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A Comparative Study between Intraoperative Scrape Imprint Immunocytochemistry and Serial Section with Combined H&E Staining and Immunohistochemistry for the Detection of Breast Cancer Cell Metastasis in the Sentinel Lymph Node

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Abstract

Background: **The multidisciplinary approach for the improvement of the accuracy of sentinel lymph node (SN) examination is beneficial as it is a less invasive procedure for breast cancer patients . The trend in avoiding axillary dissection will decrease the operative morbidity. The intraoperative technique for the detection of tumor cells in SN by imprint immunocytochemistry (ICC) was studied to avoid axillary dissection.**

Objective: **The aim of this study was to compare the results of intraoperative scrape imprint ICC with the results of serial section stained by combined hematoxylin-eosin (H&E) and subsequent immunohistochemistry (IHC). The accuracy, sensitivity and specificity of both methods were analysed.**

Material and Methods: **From April 2005 to March 2006, 33 patients with breast cancer were enrolled. The sentinel lymph nodes were removed and sent to pathologists for scrape imprint cytology. The anticytokeratin ICC was used to detect metastatic cancer cells. The sentinel lymph nodes were then routinely examined by H&E staining. If the cancer cells were not detected by the routine H&E study, the negative nodes were then serially sectioned at 20 μm and stained by anticytokeratin IHC to rule out the false negative results. The axillary contents of all cases were removed following the SN dissection for complete pathologic examination to confirm the SN node examination results.**

Results: **Seventy-two sentinel lymph nodes were removed from 33 patients with operable breast cancer. The results of imprint cytology were compared with those of the serial section of SN stained using the combined H&E and IHC methods. The sensitivity of intraoperative SN imprint ICC was 33%, the specificity 94% and the accuracy 66%. The positive predictive value of the imprint ICC was 83% and the negative predictive value 63%.**

Conclusions: **The intraoperative SN imprint ICC in this study was found to have 33% sensitivity, 66% accuracy and low negative predictive value when compared with serial section with combined H&E and IHC . The intraoperative SN imprint ICC alone is not sufficient to be used as a guide to avoid axillary lymph node dissection in operable breast cancer.**

Key words: sentinel lymph node, immunocytochemistry, breast cancer

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Introduction

Axillary lymph node metastasis in early breast cancer patients is one of the most important prognostic factors for recurrence and survival. The American Joint Cancer Committee in 2003 had modified the staging system of breast cancer.[1-3] The classification was dependent on the type of metastasis and the number and location of axillary lymph nodes. A method of sentinel lymph node (SN) biopsy was suggested about 30 years ago but substantial numbers of patients have been evaluated for approximately the last 10 years.[4] The SN biopsy has been widely used for the detection of cancer cell metastasis in breast cancer. The trend of breast cancer pattern has changed to more early-stage disease since the screening mammogram has been generally accepted. The greater accuracy in the detection of cancer cells in the SN has been used as the basis for prediction of axillary lymph node metastasis. When the SN is negative, the entire axilla is assumed to be negative for metastasis and the total axillary dissection is avoided.[5,6] The less invasive axillary assessment reduces the complications of axillary lymph node dissection.[7-9]

The negative result in the detection of cancer in the SN is the challenging guide for avoiding axillary dissection. False negative rate of SN biopsy is about 5-10% in routine pathological examination,[10-12] but for the frozen sections the false negative rate may vary up to 32%.[13] The accuracy of rapid detection of cancer cell during the operation is improved by the use of immunohistochemistry (IHC) and immunocytochemistry (ICC) staining techniques. Multiple slides of lymph node tissue and staining of imprints using the ICC process were reported to detect the tumor cells with sensitivity up to 100%.[14,15]

The rapid stain by ICC for the detection of the breast cancer cells in the SN was studied. The authors previously reported the correlation between the ICC and routine complete axillary lymph node examination.[16] This study was a comparison between the intraoperative SN scrape imprint ICC and the SN serial sectioning with combined staining by hematoxylin-eosin (H&E) and IHC technique.

Materials and Methods

Sentinel lymph node mapping

The first project of the study of sentinel lymph node mapping started in September 2004. The protocol was approved by the Ethics Committee, Faculty of Medicine, Prince of Songkla University. The sentinel lymph node mapping was performed together with subsequent axillary lymph node dissection to confirm the results of SN. The SN was sent for routine H&E staining to detect metastatic breast cancer cells. With negative result, the SN was then serially sectioned at 20 μm and stained by IHC. Step sectioning followed by cytokeratin stain was performed to confirm the true negative result.

During the first project, we designed the present study of ICC staining for intraoperative assessment of SN in breast cancer patients starting in April 2005. The subjects were patients with early breast cancer for whom mastectomy or conservative breast surgery combined with axillary lymph node dissection were planned and who consented for the SN detection. 1% isosulphan blue dye was used for the mapping of SN together with radio-colloid tracer. After general anesthesia, 4-5 cc of blue dye was injected in the peritumoral and subareolar area. The skin incision was made 5 minutes after breast massage. SN was located by the detection of blue lymph channel and hot spot detected by a gamma probe.

Immunocytological procedure

SN(s) was/were removed from the lymph node basin and then sliced along the largest diameter plane. Most of the SNs were bisected because their thickness was less than 0.5 cm. For SNs with thickness greater than 0.5 cm, attempts were made to slice more than 2 pieces in order to cover much more planes of the lymph node. The cut surfaces were scraped to obtain the metastatic tumor cells and then smeared on a glass slide to obtain a thin layer of cells. The smeared slides were immediately fixed in acetone for 1 minute and allowed to air dry. These slides were ready for immunocytological staining. The IHC method employed in this study was modified from the protocol for the ready-to-use kit (DAKO EPOS anti-human cytokeratin/HRP clone MNF 116). However, we used the routine cytokeratin antibody (AE1/AE3, Zymed) instead of the ready-to-use kit, but other reagents were similar to that method. The modification was made because the ready-to-use kit is rather expensive with limited shelf life. The IHC procedure used in this study is described in diagram 1.

Procedure	Total time (min)
Air dry slide	
↓	
Washed in PBS buffer	1
↓	
Incubated in 3% normal horse serum (at room temperature)	5
↓	
Incubated in 1:40 cytokeratin antibody (at 37 °C)	10
↓	
Washed in PBS buffer for 30 sec × 2 times	1
↓	
Incubated in EnVision (at 37 °C)	5
↓	
Washed in PBS buffer for 30 sec × 2 times	1
↓	
Incubated in DAB (at 37 °C)	5
↓	
Washed in tap water (at 42 °C)	1
↓	
Counterstained in hematoxylin	1
↓	
Washed in tap water (at 42 °C)	1
↓	
Dehydrated in graded alcohol (dipping)	1
↓	
Cleared in xylene (dipping)	1
↓	
Mounted	
↓	
Cover slipped	
↓	
Viewed	
↓	
Total time	**34**

Diagram 1 The cytokeratin immunocytological staining procedure

Brown cytoplasmic staining was considered as positive and the presence of any of positive stained cells was regarded as a positive result (Figure 1). In each run of staining, a positive control for cytokeratin was included.

Tissue section procedure

The remainders of each sentinel node were submitted for formalin fixation and routine H&E examination by pathologists who did not know the prior cytological results. Following histological examination, all sentinel nodes which were negative by routine H&E examination were subsequently examined for metastasis according to the standard protocol. The examination consisted of consecutive levels at intervals of 20 μm with the first two levels for H&E staining and

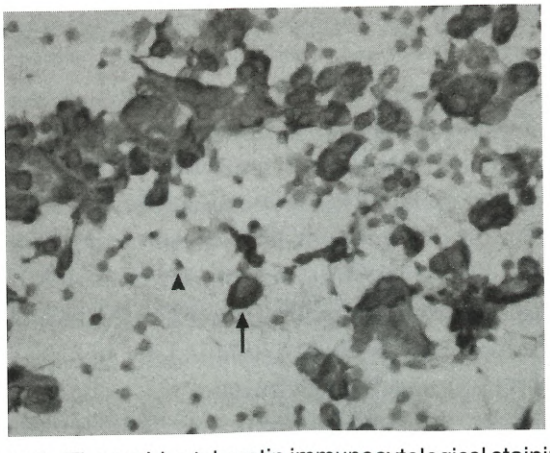

Figure 1 The rapid cytokeratin immunocytological staining of metastatic breast cancer cell of SN. The positive tumor cells are identified by brown cytoplasmic staining (arrow). The small round cells with scant cytoplasm are lymphocytes (arrow head).

the latter three ribbons for cytokeratin IHC staining. The diagnosis of macrometastases, micrometstases or isolated tumor cells was made according to the classification of the AJCC pathologic staging for breast cancer.[1]

Breast cancer operation

Modified radical mastectomy (MRM) or breast conservative surgery (BCS) was performed after the sentinel lymph basin was removed. The axillary lymph nodes were dissected at the level II. All of the axillary lymph nodes were subjected to routine completed pathological examination.

Statistical analysis

Results of ICC scrape smear staining technique was compared with the serial sectioning of the SN by H&E and IHC stain for the detection of metastatic cancer cells. Sensitivity, specificity, negative and positive predictive value were calculated.

Results

Since September 2004, 76 patients with operable breast cancer underwent surgery with SN mapping and complete axillary lymph node dissection. The result of SN mapping and identification rate was 97%. The detection of metastatic cancer cells in the SN had 87% sensitivity, 71% specificity and 94% accuracy.

The main purpose of this study was to study the intraoperative detection rate of the cancer cell by rapid ICC staining of SN. From April 2005 to March 2006, 33 patients consented and enrolled for scrape imprint and smear of the sentinel lymph node together with axillary dissection. The number of patients in Stage I, II, III was 7 (21.2%), 23 (69.7%), and 3 (9.1%) respectively. The average age of the patients was 54.3 years, (range 29-87 years). Seventy-two sentinel lymph nodes were identified in 33 patients, with a range of 1-5 nodes and an average of 2.18 nodes per patient. Axillary dissection was performed with 470 lymph nodes removed.

There were 5 patients with true positive staining cancer cell by scrape imprint ICC and 1 patient with false positive result (Table 1). There were 10 patients with false negative result in the total of 27 patients with negative stain by ICC. In the subgroup analysis of the false negative results, 4 cases were classified as isolated tumor cell metastasis, 5 as micrometastasis and 1 as macrometastasis. The sensitivity, specificity, and accuracy of imprint ICC when compared with the serial section were 33%, 94% and 66% respectively. The positive predictive value of the ICC was 83% and the negative predictive value was 63%.

Discussion

The immunocytokeratin staining for the detection of metastatic breast cancer cells is one of the most accurate methods when used in the serial section of the lymph node. The technique has been applied for use as a guide in order to avoid dissection of the axillary lymph nodes. The less invasive breast tissue operation combined with less invasive axillary detection of tumor cell metastasis are major benefits.

The technique of touch imprint ICC for detection of tumor cell metastasis has been widely studied and applied in the intraoperative evaluation of SN metastasis. This technique is explored as a possible guide to avoid axillary dissection. The advantage of ICC technique is its ability to identify the tumor cell by staining cytokeratin antigen. The tumor cell staining by this technique is more accurate than by other staining methods of imprint cytology such as Papanicolaou method. The overall reported results of the intraoperative study vary widely, which may be from various standards of laboratory protocol.

The sensitivity of ICC method in this study was 33%, which is comparable to some other reports,[15] although the sensitivity of imprint cytology varies greatly

Table 1 The results of scrape smear imprint ICC compared with the combined H&E and IHC stain

	Combined H&E and immunohistochemistry stain	
	positive	negative
Scrape smear imprint immunocytochemistry stain		
positive	5	1
negative	10	17

between 34% and 96%.[17-22] Motomura reported a 90% sensitivity in the detection of tumor cell by the same technique.[23] The different results may be due to the process in preparing the cut surface of fresh lymph node, cell lost during the process and staining technique. If the SN is too small, the slicing cut surface is difficult to handle. Another reason is the low volume of tumor cells in the lymph node.

Micrometastases or clusters of tumor cell are localized in the area adjacent to the capsule of the cut surface. Starz[24] and Viale[25] reported intranodal metastasis as opposed to capsular/subcapsular location. The scraping procedure will be more effective at the central part than at the periphery of the cut surface. In the subset analysis of 10 false negative cases, we found that 4 cases had clusters of metastatic cells and 5 cases had micrometastasis in the SN.

The accuracy in this report is 66% and the specificity is 94%. This accuracy is also low when compared with other reports.[14,15,21-26] A larger sample size may be needed for a more definite and conclusive result. The result of rapid imprint cytology in this study is comparable to the frozen section with H&E stain. Zurrida reported a 32.1% false negative rate in intraoperative frozen sections by H&E stain.[13] In this report, there was one case with false positive result; this may be explained by the displacement of epithelial cell or artifact.[27,28]

Conclusions

The intraoperative detection of tumor cell by this scrape imprint immunocytochemistry technique should be further investigated. The rapid ICC consumed about 30 minutes for completion of the process. The waiting time for the result of rapid stain ICC in the operating room is acceptable and is not different from that of the frozen section with H&E. However, the low accuracy in the present study confirms that it is not applicable for use as the routine intraoperative procedure. The standard paraffin section with H&E combined with IHC is still the most accurate procedure.

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