

# THE VALUE OF TOUCH IMPRINT CYTOLOGY AND FROZEN SECTION FOR INTRA-OPERATIVE EVALUATION OF AXILLARY SENTINEL LYMPH NODES

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**Background:** Sentinel lymph node (SLN) biopsy is a procedure of choice to determine the axillary involvement in breast cancer. The most important intraoperative evaluation methods are touch imprint cytology (TIC) and frozen section (FS). Each of these techniques has its own drawbacks and it is still unclear which is preferred.

**Material and methods:** 97 consecutive patients with recently diagnosed breast cancer and no clinical evidence of lymph node involvement were included in the study. The SLN was determined with lymphoscintigraphy. Touch imprint cytology and FS were performed and their results were compared with permanent histopathological examination.

**Results:** Using a permanent section as the gold standard TIC showed sensitivity of 71.4%, specificity of 100%, positive predictive value of 100%, and negative predictive value of 88.7%. These figures were 87.5%, 98.5%, 96.5%, and 95% for FS, respectively.

**Conclusions:** Frozen section is a reliable method for SN assessment during surgery. Using the frozen section during surgery can give the surgeon an opportunity to avoid the second surgery. Touch imprint cytology is also a reliable method for intra-operative SN evaluation. Due to its high predictive value, TIC can be used first in the surgery room and FS can be saved for cases with negative results of TIC.

**Key words:** breast cancer, touch imprint cytology, frozen section, sentinel lymph node, intra-operative biopsy, lymphoscintigraphy.

## Introduction

Breast cancer is the most common and the second leading cause of cancer death among women [1]. There is a consensus in the literature that the axillary node involvement is an independent risk factor for poor prognosis [2], and the axillary lymph node (LN) dissection is associated with remarkable complication [3]. In addition, in approximately 70% of patients with

early breast cancer the axillary lymph nodes are not involved [2]. Considering these facts, axillary lymphadenectomy is an unnecessary and even harmful surgery in many cases [4]. It seems that the concept of SN has significantly changed the diagnostic and therapeutic processes in several malignancies, especially in breast cancer and melanoma [5]. Consequently, finding and pathological evaluation of a sentinel lymph node (SLN), which is the first draining

lymph node, not only has the ability to avoid the complications of an unnecessary LN dissection [6, 7] but also offers the potential to determine the presence of nodal metastasis with high accuracy [8]. If the SN can be evaluated intra-operatively, the second surgery can be avoided, which results in reduction of morbidity as well as significant cost (and time) saving. The most important methods for SN assessment during operation are frozen section histology (FS) and touch imprint cytology (TIC). Each of these two techniques has its own advantages and disadvantages [9] and it is still unclear which technique is superior to the other.

Initially, FS was the gold standard method for intraoperative evaluation of SLN [10] but this method is difficult for interpretation in fatty nodes, brings about high costs and is labour intensive [11]. On the other hand, TIC, despite its convenience, rapidity and low cost, is operator dependent and requires significant pathologist expertise [12]. A meta-analysis [4] performed in 2005 reported that TIC despite its simplicity and rapid method of preparing the specimen, lower cost and not making artefacts or loss of tissue (which are present in FS due to freezing and thawing), is comparable in accuracy to frozen sectioning. However, some reports published after 2005 suggest the superiority of FS over TIC for assessment of SLN metastasis [13, 14]. The early reports of TIC have found a wide range of sensitivity (63-75%) and the specificity of more than 90% [2, 8]. In this study, we compared our results for each of these two procedures.

## Material and methods

97 consecutive patients with recently diagnosed breast cancer and no clinical evidence of axillary lymph node involvement who were hospitalized in Omid hospital from May 2007 to January 2010 were included in the study. Sentinel nodes were defined scintigraphically with the intradermal injection of 0.5 mCi/0.2 ml  $^{99m}\text{Tc}$ -antimony sulfide colloid in the periareolar region. Imaging was done 1 hour after injection and the surgeon found the sentinel node using the hand-held gamma probe and patent blue V injection. Any blue or hot node was harvested as SLN.

The LNs with a diameter of less than 5 mm were bisected and the remainder underwent serial sectioning (2 mm each). Touch imprint cytology (TIC) and frozen section (FS) were both performed for all cases intra-operatively. Touch imprint was done for each slice of a node on a slide and stained with haematoxylin and eosin (HE) and was examined by an experienced cytopathologist. In the next step, frozen section was made in the laboratory for sectioned nodes, using HE staining and the specimens were evaluated by the same cytopathologist.

If FS result was positive, the LN dissection was done simultaneously during the initial operation, but no decision based on the TIC result was made. Permanent section (PS) was produced by thawing the remaining tissue, fixing in formalin, embedding in paraffin and staining with HE. The permanent specimens were examined post operatively for final diagnosis and served as a gold standard for evaluating the intraoperative techniques.

The results were categorized on a per node and per patient basis.

The quantitative data were expressed as mean  $\pm$  SD. Statistical analyses were made using SPSS software version 11.5.  $2 \times 2$  table was used for sensitivity, specificity, positive and negative predictive values calculation.

## Results

97 consecutive patients with early breast cancer were evaluated with lymphoscintigraphy to localize SLNs. The age range of the patients was 26-71 years ( $47 \pm 11.88$ ) and all but one case were females. Totally 147 nodes as sentinel node with a diameter between 3 and 25 mm and the average size of  $10.2 \pm 4.88$  mm were removed. The median number of sentinel node was 1. All nodes in each case were examined separately and we analyzed our results on both a per patient and per node basis. Our results included 137 imprint cytology and 147 frozen and permanent sections. 91 cases had TIC, FS and PS and in 6 cases only FS and PS results were available.

Considering PS results, in total 97 cases, 32 patients (33%) were positive for tumoral involvement in axillary lymph nodes and 65 cases (67%) were not involved.

74% of cases had ductal carcinoma, 25% had lobulated form and one patient was reported to have poorly differentiated adenocarcinoma.

Touch imprint cytology data among 91 cases were compared with definitive histopathological examination (PS) and 63 patients were found to have true negative SLN. No-one was found to have a false positive result. A total of 20 cases (71.4%) had true positive SLN and 8 patients (28.6%) had false negative results. The test characteristics with 95% confidence intervals are as follows: sensitivity 71.4% (55-88%), specificity 100%, positive predictive value 100% and negative predictive value 88.7% (81-96%).

Frozen section results were compared with PS in 97 patients and showed 64 true negative, 28 true positive and 4 false negative SLN. There was one case where PS and TIC results were negative but FS has shown tumoral invasion in the sentinel node. The test characteristics with 95% confidence intervals are as follows: sensitivity 87.5% (76-99%), specificity

98.5% (95-100%), positive predictive value 96.5% (90-100%) and negative predictive value 95% (89-100%). All true positive cases who had a false negative result in FS were negative in TIC too.

The data analysis on a per node basis is shown in Table I.

The mean size of the sentinel node was not statistically different between nodes with and without tumoral involvement using independent sample *t*-test ( $p > 0.05$ ). This was also true for nodes with false negative and true positive results ( $p > 0.05$ ).

## Discussion

Axillary LN dissection has many complications including arm lymphedema, seroma formation, numbness and shoulder stiffness [15]. To avoid unnecessary dissection, SLN biopsy was introduced as the optimum approach to axillary metastasis evaluation [16]. SLN evaluation during surgery gives the surgeon an opportunity to perform LN dissection during the same operation. The most important intraoperative SLN examinations are TIC and FS techniques. It is not clear which one is the preferred technique yet and much controversy remains in this regard [17].

Tew *et al.* [4] in a meta-analysis included 31 studies on TIC and have found pooled sensitivity and specificity of 63% and 99% respectively for TIC and if the micrometastases were excluded, the sensitivity improved to 81%. Some other studies also agree with this notion underscoring the importance of TIC as an accurate method [2, 3]. However, some recent studies emphasized the use of FS as the preferred intra-operative SLN examination technique [18, 19]. Mori *et al.* [8] had performed a study of 138 patients and reported 47.1% and 88.2% sensitivity for TIC and FS, respectively. They have come to a conclusion that FS is superior to TIC for intra-operative SLN assessment.

In our study, negative predictive values for both techniques were high (89% for TIC and 94% for FS) and this is compelling evidence that if the intraoperative techniques show negative results, the possibility of SN tumoral involvement on PS evaluation is very low.

Similarly, our study showed excellent specificity for these two techniques (100% for TIC and 98% for FS), which shows that with positive results during surgery, axillary lymph node dissection can be performed with reasonable confidence [20].

In our study, there was a case with negative TIC but positive FS. Because axillary dissection was made on the basis of FS result, the patient has undergone dissection but the PS showed no evidence of tumoral involvement in the sentinel node. As the tumoral invasion was shown in another node, we can estimate

**Table I.** The per node analysis of the study

	TIC	FS
<b>Total number</b>	137	147
<b>Positive</b>	33	42
<b>Negative</b>	104	105
<b>Sensitivity (95% CI*)</b>	80% (68-93%)	91% (83-99%)
<b>Specificity (95% CI)</b>	100%	99% (97-100%)
<b>Positive predictive value (95% CI)</b>	100%	97.6% (93-100%)
<b>Negative predictive value (95% CI)</b>	92% (87-97%)	96% (93-100%)

\* 95% confidence interval

that the negativity of PS was due to tissue destruction during freezing and thawing processes which is one of the important limitations of FS [2].

Although the specificity of TIC and FS were almost similar in our study, sensitivity of FS was better than TIC. In the other way, a false negative rate for TIC was more than that for FS, which was similar to most other studies in this regard [2, 8, 18, 19]. Considering the results of our study, in 20 of 28 positive cases (71%), the TIC and FS results were similar to final results (PS), which constitutes the major part of our positive cases. Four patients (14%) had false negative FS and TIC and 4 cases (14%) had true positive FS and false negative TIC.

Many factors are reported to affect the false negativity of TIC results [4]. The most important ones are micrometastases and lobular carcinoma [2]. In our study, although the ductal carcinoma was about three times more frequent than the lobular form (74 vs. 25%), half of false negative cases in TIC results were lobular which is in agreement with other studies in this regard [3, 4]. On the contrary, in cases with false negative FS, 3 of 4 had ductal carcinoma which is also in agreement with previous studies [21, 22]. In brief, the histological type of the tumour affects the accuracy of TIC but not FS.

Another factor which is reported to influence the false negative rate of TIC is the use of immunohistochemistry (IHC) [23]. IHC is able to identify minimal involvement and due to showing micrometastases as well as isolated cell tumours, more false negative cases can be found by IHC compared to PS evaluation alone. As mentioned above, most of these false negative cases are micrometastases or isolated tumour cells, the importance of which is still under much debate [24, 25]. Many believe that the completion of axillary lymph node dissection in patients with breast cancer who had micrometastasis in SLN,

will not improve the outcome [26]. It is also shown that false negative results in intraoperative biopsies had a very low risk of non-sentinel axillary involvement and therefore the axillary LN dissection can be avoided [7].

As our study showed, if there is a decision to perform FS, there is no need to do TIC since TIC will not add more information than FS. It is also recommended that when the histological type of the primary tumour is lobular carcinoma, FS should be used as the method of choice regarding higher false negative TIC results in this type of tumour [2], which is also in agreement with our results.

Touch imprint cytology has the added advantage of simplicity, rapidity and not sacrificing tissues [3]. So it is possible to use TIC as a useful screening test in determining SLN involvement. If TIC is positive, considering the high specificity of this technique, the surgeon can perform LN dissection simultaneously without any need for FS. If the TIC is negative, FS can be performed as well. With this protocol, 71% of unnecessary FS will not be performed. However, in usage of TIC as a screening method, the most important factor is ability of the pathology lab to provide TIC and – if necessary – FS after that. This needs a significant skill of the technicians for preparing samples and also experience of the pathologist and coordination between pathology and surgery departments is required.

In conclusion, FS is a reliable method for assessment of SN during surgery. Using frozen section during surgery can give the surgeon an opportunity to avoid the second surgery. TIC is also a reliable method for intra-operative SN evaluation. Due to its high predictive value, TIC can be used first in the surgery room and FS can be saved for cases with negative results of TIC.

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