SLNを長軸方向に平行に半割〜4分割して，各々の組織をコンパウンドに埋め込み急速に凍結して，クライオスタットで薄切し凍結切片を作製する．凍結切片を短時間で固定しヘマトキシリン-エオジン(HE)染色を施してリンパ節当り2〜4割面の切片を作製し，術中組織診断を行う．施設やリンパ節の大きさによっては割を入れずそのまま1割面のみの切片を作る場合もある．胃癌SLNでは，数も多く2mm以下のSLNも多いので，術中病理診断の際に割を入れないことが多い．術中病理診断後のSLN組織は解凍し，一晩ホルマリン固定したのちルーチンどおりパラフィン包埋し，薄切，HE染色を行って永久病理診断に供する．

術中病理診断は30分以内に結果報告がなされ，必要に応じて腋窩郭清を追加でき，一期的にSLN生検と原発巣の手術を行うためには不可欠な方法である．事実，転移の術中病理診断は多くの場合さほど困難ではない．

しかし，術中病理診断は，病理医不在の施設では行うことができず，また件数が増えると病理スタッフの負担を増やす結果ともなる．さらに，作製された標本の質が十分ではなく，正しい評価が困難な場合もありうる．通常は，必要最小限の検索であることが多いため，転移陰性と報告したが，永久標本で癌細胞が見つかる，いわゆる転移診断の偽陰性(false negative)がしばしば起こりうる．

乳癌SLNの術中病理診断における偽陰性の頻度に関する諸家の報告を表に示す[3]．永久標本での転移陽性を真の陽性，転移陰性を真の陰性とすると，真の陽性に対する術中病理診断の陽性率は57〜89%，したがって転移見落とし率は11〜43%と報告されている．

表　センチネルリンパ節生検の術中迅速病理診断の偽陰性率(乳癌)

Hingston/Flett(1999年)	11%
Turner(1999年)	26.3%
Veronesi(1999年)	5.5%
Zurrida(2000年)	32%
Canavese(1998年)	27%
Van Diest(1999年)	13%
Rahusen(2000年)	43%
Weiser(2000年)	42%
Chao(2002年)	32%

註：Veronesi論文での偽陰性率が著しく低いのは，本文に述べたようにextensiveな術中評価を行ったことによる．
〔Chao, C., et al.：Am. J. Surg. 182；609-615, 2002[3]より〕

この頻度は胃癌，大腸癌でも同程度である．われわれの胃癌の検討でもSLN術中病理診断の転移見落とし率は22%(2/9)であった．

Ⅱ．術中病理診断の偽陰性

この項のポイント
- 微小転移の見落としを防ぐためには，SLNをなるべくこまかくスライスし，丁寧に検索するのがもっとも有効である．

偽陰性の大部分は，転移巣が検索した割面に含まれていないことで生じる(**図1**)．次いで，生検時の熱変性や圧挫によって診断がしにくい組織である場合，癌細胞が検索割面に出ていても異型や細胞数が少ないため見落とす場合，標本の質の問題などがある(**図2**)．

SNNSが試みられるようになった初期のころは，他臓器癌のリンパ節転移の病理診断と同様，一割面だけの検索を行う施設が多かった．しかし，SLNはほかのリンパ節と比べて転移

の頻度が高く，とくに微小転移巣(長径>0.2 mm，≦2 mm)が高率で検出される．SNNSにおける術中病理診断の偽陰性率を減少させるためには，SLNをなるべくこまかくスライスし，各々についてきれいな標本を作製して丁寧に検索するのがもっとも有効である．極端な例では，Veronesiらのように乳癌のSLNを最初の15枚の切片を50μmの厚さごとに，その後は100μmの厚さごとに切片を作製している施設もある[4]．以下に，術中病理診断のためのSLN標本の切り出し法について述べる．

Ⅲ．術中病理診断におけるセンチネルリンパ節の切り出し法

この項のポイント

- SLNの切り出し法は，実際にはsingle sectioningかslice sectioningが用いられる．

術中病理診断のためのSLNの切り出し法にはsingle sectioning，slice sectioning，serial step sectioningなどがある(図1)．実際にはsingle sectioningかslice sectioningが用いられる．

1．single sectioning

single sectioningとは，SLNに割を入れずに包埋して，代表一割面のみの切片を作製し検索することをいう．リンパ節全体の1%以下(厚さ5mmのリンパ節のうち5〜10μmで0.1〜0.2%)を検索しているにすぎず，微小転移のみならず2mm径より大きなマクロ転移も正確に診断できない可能性がある．

1999〜2001年の間に当施設で経験したT1-T2，N0-N1a乳癌患者131名を対象に，RI法によりSLNを同定・生検し，single sectioning法により術中病理診断を行った．1名当りSLNは1.7個(1〜6個)摘出された．106名が術中病理診断にて転移陰性とされ，そのうち11名(10.4%)に術後検索により新たな転移が判明した．真の転移陽性例は36例で，術中病理診断による偽陰性率は31%(11/36)となった．見落とされた転移巣はすべて微小転移巣であった．

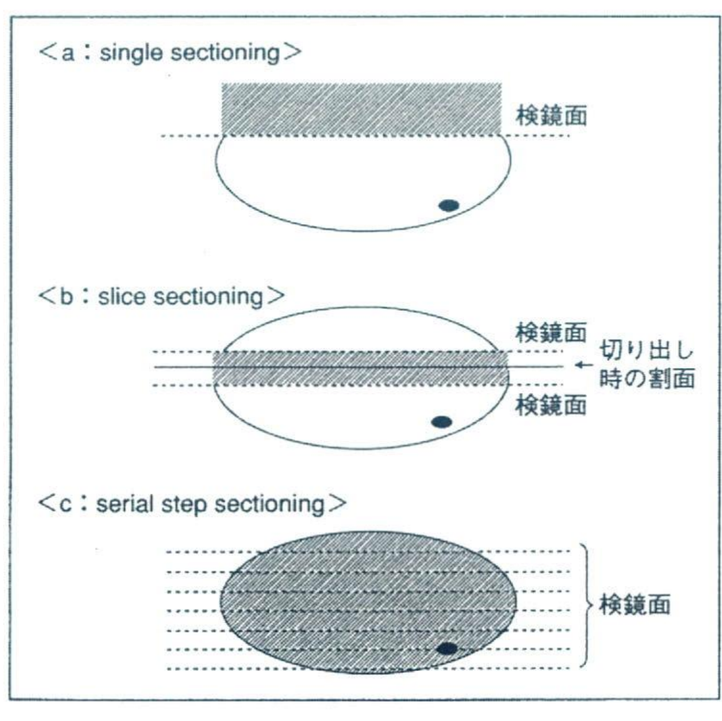

図1　センチネルリンパ節(SLN)の切り出し法
a：single sectioning．SLNをそのまま包埋し，急速凍結してクライオスタットにて面をある程度削り取り(斜線部)，面が出たら切片を作製し(破線部)，術中病理診断を行う．微小転移巣(黒玉)を見落とす可能性が高い．
b：slice sectioning(ここでは半割)．SLNで1カ所割を入れ(実線部)，二つに分かれた組織を包埋し，急速凍結してクライオスタットにてある程度面を削り取り(斜線部)，適切なところで2カ所の切片を作製(破線部)，術中病理診断を行う．この場合も微小転移を見落とす可能性はある．
c：serial step sectioning(ここでは7カ所で切片作製)．SLNをそのまま包埋し，急速凍結してクライオスタットにて，たとえば500μmごとに切片を作製していく(破線部)．二つの異なる面の切片の間の組織はクライオスタットで削り取っていき，組織がなくまるまで等間隔で切片を作っていく．微小転移はほぼ確実に見つけられるが，実際的な方法ではない．

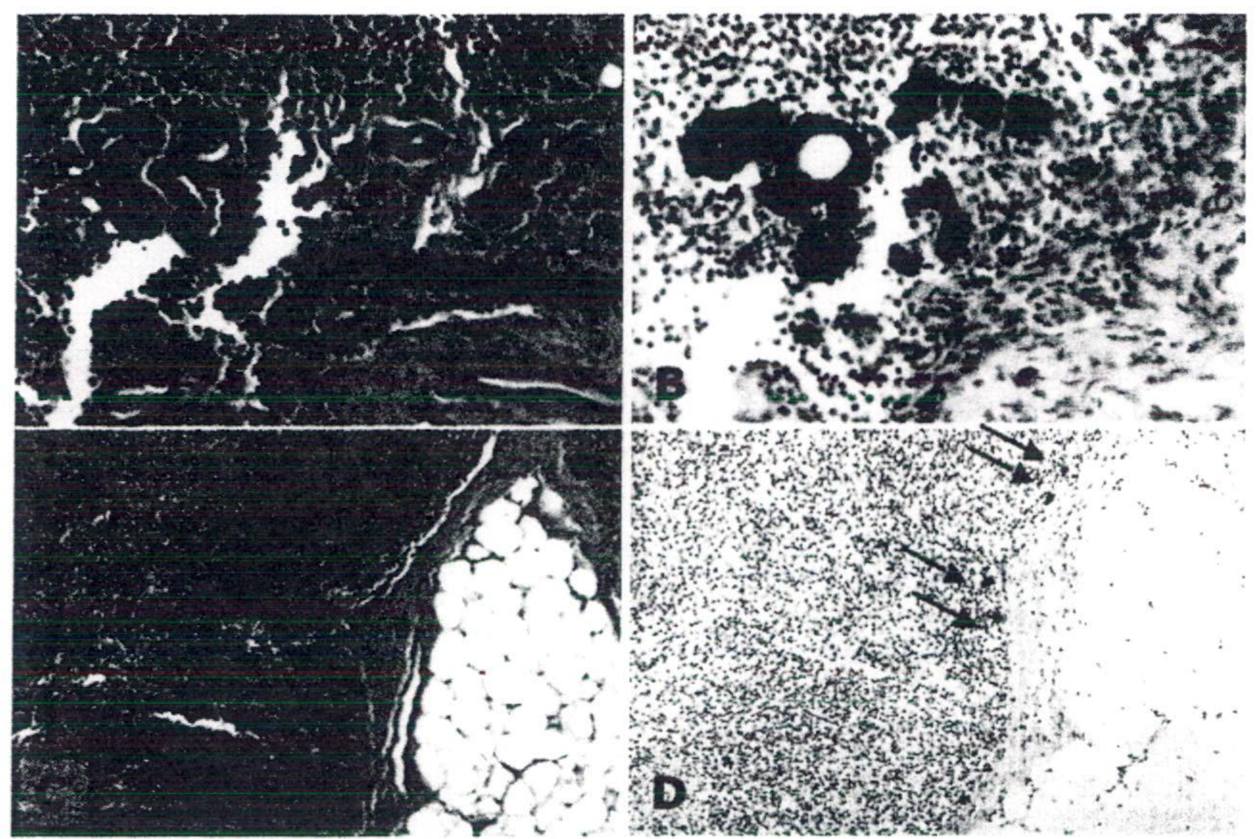

図2　センチネルリンパ節の乳癌微小転移と遊離腫瘍細胞(ITC)

A：HE で判定しにくい微小転移.
B：A と連続的な切片で，サイトケラチン(CK)の免疫組織化学(IHC)法にて癌細胞を検出したもの．微小転移巣が黒褐色に染色され判定が容易となる．(原倍率 ×200)
C：HE で判定不可能な ITC．浸潤性小葉癌の1例.
D：C と連続的な切片に IHC 法を施し，CK を検出したもの．個々バラバラに分布する癌細胞が同定された(矢印)．(原倍率 ×100)

当施設において，胃癌の SLN single sectioning による転移の見落とし率は 22%(2/9)であった.

2．slice sectioning

slice sectioning では SLN を長軸方向に平行に半割～数分割して約1～2 mm 間隔で各組織片から切片を作製して検索を行う．マクロ転移の見落としはなくなり微小転移もかなり検出できるが，微小転移を見逃す可能性は残る.

2001～2004 年の間に当施設で SNNS を施行した乳癌患者 185 名，334 個の SLN に対して半割～3分割して slice sectioning による術中病理診断を行った．139 名が術中に転移陰性と診断され，そのうち転移の見落としはすべて微小転移巣であり，7名(5.0%)にみられた．真の転移陽性は 46 名，偽陰性率は 13%(7/53)で，single sectioning による術中病理診断成績と比べて改善している.

ASCO(American Society of Clinical Oncology)ガイドラインなどでは，SLN を最大2 mm 間隔 slice sectioning で病理標本を作製することを推奨している[5),6)]．この方法は理論的に2 mm 径以上のマクロ転移を見逃さず，微小転移もかなりの頻度で検出できることと，実際にルーチンで行うことが可能である点から，現実的であると考えられる．今後，2 mm 間隔での切り出しが普及するものとみられる.

3．serial step sectioning

serial step sectioning は，SLN 全体を等間隔(間隔には 50～500 μm までさまざまな報告

がある)で，マイクロトームを用いて組織がなくなるまで連続的に組織切片を作製していく方法である[7]．国際対がん連合(UICC)のTNM分類で定義される微小転移が直径>0.2 mm～≦2 mmであるから，微小転移を見逃さないためには200 μm間隔以下でserial step sectioningを行う必要が生じる．2 mmの厚さのリンパ節であれば10枚，5 mmの厚さのリンパ節組織であれば25枚の連続切片が作製されることになる．正確な転移診断が可能になる一方で，マンパワーの限界から通常は臨床実地で行うことは難しい．

上述のように，Veronesiらは乳癌SLNを最初の15枚の切片を50 μmごとに，その後は100 μmごとに切片を作製し，術中に検索している．彼らはこのexhaustiveな方法によってSLN当り計60枚以上の切片を作製し，術中に40～50分かけて病理学的評価を行い，転移診断の偽陰性率を5.5%に低下させたと報告している(表)[4),7)]．

4. complete serial sectioning

serial sectioningはリンパ節組織全部を連続的にマイクロトームでスライスし，全部の組織切片を作製する方法である．微小転移のみならず長径0.2 mm以下の遊離腫瘍細胞(isolated tumor cells；ITC)をも検出可能となるが，実地臨床の場で行ったという報告はない．

Ⅳ．術中迅速細胞診

この項のポイント

- 細胞診における微小転移の見落としの頻度は，組織診とほぼ同程度と考えられる．

術中迅速細胞診の場合は，摘出したリンパ節を2 mm間隔にスライスし，各割面をスライドグラスに捺印してパパニコロウ染色またはギムザ染色を行い，癌細胞の有無をスクリーニングする．残りの組織はホルマリン固定し，組織切片を作製してHE染色を行い，永久病理診断に供する[8)]．

捺印細胞診は迅速病理診断と比較して，標本作製が簡単で，検査時間が短い，薄切による組織の損失がない，などの利点がある．一方で診断に熟練を要するため，経験豊富な細胞診スクリーナが常勤していることが必須である．

細胞診における微小転移の見落としの頻度は，組織診とほぼ同程度と考えられる．細胞診のほうが転移診断の偽陰性率が低いという施設もある．尾浦らは，乳癌SLN生検の際に多数切片の術中病理診断にて転移が検出不能であったが捺印細胞診で癌細胞を同定しえた症例を4%に認めたと報告している[9)]．

Ⅴ．センチネルリンパ節の微小転移，ITCの定義と意義

この項のポイント

- 微小転移は，非SLN転移を伴う確率が比較的高い．

TNM分類第6版における乳癌所属リンパ節の病理学的分類(pN)では，微小転移(長径>0.2 mm，≦2.0 mm)はpN1miと表記され，転移陽性として扱われる[10)]．一方，長径0.2 mm以下の単一の腫瘍細胞群や小さな細胞集塊はITCと定義され，転移活性の確証が得られないとして，pN0に分類されている[10)]．ITCはおもにサイトケラチン(CK)などを検出する免疫組織化学(immunohistochemistry, IHC)法にて見つかるが，HEで検出されることもある．

現在も微小転移，ITCの臨床的意義について議論がなされている．微小転移については非SLN転移を伴う確率が比較的高いことがわ

かっている[11),12)]．非SLN転移予測因子としてのSLN微小転移巣の意義についてもいくつかの検討がなされた．Vialeらは，上述のexhaustiveな術中病理検索によって，SLNに微小転移が見つかった患者の22%に非SLN転移がみられたと報告している[13)]．さらに，非SLN転移の頻度はSLN微小転移巣の長径>1 mmの例で36%，≦1 mmの例で16%であった．

私どもは臨床的にN 0-N 1 aでSLN生検と腋窩郭清が行われた91例について，レトロスペクティブにSLN転移巣の非SLN転移に対する意義を検討した．ルーチン病理診断にて転移が見つからなかったリンパ節にはすべて100 μmごとのserial step sectioningを行い，HEとIHCを併用して転移を検索した[14)]．SLNへのマクロ転移，微小転移，ITCは各々26，10，5例にみられたが，各群における非SLN転移例数はITCを含むと各々17(65%)，4(40%)，1(20%)であり，ITCを除くと15(58%)，4(40%)，0(0%)であった．例数が少ないこと，追跡時間が短いことなどから参考にとどまるが，これらの結果はSLN微小転移の臨床的重要性を表すものと考えられる．

胃癌におけるレトロスペクティブな検討でも，SLNマクロ転移の群と，微小転移あるいはITCの群において，非SLN転移の頻度は各々60%(3/5)と0%(0/4)であった．胃癌におけるSLN転移巣の長径は非SLN転移の予測因子となりうると考えられる[15)]．

Ⅵ．免疫組織化学(IHC)の意義

この項のポイント

- HEで転移診断が困難なときは，IHCを追加することがある．

術中，永久にかかわらずSLNの病理診断にはHE染色が用いられるが，HEで転移診断が困難なときは，IHCを追加することがある．乳癌や消化器癌の細胞はサイトケラチン(CK)を発現しているため，抗CK抗体を用いたIHCにてSLNの転移診断が可能である．

IHC法により，HEで転移陰性とされた症例の7～31%でリンパ節にCK陽性の癌細胞が見つかるという報告がある[16)]．HE染色では細胞数が少なかったり異型が乏しいため観察できないような癌細胞は，IHCで簡単に発見される．乳腺の浸潤性小葉癌や胃の低分化腺癌の転移巣で細胞数が少なく個々バラバラに分布している場合は，HEで転移を見つけられないことが多い(図2)．

IHCのみで検出されるリンパ節転移巣は通常ITCであるが，ITCが予後に影響するか否かについては判定材料がない[17)]．TNM分類第6版やASCOガイドラインでは，IHCや分子レベルでのみ検出されるものは微小転移に含めるべきでないとし，それらの意義は臨床試験NSABP B-32の結果をみて決定することが望ましいとしている[5),18)]．

Ⅶ．分子生物学的手法によるセンチネルリンパ節転移の検出

この項のポイント

- 分子生物学的方法は感度がきわめて高く，将来有用な検査法である．

CK 18，CK 19は上皮細胞に陽性であり，ほかのリンパ節固有の細胞には発現されないことから，リンパ節への癌転移の優れたマーカーとなりうる．近年，分子生物学的手法により，癌細胞を含むリンパ節における癌細胞特異的分子のmRNAを検出して転移診断を行う方法が開発されている．

Schoenfeldらは，RT-PCR法により乳癌患者のSLNからCK 19 mRNAを検出し転移診

断を行ったところ，ルーチンのHE染色にて転移陰性と診断された例の15%がRT-PCRで転移陽性となったと報告している[19]．

PCRを行わず一定温度で目的の遺伝子断片を増幅できる直接遺伝子増幅法(one step nucleic acid amplification；OSNA法)が開発され，注目を集めている．OSNA法は，リンパ節を可溶化し試薬と反応させるだけで目的分子のmRNAを増幅・検出でき，約30分で転移診断を行える．現行の病理診断法と比較する多施設臨床試験においても，OSNA法は感度，特異度ともに良好であった[20]．微小転移もほぼ確実に検出できるため，従来の病理診断に代わる術中リンパ節転移検査として実用化が期待される．さらに胃癌，大腸癌でも検討が計画されている．

分子生物学的方法は感度がきわめて高く，ITCも十分検出されうる．したがって，微小転移以上が選択的に検出されるようカットオフ値の設定を厳密に行っている．ITCの意義が未だ不明なことから，ITCの解釈が変わってくればカットオフ値を変更していく必要があろう．

OSNA法では現在CK 19をマーカーに用いているが，CK 19発現陰性の乳癌がごくまれに存在し，その場合，転移診断の結果が偽陰性となる可能性がある．このような例に対しては，別のマーカーとの組み合わせなどを考慮する必要がある．また，全部のSLN組織を分子生物学的手法で用いてしまうと転移巣の長径などの情報が失われてしまうので，現時点では，組織を4等分し2片を分子生物学的手法に用い，残り2片をルーチンのHEに供して形態学的評価と合わせて判断するなどの併用法が良いと考えられる．

おわりに

SLNにおける微小転移の臨床的意義が示されたことから，現時点では病理部門の最重要課題は微小転移の見落としを減少させることであろう．そのためには，SLNの2 mm間隔切り出しなど，術中病理検索をより詳しく丁寧に行うのが最善と考える．また近い将来には分子生物学的手法の導入により，術中転移診断を行える施設の増加も期待できる．病理部門のマンパワーの問題など課題は多いが，各施設で可能な策を講じることで，術中病理診断の精度向上は十分可能であると考えられる．外科，放射線科と病理部門とが協力関係を維持して診療に当たることが肝要と考えられる．

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Summary

How to Improve the Accuracy of Intraoperative Diagnosis of Lymph Node Metastasis

Shigeto Ueda* and Hitoshi Tsuda**

In Japan, sentinel node navigation surgery for breast cancer, the presence or absence of metastasis into the sentinel lymph nodes (SLN), is diagnosed by intraoperative histopathological diagnosis. Intraoperative histopathological diagnosis gives rise to false negative results in approximately 10% to 40% of truly metastasis-positive cases. It is reported that micrometastasis (>0.2 mm and ≦2.0 mm in diameter) is frequently detected in SLNs. Micrometastasis is relatively frequent and accompanied by metastases to non-SLNs. In order to decrease false-negative results in intraoperative histopathological diagnosis, slice sectioning into 2 mm segments is recommended. The significance of isolated tumor cells (≦0.2 mm in diameter) in SLNs is undetermined. Recently, molecular biological methods to detect metastases to SLN at the mRNA level intraoperatively have been developed and their clinical application is expected.

Key words : breast cancer, sentinel lymph nodes

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An individual patient data meta-analysis of adjuvant therapy with uracil–tegafur (UFT) in patients with curatively resected rectal cancer

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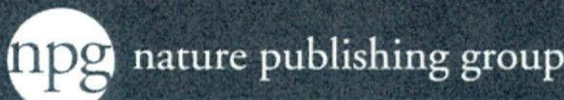

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An individual patient data meta-analysis of adjuvant therapy with uracil–tegafur (UFT) in patients with curatively resected rectal cancer

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Uracil–Tegafur (UFT), an oral fluorinated pyrimidine chemotherapeutic agent, has been used for adjuvant chemotherapy in curatively resected colorectal cancer patients. Past trials and meta-analyses indicate that it is somewhat effective in extending survival of patients with rectal cancer. The objective of this study was to perform a reappraisal of randomised clinical trials conducted in this field. We designed an individual patient-based meta-analysis of relevant clinical trials to examine the benefit of UFT for curatively resected rectal cancer in terms of overall survival (OS), disease-free survival (DFS), and local relapse-free survival (LRFS). We analysed individual patient data of five adjuvant therapy randomised clinical trials for rectal cancer, which met the predetermined inclusion criteria. These five trials had a combined total of 2091 patients, UFT as adjuvant chemotherapy compared to surgery-alone, 5-year follow-up, intention-to-treat-based analytic strategy, and similar endpoints (OS and DFS). In a pooled analysis, UFT had significant advantage over surgery-alone in terms of both OS (hazard ratio, 0.82; 95% confidence interval (CI), 0.70–0.97; $P=0.02$) and DFS (hazard ratio, 0.73; 95%CI, 0.63–0.84; $P<0.0001$). This individual patient-based meta-analysis demonstrated that oral UFT significantly improves both OS and DFS in patients with curatively resected rectal cancer.
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Keywords: rectal cancer; UFT; adjuvant chemotherapy; randomised clinical trials; individual patient data meta-analysis

Colorectal cancer accounts for 10–15% of all cancers and is the second leading cause of cancer deaths in developed countries (Pisani *et al*, 1993). In Japan alone, nearly 56 000 new cases are diagnosed and this disease causes 36 000 deaths every year (Statistics and information department, Ministry of Health and Welfare, 1996). Surgical treatment is the primary management of colorectal cancers, with 75–80% of the patients being operable at the time of diagnosis (Boring *et al*, 1991; Vernaba *et al*, 1994). However, even if a curative resection is performed, those patients with regional lymph node involvement (Dukes' C, Stage III) have a 40–50% 5-year survival rate.

Recently, in the field of Stage III colon cancer treatment, adjuvant chemotherapy by 5-fluorouracil (5-FU)/levamisole was proved to be superior to surgery-alone therapy, and then various 5-FU/leucovorin (LV) regimens were confirmed to be effective from the results of numerous large-scale randomised trials and from the pooled analysis of clinical trials (Wolmark *et al*, 1993; International Multicentre Pooled Analysis of Colon Cancer Trials (IMPACT) investigators, 1995; O'Connell *et al*, 1997). In 2004, results from the Multicenter International Study of Oxaliplatin/5-FU/Leucovorin in the Adjuvant Treatment of Colon Cancer (MOSAIC) trial demonstrated that combination chemotherapy with 5-FU/LV (de Gramont regimen) plus oxaliplatin was significantly superior to 5-FU/LV alone (André *et al*, 2004). With regard to adjuvant chemotherapy for colon cancer, therefore, solid evidence has been accumulated from relevant clinical trials, and steady evolution of the new treatment modalities has been achieved.

However, the situation is still uncertain focusing on adjuvant therapy for rectal cancer. Despite apparently curative surgery, rectal cancer recurs in more than 55% of the patients, including local recurrence rates of 25% (Vernaba *et al*, 1994). Despite the recommendation of the consensus conference by the National Institute of Health (NIH consensus conference, 1990) that concluded that adjuvant radiotherapy and chemotherapy should be given to all patients with locally advanced rectal cancer, recent findings by a large-scale randomised trial and meta-analysis have failed to prove significant benefit of radiotherapy for survival (Fisher *et al*, 1988; Vernaba *et al*, 1994). In this regard, the quest for an effective adjuvant treatment with a robust advantage on the

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outcome of resected rectal cancer remain an important task for gastrointestinal oncologists.

In Japan, mesorectal excision is standard surgical procedure. Radiotherapy is not routinely performed as adjuvant therapy.

In Japan, adjuvant therapy after resection of colorectal cancer was developed primarily using oral fluorinated pyrimidines (O-FPs). A meta-analysis of three old trials (Sakamoto *et al*, 1999) and a more sophisticated analysis of four recent pivotal randomised trials (Sakamoto *et al*, 2004) demonstrated a statistically significant benefit of O-FPs on the outcome of colorectal cancers over surgery alone. However, the survival benefit shown in that meta-analysis was more pronounced in colon cancers. The risk reduction in terms of rectal cancer was only 8% and the result of those previous meta-analyses that analysed various types of oral fluorinated pyrimidine clinical trials was not sufficient to show a significant effect on survival.

Uracil–tegafur (UFT) is one of the O-FPs. In colon cancer, the majority of recurrences occurred in the liver, whereas in rectal cancer many recurrences occurred in the lung and locally in addition to the liver. Treatment effect may thus differ between colon cancer and rectal cancer. As the previous meta-analysis, two trials of UFT in patients with rectal cancer have been reported. The present study focused on rectal cancer, which lacked a clear-cut survival benefit in our previous meta-analysis. Unlike oral fluoropyrimidines such as carmofur and capecitabine, the formulation of UFT includes a dihydropyrimidine dehydrogenase inhibitor (Diasio, 1999), designed to enhance the bioavailability of FU. This combination of uracil and tegafur was shown, in an animal tumour system, to increase the anti-tumour activity compared with tegafur alone (Ooi *et al*, 2001). UFT also produced an enhanced intratumoural concentration of fluorinated pyrimidine, 5–10 times greater than that achieved with Tegafur alone (Fukunaga *et al*, 1987). Preclinical studies established that the optimal molar ratio of uracil to Tegafur is 4:1, which resulted in the highest 5-FU tumour: blood and tumour: normal tissue partition coefficients (Kawaguchi *et al*, 1980). UFT has now been clinically tested for lung cancer (Kato *et al*, 2004), breast cancer (Noguchi *et al*, 2005), and for gastric cancer (Kinoshita *et al*, 2005) in an adjuvant setting in Japan. Recently, UFT has also been tested in Western countries, regarding its efficacy for both advanced and curatively resected colon cancer (Carmichael *et al*, 2002; Douillard *et al*, 2002; Lembersky *et al*, 2006).

Here, we present an individual patient data meta-analysis of five centrally randomised trials recently performed in Japan to compare rectal cancer patients treated with UFT, with the surgery-alone control group. This meta-analysis includes data from more than 2000 patients and therefore provides a more reliable assessment of the effect of UFT on the survival, disease-free survival (DFS), and local relapse-free survival (LRFS) of the patients with rectal cancer than is available from any of the individual studies.

PATIENTS AND METHODS

Selection of trials

Trials that randomly assigned patients to either long-term (12 months) administration of UFT or surgery-alone treatment after curative resection of rectal cancer were eligible for meta-analysis. The randomisation technique used in these trials was the centralised randomisation that precluded the possibility of prior knowledge of the treatment to be allocated.

Five relevant trials identified as Japanese Foundation for Multi-disciplinary Treatment of Cancer (JFMC) 7-1 (Kodaira *et al*, 1998), JFMC15-1, JFMC15-2 (Watanabe *et al*, 2004), Tokai Adjuvant Chemotherapy Study Group for Colorectal Cancer (TAC-CR) (Kato *et al*, 2002), and National Surgical Adjuvant Study of Colorectal Cancer (NSAS-CC) (Akasu *et al*, 2006) were included in the meta-analysis involving a total of 2091 patients. In trials JFMC7-1, JFMC15-1, and JFMC 15-2, patients who were randomly assigned to the experimental group received intravenous mitomycin C (6 mg m^{-2}) at 1 week and once monthly for 6 months. In the JFMC15-1 and 15-2 trials, patients who were randomly assigned to the experimental group additionally received an induction course of intravenous 5-FU (250 mg daily^{-1}) during 7 postoperative days (Table 1).

Protocol and data collection for the meta-analysis

In December 2003, a protocol for the meta-analysis, describing the study rationale, statistical methods, and guidelines for publication, was distributed to the principal investigators of the five trials. Investigators were asked to provide individual data for every randomised patient, whether eligible or not, assessable or not, and

Table 1 Details of the randomized controlled trials included in the individual patient data meta-analysis

Category	JFMC7-1	JFMC15-1	JFMC15-2	TAC-CR	NSAS-CC	Total
Additional chemotherapy	Mitomycin C	Mitomycin C+FU IV	Mitomycin C+FU IV	None	None	—
Radiotherapy	None	None	None	None	None	—
UFT dose/day	400 mg	400 mg	400 mg	400 mg	600 mg[a]	—
Period	12 months	12 months	12 months	24 months	12 months	—
Dates of accrual	1986–1988	1989	1990	1991–1994	1996–2001	—
No. of patients	834	447	391	143	276	—
Duration of accrual, months	35	24	24	36	54	—
Sex, No. of patients (male–female ratio)						
Male	521 (62.4%)	260 (58.1%)	244 (62.4%)	93 (65.0%)	167 (60.5%)	1285 (61.4%)
Female	313 (37.6%)	187 (41.9%)	147 (37.6%)	50 (35.0%)	109 (39.5%)	806 (38.9%)
Duke's stage, No. of patients						
A	135	67	62	12	0	276
B	326	175	139	53	0	693
C	373	205	189	78	276	1121
Median age	57	60	59	62	58	58
Upper age limit, years	70	75	75	75	75	—

JFMC = Japanese Foundation for Multidisciplinary Treatment of Cancer; NSAS-CC = National Surgical Adjuvant Study of Colorectal Cancer; TAC–CR = Tokai Adjuvant Chemotherapy for Colorectal Cancer; UFT = Uracil–Tegafur. [a]400 mg m^{-2} day^{-1} for 5 days every 7 days.

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properly followed up or not. Items requested for every patient were as follows: patient identification, date of surgery, eligibility, allocated treatment by random assignment, age, sex, primary tumour site, Dukes' stage, induction chemotherapy, dates of recurrence, death, or last visit. Disease-free survival was calculated from the date of surgery to the date of recurrence, second primary cancer or death, whichever occurred first. Survival was calculated from the date of surgery to the date of death, regardless of the cause of death. Local relapse-free survival was calculated from the date of surgery to the date of local recurrence. Data from patients with only distant recurrence and those who were died without recurrence were censored. Patients enrolled in these trials had been followed up for 5–7 years. Toxicity data were not collected, because detailed analysis of side effects can be found in the published reports of the individual trials (Kodaira *et al*, 1998; Kato *et al*, 2002; Watanabe *et al*, 2004; Akasu *et al*, 2006).

All investigators and the Clinical Trial Committee of all the trials agreed to join in the meta-analysis. Individual patient data were received by the independent secretariat by February 2004 and October 2006.

Pretreatment patient characteristics

All 2091 patients had curatively resected rectal cancer without evidence of distant metastasis by diagnostic imaging criteria or by macroscopic examination of the abdominal organs during surgery. Patients with severe postoperative complications were excluded from all trials, as were patients with any previous chemotherapy or radiotherapy or with a synchronous or metachronous second cancer. Median patient age was 61 years at the time of random assignment. The male/female ratio was approximately 3 : 2. Performance status was less than 2 on the Japan Clinical Oncology Group scale for all patients.

Statistical analysis

The method used for the meta-analysis and the format for the presentation of the results have been described in detail elsewhere (Advanced Colorectal Cancer Meta-Analysis Project, 1992). All analyses were based on individual patient data. Treatment effects on DFS, LRFS, and survival were first estimated within each trial and then combined using classical meta-analytic methods (Colorectal Cancer Collaborative Group, 2001). Treatment effects were displayed as hazard ratios. These ratios were estimated by univariate Cox's proportional model as relative risks of having an event in the UFT group as compared with having the same event in the surgery-alone control group. A ratio less than unity indicates benefit from UFT, and this benefit is statistically significant when the 95% confidence interval (CI) of the ratio does not include unity. The overall effect of treatment was assessed through a χ^2_1 d.f. and the heterogeneity between five trials through a χ^2_4 d.f. (Colorectal Cancer Collaborative Group, 2001). Additional analyses were carried out to determine which of the following prognostic features, if any, were predictive of the treatment effect: Dukes' stage (A *vs* B *vs* C), sex (male *vs* female), and age (three groups of increasing age). Tests for interaction were applied to detect departures from the homogeneity of treatment effects. Multivariate analyses were performed with the use of the Cox proportional hazards regression model for DFS, LRFS, and survival to assess the robustness of the observed effects to adjustments for important covariates and the magnitude of interaction between treatment effect and covariate (Advanced Colorectal Cancer Meta-Analysis Project, 1992). All *P*-values resulted from use of two-sided statistical tests. The significance level was set at 5% for all tests.

RESULTS

Survival

Survival hazard ratios for all the trials are presented in Figure 1. The overall hazard ratio was 0.82 (95% CI, 0.70–0.97; $P = 0.02$) with no significant heterogeneity between the treatment effects in different trials (χ^2_4 for heterogeneity = 4.31; $P = 0.37$). UFT showed significant effect on survival of curatively resected rectal cancers with a 5-year survival benefit of approximately 5%.

Figure 2 shows the breakdown of the survival hazard ratio stratified by various patient characteristics. There was a slight trend toward larger treatment benefits in earlier Dukes' stages (Hazard ratio; Dukes' $A = 0.60$, Dukes' $B = 0.79$, Dukes' $C = 0.86$) but heterogeneity tests did not show any significant difference ($\chi^2_2 = 1.41$; $P = 0.495$). There was no statistically significant difference in sex (χ^2_1 for interaction = 1.62; $P = 0.204$) or age (χ^2_2 for interaction = 0.22; $P = 0.898$).

Figure 3 shows survival curves by treatment and disease stage. These curves confirm the hazard ratio analysis shown in Figure 2 and point to favourable effects of UFT in all Dukes' stages.

Disease-free survival

Disease-free survival hazard ratios are presented in Figure 4 for all the trials. These figure show a somewhat larger effect of treatment on DFS than on survival, with an overall DFS ratio of 0.73 (95%CI,

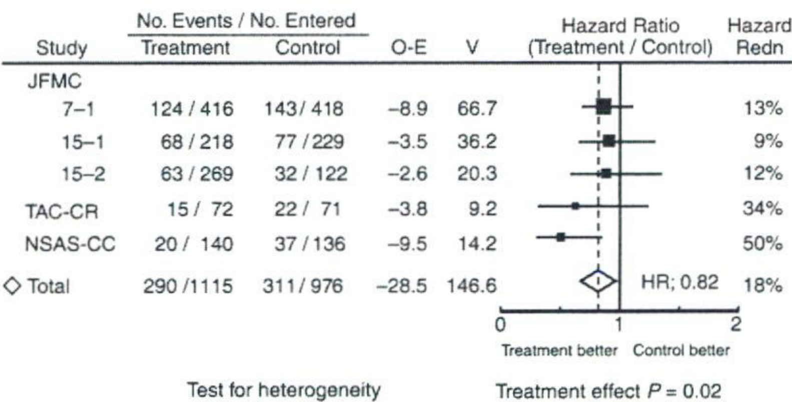

Figure 1 Survival hazard ratios by individual trial (Abbreviations: O/N = observed number of events/number of patients; O−E = Observed minus Expected number of events; V = variance of (O−E); Hazard Redn = hazard reduction; SE = standard error of hazard reduction).

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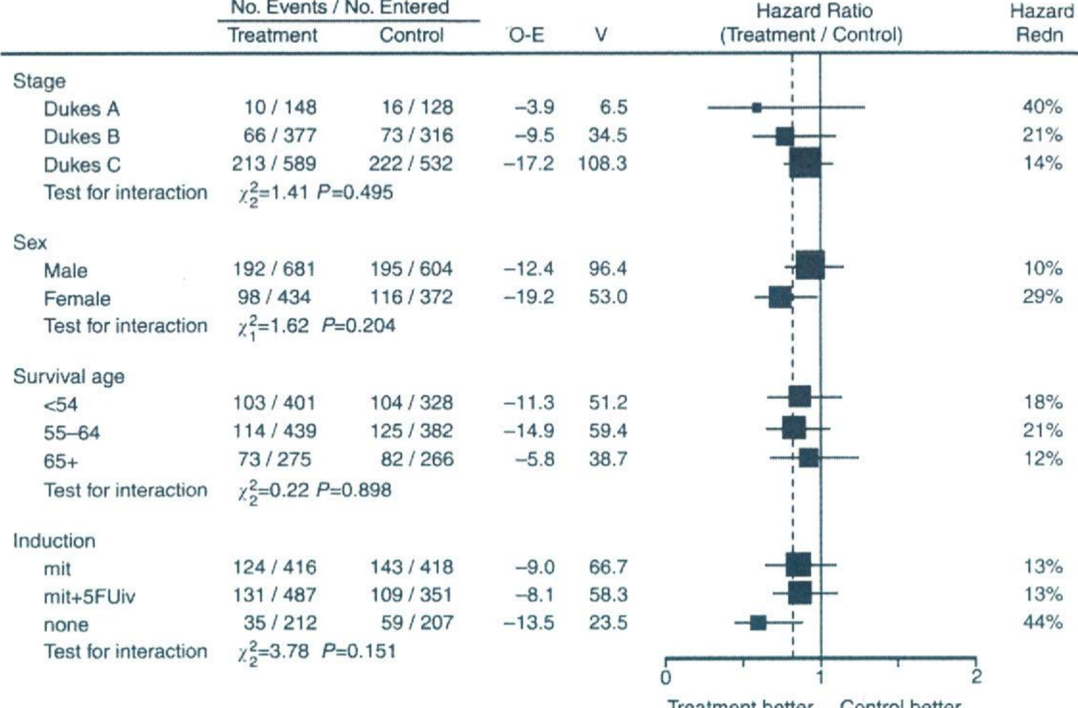

Figure 2 Survival hazard ratios by patient and treatment characteristics (Abbreviations as in Figure 1).

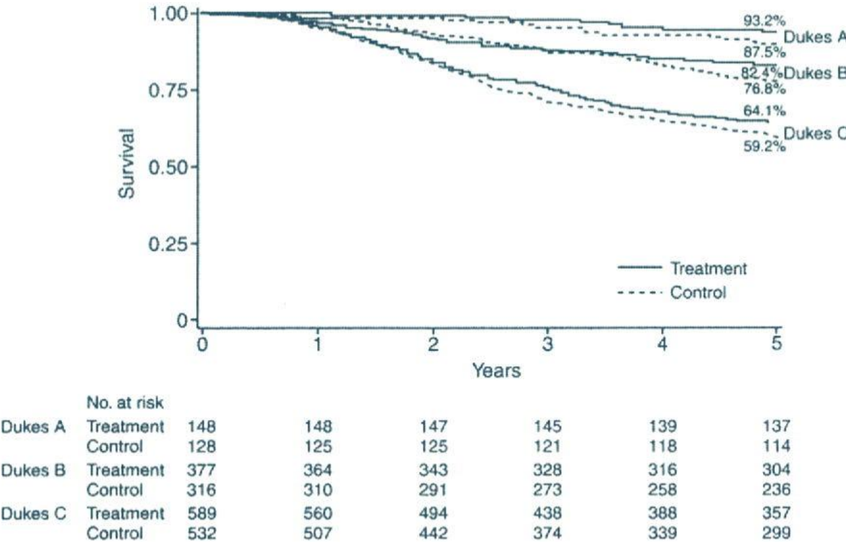

Figure 3 Survival curves by tumour stage and by treatment.

0.63–0.84; $P<0.0001$) with a 5-year DFS benefit of 9.7%, but demonstrating some heterogeneity among the treatment effects in different trials (χ^2_4 for heterogeneity $=7.85$; $P=0.097$). Additionally, random effect model assuming the variation between trials was applied. The results of the random effect model still revealed highly significant differences owing to the relatively high effect in TAC-CR and NSAS-CC trials.

Figure 5 lists the DFS hazard ratios by various patient and treatment characteristics.

Figure 6 shows DFS curves by treatment and disease stage. These curves again point to benefits of UFT in Dukes' A, B and C stages. Roughly identical effect extended across all Dukes' stages: the DFS benefits at 5 years in terms of risk reduction were 0.42, 0.33, 0.23.

Local relapse free survival

The overall hazard ratio was 0.68 (95%CI, 0.53–0.87; $P=0.0026$), and demonstrating some heterogeneity among the treatment

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