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Identification of Sentinel Lymph Nodes in Patients with Lymph Node-Negative (N0) Breast Cancer

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ABSTRACT

We identified sentinel lymph nodes in a series of 20 consecutive patients with breast cancer who had no clinical evidence of lymph node metastases (stage N0). We then determined the rate of lymph node metastasis on histologic examination and the rate of micrometastases, consisting of metastatic foci on cut surfaces other than the largest cut surface. Histologic examination included cytokeratin immunostaining. We also evaluated the relationship of clinical prognostic factors, such as age, long diameter of the tumor, and degree of histologic malignancy, between patients with and without metastases. In patients with N0 disease the rate of N1 lymph node metastasis was 25% and the rate of micrometastasis was 5%. The rates of metastasis were not correlated with any clinical prognostic factors. By examining the relationship of risk factors and prognostic factors, sentinel lymph node biopsy may eliminate the need for further axillary node dissection and its associated discomfort and complications in many patients with breast cancer.

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Key words : sentinel lymph node, micrometastasis, cytokeratin immunostaining

INTRODUCTION

Axillary lymph node dissection is performed in cases of operable breast cancer to stage the tumor, to acquire prognostic information, and to provide local disease control by preventing axillary recurrence, thus ultimately increasing survival rates. However, level 1 and level 2 axillary lymph node dissections are associated with complications, including permanent lymphedema, wound infection, loss of muscle strength in the upper extremities, limitation of shoulder mobility, and neurologic changes. These functional sequelae and the psychological distress commonly associated with them can increase the costs of treatment.

In 1995, a meta-analysis by the Early Breast Cancer Trialists' Collaborative Group[1] suggested that axillary node dissection does not prolong survival, particularly in patients with early-stage breast cancer. Sixty percent to 70% of patients without clinical evidence of axillary lymph node enlargement (N0) have lymph nodes histologically negative for metastasis (n0). Because axillary node dissection is associated with significant postoperative complications in these patients, this procedure should be avoided whenever possible.

In 1994 Giuliano[2] proposed the concept of sentinel lymph nodes, which are the first lymph nodes to

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receive drainage from the primary tumor. Axillary lymph node metastases were previously thought to occur in a random fashion, but in a study by Cox et al.[3] only 1 of 446 patients had skip metastases. This finding suggests that breast cancer cells are "captured" by the first lymph node in the path of the primary tumor (i.e., sentinel nodes). Therefore, sentinel node biopsy (SNB) in patients with stage N0 may provide sufficient information to eliminate the need for further axillary node dissection.

In 2001, Higaki et al.[4] investigated whether the indications for SNB could be expanded to avoid unnecessary axillary dissection. Several methods have been used to identify metastasis to sentinel nodes, including staining with indocyanine green[5], identification with a gamma probe using radioisotopes[6,7], or a combination of both techniques[8–10]. However, procedures remain unstandardized and vary among surgeons[11]. Cytokeratin immunostaining has also been used to identify metastasis to sentinel nodes, particularly micrometastases[12,13]. Among patients with breast cancer, two high-risk groups are those with clinical N0 disease who are found to have n1 histologic disease and those with n0 disease staged with standard methods who are found to have micrometastases. Identifying such patients and investigating the relationship between these findings and other prognostic factors can facilitate the selection of more-appropriate surgical procedures and enable unnecessary axillary node dissection to be avoided. Surgeries that are more appropriate and less invasive can in turn reduce postoperative complications and improve risk management and the effectiveness of follow-up.

Therefore, we investigated the rate of histologic metastasis (n1 disease) and the rate of micrometastases in axillary sentinel nodes in patients with N0 breast cancer. Histologic examination was done with both standard hematoxylin-eosin (H & E) staining and with cytokeratin staining. We also examined the relationship of various prognostic factors, such as age, tumor diameter, and degree of histologic malignancy, between patients with lymph node metastasis and those without.

Patients and Methods

1. *Patients*

The subjects were 20 consecutive patients with N0 breast cancer who underwent surgery and SNB without further axillary node dissection from October 2000 through December 2001 at Sanikukai Hospital. All patients gave written informed consent.

Table 1. Nottingham method for degree of malignancy

Tubular formation
1. The great majority is composed of formed tubules with clearly visible lumina.
2. Definite tubule formation is seen in moderate amounts of but there are also clear areas of solid tumor growth.
3. Little or no tubule formation is seen, the cells growing insheets or cords. With good fixation and processing clefts in tumor tissue due to shrinkage artifact should be reduced to a minimum. Their presence should not be mistaken for tubular structures.

Nuclear pleomorphism
1. Nuclei are regular and show little variation in size and shape.
2. A moderate variation is seen, without extremes of cell size of shape.
3. Marked variation, particularly when very large and bizarre nuclei are present. Multiple nucleoli in a nucleus favour a score of 3.

Mitotic rate
Using a magnification of approximately 300 times.
1. Less than 10 mitosis per 10 fields.
2. 10–19 mitosis per 10 fields.
3. 20 or more mitosis per 10 fields.

Total score 3–5 points is grade 1, low malignancy ; 6–7 points is grade 2, intermediate ; and 8–9 points is grade 3, high.

2. *Methods*

Surgery was performed under general anesthesia. In a sterile surgical field, 5 ml of indocyanine green was injected with a 22-gauge needle into the subcutaneous tissue just above the tumor site. The area was then manually massaged for 5 minutes. The stained lymph duct was followed to identify the first node to receive lymph flow. If an approach from the skin incision line was not possible during partial mastectomy, a 5 cm incision was made along the skin cleavage from the intersection of the lateral margin of the pectoralis major muscle and the anterior axillary line. Using the stained lymph fluid as a reference, we identified and resected 1 or 2 sentinel lymph nodes. When a clearly stained lymph node could not be detected, the associated fat tissue was resected instead.

The resected sentinel lymph nodes were cut in half and embedded in paraffin for histologic examination. A thin section of the largest cut surface was prepared, stained with H & E and cytokeratin, and then examined for the presence or absence of metastases. If no metastases were observed on the largest cut surface, thinner sections of the lymph node were cut at 2-mm intervals. Each section was then stained with H & E and cytokeratin and examined for the presence of micrometastases. The primary lesion was evaluated with standard histopathologic criteria. Lesions were also scored with the Nottingham method for degree of malignancy (Table 1). We then compared age, tumor diameter, and degree of malignancy between patients in whom lymph nodes were histologically positive or histologically negative for metastasis. Differences between patient groups were analyzed with the *t*-test.

Results

From October 2000 through December 2001, we evaluated findings in 20 patients with breast cancer and clinically negative lymph nodes (N0) who underwent surgery. Stained lymph nodes or associated fat tissue was obtained in all cases (Table 2). The patients ranged in age from 25 to 70 years (mean, 54.3 years). The procedure selected was mastectomy+SNB in 7 patients and partial mastectomy+SNB in 13 patients. Of the 20 patients with clinically negative lymph nodes (N0 disease), 5 (25%) had lymph nodes histologically positive for metastasis (n1). In 1 patient (5%), no metastases were present on the largest cut surface, but micrometastases were present on thinner sections cut at 2-mm intervals (Fig. 1).

In all 5 patients with lymph nodes histologically

Table 2. Patients' clinical information and Nottingham score

Case	Age	Operation	n	cyk	micro	T+N+M= ; G	tumor size (mm)
01	62	Bp+SNB	0/2	0/2	−	2+2+1=5 ; I	12
02	61	Br+SNB	0/2	0/2	−	2+2+1=5 ; I	27
03	68	Bp+SNB	0/2	0/2	−	3+2+1=6 ; II	24
04	41	Bp+SNB	0/1	0/1	−	3+2+2=7 ; II	10
05	33	Bp+SNB	2/2	2/2	−	2+1+1=4 ; I	24
06	56	Bp+SNB	2/2	2/2	−	3+3+2=8 ; III	20
07	57	Br+SNB	0/1	0/1	−	3+3+1=7 ; II	45
08	47	Bp+SNB	0/2	0/2	−	2+1+1=4 ; I	18
09	53	Br+SNB	1/2	1/2	1/2	2+2+2=6 ; II	30
10	70	Br+SNB	1/2	1/2	−	3+2+1=6 ; II	40
11	38	Bp+SNB	0/2	0/2	−	2+2+1=5 ; II	25
12	41	Bp+SNB	0/2	0/2	−	2+1+1=4 ; I	15
13	43	Bp+SNB	0/2	0/2	−	1+2+2=5 ; I	10
14	25	Br+SNB	2/2	2/2	−	3+2+2=7 ; II	60
15	57	Bp+SNB	0/1	0/1	−	2+2+1=5 ; II	20
16	44	Br+SNB	0/2	0/2	−	2+3+2=7 ; II	25
17	54	Bp+SNB	0/2	0/2	−	1+3+3=7 ; II	12
18	44	Bp+SNB	0/2	0/2	−	3+3+3=9 ; III	18
19	58	Bp+SNB	0/2	0/2	−	2+2+2=6 ; II	10
20	35	Br+SNB	0/2	0/2	−	2+3+2=7 ; II	9

Bp, partial mastectomy ; Br, mastectomy ; SNB, sentinel lymph node biopsy ; n, pathogical lymph node metastasis ; cyk, cytokeratin imunostaining ; micro, micrometastasis ; T, N, M, G, Nottingham score and grade.

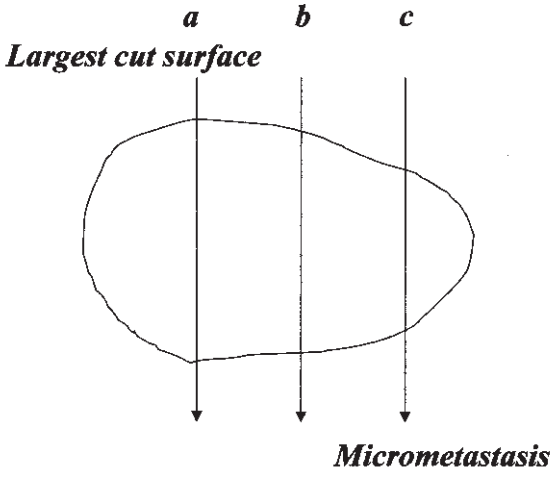
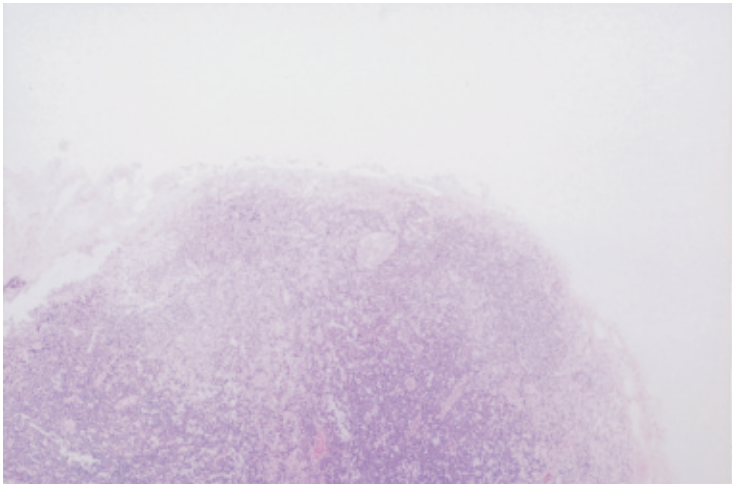
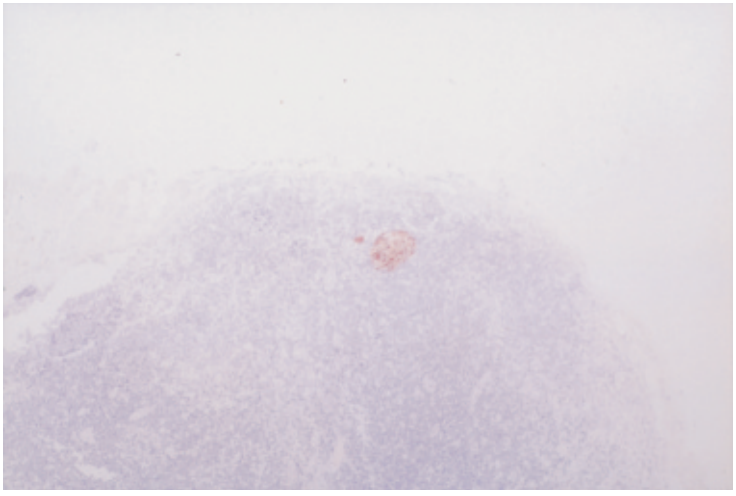


Fig. 1. Multiple-level sectioning.



H & E staining, 3.3×10.



Cytokeratin staining, 3.3×10.
Micrometastasis was visualized more clearly with cytokeratin staining.

Fig. 2. Histopathologic findings.

Table 3. Comparison of Clinical Prognostic Factors in Histologically Negative and Positive Lymph Node Metastases Groups

	N0n0	N0n1	p Value
Mean age (years)	50.0±10.1	47.4±18.2	$p=0.45$ NS
Mean long diameter of tumor (mm)	18.7±4.4	34.8±16.0	$p=0.36$ NS
Mean Nottingham score	5.93±1.29	6.20±1.48	$p=0.44$ NS

t-test was used to compare groups, and differences were considered significant when $p<0.05$.

positive for metastasis, findings on H & E staining agreed with those on cytokeratin staining. Cytokeratin staining was particularly useful in providing a clear microscopic image of the micrometastases in 1 patient (Fig. 2).

The degree of histologic malignancy was grade I in 7 patients, grade II in 11 patients, and grade III in 2 patients.

Clinical prognostic factors did not differ significantly between the 5 patients with histologic lymph

node metastasis and the 15 patients without (Table 3).

Discussion

Standard procedures for radical cancer surgery often include the removal of a margin of normal tissue around the lesion and dissection of the draining lymph nodes. The postoperative complications associated with lymph node dissection are accepted by patients for the sake of a radical cure. However, in our study of patients with N0 breast cancer, SNB alone was performed to avoid the complications, such as upper extremity edema and muscle weakness, associated with axillary node dissection. None of our 20 patients had postoperative complications.

In patients who undergo SNB without further axillary node dissection, a primary risk factor is the discrepancy between preoperative clinical lymph node positivity (N0) and histopathologic lymph node positivity (n1). Such a discrepancy was present in 5 of our 20 patients (25%). Patients with metastasis to axillary nodes have systemic disease and require further treatment, which may include local tumor control with radiation therapy, chemotherapy, and hormone-receptor therapy, or surgery to dissect axillary lymph nodes. In addition, this high-risk group of patients requires careful follow up. Therefore, this represents a primary risk factor when further axillary node dissection is not performed.

In patients without metastases on the largest cut surface on standard histopathologic assessment (n0), the presence of occult metastases in other regions ("micrometastases") is a secondary risk factor. In our study, micrometastases were found in 1 patient (5%). This patient required management similar to that of other patients with metastasis to lymph nodes, including further treatment and careful follow-up. When considering whether to forgo further axillary lymph node dissection, it is important to adequately recognize primary and secondary risk factors to plan postoperative management, including radiation therapy, chemotherapy, hormone therapy, or the need for further surgery. Cytokeratin immunostaining was used in our study for histopathologic examination to determine the presence or absence of lymph node metastases.

Cytokeratin immunostaining, which targets epithelial components, was useful, even in histopathologically positive lymph node specimens, and agreed with results of H & E staining in the 5 patients. Cytokeratin immunostaining was particularly useful in providing a clear microscopic image of the micrometastases present in 1 patient (Fig. 2). However, cytokeratin immunostaining was done postoperatively in all patients; intraoperative assessment of axillary node metastases was not performed.

Masuda[14] reports using intraoperative reverse transcription-polymerase chain reaction, which takes about 1 hour, but has the advantage of providing results during surgery to allow proper selection of a procedure. More widespread use of the polymerase chain reaction during surgery may be promising. In our study, examination of serial 2-mm sections revealed micrometastases in 1 of the 20 patients. Suggested optimal section thickness for lymph nodes varies among reports[14-17]. To determine if thinner sections and more sensitive detection of micrometastases are indeed clinically useful, long-term follow-up and cost-benefit analysis must done[18]. The thinner the sections, the more likely that smaller micrometastases will be detected, but the increased time and labor required in actual clinical practice must also be considered.

We also examined clinical prognostic factors (age, long diameter of tumor, and degree of histologic malignancy) and found no differences between the patients with and without histologic evidence of lymph node metastasis (Table 3). However, our series 20 patients was small, and a larger number of cases, such as the 4,782 cases of sentinel lymphadenectomy reviewed by Sandrucci et al.[19] is required for more meaningful statistical analysis.

A 2000 study of patients who had negative SNB results but did not undergo further axillary dissection[20] found no local tumor recurrence after an average of 39 months. If a primary risk factor group (N0 and n1), secondary risk factor group (micrometastases), and other risk factors can be identified and correlated with clinical prognostic factors and specific clinical findings in a larger number of patients, decid-

ing which patients do not require axillary node dissection will be easier. On the basis of these risk factors, patients can be better classified after surgery for more effective risk management and follow-up. Although some risk was present in this study, the information gained from SNB was useful for identifying patients who did not require further axillary node dissection. Forgoing extensive axillary node dissection reduced postoperative complications and thus prevented further physical discomfort and psychological distress in many of these patients.

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