Frozen Section in Axillary Sentinel Lymph Nodes for Diagnosis of Breast Cancer Micrometastasis

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Abstract. *Background: Sentinel lymph node (SLN) biopsy plays a major role in the surgical management of primary breast cancer. The aim of this study was to assess the diagnostic accuracy of the assessment of axillary frozen sections of SLNs for micrometastasis diagnosis. Patients and Methods: This study focused on 278 SLNs from 149 patients. Each lymph node was fully analyzed by frozen section. After fixation, serial sections were cut and stained by hematoxylin and eosin (HE) and for pan-cytokeratins by immunohistochemistry (IHC). Results: Tumor cells were detected in 63 SLNs, 41 on frozen sections and 22 on controls. Of these 63 positive SLNs, 42 contained metastases, 10 contained micrometastases and 11 contained isolated tumor cells. The specificity and positive predictive value of SLN frozen sections for micrometastasis was 100%. The sensitivity was 83.3% for metastasis, 40% for micrometastasis; the false-negative rate was 16.7% for metastasis and 60% for micrometastasis. Conclusion: Analysis of frozen section of SLNs is an accurate method for metastasis detection, allowing concurrent axillary dissection when positive. The protocol for SLN analyses described herein shows good sensitivity for micrometastasis detection.*

Axillary lymph node status remains one of the most important prognostic factors in primary breast carcinoma, despite the development of a range of other prognostic markers (1). The sentinel lymph node (SLN) biopsy technique was introduced to permit an accurate staging of the axillary lymph node status for T1-2, N0 breast carcinoma (2, 3).

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Key Words: Sentinel lymph node, breast cancer, frozen sections, micrometastasis, immunohistochemistry.

Examination of the SLN by frozen section is known to be less accurate than analysis of permanent sections, but allows re-intervention for axillary lymph node dissection to be avoided if metastases are detected. Some controversies remain in the literature concerning the utility of routine SLN frozen section in breast cancer. Some centers prefer touch imprinting or delayed histopathologic of analysis.

Recently, studies have focused on the implication of SLN micrometastasis (3-6), Viale *et al*. found that axillary lymph node metastases were present in 21.6% of cases, and they discussed whether axillary lymph node dissection should undertaken in cases of sentinel lymph node micrometastasis (1).

In our institution, SLN biopsy frozen section was introduced in the late 1990s, in a multidisciplinary setting, in order to avoid unnecessary axillary lymph node dissection. At the end of 2003, we modified our SLN protocol following the recommendation of Turner *et al*. (3).

The aim of this study was to estimate the sensitivity, specificity, negative predictive value and false-negative rate of frozen section in the detection of SLN micrometastasis.

Patients and Methods

Patients. For this retrospective study, we collected 361 SLNs from 160 patients with breast carcinoma who underwent SLN mapping during the two years following the introduction of an SLN protocol for frozen section. The median number of SLNs examined per patient was 2.25 (range 1 to 9). There were 14 cases of mastectomy and 146 cases of lumpectomy. In 10 cases, no invasive carcinomas were retrieved, but ductal carcinoma *in situ* was present in 7 cases and no residual tumor was found in 3 cases of neo-adjuvant therapy. These 10 cases were excluded from the study, as well as one case of a pT3 which was not an indication for SLN detection. A total of 278 SLNs were thus included in this study.

Histopathological analysis of the tumor. The relevant clinicopathological characteristics were tumor type, Bloom-Richardson grade, lymphovascular invasion, perineural invasion, proliferation index (MIB-1), estrogen and progesterone receptor status as analyzed by immunohistochemistry and scored with Allred score (4), *HER2/neu* amplification assessed by fluorescence *in situ*

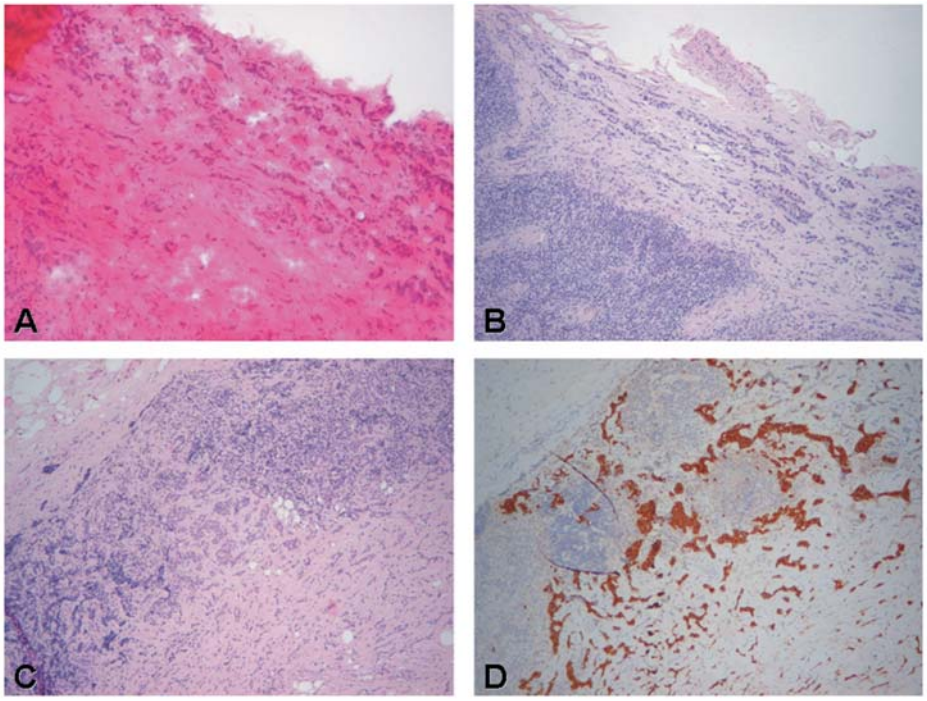

Figure 1. *Sentinel lymph node metastasis. A, Frozen section of SLN metastasis greater than 2 mm as compared with HE control (B). C, Frozen section of SLN lobular carcinoma metastasis missed during intraoperative examination and revealed on the control with anti-cytokeratine IHC (D).*

hybridization (FISH) analysis and gene amplification determined by a ratio of *HER2/CEP17>2*. All results are reported in Table I.

The study was approved by the Geneva University Hospitals Ethics Committees (protocol NAC 06-054R).

Histhopathological analysis of the lymph nodes. Axillary SLNs were analyzed by frozen section intra-operatively, using a protocol modified from Turner *et al*. (3). Lymph nodes less than 4 mm in diameter were included in totality for frozen section; lymph nodes between 5 and 7 mm in diameter were bisected and each part was included for frozen section; lymph nodes equal or greater than 8 mm in diameter were cut into 4 mm slices and totally included for frozen section. Control sections were performed after formalin fixation and paraffin embedding; every paraffin block was totally cut into 4 μm-thick serial sections at 250 μm intervals. These sections were stained with hematoxylin and eosin (HE). If no metastasis was visible on HE staining, immunochemistry was performed on 3 sections separated by 500 μm as follows. A pool of monoclonal mouse anti-human cytokeratin antibodies was used from Dako, Baar, Switzerland (AE1-AE3, M3515; dilution 1:100) and from BioGenex, San Ramon, USA (anti-cytokeratin 8 and 18 (5D3), AM131; dilution 1:25), with microwave antigen retrieval and 3,3-diaminobenzidine as chromogen.

Metastasis was defined as a tumor infiltrate larger than 2 mm and micrometastasis defined as tumor involvement ≥0.2 mm but <2 mm. The presence of isolated tumor cells (ITCs) was defined as existence of single tumor cells or small cell clusters not greater than 0.2 mm according to the AJCC TNM Classification of Malignant Tumors, 6th Edition (5). All slides of SLNs frozen section (range from 2 to 5) and control section (rage from 10 to 15) were retrospectively reviewed by three experienced breast pathologists

Table I. *Clinicopathological characteristics of the patients with breast cancer.*

		Number
Patients		149
Age (years)	mean 55	range 21 to 90
Tumor stade	pT1 pT1a	3
	pT1b	33
	pT1c	80
	pT2	33
Tumor grade	1	46
	2	84
	3	19
Histology type	Ductal	119
	Lobular	15
	Tubular	7
	Other	8
Lymphovascular invasion	Yes	18
	No	131
Perineural invasion	Yes	10
	No	139
Hormonal status*	ER +/PR +	113
	ER +/PR –	17
	ER –/PR +	0
	ER –/PR –	19
MIB-1 index	<30%	118
	>30%	31
HER2/neu amplification$^{\pi}$	Amplification	21
	No amplification	128

*Estrogen receptor (ER) and progesterone receptor (PR) considered positive if Allred score >3. $^{\pi}$HER2 amplification determined by FISH with *HER2/CEP*17>2.

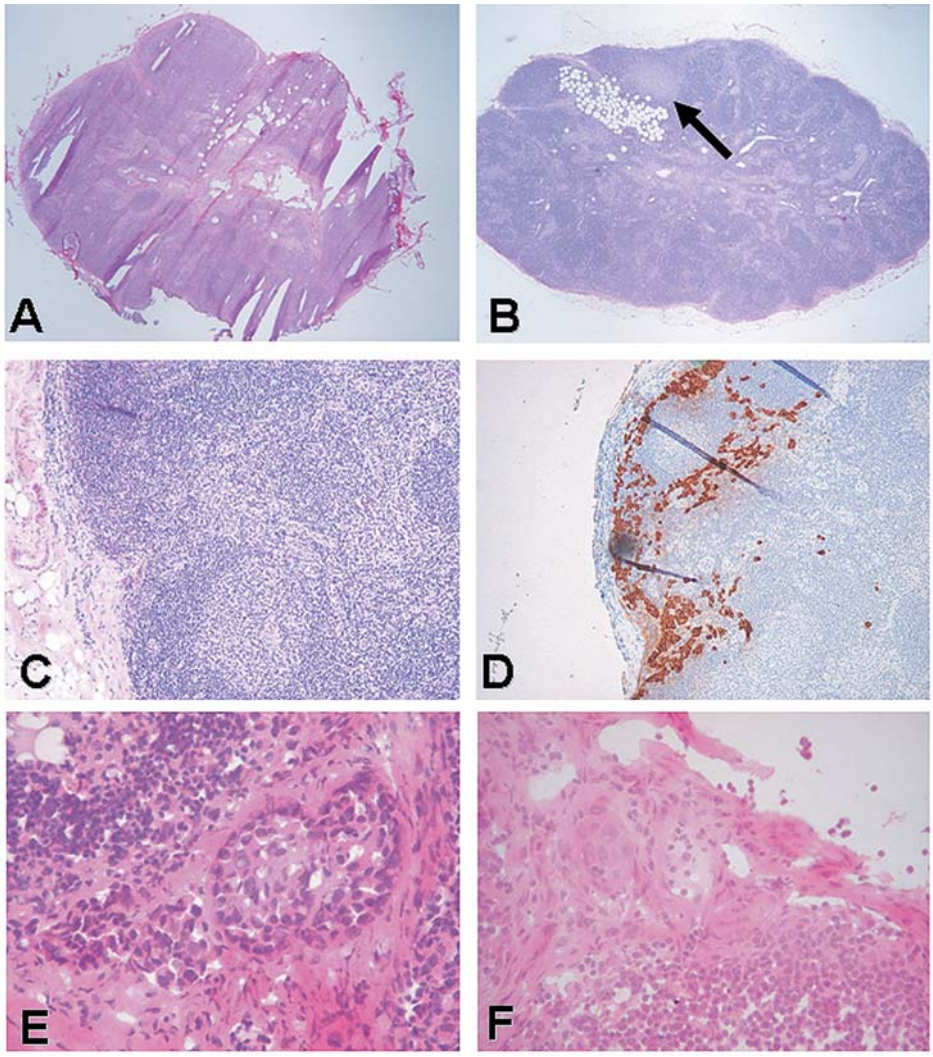

Figure 2. *Sentinel lymph node micrometastasis and isolated tumor cells. A, Frozen section with no micrometastasis at the intraoperative examination. B, Control section of the same sample showing a micrometastasis found on the last level, indicated with an arrow. C, Control with HE staining with no evidence of tumoral cells and in D, the anti-cytokeratin IHC. E and F, Two cases of isolated tumor cells found on frozen section with HE*

(JCT, JFE and MFP) and exact measurement in millimeter of the SLN metastasis or micrometastasis was made. In all reviewed slides, the final diagnoses were confirmed and we were not aware of the patient's outcomes.

Results

SLN metastases. Over a two year period, we performed the same SLN protocol as described in the Materials and Methods section. Of a total of 278 SLNs retained for this study, 63 were positive (22.7%): 41 were diagnosed using the intraoperative frozen section (65%) and 22 positive SLNs were found on control sections (35%).

Metastases greater than 2 mm were present in 42 SLNs: 35 diagnosed on frozen section and 7 on control sections (Figure 1A and 1B). SLN metastasis that were not diagnosed on frozen section were considered as false-negative SLN, so 7 out of 42. Of the seven false-negative SLN frozen sections, in 3 cases the slides were sub-optimal, rendering a correct diagnosis impossible; in 2 cases, the metastases were undiagnosed on frozen section and in 1 case the metastasis appeared only at the levels performed for the control sections. In one case of lobular carcinoma the lymph node metastasis was not seen but was revealed with cytokeratin IHC (Figure 1C and 1D).

Micrometastases were diagnosed in 10 SLNs: 4 on frozen section and 6 on control sections. There was 6 false-negative SLN on frozen section. The size of the micrometastases ranged from 0.25 mm to 1.9 mm. Of the 6 cases diagnosed

Table II. *Review of the literature on micrometastasis.*

Studies	Number of patients	Intraoperative technique	Final pathology technique	Sensitivity for micrometastasis (%)
Current study	161	HE	HE+IHC	40
Turner *et al.* (3)	278	HE	HE+IHC	28
Weiser *et al.* (17)	890	HE	HE+IHC	17
Brogi *et al.* (18)	133	HE	HE+IHC	20
Van de Vrande *et al.* (19)	615	HE	HE+IHC	61
Chao *et al.* (20)	200	HE and levels	HE+IHC	28
Grabau *et al.* (21)	108	HE and levels	HE+IHC	14
Leidenius *et al.* (22)	84	HE and levels	HE+IHC	46
Ryden *et al.* (23)	174	HE and levels	HE+IHC	9
Langer *et al.* (24)	648	HE and levels	HE+IHC	10
Celebioglu *et al.* (6)	102	HE	HE+IHC	35
		HE+IHC	55	
Total				30

HE: Hematoxylin-eosine; IHC: immunohistochemistry using anti-cytokeratin.

on control sections, 3 were found at successive levels (Figure 2A and 2B) and 3 only by IHC (Figure 2C and 2D). For the 3 cases diagnosed at successive levels, micrometastases were first found in section 1 of 5, section 2 of 4 and section 7 of 7. This last example illustrates the usefulness of serial sections for micrometastasis detection.

ITCs were found in 11 cases, two on frozen sections (Figure 2E and 2F) and 9 cases on controls sections, only by IHC. There was 9 false-negative SLN on frozen section.

Sensitivity and specificity. The specificity of frozen section analysis was 100% for metastasis, micrometastasis and ITC. The sensitivity of frozen section in metastasis was 83.3% and decreased to 40% for micrometastasis and 18.2% for ITC. The negative predictive value was 91.9% for metastasis, 80.6% for micrometastasis and 70% for ITCs. The false-negative rate was 16.7% for metastasis, 60% for micrometastasis and 81.8% for ITCs. There was no false-positive case.

Axillary lymph node dissection. An axillary lymph node dissection was performed in 25 out of the 28 patients (89.3%) with SLN metastasis: 22 during the same surgery and 3 during a second operation. In 10 cases, there were positive axillary lymph nodes. Three out of the nine patients with SLN micrometastasis underwent axillary dissection: 1 during the same operation and 2 in a second intervention. All axillary lymph nodes were negative. Only 1 out of the 10 patients with SLN ITCs underwent axillary lymph node dissection during the same operating time, which one was positive.

Extracapsular SLN infiltration. Interestingly, of the 29 axillary dissections performed, 11 patients had axillary lymph node metastasis. Of them, in 7 cases (63.6%) the SLN had metastatic extra capsular growth and adipose tissue infiltration. Furthermore, in the 18 cases of negative axillary dissection, only 4 cases (22.2%) had SLN metastatic extra capsular growth and adipose tissue infiltration.

Discussion

While the detection of rate of SLN and the accuracy of SLN histology as a predictor of axillary lymph node status have been extensively studied (6, 8-10), information about the utility of intra-operative frozen section for SLN micrometastasis is sparse. In this study of 278 SLN frozen sections, 63 SLNs were infiltrated by tumoral cells including 66.7% SLN metastasis, 15.9% of SLN micrometastasis and 17.4% of ITCs (Figure 3).

We demonstrated a sensitivity of 83.3% for this method in detecting metastasis, but a lower sensitivity, 40%, for micrometastasis. Previous studies published on the sensitivity of frozen section for micrometastasis (Table II) confirmed the variable rate, ranging from 10% to 61%. This large variation depends on the different histopathological techniques used for frozen section analysis, including the cutting intervals, the number of sections and the use of IHC on control, as well as if perioperative IHC was used (6). In our opinion, IHC during intraoperative SLN examination is time consuming and not practicable for most small institutions. Recently, reverse-transciption polymerase chain reaction (RT-PCR) assay for cytokeratin-19 and mammaglobin are beginning to be evaluated during intraoperative SLN exam (7-9).

At the present time, no clear international directive exists regarding the use of frozen section. In 2004, the European Working Group for Breast Screening Pathology (EWGBP)

reported a high degree of variability among European pathology institutes for SLN examination (10). Two years later, Cserni *et al*. and the EWGBSP formulated guidelines which are in accord with our protocol (11, 12). They stipulated that intraoperative assessment of SLN can be carried out by frozen section and/or imprinting cytology based on institutional resources and preferences, since both methods have similar sensitivity and specificity for metastasis detection.

Our SLN protocol for frozen and permanent sections consists of 4 mm slices for frozen section analysis followed by consecutive sections at 250 μm intervals for histology and IHC until the material is exhausted. We show that this protocol results in good sensitivity and specificity compared to the literature for detection of micrometastsis (Table II). For metastasis, sensitivity was of 83.3%, while it was 40% for micrometastasis, which is better than the published mean of 30%. After fixation, analysis of serial sections resulted in detection of 50% more cases of SLN micrometastasis. In one case micrometastasis was obtained only on the last section, which is an argument for exhaustion of the lymph node on permanent sections. Furthermore, IHC increased the sensitivity, resulting in the diagnosis of 50% more micrometastasis, confirming data of the literature (13, 14) and supporting the usefulness of IHC on HE-negative lymph node samples.

With regards to axillary lymph node dissection, this was positive in 10/25 cases of SLN metastasis, 0/3 cases in SLN micrometastasis and 1/1 case in SLN ITC. These results should be interpreted with caution because this small series of axillary lymph node dissection for micrometastasis and ITC is not representative of the literature data with an involvement of 3% to 21% for micrometastasis (1, 15) and from 1.6% to 14% for ITC (1, 16).

An important point is the higher prevalence of axillary lymph node metastasis in cases of SLN showing capsular rupture (63.6% *versus* 22.2%), supporting the recent evidence from the literature concerning the biological significance, in breast carcinoma, of perilymph node fat tumoral invasion and an increased axillary lymph node metastasis (15-17).

Conclusion

Frozen section analysis is a reliable and safe procedure for diagnosis of SLN metastasis, allowing immediate axillary dissection in a one-stage surgical procedure. Furthermore, our SLN protocol demonstrated a good sensitivity for micrometastasis detection, although as yet, this is not a reliable indication of axillary lymph nodes dissection.

Acknowledgements

We thank Dr. P. Schafer for constructive discussion and Dr. T McKee for his critical review of the manuscript.

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Received July 27, 2009
Revised October 12, 2009
Accepted October 15, 2009