Role of Axillary Ultrasound, Fine Needle Aspiration Cytology and Sentinel Lymph Node Biopsy in clinically N0 Breast Cancer

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Received: 14 July 2015 / Accepted: 19 April 2016 / Published online: 23 April 2016
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Abstract This study evaluates the combined role of axillary ultrasound, fine needle aspiration cytology and sentinel lymph node biopsy in clinically N0 axilla. Between January 2014 and June 2015, 150 women with early breast cancer underwent axillary ultrasound as a first investigation for nodal status. Suspicious nodes were subjected to image guided fine needle aspiration cytology. Non-suspicious and fine needle aspiration cytology negative axillary nodes proceeded to sentinel lymph node biopsy at time of primary breast surgery. All confirmed positive (cytology and frozen) cases proceeded to axillary lymph node dissection. 52 women had positive axillary nodes at final histology. Axillary ultrasound with fine needle aspiration cytology identified 27 patients with positive axillary nodal status and had a sensitivity of 84.36 % (27/32) and specificity of 87.5 % (14/16). Intraoperative frozen analysis identified a further 13 cases with sensitivity of 56.52 % (13/23) and specificity of 97.56 % (80/82). Overall 76.92 % (40/52) patients with positive axillary metastasis were identified peri-operatively using combination of axillary ultrasound, cytology and sentinel lymph node biopsy.

Keywords Axillary ultrasound · Fine needle aspiration cytology · Sentinel lymph node biopsy · Breast cancer

Introduction

Axillary lymph node metastasis remains the key prognostic factor in breast cancer [1, 2], with the presence of metastatic disease in axilla guiding decisions both on adjuvant therapy and surgery. As a result of increased awareness and improved imaging modalities patients with breast cancer are presenting at an earlier stage and the number of patients with lymph node involvement are correspondingly less [3]. In our institute over a period of 8 years from 2006 to 2013 we had total 2716 breast cancer patients out of which 779 (29 %) were clinically N0.

Earlier axillary lymph node detection (ALND) was the norm to detect axillary metastasis, however with availability of better imaging and minimal biopsy techniques like sentinel lymph node biopsy (SLNB) it is no more considered as a definitive method to detect axillary metastasis. Now SLNB has emerged as an equally effective method of evaluating axilla in cases of early stage breast cancer because of significantly lower rate of morbidity than does ALND [4–6].

Axillary ultrasound (Ax US) is the primary non-surgical method for evaluating axillary nodes [7]. It is moderately sensitive especially when morphological criteria's are used to detect axillary metastasis [8, 9]. However, Ax US is subjective and the experience of the Radiologist is important for the diagnostic accuracy. Therefore, the accuracy of the Ax US has appropriately been questioned. It is well known that overall sensitivity and positive predictive value (PPV) of preoperative Ax US alone is low [10]. With the addition of fine-needle aspiration cytology (FNAC) of suspicious lymph nodes, specificity for detecting metastatic lymph nodes can be increased [11–13]. Recent studies have reported successfully diagnosing

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7.8–16.2 % of patients with axillary involvement preoperatively via US guided FNAC [13, 14].

Peri-operatively axillary metastasis can be successfully detected either by doing Ax US and FNAC of suspicious lymph node, or intra-operative frozen section analysis of the sentinel node (FS SN) in Ax US benign nodes. Surgeon can thereby perform ALND at the time of primary breast surgery in FNAC and frozen positive cases [15]. Advantage of above combination is we can directly plan definitive surgery, reduces operating time and cost effective as SLNB can be omitted in FNAC positive cases.

For early detection of axillary lymph node metastasis, we routinely utilize a combination of both Ax US $\pm$ FNAC and intra-operative FS SN. We have a dedicated breast radiologist since last 3 years and have also been practicing sentinel node biopsy using a combination of iso-sulphan blue dye and Tc 99 labelled sulphur colloid. The aim of present study was to evaluate the combined role of Ax US $\pm$ FNAC and SLNB in detecting axillary metastasis in clinically N0 axilla.

Materials & Methods

All the cases with clinically N0 axilla presenting to Surgical Oncology department at Amrita Institute of Medical Sciences from January 2014 to June 2015 were prospectively included in the present study. Ethical approval was taken from institutional Ethical committee and informed written consent was taken from all the patients. Patient demographics, clinico-pathological features, Ax US $\pm$ FNAC findings, intra-operative FS SN analysis findings and final histology were recorded.

Exclusion Criteria

Patients with Locally advanced breast cancer, previous breast & axillary surgery, prior breast irradiation, proven distant metastasis, inflammatory breast cancer & Neo-adjuvant chemotherapy were excluded from the present study.

During the pre-operative evaluation, after clinical examination, targeted Ax US was carried out by a trained breast radiologist and patients were categorized into the benign appearing or suspicious axillary lymph nodes on the basis of morphologic criteria seen on imaging. Suspicious looking nodes were subjected to image guided FNAC and if a positive nodal status was confirmed by FNAC, an ALND was scheduled along with primary breast surgery. For a negative Ax US i.e. benign appearing nodes or a negative FNAC, a SLNB procedure with intra-operative FS SN was performed at the time of surgery. Similarly, ALND was done if a positive node is identified by intra-operative FS SN or if no sentinel node was detected intra-operatively. Axillary sampling was done in patients with grossly enlarged lymph node on table even if frozen was reported negative for malignancy. Micro metastasis detected during intra-operative frozen section analysis was considered positive and ALND was done in all such cases. All the nodal tissue along with the Breast specimen was then subjected to final histo-pathological examination to assess the final status of lymph nodes. Figure 1 demonstrates above protocol for peri-operative diagnosis of axillary metastasis.

Axillary Ultrasound (Ax US)

Axilla was scanned using a high frequency linear 12 MHz transducer. Axillary lymph node which was oval, smooth, with well-defined margin, hypoechoic, with uniformly thin cortex measuring 3 mm or less was considered as benign. Nodes with any of the features like diffuse cortical thickening, focal cortical bulge, eccentric cortical thickening, complete or partial effacement of the fatty hilum, complete or partial replacement of the node with an ill-defined or irregular mass, and micro-calcifications in the node was considered as suspicious on imaging [6]. Figure 2 shows the sonological images for suspicious lymph nodes. Using a 22 gauge needle, under real time ultrasound guidance, fine needle aspirate samples was obtained from the cortex of the lymph node by applying slight negative pressure for few seconds. The aspirate was then sent for subsequent analysis by a cytopathologist.

Fig. 1 Peri-operative diagnosis of axillary metastasis

Fig. 2 Axillary ultrasound images for suspicious nodes

Sentinel Lymph Node Biopsy (SLNB)

Sentinel lymph node biopsy was performed using both Tc 99 labelled sulphur colloid & iso-sulphan blue dye. Sentinel lymph node intra-operatively was identified with the use of a handheld gamma probe & blue colour. SLNB was done and sent for frozen evaluation. Other lymph nodes which were having count higher than 10 % of maximum count & blue in colour were also sent for evaluation. All grossly enlarged and suspicious LN intra-operatively were sampled and sent for final HPE.

Intra-Operative FS SN Analysis

For frozen section analysis Nodes are measured in three dimensions and bivalved parallel to the long axis through the hilum. If the node measures less than 1.5 cm in any of the dimensions, both the sections are submitted for frozen sectioning after taking imprints from both halves. Node was step sectioned at particular intervals in 5 μ m thickness so that 6 sections are studied per half of the node; i.e., after trimming, 5 μ m sections are taken on slide 1 with an interval of 15 and 25 μ m between first and second, and second and third sections respectively. Second slide will have next three sections separated by 5 μ m between each of them. If negative for macro-metastasis, remaining nodal tissue from frozen tissue is processed and is sectioned at 5 μ m intervals again in single slide. If the node is larger than 1.5cms, one of the bisected half is frozen and sectioned as above. Imprints are taken from the other half. If negative for macro-metastasis; the remaining tissue from frozen tissue is processed in formalin and sectioned as above while other half is also step sectioned in same manner as we take for frozen tissue. Nodes were evaluated with hematoxylin and eosin staining only.

Statistical Analysis

Using Software- SPSS Version 20, validity parameters namely sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) & overall accuracy of Ax US was computed.

Results

During the study period between January 2014 to June 2015, 347 patients were treated for invasive breast cancer including early and locally advanced breast cancer, out of which 150 patients were having clinically N0 axilla and underwent Ax US prior to definitive surgery. Table 1 demonstrates patient's demographic and histo-pathologic characteristics.

The median age of the patient in the study was 56.37 years (range 24–88). There were 110 patients with invasive ductal carcinoma, 14 with invasive lobular carcinoma & 26 with other histologies (invasive tubular, medullary, mucinous, papillary, and mixed carcinoma). Out of 150, 49 patients were diagnosed with T1, 92 with T2, 7 with T3 and 2 with T4 disease on final histology. Out of 150 patients, 59 had grade I, 67 had grade II, and 24 had grade III disease.

Ax US Results

As shown in Table 2 pre-operative Ax US judged 51 patients as having suspicious axillary lymph nodes, while 99 patients were sonologically benign. Final histological diagnosis showed that 52 patients (34.66 %) had metastatic disease. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of Ax US alone were 65.38 % (34/52), 82.5 % (81/98), 66.7 % (34/51), and 81.89 % (81/99) respectively. False negative rate for Ax US

Table 1 Patient Demographic and Histo-pathologic Characteristics

Characteristics	N
Age (Years)	
Median	56.37
Range	24–88
Tumor Histology	
Ductal	110
Lobular	14
Others	26
T Stage	
T1	49
T2	92
T3	07
T4	02
Tumor Grade	
I	59
II	67
III	24
Breast Surgery	
Mastectomy	115
Wide local excision	35

was 18.18 % (18/99). The overall diagnostic accuracy of Ax US was 76.67 % (115/150).

Ax US + FNAC Results

In our series, out of 51 patients with suspicious axillary lymph node at Ax US, 48 had FNAC performed. In 3 patients with sonographically suspicious axilla FNAC was not performed as the lymph node was close to axillary vessel. Of these 48 patients, 29 were confirmed as having malignant cytology on FNAC and they underwent ALND.

Table 2 shows the association between the cytological and histological diagnosis of axillary lymph nodes. Final histology of 2 patients with cytologically positive lymph node did not

Table 2 Shows association between Ax US ± FNAC, SLNB + FSSN with final histo-pathology

Investigation	Category	Final Histo-pathology		Total
		Positive n(%)	Negative n(%)	
Ax US	Suspicious(51)	34 (66.7)	17 (33.3)	51
	Benign(99)	18 (18.20)	81 (81.80)	99
Ax US + FNAC	Positive(29)	27 (93.1)	2 (6.9)	29
	Negative(19)	5 (26.3)	14 (73.7)	19
SLNB + FSSN	Positive(15)	13 (86.7)	2 (13.3)	15
	Negative(90)	10 (11.10)	80 (88.90)	90

Ax US, FNAC, SLNB and FS SN stands for axillary ultrasound, fine needle aspiration cytology, sentinel lymph node biopsy and frozen section sentinel node analysis respectively

confirm the metastasis. Thus the sensitivity, specificity, PPV, and NPV of Ax US + FNAC were 84.36 % (27/32), 87.50 % (14/16), 93.10 % (27/29), and 73.68 % (14/19) respectively. False negative rate was 26 % (5/19). The overall diagnostic accuracy of Ax US + FNAC was 85.41 % (41/48).

Intra-Operative FS SN Analysis

Out of total 121 patients, (including 99 with benign appearing Ax US, 19 Ax US + FNAC negative and 3 patients where FNAC could not be performed) only 105 patients successfully underwent sentinel lymph node biopsy followed by frozen section analysis. 16 patients directly underwent axillary clearance due to technical problems like non availability of blue dye or isotope and failure to detect sentinel lymph node.

Table 2 shows association between intra-operative FS SN analyses and final histology. The sensitivity, specificity, PPV, and NPV of intra-operative FSSN analysis were 56.52 % (13/23), 97.56 % (80/82), 86.67 % (13/15), and 88.89 % (80/90) respectively. Overall diagnostic accuracy was 88.57 % (93/105). As shown in above Table 2, there were 10 patients with frozen negative axilla where final histology was positive for metastasis i.e. false negative rate was 11.10 % (10/90). Above false negative rates are comparable with acceptable standards for procedure. In all 10 patients a non sentinel LN was positive which was picked up due to our protocol of LN sampling in grossly enlarged or suspicious LN intra-operatively even in cases where frozen was reported negative.

Discussion

In patients with clinically N0 axilla, peri-operative detection of axillary metastasis is of paramount importance because positive axillary lymph node metastasis changes many of the treatment and surgical options offered to patients. With the availability of Ax US & SLNB techniques, ability to gather this information peri-operatively has improved greatly [16].

Leenders et al. (2012) reported sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of axillary ultrasound alone as 43.8 % (188/429), 80.7 % (582/721), 57.5 % (188/327) and 70.7 % (582/823), respectively. They also reported sensitivity, specificity, PPV, NPV of Ax US + FNAC as 24.7 % (106/429), 99.9 % (720/721), 99.1 % (106/107) and 69.0 % (720/1043) respectively [17]. Similarly Fernandez et al. (2012) found that when compared with final axillary histology, ultrasound fine-needle aspiration showed positive predictive value of 87 %, negative predictive value of 82 %, sensitivity of 53 % and specificity of 100 % [12].

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of Ax US alone were 65.38 % (34/52), 82.5 % (81/98), 66.7 % (34/51), and

81.89 % (81/99) respectively in the present study. Above results are comparable to previous studies investigating the role of Ax US alone in pre-operative detection of axillary metastasis. However after combining the Ax US with FNAC in our study, we were able to identify 27 of the 52 patients with positive axillae in pre-operative period thereby avoiding sentinel lymph node biopsy procedure in 18 % of cases. The sensitivity, specificity, PPV, and NPV of Ax US + FNAC were 84.36 % (27/32), 87.50 % (14/16), 93.10 % (27/29), and 73.68 % (14/19) respectively in present study, which was again in agreement to previous studies.

Carroll et al. (2011) in their study evaluated the combined role of axillary ultrasound (Ax US), fine needle aspiration (FNAC) and intra-operative frozen section analysis of the sentinel node (FS SN) and concluded that out of 188, 93 women had positive axillary nodes at final histology. Ax US & FNAC identified 59 positive axillae and had a sensitivity of 63.4 % and specificity of 100 %. FS SN identified a further 26 cases with a sensitivity of 76.5 % and specificity of 100 %. Overall, only 8 women required reoperation for axillary clearance. Sensitivity for the combined procedures was 91.4 % [15]. In Our study 52 patients had positive axillae at final histology. Ax US & FNAC identified 27 positive axillae and had a sensitivity of 84.36 % and specificity of 87.50 %. FS SN identified a further 13 cases with a sensitivity of 56.52 % and specificity of 97.56 %.

Our study demonstrates the merit of performing Ax US ± FNAC along with intra-operative FS SN analysis in early breast cancer patients. We were able to identify 51.92 % (27/52) patients with positive axillary metastasis in pre-operative period with Ax US + FNAC alone. Another 25 % (13/52) patients with positive axillary metastasis were detected with intra-operative frozen section analysis of sentinel lymph node. Sentinel Lymph node procedure was avoided in 18 % of cases. Overall 76.92 % (40/52) patients with positive axillary lymph node metastasis were identified peri-operatively using combination of Ax US ± FNAC & intra-op FS SN analysis.

Conclusions

From the results of our prospective study we conclude that combining Ax US ± FNAC and intra-operative FS SN analysis results in timely identification of axillary metastasis and helps us to plan our first surgery accordingly. We also conclude that Ax US can be complementary to physical examination in axillary evaluation however SLNB and intra-operative FS SN to be considered as standard of care for peri-operative detection of axillary metastasis. We recommend sampling of grossly enlarged axillary lymph nodes intra-operatively as such nodes may have altered lymphatic channels where we may not sample the true sentinel lymph node leading to incomplete axillary staging.

Acknowledgments I would like to acknowledge the support of Mr. Unnikrishnan UG who helped me with the statistical analysis.

Compliance with Ethical Standards

Ethical Approval Ethical approval was taken from institutional ethical committee.

Funding No funding source.

Conflict of Interest Statement None.

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