

Diagnostic value of fine needle aspirates processed by ThinPrep® for the assessment of axillary lymph node status in patients with invasive carcinoma of the breast

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Objective: To evaluate the utility of ThinPrep® as an optional specimen processing method for the detection of axillary lymph node metastasis of invasive breast carcinoma.

Methods: A computer SNOMED search from the file at our institution between January 2003 and August 2011 retrieved a total of 209 fine needle aspiration (FNA) specimens of axillary lymph nodes prepared by ThinPrep and followed by axillary lymph node biopsy and/or dissection. Original cytological diagnoses and corresponding histological diagnoses were documented. Using the histological diagnoses as the gold standard, the diagnostic parameters including sensitivity, specificity, positive (PPV) and negative predictive values (NPV) and diagnostic accuracy were calculated. Both cytology and histology slides from cyto-histologically discrepant cases were reviewed.

Results: Out of a total of 209 specimens, 193 (92%) had adequate diagnostic material while the remaining 16 specimens (8%) were inadequate for cytological assessment. The diagnostic specimens included 168 invasive ductal carcinomas (IDC), 15 invasive lobular carcinomas (ILC) and 10 mixed carcinomas (IDC and ILC). Excluding 19 cases with malignant cells on FNA in which no residual tumour was found in fibrotic lymph nodes after neoadjuvant therapy (cytology and histology confirmed on review) ThinPrep detected nodal metastasis with an overall sensitivity of 77.5%, specificity of 100%, PPV of 100% and NPV of 53.7%. Diagnostic accuracy was 82.2%. There was no difference in Bloom–Richardson grade or the number or size of metastases between tumours with true-positive and false-negative cytology. Sampling error was the sole factor contributing to cyto-histological discrepancy.

Conclusions: ThinPrep is a good alternative to the conventional smear for cytological assessment of axillary lymph node status in patients with invasive breast carcinoma, particularly when specimens are collected at remote sites or when cytologists are not available for assistance during FNA.

Keywords: fine needle aspiration, liquid-based cytology, ThinPrep, nodal metastasis, invasive breast carcinoma

Introduction

Fine needle aspiration (FNA) has been widely utilized as an important modality in the determination of status of locoregional lymph nodes including axillary lymph nodes during the initial staging and subsequent

management of patients with invasive breast carcinoma. Various degrees of diagnostic sensitivity (37–89%) and 99.6–100% of specificity were reported.^{1–5} In these previous studies, the aspirates were processed as conventional smears. Subsequently, some smears were stained with rapid haematoxylin and eosin (H&E) stain combined with an immunocytochemical stain,⁶ while others were stained with Diff-Quik and/or Papanicolaou (Pap) stains.^{7,8}

Generally, ThinPrep® [Cytoc Corporation (now Hologic®), Marlborough, MA, USA] has a number of advantages over conventional smear preparation,

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such as easier submission of the specimen, which is directly rinsed in the CytoLyt®-collecting medium (Cytyc Corporation; now Hologic), fewer numbers of slides examined, well-preserved nuclear details and a cleaner background. On the other hand, for each specimen, only one ThinPrep slide is routinely prepared and it randomly represents the sample obtained from multiple needle passes. A ThinPrep preparation produces cytological artefacts including apparent discohesion with smaller cellular clusters, fragmented cellular sheets and more single cells, as well as cellular shrinkage.⁹ These artefacts could possibly cause cytological misinterpretation, especially for low-grade invasive ductal and lobular carcinomas. In the current study, we evaluated the diagnostic utility of ThinPrep as an optional specimen processing method for the detection of axillary lymph node metastasis of invasive breast carcinoma.

Methods

A computer SNOMED search from the files at our institution between January 2003 and August 2011 was conducted to identify patients with invasive breast carcinoma who were worked up by FNA of axillary lymph node and followed by axillary lymph node biopsy and/or dissection. The study consisted solely of cases in which FNA cytology specimens of axillary lymph node were submitted in the CytoLyt solution from which ThinPrep slides and cell blocks were prepared. All FNA procedures were performed under ultrasound guidance by radiologists. For each FNA procedure, 21-gauge needles were routinely used and an average of four passes were collected directly into the CytoLyt solution, from which one Pap-stained ThinPrep slide and one H&E-stained cell block slide were prepared and evaluated. Confirmatory immunostains for AE1/AE3 (Chemocon 1:200 dilution) were performed on cell blocks in only four specimens at the time of the initial diagnosis.

For each case, available ultrasound measurement of lymph node, original cytological diagnosis, results of corresponding histological examination including type and grade of primary breast carcinoma along with number of positive axillary lymph nodes and available maximum size of metastatic foci were collected. Using the histological diagnosis as the gold standard, the diagnostic parameters including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy were assessed. Both cytology and histology slides from cases with discrepancies were reviewed.

Results

We retrieved a total of 209 ThinPrep specimens prepared from FNAs of axillary lymph nodes that were suspicious for breast carcinoma metastasis. Ultrasound measurement of the FNA-targeted lymph nodes ranged from 0.5 to 3.9 cm. Adequacy was defined as the presence of lymphocytes representative of a lymph node in negative cases and the presence of malignant cells with or without lymphocytes in positive cases. Out of a total of 209 specimens, 193 (92%) were defined as adequate: each of the ThinPrep slides revealed lymphocytes with or without tumour cells. The remaining 16 specimens (8%) were inadequate for cytological assessment as neither tumour cells nor lymphocytes were identified on the ThinPrep slides. The study cohort excluded cases in which diagnostic material were present solely in cell blocks.

Among the 193 diagnostic specimens, 168 and 15 were obtained from the patients with invasive ductal carcinoma (IDC) and invasive lobular carcinoma (ILC), respectively. The remaining 10 specimens were from the patients with invasive breast carcinoma showing combined features of IDC and ILC. Out of 16 non-diagnostic FNAs, nine were from the patients with IDC whereas three and four were from ILC alone and combined IDC and ILC, respectively. With regard to the corresponding surgical specimens, Bloom–Richardson grade of primary breast carcinoma ranged from grade 1 to 3; metastatic breast carcinoma was detected in various numbers (from 1 to 21) of lymph nodes and the available maximum size of foci of nodal metastasis measured from 0.2 to 2.6 cm.

Lymphocytes and/or malignant cells were present in all diagnostic ThinPrep slides allowing a cytological diagnosis upon morphological evaluation of slides regardless of the accompanying cell block slides. There were 19 ThinPrep specimens obtained from patients with IDC ($n = 16$) or with ILC alone and combined features of IDC and ILC ($n = 3$), which appeared to be false positive as a result of the presence of carcinoma cells which completely disappeared on the follow-up surgical specimens. However, all 19 specimens were obtained from the patients who received neoadjuvant therapy after a FNA procedure. The subsequent histological examination of lymph nodes revealed not only the absence of carcinoma cells, but also fibrosis and collections of histiocytes (Figure 1). The histological findings were consistent with therapy-related changes, which suggested that the positive lymph nodes showed a complete pathological

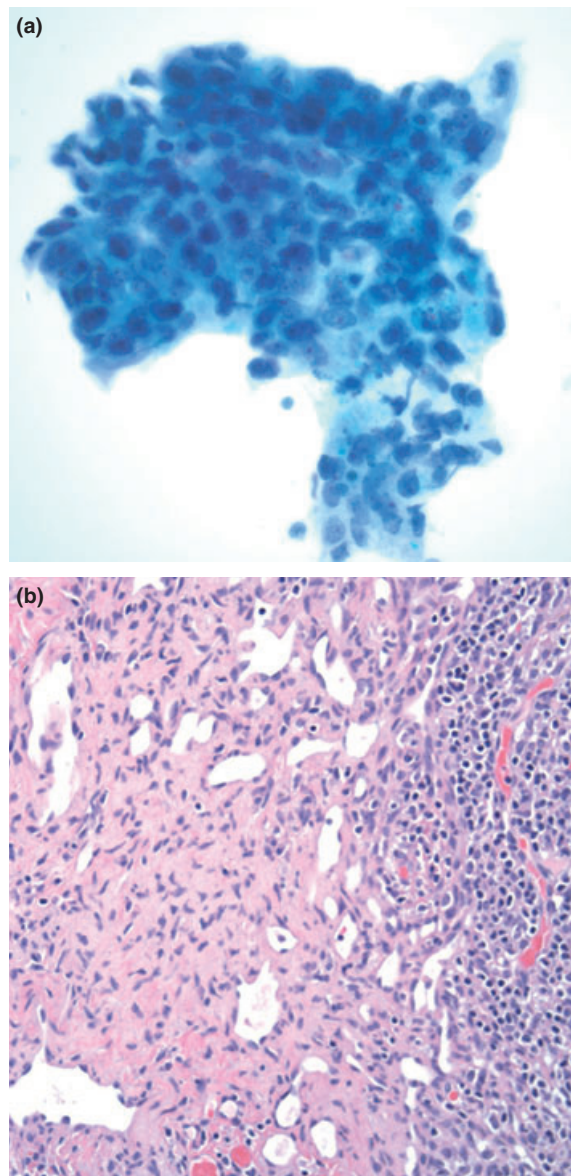


Figure 1. (a) Cytology of metastatic ductal carcinoma in the axillary lymph node (ThinPrep, $\times 600$). (b) The subsequent surgical specimen revealed fibrosis and a collection of histiocytes without cancer cells, which corresponded to the patient's history of neoadjuvant therapy after the fine needle aspiration procedure (haematoxylin and eosin $\times 200$).

response to neoadjuvant therapy. While the presence of metastatic carcinoma on the follow-up surgical specimens was considered the only gold standard for true positives, the aforementioned diagnostic parameters were calculated after excluding these 19 potential true positives.

As can be seen in Table 1, excluding the 19 cases described above, follow-up histological examination confirmed the presence of nodal metastasis in 94 (true positive) out of 168 specimens with IDC (Figure 2). Although the absence of nodal metastasis was confirmed on histological examination in 32 specimens (true negative), ThinPrep preparation failed to reveal nodal metastasis in 26 IDC (false negative), which were detected in the corresponding surgical specimens. Among the 22 specimens obtained from the patients with ILC alone and combined features of IDC and ILC, histological examination confirmed true-positive status in 13 specimens. Histological examination proved true-negative and false-negative findings in four and five cases, respectively. ThinPrep preparation provided an overall sensitivity of 77.5%, specificity of 100%, PPV of 100%, NPV of 53.7% and 82.2% diagnostic accuracy (Table 1).

Review of the false-negative cases revealed that the ThinPrep slides contained an optimal amount of lymphocytes without carcinoma cells present. The ultrasound measurement of the FNA-targeted lymph nodes ranged from 1.0 to 3.8 cm and corresponding surgical specimens proved the presence of metastasis involving 1 to 21 lymph nodes with the maximum size of metastatic foci ranging from 0.4 to 2.6 cm. The follow-up histological examination revealed that true-positive and false-negative cases shared similarities with regard to Bloom–Richardson grade of primary breast carcinoma, total number of positive lymph nodes and available maximum size of metastatic foci (Table 2). Thus, FNA sampling errors were considered the sole factor leading to the cytohistological discrepancy.

Discussion

It is crucial to evaluate the status of metastasis to axillary lymph nodes during the initial staging and subsequent management of invasive breast carcinoma. During the initial work up, patients with evidence of nodal metastasis are selected for a complete axillary lymph node dissection without going through a sentinel lymph node biopsy. The latter procedure could result in not only additional operative time and cost, but also possible medical and cosmetic side effects. It has been reported that preoperative ultrasound-guided FNA can help in avoiding sentinel lymph node biopsy in 24–30% of patients with early stage breast carcinoma.^{10–12}

Table 1. ThinPrep for the assessment of axillary nodal status in invasive carcinoma ($n = 174$, excluding 19 with positive cytology and negative lymph nodes after neoadjuvant therapy)

Parameters	IDC	ILC and mixed	
		ILC & IDC	Total
True positive	94	13	107
True negative	32	4	36
False positive	0	0	0
False negative	26	5	31
Totals	154	22	174
Sensitivity (%)	78.3	72.2	77.5
Specificity (%)	100	100	100
PPV (%)	100	100	100
NPV (%)	55.2	44.4	53.7
Accuracy (%)	82.9	77.3	82.2

IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; PPV, positive predictive values; NPV, negative predictive values.

There are no guidelines for optimal processing of FNA specimens. As previously reported, the aspirates obtained from FNA of axillary lymph node were almost exclusively prepared as conventional smears. The present study consisted of specimens collected directly into CytoLyt solution by the radiologists who performed ultrasound-guided FNA of suspicious lymph nodes. It avoided artefacts associated with preparation of direct smears such as air drying, obscuring blood and thick smears, as well as time and cost associated with on-site assessment. We herein evaluated the diagnostic utility of ThinPrep preparation as an optional specimen processing method for the detection of axillary lymph node metastasis of invasive breast carcinoma.

Sixteen out of 209 (8%) ThinPrep specimens were inadequate for cytological assessment, mainly owing to the fact that neither lymphocytes nor carcinoma cells were found in the slides. The proportion of inadequate specimens was comparable with that (5–10%) of conventional smears as reported in the literature.^{6,7,13}

The majority of the positive ThinPrep specimens resulted from IDC with 78.3% sensitivity. ThinPrep appeared to be slightly less sensitive (72.2%) for detecting ILC and carcinoma with combined features of ILC and IDC but comparison of sensitivity between IDC and ILC is beyond the scope of this study as a result of the limited size of the cohort of the latter diagnoses. The present study demonstrated an overall sensitivity of 82.2%, which is compatible with the previously

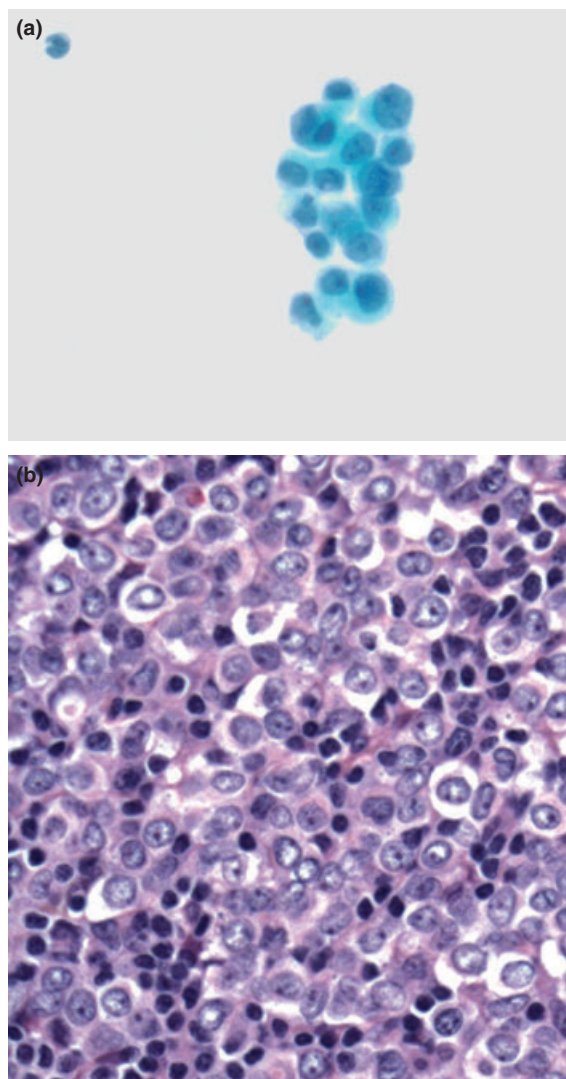


Figure 2. (a) Cytology of the axillary lymph node involved by invasive lobular carcinoma (ThinPrep, $\times 600$). (b) The subsequent histological examination confirmed the nodal metastasis (haematoxylin and eosin $\times 400$).

reported maximum (89%) of conventional smear preparations.^{1–5} Similar to previous reports, ThinPrep preparation offered excellent specificity and PPV.

In spite of the well-documented cytological artefacts produced by ThinPrep,⁹ none of the false-negative diagnoses resulted from misinterpretation; sampling errors were the major factor contributing to false-negative cytology interpretation. There was no difference in the Bloom–Richardson grade, or the number or size of metastases between tumours with true-positive and false-negative cytology. Although not a

Table 2. Comparison between tumours with true-positive and false-negative cytology

Parameters	True positive ($n = 107$)	False negative ($n = 31$)
Ultrasound measurement of targeted lymph nodes (cm)	0.8–4.0	1.0–3.8
Corresponding histology findings		
Bloom–Richardson grade		
I	5 (4.6%)	1 (3.0%)
II	48 (45.0%)	14 (45.0%)
III	54 (50.4%)	16 (52.0%)
Total number of lymph nodes with metastasis (n)	1–19	1–21
Maximum size of metastases (cm)	0.2–2.5	0.4–2.6

primary goal of present study, our data did not reveal any relation between the FNA result and histology grade of primary breast carcinoma or nodal stage.

In conclusion, ThinPrep is a good alternative to the conventional smear for cytological assessment of axillary lymph node status in patients with invasive breast carcinoma, particularly when specimens are collected at remote sites or when cytologists are not available for assistance during FNA.

References

- Jain A, Haisfield-Wolfe ME, Lange J *et al.* The role of ultrasound-guided fine-needle aspiration of axillary nodes in the staging of breast cancer. *Ann Surg Oncol* 2008;**15**:462–71.
- Koelliker SL, Chung MA, Mainiero MB, Steinhoff MM, Cady B. Axillary lymph nodes: US-guided fine-needle aspiration for initial staging of breast cancer—correlation with primary tumor size. *Radiology* 2008;**246**:81–9.
- Alkuwari E, Auger M. Accuracy of fine-needle aspiration cytology of axillary lymph nodes in breast cancer patients: a study of 115 cases with cytologic-histologic correlation. *Cancer* 2008;**114**:89–93.
- Swinson C, Ravichandran D, Nayagam M, Allen S. Ultrasound and fine needle aspiration cytology of the axilla in the pre-operative identification of axillary nodal involvement in breast cancer. *Eur J Surg Oncol* 2009;**35**:1152–7.
- Park SH, Kim MJ, Park BW *et al.* Impact of preoperative ultrasonography and fine-needle aspiration of axillary lymph nodes on surgical management of primary breast cancer. *Ann Surg Oncol* 2011;**18**:738–44.
- Sapino A, Cassoni P, Zanon E *et al.* Ultrasonographically-guided fine-needle aspiration of axillary lymph nodes: role in breast cancer management. *Br J Cancer* 2003;**88**:702–6.
- Ciatto S, Brancato B, Risso G *et al.* Accuracy of fine needle aspiration cytology (FNAC) of axillary lymph nodes as a triage test in breast cancer staging. *Breast Cancer Res Treat* 2007;**103**:85–91.
- krishnamurthy S, Hynek I, Hunt KK, Yang WT. Utility of ultrasound-guided fine needle aspiration of indeterminate and suspicious axillary lymph nodes in the management of patients with early breast cancer: a study of 278 patients. *Cancer Cytopathol* 2008;**114**:2.
- Michael CW, Hunter B. Interpretation of fine-needle aspirates processed by the ThinPrep technique: cytologic artifacts and diagnostic pitfalls. *Diagn Cytopathol* 2000;**23**:6–13.
- Gilissen F, Oostenbroek R, Storm R, Westenend P, Plaisier P. Prevention of futile sentinel node procedures in breast cancer: ultrasonography of the axilla and fine-needle aspiration cytology are obligatory. *Eur J Surg Oncol* 2008;**34**:497–500.
- Altomare V, Guerriero G, Carino R *et al.* Axillary lymph node echo-guided fine-needle aspiration cytology enables breast cancer patients to avoid a sentinel lymph node biopsy. Preliminary experience and a review of the literature. *Surg Today* 2007;**37**:735–9.
- Sahoo S, Sanders MA, Roland L, Pile N, Chagpar AB. A strategic approach to the evaluation of axillary lymph nodes in breast cancer patients: analysis of 168 patients at a single institution. *Am J Surg* 2007;**194**:524–6.
- Deurloo EE, Tanis PJ, Gilhuijs KG *et al.* Reduction in the number of sentinel lymph node procedures by preoperative ultrasonography of the axilla in breast cancer. *Eur J Cancer* 2003;**39**:1068–73.