Testing the feasibility of intra-operative sentinel lymph node touch imprint cytology

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ABSTRACT

INTRODUCTION Sentinel lymph node biopsy is emerging as the new standard for axillary staging in breast cancer. Intra-operative assessment of the sentinel lymph nodes allows immediate completion of axillary dissection during the same anaesthetic. This project was a quality assurance practice to establish feasibility, time-to-report, as well as accuracy of performing intra-operative assessment of sentinel lymph nodes using touch imprint cytology in our centre.

PATIENTS AND METHODS This prospective audit included 146 sentinel lymph nodes from 74 consecutive patients with invasive breast cancer. All patients underwent axillary sentinel lymph node biopsy using combined blue dye and radiocolloid technique. Results of intra-operative touch imprint cytology using haematoxylin and eosin staining were compared with the definitive histopathology results.

RESULTS Mean time to report touch imprint cytology was 25.7 ± 6.4 min (range, 15–40 min). Histopathology demonstrated metastasis in 25 sentinel nodes from 17 (23%) patients. Intra-operative touch imprint cytology detected 15 nodes in 11 patients, giving a sensitivity of 60% (nodes) and 66.7% (patients) and specificity of 99.2% (nodes) and 98.2% (patients) based on the number of nodes and patients involved, respectively. Touch imprint cytology failed to show metastatic involvement in 10 nodes from 6 patients; of these, five nodes had micrometastasis (< 2 mm) and the other five had macrometastasis. One touch imprint cytology positive node contained isolated tumour cells only. Using intra-operative touch imprint cytology made a change in treatment of 11 (14.9%) patients, and spared second axillary procedure in 7 (9.4%) patients.

CONCLUSIONS Intra-operative sentinel lymph node assessment using touch imprint cytology is feasible within a busy NHS practice. We now offer touch imprint cytology to patients following appropriate counselling.

KEYWORDS

Touch imprint cytology – Sentinel lymph node – Breast cancer

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Sentinel lymph node biopsy (SLNB) is replacing routine axillary dissection and is the new standard for axillary staging in early breast cancer supported by some uni-centre and multi-centre randomised controlled trials.^{1,2} Sentinel lymph nodes are generally evaluated postoperatively, requiring 5–7 days for pathological assessment. Axillary clearance remains standard treatment for patients with axillary metastases identified on sentinel lymph node biopsy.³

Intra-operative assessment of the sentinel lymph nodes allows immediate and therapeutic completion of axillary dissection during the same anaesthetic session if nodes are found to be tumour-positive.⁴ In addition, delayed axillary clearance after sentinel lymph node biopsy can be technically challenging with the potential risk of increased morbidity

(particularly infection and seroma) and lower overall nodal yield. Intra-operative touch imprint cytology (TIC) will also help by avoiding the economic and psychological costs of a second operation and prevents further delay in the commencement of adjuvant systemic treatment, chemotherapy and radiotherapy for high-risk patients with confirmed node involvement.⁵

Frozen-section examination and TIC are two methods used for intra-operative evaluation of sentinel lymph nodes. Although frozen section examination is more expensive than TIC and has disadvantages (*i.e.* it is more difficult to perform, requires more time for processing and is associated with tissue loss, which might affect further histological examinations) it is still more commonly used than TIC.⁶

Table 1 Touch imprint cytology versus histopathology results based on the number of the nodes and patients studied

	Number of nodes		Number of patients	
	TIC positive	TIC negative	TIC positive	TIC negative
Histopathology positive	15 (10.3%)	10 (6.8%)	11 (14.9%)	6 (8.1%)
Histopathology negative	1 (0.7 %)	120 (82.2%)	1 (1.3%)	56 (75.7%)

TIC, touch imprint cytology.

Touch imprint cytology is quicker to perform, requires minimal tissue preparation, can be done using simple equipment and will provide acceptable details for interpretation. However, it requires experienced cytopathologists.

This is a quality assurance audit to establish overall feasibility, time-to-report, as well as accuracy of performing intra-operative assessment of sentinel lymph nodes using TIC in our centre.

Patients and Methods

This prospective audit included 74 patients with invasive breast cancer, who were treated in the Royal Marsden Hospital in London, UK between May 2006 and February 2007. All patients had pre-operative diagnosis of invasive breast carcinoma with clinically normal and image-negative axillae. They underwent axillary sentinel lymph node biopsy using combined technique of blue dye (Patent Bleu V; Guerbet Laboratories, Issy Les Moulineaux, France) and radiocolloid identification (^{99m}Tc -Labelled Nanocoll; Amersham Health, Little Chalfont, UK), with routine pre-operative sentinel node imaging.

A sentinel lymph node was defined as any blue or hot node with a total count of > 10% of the highest node count. Sentinel lymph node biopsy was performed before removal

of the primary tumour. Intra-operative TIC using standard hematoxylin and eosin staining of the freshly taken sentinel nodes were performed for all patients.

Sentinel nodes were bivalved along their long axis and imprinted directly on to coated microscopy slides. At least two imprints from each bivalved node were made on a separate microscope slide. Sentinel lymph node removal-to-report time was recorded in all patients. Indeterminate or suspicious TIC results were considered as negative results.

Results of the TIC were compared with the definitive postoperative histopathology results. Postoperative histopathology assessment included hematoxylin and eosin staining for all of the specimens. Immunohistochemical staining was used for equivocal results.

Results

In this study, 146 nodes were examined from 74 patients with invasive breast cancer (average node yield, 2 nodes per patient). Mean age of the patients was 49.59 ± 13.6 years (range, 23–78 years) and mean time-to-report TIC was 25.7 ± 6.4 min (range, 15–40 min). Touch imprint cytology and postoperative histopathology results are shown for number of nodes and patients in Table 1.

Postoperative histopathology results demonstrated metastasis within 25 sentinel lymph nodes from 17 (23%) patients. Seventeen nodes showed macrometastasis (> 2 mm) and eight nodes showed micrometastasis involvement (< 2 mm). Intra-operative TIC detected 15 of the involved nodes in 11 patients, giving this technique sensitivity of 60% and 66.7% and specificity of 99.2% and 98.2% based on the number of lymph nodes and patients involved, respectively.

Sensitivity, specificity, positive predictive value and negative predictive value have been calculated separately based on the number of the sentinel lymph nodes and patients and are summarised in Table 2.

Intra-operative TIC failed to show metastatic involvement in 10 examined lymph nodes from six patients; of these, 5 nodes had micrometastasis (< 2 mm) and the other 5 had macrometastasis (> 2 mm) on postoperative hema-

Table 2 Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) based on the number of sentinel nodes and patients studied

	Based on number of patients	Based on number of nodes
Sensitivity	66.7% (0.43–0.83)	60.0% (0.40–0.76)
Specificity	98.2% (0.90–0.99)	99.2% (0.95–1.00)
PPV	92.3% (0.66–0.99)	93.8% (0.71–0.99)
NPV	92.3% (0.80–0.95)	92.3% (0.86–0.95)

Figures in parenthesis are 95% confidence intervals.

toxylin and eosin analysis. One TIC positive node (the only sentinel lymph node removed for that patient) contained isolated tumour cells only.

Discussion

Axillary status has been proven to be the strongest prognostic factor for women with early breast cancer.^{7,8} Accurate staging has traditionally been accomplished with a Level I and II axillary lymph node dissection (ALND).

Although ALND provides good prognostic information as well as local control in patients with metastatic axillary disease, morbidity can be high (*e.g.* lymphoedema, loss of function, *etc.*). Consequently, less invasive staging with lower morbidity risk (sentinel lymph node biopsy) has been promoted.

Multiple trials have documented that lymphatic mapping and subsequent SLNB reflects the stage of axillae.^{9–11} However, the extent to which SLNB provides local control is currently unknown. Therefore, completion ALND is still indicated for patients with a node-positive specimen.⁵

Intra-operative assessments of the sentinel lymph nodes to ascertain lymphatic metastasis allows immediate completion of ALND during the same operative session. Different advantages of intra-operative assessments of the sentinel nodes include: (i) avoiding the technical challenge of ALND after sentinel lymph node biopsy; (ii) its increased morbidity risk; (iii) avoiding economic and psychological costs of a second operation; and (iv) preventing further delay in systemic treatment and radiotherapy.

However, it has its own disadvantages as well: the risk of over treatment, uncertainty for patient regarding outcome of surgery, difficulties in consenting the patients, planning theatre lists, in-patient beds, and its requirement for availability of cytopathological expertise.

Many strategies have been investigated for intra-operative assessment of the sentinel nodes including extensive evaluation of intra-operative frozen sections, intra-operative TIC and intra-operative TIC with the adjunct of immediate immunohistochemical staining for cytokeratin. Different studies have compared these techniques with regard to the following three considerations – speed (required time-to-report), reliability and cost-effectiveness.^{12,15}

In our study, time-to-report TIC fitted comfortably with average times for completion of breast surgery. Although the average time taken for reporting TIC appears acceptable, the additional anaesthetic time in a minority of patients must be offset against the increased operating time involved in a delayed axillary clearance.

Accuracy of TIC in predicting node involvements is ‘operator dependent’ as reflected in very variable range of reported accuracy for this method in different studies.⁶ Reported sensitivity of TIC varies from 33.3 to 100%.^{14–16}

Overall sensitivity and specificity of TIC in the present study based on the number of the patients was 66.7% and 98.2%, respectively. In this study, the pressure of immediate intra-operative reporting and the fact that the plan for continuation of surgical procedure would be based on the report, did not affect the accuracy. Our TIC accuracy was consistent with other studies.^{6,17}

Sensitivity of TIC in our practice may be affected by pre-selection of gross node-positive patients by axillary ultrasound. In these situations, we normally proceed with ALND directly. All of our patients had pre-operative clinically normal and image-negative axillae. Touch imprint cytology sensitivity in unselected patients would be much higher.

Many of our patients have early disease so the burden of nodal disease is small and, therefore, less amenable to TIC. Intra-operative TIC failed to show metastatic involvement in 10 examined lymph nodes from 6 patients; of these, 5 nodes had micrometastasis (< 2 mm) on postoperative hematoxylin and eosin analysis.

Touch imprint cytology sensitivity is dependent on the burden of disease within a node. In early disease, the nodal metastatic cell count is, therefore, relatively ‘low’ demonstrating the limitations of TIC. Hence, in these situations, there is a drive towards more sensitive techniques of nodal analysis such as polymerase chain reaction techniques.^{18,19}

A false-positive intra-operative TIC would make the surgeon proceed with ALND while it might not be necessary, thereby the initial intention of SLNB to decrease morbidity caused by ALND would not be achieved.⁶ In the current study, indeterminate or suspicious TIC results were considered as negative to maintain high specificity and avoid unnecessary ALND.

In our practice, pre-operative axillary scanning selects out gross node-positive patients so the benefits of TIC in reducing the number of second operations is potentially limited. Using intra-operative TIC changed treatment in 11 (14.9%) patients, and spared second axillary procedure in seven (9.4%) patients. The remaining four patients still required another surgical intervention for positive margins. However, they proceeded with axillary clearance at an earlier time and in the setting of their initial operation. One patient had an ALND for isolated tumour cells.

Conclusions

Intra-operative sentinel lymph node assessment using TIC is feasible within a busy NHS practice but it only changed management in 15% of our patients. Therefore, its application needs careful consideration. We now offer TIC to suitably counselled patients. Patients predicted to have significant risk of node-positive axillae, considering pre-operative core biopsy results and tumour size, patients with high risk of anaesthesia (American Society of Anesthesiologists [ASA] Class

> III) and patients undergoing mastectomy who are not likely to require further breast surgery are examples of patients who would benefit more from intra-operative TIC.

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Erratum

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A new technique for ensuring local anaesthesia in tunnelled central venous catheters

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We apologise for the incorrect spelling of Mr Ram's middle name in the above Technical Note.

The correct name is Daya, as given above.