Intraoperative estimation of sentinel lymph nodes in breast cancer by imprint cytology

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Summary

Background: Frozen section biopsy has been widely used for intraoperative diagnosis and evaluation of sentinel lymph nodes, so a decision can be made regarding whether to perform axillary clearance during primary surgery. This study aims to discuss the reliability of a simpler and faster method – touch imprint cytology – in the interpretation of metastasis from breast cancer. *Methods:* A retrospective review of 41 sentinel lymph node biopsies from patients with breast cancer were examined by intraoperative imprint cytology using rapid Diff-Quick staining. Paraffin-embedded permanent sections were examined using hematoxylin and eosin stained sections from the sentinel lymph nodes in collaboration with the employment of an anti-cytokeratin antibody. *Results:* Sixteen of all sentinel nodes harbored metastases in the paraffin sections, of which all 16 were identified by imprint cytology (sensitivity 93%). *Conclusion:* Touch imprint cytology is a fast and reliable alternative for intraoperative evaluation of sentinel lymph nodes in breast cancer patients.

Key words: Breast cancer; Sentinel lymph node; Imprint cytology.

Introduction

Axillary lymph node examination is necessary for breast cancer staging however it has relatively high morbidity. Sentinel node (SLN) biopsy was developed to evaluate nodal status without removing most of the axillary contents [1]. SLN is the first node in the lymphatic chain that receives the primary lymphatic flow. When the SLN is tumor free, the risk that any other axillary node is involved is near zero and complete dissection can be avoided. The SLN can be examined intraoperatively by frozen section and touch imprint smears. Some studies have found lower sensitivity of imprint diagnoses than frozen sections, whereas others have found it similar to that of frozen sections [1].

Intraoperative imprint cytology is a method by which imprints of a cut surface of a lymph node can be examined rapidly in the operating room for evidence of metastatic disease [2]. In theory intraoperative imprint cytology should be advantageous over intaoperative frozen sections because it is faster, cheaper, does not waste tissue in the cryostat, does not introduce freezing artifacts into the tissue, and avoids the inherent problems associated with attempting to cut frozen sections from lymph nodes that have been largely replaced by fat [2]. These qualities have resulted in many investigators using intraoperative imprint cytology to evaluate SLNs in breast carcinomas [2-6].

Our purpose in the current study on the cytopathologic procedure for intraoperative diagnosis was to assess it by analyzing the diagnostic accuracy of the sentinel node in patients with breast cancer.

Patients and Methods

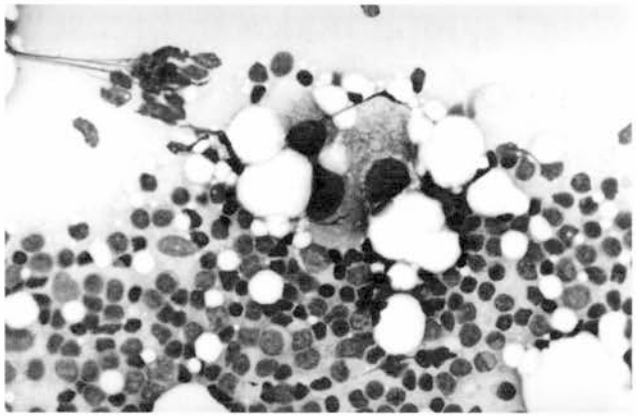
Between January 1999 and April 2003, 65 patients with breast carcinoma underwent lymph node biopsy at the Obstetrics and Gynecology Department of the University of Thrace Medical School. Forty-one sentinel lymph nodes from 18 of these patients were intraoperatively evaluated by touch imprint cytology; the remaining cases were not included in the current study. The median age of patients was 58 (range 30-78) years. The median pathological tumor size was 2.5 (range 0.7-3.9) cm.

SLN biopsy was performed according to the Dutch guidelines using patent blue dye (Blue patent V, Guerbet, Aulnay-sous-Bois, France) for lymphatic mapping. The dye was injected around the tumor or around the scar where there had been previous excision biopsies [7]. At surgery, a maximum of five SLNs were sent to the Cytopathology Department for examination by touch imprint cytology.

If more SLNs were identified, only the most suspicious were imprinted. Each SLN was bisected, after which cells from both sides were scraped with a scalpel blade onto a slide. Then the material was smeared onto a second slide. The second slide, the touch imprint slide, was stained with the rapid Diff-Quick stain. Each SLN was bisected along the long axis. Care was taken to obtain complete cross sections of the maximum diameter, preferably including the hilum and the marginal sinus. For each lymph node half, a pair of imprints was made by gently touching the cut surface of the SLN to a glass slide. One imprint from each pair was air dried and stained with the Diff-Quik stain. The second imprint from each surface was immediately fixed in 95% ethanol for 3 min and then stained with the Papanicolaou stain. Cytologically, SLN metastases were detected as cohesive

Revised manuscript accepted for publication April 24, 2008

Fig. 1



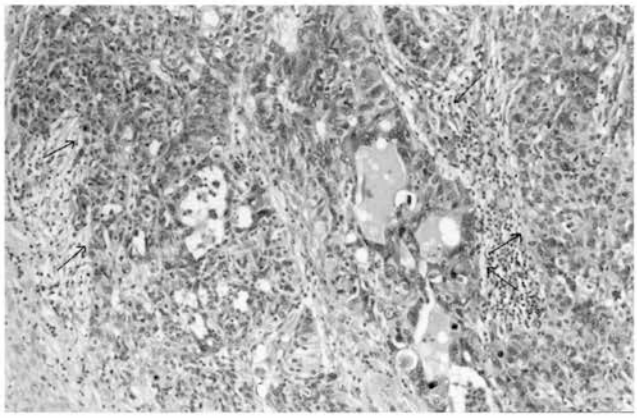


Fig. 2

Figure 1. — Sentinel lymph node: touch preparation. Dispersed neoplastic cells originating from a breast primary lying against lymphoid cellularity (Diff-Quick stain x 200).
Figure 2. — Neoplastic cell clusters in the sentinel lymph node (arrows) (H&E x 200).

epithelial cells or isolated metastatic cells scattered throughout a background of lymphoid cellularity (Figure 1). Assessment of SLN imprint cytology preparations was performed by an experienced cytopathologist, who assigned each case to one of two possible diagnostic categories: SLN negative for metastatic tumor or SLN positive for metastatic tumor. To achieve a more complete cytologic evaluation, preparations from the diagnostic procedure of the primary lesion (fine-needle cytosmears) were retrieved and used as a backup support to help analyze the cytomorphology of the SLNs. Processing and assessment of imprint cytology preparations on Diff-Quick stains was completed within 8-10 min.

Paraffin-embedded sections stained with hematoxylen-eosin (Figure 2) and a cytokeratin immunohistochemical (CK-IHC) preparation (clone product MNF-116, Dakopatts) was performed postoperatively on all samples as well.

Results

In a number of 41 sentinel nodes examined by cytology, there were 16 reported as positive. The mean number of SLNs was 2.5 (range 1-5). Intraoperative cytological examination was performed in all cases. Sixteen of all sentinel nodes were judged to be tumor-positive in paraffin sections. There was one false-negative result by cytology which resulted from a lobular invasive carcinoma.

Our method showed high diagnostic accuracy with 93% sensitivity, 96% specificity, positive predictive value 93% and negative predictive value 96%.

Cytokeratin immunostaining was performed on all samples and confirmed the results mentioned above.

Discussion

The concept of SLN biopsy in the treatment of breast carcinoma has increasingly evolved from its first description by Giuliano *et al.* in 1994 to its current routine practice in many centers [8]. The ability of the SLN to predict the histopathology of the regional lymph nodes was proven by Turner *et al.* in 1997 [9].

The aims of SLN biopsy are to obviate the need of lymph node dissection in node-negative patients, and possibly to improve lymph node staging in other patients by increasing the detection of micrometastases to lymph nodes or of metastases to lymph nodes not included in the standard specimen of lymph node clearance, such as apical, intramammary, or internal mammary nodes. To achieve the first goal there must be a dependable method for the intraoperative evaluation of the SLN, so that the host can be spared axillary dissection performed during primary surgery, without exposing many patients to a second operation because of undetected SLN metastases. Viale *et al.* [10], used serial frozen sections of SLNs for intraoperative examination in a series of 155 patients in whom complete dissection was also performed. They concluded that the procedure was effective in predicting axillary nodal status. SLN metastases were detected in 45% of their patients, but they noted five hosts with false-negative results (negative SLN and positive complete dissection). Perhaps the problem with frozen section analysis is that a significant part of the SLN is wasted and cannot be reprocessed later on to provide us with a more definitive result with the use of cytokeratin immunohistochemistry. In our cases there was one cytologically false-negative result. The case resulted from a lobular invasive carcinoma. Metastatic lobular carcinoma is difficult to identify in SLN because of its low-grade cytomorphology, its tendency to infiltrate lymph nodes in a single-cell pattern, and because individual cells can resemble lymphocytes. We are unaware of any large published studies, using any technique, to evaluate SLN for lobular carcinoma [11-18]. There was also a false-positive result resulting from a benign glandular inclusion.

In conclusion imprint cytology is a sensitive and specific method in the diagnosis of sentinel lymph node metastasis of breast cancer.

Further investigations on larger numbers of samples are needed to establish the method in common clinical practice.

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