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Intraoperative imprint cytology of sentinel lymph nodes in breast cancer: initial experience and lessons learnt in establishing a new practice

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Summary

Aims: The initial 18 months experience of performing intraoperative imprint cytology for patients with breast cancer undergoing sentinel lymph node biopsy is described for a single institution. The learning process is compared with published results from institutions with many years of experience in order to assess progress in reaching those ideal results, and the methodology used by these institutions is reviewed.

Methods: A retrospective review was undertaken of the intraoperative imprint cytology results from 103 patients with breast cancer (yielding a total of 170 lymph nodes) who underwent imprint cytology of their sentinel lymph node. The intraoperative imprint cytology results were compared with the final histopathological results. Details regarding the primary tumour characteristics and metastatic deposit size were recorded.

Results: The sensitivity for imprint cytology was 31.1%, with a specificity of 100% and overall accuracy of 77.8%. The sensitivity for detecting macrometastases (>2 mm diameter) was 61.9% and the sensitivity for micrometastases (<2 mm diameter) and including isolated tumour cells was 4.2%.

Conclusions: The differences in sensitivity in comparison with many studies in the literature are multifactorial, and include technical aspects, such as the methodology used in the final histopathological and intraoperative evaluation of the sentinel lymph nodes, interpretative difficulties, and much lower case numbers. Furthermore, these numbers represent early experience and methods to improve sensitivity and overall accuracy are detailed in this paper.

Key words: Cytology, breast, imprint cytology, sentinel lymph node.

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INTRODUCTION

The use of intraoperative imprint cytology appears to have been first described in the literature by Dudgeon and Patrick in 1927, who examined a series of 200 cases using this method for rapid diagnosis of tumours.[1] Subsequently, the method received little attention in the literature until 1978 when Suen *et al.*[2] reported a large series of fresh surgical specimens (1306 cases), and compared them to frozen section (which was done concurrently). Their results supported the use of imprint cytology with a false positive rate of only 0.24% and a false negative rate of only 6% and overall accuracy of 93.6%. These numbers are impressive and support the use of the technique.

The use of sentinel lymph node biopsy in the management of breast cancer was pioneered in the early 1990s.[3,4] It is now acknowledged and accepted as standard management practice in many centres worldwide. Since this time, we have entered a new era in intraoperative diagnoses, which brings with it new challenges for the pathologist. One of these challenges is intraoperative evaluation of sentinel lymph nodes. This procedure permits an axillary dissection to be performed during the initial operation if the node is positive.

The benefits of this procedure are clear, and intraoperative diagnosis in these patients has the added advantage of enabling the patient to have a one-step procedure, thereby eliminating the risk and inconvenience of a second surgical procedure as well as saving cost and theatre time. Intraoperative imprint cytology is a quick and effective method and clearly does not compromise the examination of paraffin-embedded sections, which are the reference standard.

METHODS

Patients and data collection

A search for patients who had undergone sentinel lymph node biopsy for breast cancer over the past 18 months was made through the PathNet system (Cerner, USA) with the SNOMED diagnostic retrieval function, using 'sentinel lymph node' and 'breast' as search criteria (freetext string). The reports of these cases were then reviewed and those cases in which imprint cytology was reported were selected, yielding 103 consecutive cases with a total of 170 lymph nodes. In these cases the reports were reviewed to obtain details of intraoperative imprint cytology result and final sentinel lymph node diagnosis, including size of deposit and whether the metastasis was detected in the H&E stained sections or on immunohistochemistry.

In those patients for which the result was found to be a false negative (i.e. positive for metastatic disease on final histopathology), the original imprint slides were reviewed (WJ and NP). No false positive cases were identified. The imprint slides of cases in which a positive intraoperative diagnosis was obtained were also reviewed (WJ and NP). In these cases details of patient age and primary tumour characteristics were also obtained.

Surgical protocol

A standard protocol was used to identify the sentinel lymph node in our institution and the procedure was performed in all cases by surgeons who

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were certified to undertake the procedure. In all patients, the tumour bed was infiltrated using radioactive colloid and a lymphoscintigram was obtained prior to the surgical procedure. At operation, blue dye was injected around the tumour bed for visual identification and, using a gamma probe for confirmation, sentinel lymph nodes were harvested and sent fresh to the pathology department for intraoperative sentinel lymph node assessment.

In cases where the lymph node was reported to be positive for malignancy, the patients underwent axillary lymph node dissection.

Intraoperative imprint cytology method

The lymph nodes were received fresh in the pathology department. Following trimming of excess fatty tissue they were sectioned into 3 mm slices (or bisected if under 6 mm in maximum dimension). Touch imprints were made on glass slides of each side of each slice of the lymph node and these were then stained using a rapid H&E stain (Fig. 1). A few of the initial cases were also stained with a Diff-Quik stain. These cases were reported by cytopathologists with varying experience in breast cytology (some of the pathologists rated breast cytology as one of their primary interests and had a high degree of expertise in the area), as either positive or negative (cases that were called atypical or suspicious were regarded as negative, as this did not effect a change in intraoperative management). In a few of the cases (Cases 9 and 3 of the true positive cases and Case 23 of the false negative cases), at the discretion of the reporting pathologist when only a few atypical cells were seen, a 'scrape smear' was made, using a fresh scalpel blade to gently scrape the surface of suspicious slices and then smear this material on glass slides to increase the cell yield. The intraoperative imprint cytology (IOIC) process took approximately 7–10 min per lymph node.

Histopathological evaluation

Once the intraoperative procedure was completed, the lymph node slices were fixed in 10% neutral buffered formalin overnight, followed by paraffin embedding of the entire specimen. Our protocol for assessment of sentinel lymph nodes involves H&E sections taken at six levels of each slice, 150–200 μm apart, followed by a further level for immunohistochemistry which was done routinely regardless of whether the node was positive on the H&E section. The immunohistochemical stains consisted of a keratin stain (AE1/AE3) and an epithelial membrane antigen (EMA) stain in the earlier cases, and AE1/AE3 alone in the later cases (the protocol was amended as there was found to be little gain and poor staining/artefacts with the EMA stain).

Statistical methods

Each case was classified as being true positive (TP; i.e., concordant results in the IOIC and final pathological evaluation), false positive (FP; i.e., where IOIC is positive and the final histopathological evaluation is negative), true negative (TN) or false negative (FN). Only those cases in which there was metastatic disease proven on final histopathological diagnosis were classified as true positive.

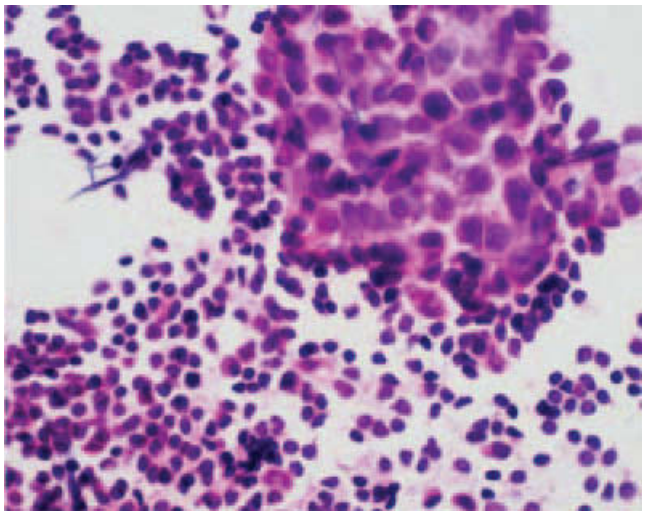

Fig. 1 Imprint from a true positive case of high grade ductal carcinoma showing a cohesive sheet of malignant cells (H&E, ×400).

The following formulae were used to calculate statistical parameters:[5]

$$\text{Sensitivity} = TP/(TP+FN)$$
$$\text{Specificity} = TN/(TN+FP)$$
$$\text{Accuracy} = (TP+TN)/(TP+FP+TN+FN)$$

RESULTS

There was a total of 103 patients (yielding a total of 170 lymph nodes), who underwent sentinel lymph node biopsy procedures with intraoperative imprint cytology evaluation. There were 11 patients (yielding 14 lymph nodes) with true positive results (Table 1). False negative results (Table 2) were seen in 29 patients (yielding 31 lymph nodes). The remaining 91 patients (125 lymph nodes) were true negatives. No false positive cases were identified.

All false negative and true positive cases were reviewed and the adjusted figures following review are seen in Table 3.

No false positive cases were diagnosed, although one case was diagnosed as positive on imprint cytology and a subsequent frozen section (this was the only case in which a frozen section was done) revealed an axillary rest of breast tissue, so the final intraoperative diagnosis was

TABLE 1 True positive cases

True positive	Imprint cytology	Deposit size (mm)	H&E	IHC	Tumour type	Size (mm)	Grade	Cytology review
1	2 positive LN	Both 2	+	+	Lobular, classical	55	2	Both positive
2	2 positive LN	7, 8	+	+	Ductal nst	18	1	Both positive
3	1 positive LN	5	+	+	Ductal nst	15	1	Positive
4	1 positive LN	19	+	+	Ductal nst	40	3	Positive
5	1 positive LN	8	+	+	Ductal nst	30	3	Positive
6	1 positive LN	9	+	+	Ductal nst	9, 8, 2	3	Positive
7	1 positive LN	2	+	+	Ductal nst	16	2	Positive
8	1 positive LN	10	+	+	Ductal nst	35	2	Positive
9	1 positive LN	7	+	+	Ductal nst	7	2	Positive
10	2 positive LN	5, 10	+	+	Metaplastic ca	22	3	Both positive
11	1 positive LN	1	+	+	Ductal nst	42	3	Positive

Total patients, 11; total number of LN, 14; total true positive cases, 14.
IHC, immunohistochemistry; LN, lymph node; nst, no special type; ca, carcinoma.