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Original Articles

Evaluation of Intraoperative Frozen Section Diagnosis of Sentinel Lymph Nodes in Breast Cancer

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Background: Intraoperative frozen sections (FS) of sentinel lymph nodes (SLNs) can be used to detect metastatic disease, allowing immediate axillary lymph node dissection (ALND). However, pathological inconsistency in the SLNs diagnosis is sometimes encountered when the results of FS and permanent sections are compared. The purpose of this study was to reveal the usefulness and limitations of FS for the diagnosis of SLNs in patients with breast cancer.
Methods: We reviewed the results for 569 patients with breast cancer at stage 0–II who underwent a sentinel node biopsy between February 1998 and December 2002. SLNs were analyzed using standard FS procedures and a single section stained with hematoxylin and eosin was examined. Patients determined to have positive SLNs based on the results of the FS diagnosis immediately underwent ALND. Permanent sections were later prepared from the remaining frozen tissues and examined using hematoxylin and eosin staining without additional immunohistochemical staining.
Results: Seven cases (1%) with atypical cells were found in the FS diagnosis intraoperatively, which were counted as ‘negative’ by the following analysis. The final pathology results showed metastasis in the SLN sections in 159 patients (28%), of whom 26 were diagnosed as negative by the FS diagnosis. Accuracy, specificity and the false-negative rate were 95, 100 and 16%, respectively. The mean size of the nodal metastases in the false-negative cases was significantly smaller than that in the true-positive cases ($n = 72$) ($P < 0.01$). False-negative rates for T1b, T1c and T2 were 33, 19 and 14%, respectively. The rate of micrometastasis in T1 (43%) was significantly higher than that of T2 (13%) ($P < 0.01$).
Conclusions: FS diagnosis for SLNs is reliable. Patients with negative SLNs by the FS diagnosis can avoid reoperation for ALND. However, FS may fail to detect micrometastases, especially in cases with small tumors.

Key words: breast cancer – sentinel lymph node – sentinel node biopsy – frozen section – micrometastasis

INTRODUCTION

The status of axillary lymph nodes is the most powerful independent predictor of survival and the risk of recurrence in patients with breast cancer (1). Therefore, axillary lymph node dissection (ALND) has been adopted as a standard procedure to determine the status of lymph nodes. Sentinel node biopsy (SNB) represents a new standard of care for patients with clinically node-negative breast cancer. Numerous studies have confirmed the reliability of SNB, which has a high detection rate and overall accuracy (2–4). If sentinel lymph nodes (SLNs) are histologically negative, many investigators have performed observational studies on the results of SNB alone and the results of a validation study on the efficacy and safety of SNB have recently been reported (5). Consequently, unnecessary arm morbidity caused by ALND can be avoided.

With regard to the pathological examination of SLNs, the value of intraoperative frozen section (FS) diagnosis still

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remains controversial. If positive, FS has the obvious advantage of enabling ALND to be performed immediately, thereby avoiding the need for reoperation. However, the procedure is costly and increases the operation time. Furthermore, pathological inconsistencies in the SLN diagnosis are sometimes encountered when the results of FS and permanent sections are compared. It is well known that with conventional FS techniques it is easier to miss the metastases in SLN than with permanent section techniques. Hence the intraoperative assessment of SLNs must be performed with accuracy and efficiency.

In this paper, we describe the results of routine intraoperative FS diagnosis and evaluate the reliability of intraoperative FS diagnosis of SLNs in early breast cancer patients, in which we used the same technique of frozen and permanent diagnosis by a single section staining hematoxylin and eosin (HE) without immunohistochemistry (IHC).

PATIENTS AND METHODS

We reviewed the outcome for 568 consecutive female patients with stage 0–II breast cancer who underwent SNB between February 1998 and December 2002. A total of 569 cases of axillary nodal basins for which an intraoperative pathology consultation was performed at the National Cancer Center Hospital East were evaluated in this series. Lymphatic mapping and the SNB procedure were explained to the patients and informed consent was obtained prior to the surgical procedure.

SNB was performed using a dye-guided method alone (97 cases) or a combination of dye- and radio-guided methods (472 cases). These techniques have been described in detail elsewhere (6,7). Briefly, 30–50 MBq (0.8–1.4 mCi) of technetium-99m human serum albumin (^{99m}Tc-HSA) and technetium-99m tin colloid (^{99m}Tc-TC) (Nihon Medi-Physics, Tokyo, Japan) were injected subcutaneously at 1–3 sites around the primary tumor 1 day prior to the surgical procedure. A preoperative lymphoscintigraphy was performed using a large-field scintillation camera.

Under general anesthesia, 4–5 ml of indigocarmine (4 mg/ml) (Daiichi Pharmaceutical, Tokyo, Japan) were injected subcutaneously at 1–3 sites around the primary tumor and the breast lesions were rubbed well. SNB was then performed using only the dye as a guide or a combination of the dye and a hand-held gamma-ray detection probe (Navigator; USSC, Norwalk, CT).

In all cases, the excised SLNs were submitted for immediate FS diagnosis. While awaiting the results, a partial mastectomy or total mastectomy was performed. Patients diagnosed as having positive SLNs on the basis of the FS diagnosis immediately underwent an ALND of level I, II or higher. However, 126 patients had a back-up axillary dissection scheduled in advance as part of a feasibility study and 11 dissections were performed in keeping with the patients' wishes, regardless of the result of FS diagnosis.

SLNs for intraoperative evaluation were examined using a 4 μm thick single section (one level) stained with HE. The remaining frozen tissues were fixed in formalin, embedded in paraffin and sectioned at one level. The SLNs were later diagnosed using permanent sections and HE staining without additional IHC or serial sections. Other dissected non-SLNs were also examined using the same methods.

The results of FS and permanent section were compared with regard to the pathological diagnosis for the SLNs. The standard was based on the results for the permanent section. Statistical significance was determined using a chi-squared test and an

Table 1. Patients' characteristics

Cases	569
Age: median (range) (years)	54 (21–84)
SNB methods	
Dye alone	97
Combined method	472
Dominant primary site	
UOQ	309
UIQ	119
Central	62
LOQ	51
LIQ	28
T size	
Tis	24
T1a	5
T1b	37
T1c	204
T2	299
Nodal status	
N0	549
N1	20
Stage	
0	24
I	242
IIA	289
IIB	14
Type of surgery	
Mastectomy	215
Partial mastectomy	354
Histopathological type	
Non-invasive	24
IDC	448
ILC	25
Apocrine	23
Mucinous	21
Other	28

SNB, sentinel node biopsy; U, upper; L, lower; O, outer, I, inner; Q, quadrant; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma.

Table 2. Comparison of the results for frozen section with permanent section on a patient basis

		Permanent section		
		Positive	Negative	Total
Frozen section	Positive	133	0	133
	Negative	26	410	436
	Total	159	410	569

A number in the table shows the number of cases. Seven atypical cases diagnosed by FS intraoperatively were considered as ‘negative’ in this table. Sensitivity, 84%; specificity, 100%; positive predictive value, 100%; negative predictive value, 94%; accuracy, 95%; false-negative rate, 16%.

Table 3. Comparison of the results for frozen section with permanent section on a lymph node basis

		Permanent section		
		Positive	Negative	Total
Frozen section	Positive	268	0	268
	Negative	30	800	830
	Total	298	800	1098

A number in the table shows the number of lymph nodes. Eight SLNs with atypical cells diagnosed by FS intraoperatively were considered as ‘negative’ in this table. Sensitivity, 90%; specificity, 100%; positive predictive value, 100%; negative predictive value, 96%; accuracy, 97%; false-negative rate; 10%.

unpaired Student’s *t*-test. Accuracy, sensitivity, specificity and false-negative rate (FNR) value were calculated using standard equations (3).

RESULTS

The patient characteristics are listed in Table 1. The age of the patients ranged between 21 and 84 years (median, 54 years). A total of 568 patients underwent SNB for a total of 569 axillary nodal basins. One patient had bilateral breast cancer. A total of 1098 SLNs were removed, with an average of 1.9 lymph nodes per patient.

The results of the FS diagnosis were judged to be indeterminate in seven cases (1%) because of the presence of atypical cells in the SLNs (atypical cases). Table 2 compares the results of FS and permanent section on a patient basis. Seven atypical cases diagnosed by FS intraoperatively were considered as ‘negative’ in Table 2. The seven atypical cases were finally diagnosed as five positive and two negative cases. Overall, 159 out of 569 cases (28%) were found to have nodal metastases in the final pathology. In 26 cases, the FS diagnoses were negative but the permanent section diagnoses were positive (FNR, 16%). The FS diagnosis correctly identified the lymph nodes as either positive or negative in 543 cases (accuracy, 95%). Overall, 410 cases showed no signs of metastasis in the FS diagnosis and the final pathology (specificity, 100%). Thirteen of the 26 false-negative cases subsequently underwent ALND; of these 13 cases, 11 had a metastasis only in the SLN.

Table 3 compares the results of FS and permanent section on a lymph node basis. Eight SLNs with atypical cells diagnosed by FS intraoperatively were considered as ‘negative’ in Table 3. The eight atypical SLNs were finally diagnosed as six positive and two negative lymph nodes. In the final pathology, metastases were found in 298 of the 1098 nodes (27%). Thirty of these nodes were evaluated as negative by the FS diagnosis but were later judged to be positive by the final pathology (FNR, 10%). On a lymph node basis, the sensitivity, specificity and accuracy were 90, 100 and 97%, respectively.

Table 4 lists the number of true-positive and false-negative results according to primary tumor size. The final metastatic rate increased as the primary tumor size increased. FNR for

Table 4. Frozen section analysis by primary tumor size

	n	True positive	Final metastatic rate (%)	False-negative	Accuracy	False-negative rate
Tis–T1a	29	1	3	0	29/29 (100%)	0/1 (0%)
T1b	37	6	16	2	35/37 (95%)	2/6 (33%)
T1c	204	53	26	10	194/204 (95%)	10/53 (19%)
T2	299	99	33	14	286/299 (96%)	14/99 (14%)
Total	569	159	28	26	544/569 (96%)	21/154 (16%)

Seven atypical cases diagnosed by FS intraoperatively were considered as ‘negative’ in this table.

Table 5. Metastatic size in SLN

SLN frozen section result	*n*	Metastasis size (mm) in SLN (mean ± SD)		% SLN micrometastasis (≤2 mm)	
False-negative	26	2.4 ± 2.8	*P* < 0.01*	62%	*P* < 0.01**
True-positive	72	9.5 ± 5.1		4%	

*Unpaired Student’s *t*-test; **chi-squared test.

Table 6. Results of frozen section diagnosis of sentinel lymph node in breast cancer in the literature

Reference	Year	No. of patients/axilla	Intraoperative technique		Final histopathology technique		FNR (%)	Accuracy (%)
			Stains	No. of level	Stains	No. of level		
Chao et al. [11]	2001	200/203	HE	2	HE + IHC	5	32	91
Tanis et al. [12]	2001	262/265	HE	1	HE + IHC	3	26	90
Weiser et al. [13]	2000	890/–	HE	1	HE + IHC	HE 5, IHC 2	42	78
Rahusen et al. [14]	2000	100/106	HE	≥1	HE + IHC	≥1	43	84
Veronesi et al. [15]	1999	192/–	HE	≥3	HE + IHC	≥3	32	86
Noguchi et al. [16]	1999	62/–	HE	1	HE + IHC	2	32	85
Canavese et al. [17]	1998	–/96	HE	3	HE	≥3	14	96
This study	2004	568/569	HE	1	HE	1	16	95

FNR, false-negative rate; HE, hematoxylin and eosin; IHC, immunohistochemistry.

T1b, T1c and T2 were 33, 19 and 14%, respectively, excluding the Tis–T1a tumors because of their low final metastatic rate.

We also evaluated the impact of the size of the metastatic deposit within the SLNs on the accuracy of the FS analysis. The largest transverse diameter of the metastasis was measured in all of the false-negative cases ($n = 26$) and in a portion of the true-positive cases ($n = 72$). As shown in Table 5, the mean size of the nodal metastasis was 2.4 and 9.5 mm for the false-negative and the true-positive cases, respectively ($P < 0.01$). The rates of micrometastasis of ≤2 mm in size in the false-negative and the true-positive cases were 62 and 4%, respectively. This difference was significant ($P < 0.01$). Moreover, in 98 cases, which were measured metastatic foci, the rate of micrometastasis in T1 (43%; 12/28) was significantly higher than that of T2 (13%; 9/70) ($P < 0.01$). Most of the SLNs with micrometastasis were found among the false-negative or atypical cases.

DISCUSSION

The sentinel node theory postulates that SLNs are the most likely sites of lymphogenic metastases. Accordingly, a more detailed pathological work-up of SLNs is more likely to reveal metastases undetected by conventional histology than the investigation of other lymph nodes (8–10). Many institutions are beginning to offer SNB on its own, with no further axillary surgery for SLN-negative patients.

Routine intraoperative FS diagnosis of SLNs can detect metastatic disease, allowing immediate axillary dissection, if necessary, and avoiding the need for reoperation. The main disadvantages of this procedure are its cost and the additional surgical time required for the diagnosis. Hence the intraoperative diagnosis of the harvested nodes must be performed quickly, with the highest degree of accuracy possible.

This study evaluated the reliability of intraoperative FS diagnosis with the widely used conventional method, which uses single section analysis by HE staining without IHC evaluation. This procedure is easy to apply in many institutions.

This study showed an accuracy and FNR of 97 and 16%, respectively. FNR is the rate at which metastases of SLNs recognized in the permanent section diagnosis are not recognized in the FS diagnosis. To minimize the possibility of false-positive results at our institution, intraoperatively indeterminate (atypical or suspicious) results, which accounted for 1% of the present FS series, can only be considered negative at the time of surgery, unless confirmed as positive in the permanent section diagnosis. In two of our seven atypical cases, the permanent results were negative. Atypical cases should be confirmed using permanent sections to avoid unnecessary ALND. We need to approval the number of atypical cases to some extent.

A review of currently available data (Table 6) shows variable results for FS diagnosis, with accuracy ranging from 78 to 96% and FNR from 14 to 43% (11–17). However, the methods used were heterogeneous and the interpretation of the results probably included any type of nodal involvement as metastasis. Hence these results cannot be directly compared with our results. Detailed histopathological work-ups of SLNs using serial or step sectioning and IHC increase the rate at which metastatic cells and micrometastases are detected. The examination of multiple permanent sections would probably lead to a poorer FNR (18). The best FNR performances are usually reported when the final histological evaluation considers only the same surface as that used in the intraoperative assessment (19). The results of our study were based on a comparison of a single FS and a single permanent section without IHC. We have to check the SLNs by using multi sections and IHC again.

Most of the metastatic foci in the false-negative cases were found on a permanent section that was adjacent to the FS surface and thus belonged to the category of isolated tumor cells or micrometastasis (≤2 mm). The rate of micrometastases in the false-negative cases was 62%, which was significantly higher than that in the true-positive cases (4%) in this study. However, these results suggest that FS is most accurate at detecting the foci of nodal metastases that are >2 mm in size. The failure of routine intraoperative FS is largely a result of the failure to detect micrometastatic disease (13).

Undoubtedly, the use of step sectioning and IHC would have increased the chances of finding occult metastases and micrometastases in SLNs (9,15,20,21). However, the significance of these procedures is not clear in terms of predicting further nodal involvement, prognosis and indications for adjuvant systemic treatment. The significance of isolated tumor cells detected only by IHC may be even less. It remains unclear how often these isolated tumor cells reflect metastases capable of further growth and dissemination. Most institutions experience only a limited number of cases where the SLN metastasis is first discovered by IHC and then retrospectively found on HE-stained slides. Therefore, the cost of IHC may not justify its use as a routine procedure. T1 tumors had significantly more micrometastases than T2 tumors in this study. Smaller tumors are more easily overlooked, which may lead to false-negative results Hence the use of IHC may be advisable for patients with small tumors. More sensitive intraoperative methods are needed to avoid reoperation. Imprint cytology is another reliable method for the intra-operative investigation of SLNs that is equivalent to FS (18,22,23). Imprint cytology is probably a better choice, owing to its lower cost, shorter time requirement and the lack of tissue damage.

In conclusion, FS is reliable for SLN examinations in patients with breast cancer and can permit immediate decisions regarding the need for ALND. There were more micrometastases in T1 than T2 tumors. We therefore postulate that a relationship exists between micrometastasis and false negativity. In such cases, detailed examination of SLNs using multiple sections and IHC will produce good results.

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