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Peer Review Report for "The Accuracy of Preoperative Axillary Ultrasound in the Diagnosis of Lymph Node Involvement at Motamed Cancer Institute, Iran"

Author(s): Asiie Olfatbakhsh, Shahpar Haghighat, Fateme Sari, Toktam Beheshtian, Esmat Alsadat Hashemi, Maryam Jafari

Review Timeline:

Submit Date: 15 Jul 2019
Revised Date: 24 Feb 2020
Accepted Date: 26 Mar 2020

Revision (0)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: Reviewer | **Revision** (0) 18 Aug 2019

Dear AE,
1- DOI & PMID must be added for each reference.
2- What was your sampling method?
3- Please, insert your ethical code or number in the methods section.
4- What P-values are significant in this essay?
5- You should state the version of spss software.
6- It is better to include a conclusion as the last part of the discussion, not apart of that.
7- We are not allowed to use any contraction in a scientific paper: "didn`t".
Thanks.

REFEREE: Associate Editor | **Revision** (0) 26 Aug 2019

Dear EIC,
My comments are as follows:
1) Article TITLE is properly selected.

2) The title reflects the content appropriately.
3) There is no abbreviation in the TITLE.
4) Authors' names and institutional affiliations mentioned according to the journal style.
5) There are about 233 words in the ABSTRACT and the word count is in a suitable range.
6) The abstract describes the content accurately.
7) INTRODUCTION is fair and does include: "What we know", "What we don't know" and "Aims".
8) Abbreviations are defined the first time they appear in the text.
9) The manuscript contains Ethical Considerations.
10) The methodology is GOOD.
11) DOI & PMID must be added for the each reference.
12) What was your sampling method?
13) Please, insert your ethical code or number in the methods section.
14) What P-values are significant in this essay?
15) You should state the version of spss soft ware.
16) It is better to include conclusion as the last part of the discussion,not apart of that.
17) We are not allowed to use any contraction in a scientific paper: "didn`t".
Thanks.

REFEREE: EIC | **Revision (0)** 27 Aug 2019

Dear Author,
please revise your manuscript according to the comments and resubmit again. also Please add the ORCID id of at least the first and corresponding authors in the authors section when submitting the revised manuscript. You can get an ORCID id from the ORCID website at https://orcid.org.
Thank you.

OPEN PEER REVIEW

Revision (1)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Dear Associate Editor

Thanks for the grateful comments of your reviewers. I have changed the article based on these comments and highlited the corrected parts. I am waiting for your new comments about the article.

Sincerely,

The corresponding editor

Reviewer 1:

1-DOI & PMID must be added for the each reference.

They were added except for those that we could not find the PMID

2-What was your sampling method?

all patients who presented to the imaging department of Motamed Cancer Institute (MCI) between Sep 2015 and August 2016 with a pathologic diagnosis of breast cancer were included, among them patients with available AUS and axillary pathology results were evaluated.(We have added the sampling method to the paper)

3-Please,insert your ethical code or number in the methods section.

It is added

4-What P-values are significant in this essay?

It is added to the statistical analysis section

5-You should state the version of spsssoft ware.

It is added to the statistical analysis section

6-It is better to include conclusion as the last part of the discussion,notapart of that.

It is done

7-We are not allowed to use any contraction in a scientific paper: "didn`t".

It is corrected

OPEN PEER REVIEW

Revision (1)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: Associate Editor | **Revision** (1) 31 Oct 2019

Dear EIC,
There are still some points for revision:
- Sampling method means:
simple random
purposive or target sampling
convenience sampling
consecutive sampling
respondent-driven sampling
So, what was the sampling method?
Thanks.

REFEREE: Reviewer | **Revision** (1) 17 Dec 2019

Dear AE,
The topic could be an interesting one however there are some problems in the manuscript that should be considered for revising.
- The title of this article has been selected properly and reflects content appropriately.
- Abstract describes the content accurately but all of the keywords must be selected from MeSH.
- Introduction : this part doesn't include what we know and what we don't know clearly. Please refer to the studies about your aims and explain that what novelty there is in your study.
- This part:" Furthermore, a 15% false negative has been reported for sentinel node biopsy, and in these
cases axillary lymph node dissection may be omitted although it is necessary" must be revised compatible with new published articles.
- Methods:
1: Inclusion criteria :
- In breast cancer stage 0 referred to Tis(dcis), N0, M0, and DCIS is not invasive carcinoma. So ,if your main inclusion criteria is invasive carcinoma then stage 0 must be excluded.
2: Physical examination and U.S: It must be cleared that P/E and A.U.S have performed by people with the same experience and skills.
- Please explain diagram No:1 (The diagram of the patients' management).

Statistical Analysis:

- This part is unclear. There Is nothing about accuracy, Positive Likelihood Ratio and Negative Likelihood Ratio of AUS, PE and AUS+PE.
- Also, True Positive (TP) was defined as positive AUS+ PE results with a positive final pathology. Which part of AUS+PE is main stem?Both of them? Please explain.
- Results :
- Table 2. The absolute and relative distribution of the diagnoses made by the AUS, PE, and the combination of them in comparison with the pathology results:

It seems that numbers of the third row aren't reasonable. please recheck them.

- An obese patient is a patient with BMI>30 and BMI between 25-30 is defined as overweight. please revise it.
- The manuscript should be edited by a native translator.

Kind Regards

REFEREE: Associate Editor | **Revision (1)** 22 Dec 2019

Dear EIC,

The authors should see the reviewers' comments and consider proper correction.

Thanks.

REFEREE: EIC | **Revision (1)** 22 Dec 2019

Dear Authors,

Please revise your manuscript according to the associate editor's and reviewers' comments.

Thanks.

OPEN PEER REVIEW

Revision (2)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Dear Reviewers,

Dear chief editor

Thanks for your valuable **comments about this manuscript. In following you will find our response to comments and the corrected manuscript with highlighted subjects.**

Reviewers' Comments:

Reviewer 1:

So,what was your sampling method?

Our sampling method was "nonprobability convenience sampling". It is added in the manuscript
Reviewer 2:
The topic could be an interesting one however there are some problems in the manuscript which should be considered for revising.
The title of this article has been selected properly and reflects content appropriately.
Abstract describes the content accurately but all of the keywords must be selected from MeSH.

The keywords are corrected
Introduction : this part doesn't include what we know and what we don't know clearly. Please refer to the studies about your aims and explain that what novelty there is in your study.

The introduction is corrected and more explained based on new references.(Referrences no 2 and 4)
This part:" Furthermore, a 15% false negative has been reported for sentinel node biopsy, and in these cases axillary lymph node dissection may be omitted although it is necessary" must be revised compatible with new published articles.

This part is corrected based on the reference No 2.
Methods:
1: Inclusion criteria :
In breast cancer stage 0 referred to Tis(dcis),N0,M0 and DCIS is not invasive carcinoma. So ,if your main inclusion criteria is invasive carcinoma then stage 0 must be excluded.

The main criteria was breast cancer including Insitu and Invasive, because the aim of the study was evaluation of axillary LN. This sentence is corrected and highlighted in the manuscript.
2: Physical examination and U.S : It must be cleared that P/E and A.U.S have performed by people with the same experience and skills.

The criteria of physicians is explained in the materials/ Physical exam and Axillary Ultrasound part and highlighted
Please explain diagram No:1 (The diagram of the patients' management).

We changed and simplified the diagram to show which variables were compared in this study.
Statistical Analysis:
This part is unclear. There Is nothing about accuracy, Positive Likelihood Ratio and Negative Likelihood Ratio of AUS, PE and AUS+PE.

The accuracy, Positive and Negative Likelihood Ratios of AUS, PE and combination of them were calculated through their specific formula
Also, True Positive (TP) was defined as positive AUS+ PE results with a positive final pathology. Which part of AUS+PE is main stem?Both of them? Please explain.

AUS+PE means combination of them.(The sentence is corrected in the statistical part)
Results :
Table 2. The absolute and relative distribution of the diagnoses made by the AUS, PE and the combination of them in comparison with the pathology results:
It seems that numbers of the third row aren't reasonable. please recheck them.

We rechecked the data and the numbers were true.
An obese patient is a patient with BMI>30 and BMI between 25-30 is defined as overweight. please revise it.

It is modified in the text.
The manuscript should be edited by a native translator.
Kind Regards

The accuracy of preoperative axillary ultrasound in the diagnosis of lymph node involvement at Motamed Cancer Institute, Iran

Abstract

Background: Axillary lymph node status plays an important role onthe local and systemic treatment of breast cancer patients. Preoperative ultrasound is routinely used for evaluation of axillary lymph nodes as a non-invasive method.

Objectives: The purpose of this study was to evaluate the sensitivity and specificity of axillary ultrasound (AUS) in comparison with pathology in a referral breast center.

Methods: During a cross-sectionalstudy all breast cancer patients presenting to the imaging department between September 2015 and August 2016 were evaluated for axillary lymph node (LN) status by ultrasound. After comparing these results with pathology of LNs, the sensitivity, specificity and accuracy of the ultrasound in diagnosis of axillary lymph node involvement were measured.

Results: Overall 140 patients were enrolled in the study. The mean age of patients were 48.87 (±10.46) with age range between 25-81 years. The sensitivity, specificity and accuracy of the ultrasound were reported as 56%, 88% and 76%respectively.The highest sensitivity rate was related to AUS+ Physical examination with 70%. The best specificity was for AUS, about 88% and the accuracy of AUS+PE was the highest about 80%.

Conclusions: The sensitivity of the AUS in the diagnosis of axillary involvement in our center was moderate and the specificity was good. The combination of physical examination and ultrasound could improve the sensitivity in comparing to each one alone. If both the physical examination and AUS are suspicious, axillary dissection could be considered when FNA or CNB of the lymph node is not available.

Keywords: Breast cancer,Axilla, Lymph node,Ultrasonography

Background

Axillary staging is one of the most important prognostic factors in breast cancer that plays asignificant role in local and systemic treatment planning. The traditional method for axillary staging is axillary lymph node dissection (ALND).Nonetheless, there is some controversy about the effect of axillary dissection on the survival of breast cancer patients. In a randomized trial with a long follow-up period, Fisher et al. showedthat removing occult positive nodes at the time of the initial surgery has no significant survivaladvantage for patients who have undergone ALND(1).With this background, sentinel lymph node biopsy (SLNB) was selected as the standard methodfor staging of the axilla in patients with clinically negative lymph node.SLNB is safe and accurate alternative to ALND with less morbidity, however about7% false negative rate has been reported for this procedure, and in these casesaxillary lymph node dissection may be omitted although it is necessary(2).

Although Physical Examination (PE) is the first step in evaluation of axillary LNs, most studies have reported a low sensitivity (8.3%-41%), specifity of 94.8% and a negative predictive value (NPV) of 46.6% for this method(3, 4). Detecting whether apalpable lymph node is inflammatory, reactive or metastatic is difficult. The rate of false positiveresults is about 53% in this method(5).
Axillary Ultrasound (AUS) is a non-invasive, inexpensive and available method that is routinelyused in breast cancer patients to determine the status of the LNs. Several studies have shown thatAUS with or without Fine-Needle Aspiration Cytology (FNAC) is a suitable method fordetermining metastatic LNs with acceptable sensitivity and specificity.
According to studies, the diagnostic specificity of lymph node sampling under ultrasound guidance for Core Needle Biopsy (CNB) or Fine Needle Aspiration (FNA) might be as high as 100%(5).
The sensitivity of these methods is reported as 65% to 70% (6), and if positive, axillary dissection can be performed directly for the patient. These practices cannot be performed in all imaging centers and are highly dependent on the radiologists' skill and available facilities.
The aim of this study was to evaluate the sensitivity and specificity of axillary ultrasound with and without physical examination in the diagnosis of lymph node involvement in breast cancer patients when there is no experience for sampling to confirm malignant involvement.

Objectives

This study was evaluation of the sensitivity and specificity of AUS with or without physical examinationin the preoperative diagnosis of axillary lymph node involvement in breast cancer patients.

Methods

Patients

During a retrospectivecross-sectional study, through a nonprobability convenience sampling, all patients referred to the Motamed Cancer Institute (MCI) between Sep 2015 and August 2016 with a pathologic diagnosis of breast cancer included.The ultrasound report and pathological diagnosis of the axillary LNs were recorded. The inclusion criteria consisted of breast carcinoma, stage 0, I, II and IIIA who had not received neoadjuvant chemotherapy.

Since the aim of this study was only to evaluate the sensitivity andspecificity of AUS, without FNA or CNB, so those patients who had been subjected to these invasive procedures were excluded. Regarding to the previous studies(7)that reported the sensitivity and specificity of AUS about 78% and 79% respectively, $\alpha<0.05$ with assumption of PLR>2.5, the predicted sample size was at least 100 patients. Informed consents were obtained routinelyfrom patients for using data with considering their privacy. This study was accepted in scientific committee and received the ethics committee approval. The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki)

Physical exam and Axillary Ultrasound

All patients underwent axillary palpation at Motamed Cancer Institute by two breast surgeons with more than ten years of experience in breast surgery.Positive PE is defined as clinically suspicious LN, it means a palpable firm or hard LN.The criteria for suspicious LNs by AUS were observing at least one of these morphological changes, including Non-round shape, Hypoechogenicity, Cortical thickness>2.5 mm, Obliteration of the hilum and Lobulation. Axillary ultrasound were performed by two radiologists who passed breast imaging course.

Axillary surgery and Histological exam

Axillary surgeries including SLNB or ALND were done based on the criteria, it means that ALND was selected in subjects with highly suspicious LN during palpation or AUS or in situations of positive SLNB. Before SLNB, lymphoscintigraphy was performed with a TC-99m with or without blue dye by subdermal, periareolar injection. Intraoperative detection of SLNs were carried out using a Gamma probe and hot LNs as well as the blue-colored LNs were excised and evaluated by frozen section.The SLNs were evaluated using H&E staining. A metastasis ≥2

mm was taken to indicate macrometastasis and <2 mm was taken as micrometastasis. Axillary Lymph Node Dissection (ALND) was not performed in the cases with micrometastasis. The results were analyzed by comparing the PE and AUS results of LNs by permanent pathology. (Figure 1)

Statistical Analysis

The data analysis was performed using the SPSS software version 22.True Positive (TP),True Negative (TN), False Positive (FP) and False Negative (FN) of Physical Examination, AUS and a combination of them were identified.The sensitivity [TP/ (TP + FN)],specificity [TN/ (TN + FP)], positive predictive value (PPV) [TP/ (TP + FP)] and negativepredictive value (NPV) [TN/ (TN + FN)] were also calculated.The accuracy, Positive and Negative LikelihoodRatios of AUS, PE and combination of them were calculated through their specific formula. Statistical analysis was carried out by SPSS20, using the significance level of 0.05 for P-value.The study was approved by the Ethics Committee of Motamed Cancer Institute (MCI) with code numberIR.ACECR.IBCRC.REC.1394.5.

Results

A total of 140 patients were eligible for participation in the study. The mean age of the patientswas 48.87 years (±10.46) and their median age was 48 years (range: 25-81). The mean BMI was 27.55 (±4.55) and 67.8% had a BMI ≥25 (range:16-40).

The clinical and pathological characteristics of the subjects are presented in Table 1.

Among 62 patients who underwent ALND, 18 patients had positive sentinel node and 44 patients had suspicious LN in physical exam. The flowchart of the management plan is showed in figure1.

Table 2 represents summary of the AUS, PE and combination of them compared to the pathology results. According to the table, the highest true positive was for combination of AUS and PE and the best true negative was related to PE.

Table 3 shows the comparison of Sensitivity, Specificity, PPV, NPV, Positive Likelihood Ratio and Negative Likelihood Ratio of AUS, PE and AUS+PE. The highest sensitivity rate was related to AUS+PE with 70%. The best specificity was for AUS, about 88% and the accuracy of AUS+PE was the highest, 80%.

The accuracy of AUS was compared between the patients with BMI<25 and ≥ 25. Thesensitivity of AUS was 60% for the patients with BMI<25 and 52.9% for the patients who were overweight; the specificity was 83.3% and 86.48%, respectively. (not shown in tables)

Another additional finding was that of 53 patients with pathologically involved LNs, 36 (66.6%) had 1-2 (low-burden) and 18 (33.4%) had three or more (high-burden) involved LNs. Twentyfour

patients had falsenegative results by AUS, including six patients in the high-burden group (25%) and the rest (75%) in the low-burden group.

Discussion

The sensitivity of AUS in the diagnosis of axillary involvement was moderate (56%), its specificity was good (88%) and the accuracy was obtained 76% when lymph node morphology was used. The sensitivity increased up to 70% and the sensitivity decreased (86%) when a classification of combined physical examination and AUS was usedin comparison to each of them alone.

Evaluation of axillary LN in breast cancer is crucial in determining the plan of treatment, including type of breast and axillary surgery, immediate reconstruction and the choice of systemic therapy. Insuspicious situations, the patient may become a candidate for neoadjuvant chemotherapy, or directly undergone ALND without SLNB. If this probability is negligible, the patient becomes a candidate for SLNB and should be prepared in advance for the procedure (6).

What is the best method for determining the axillary LN status before surgery? Various studies have considered this challenge. Feng et al, showed that the sensitivity and specificity of axillary palpation are 32% and 95.5% respectively(3). Other studies have reported a sensitivity of 8-35.5% and a specificity of 93-98.8% (7-9). The results of the present study showed a sensitivity and specificity of 31% and 98% for PE, which is consistent with previous findings. The false positive rate for PE was 1.4% in this study, which is different from the results obtained by Specht et al., who estimated the rate as 23% in very suspicious cases and up to 53% in relatively suspicious cases. They reported overallfalse negative rate of 25.7% for PE(5).

Imaging techniques can improve the diagnostic accuracy of physical examinations. AxillaryUltrasound (AUS) is one of the most common methods that is inexpensive and available, however largely dependent on radiologists' experience. The sensitivity and specificity of ultrasound were reported as 63.8% and 73.6% by Gurleyik et al., 45% and 85% by Jackson et al., 54.3% and 100% by Gipponni et al., 58.6% and 89.4% by Feng et al., and 72% and 79% by Omranipour et al.(3, 10-12).

The ultrasonographic criteria for LN involvement may be related to size or morphology. Previous studies which have used morphological criteria, reported AUS as an acceptable method for detecting involved LN. Alvarez et al reported the sensitivity and specificity of the ultrasound to

differentiate between benign and suspicious LNs as 54.7-92.3%and 80.4-97.1% respectively. In our study, the sensitivity was 56% and specificity was 88%, which is consistent with the results reported by Alvarez et al.

The only similar study in Iran was performed by Omranipour et al., who reported a sensitivityand specificity of 72% and 79% for AUS. In this study the specificity of AUS was lower than ours. Like them, in some studies only patients with normal physical examinations were included(10-12), while in the present study, the patients had both positive and negative examinations.

One of the most important reasons for the low sensitivity rate of the AUS in this study may be the selection ofearly-stage patients. Micrometastasis to the LNs is possible in these patients and may not lead toclear changes in morphology or tumor size in ultrasound.

The combination of PE and AUS improved sensitivity up to 70%, but the specificity did not change significantly (86% instead of 88%). In the case of positive PE and AUS, the probability of axillary involvement was76% (38/50). The false negative rate of the combination test was 8.6% and these patients could be directly candidates for axillary dissection if FNA/ CNB of LN is not accessible.

In 2011 with the publication of the results of the Z0011 trials, a new type of categorization was made for the first time which showed that patients with 1-2 sentinel lymph nodes with specific criteria (breast conserving surgery, postoperative radiotherapy and positive hormone receptors) should not undergo axillary dissection. In the present study, the false negative rate of AUS was reported in 24 patients, including six cases who had more than three (high-burden) and 18 who had 1-2 (low-burden) involvement. On the other words, in 75% of those who had false negative axilla, axillary involvement was low–burden. It means that negative AUS predicts negative or low burden nodal involvement and these patients rarely have high-burden axillary involvement.Farshid et al. showed that, of those who had a false negative AUS, LN involvement was low-burden in 86.8% (13).Jackson et alin another study showed the false negative rate of AUS in detecting≥3 nodal involvement is 4% (12).

In most studies, when FNA is added to the AUS, sensitivity is reduced, but specificity is increased. Gipponi et al. (10) reported sensitivity as 44.1% and specificity as 100%, and they concluded that AUS alone might be suggested for early stage breast cancer. FNA- positive

findings are more reliant when there is a plan for Neoadjuvant chemotherapy or ALND.Gurleyik et al. reported a 100% PPV for AUS+FNA (11).

Regarding the specificity of ultrasound and false negative results, it is impossible to eliminate axillary surgery based on negative ultrasound results until the results of Sound and INSEMA trials are released. Exclusion of axillary surgery for early-stage breast cancer may be possible in near future.

Although axillary palpation were performed by expert breast surgeons and axillary ultrasound by breast radiologists,we did not interfere the difference between clinicians' experience on the results and the lack of the reliability assessment could be a limitation of this study.

In conclusion, the axillary ultrasound is a moderately sensitivewith good specificity for diagnosis of lymph node involvement. The combination of physical examination and ultrasound could improve the sensitivity in comparing to each one alone. If both the physical examination and AUS are suspicious, axillary dissection could be considered when FNA or CNB of the lymph node is not available. Patients with a negative ultrasound and even the false negative cases often have low-burden LN metastases and better prognosis even without ALND.

Acknowledgements: This research was funded byresearch deputy of JahadDaneshgahi, ACECR. No 2268-20. There was no financial disclosure in this project.

Ethical considerations: The study was confirmed by Ethics Committee of Motamed Cancer Institute, ACECR. No IR.ACECR.IBCRC.REC.1394.5

Conflict of Interests: There was no conflict of interests for any of the authors

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Table 1. The clinical and pathological characteristics of the patients.

Variable	Number (%)
Stage	
0 (LCIS, DCIS)	9 (6.4%)
I	48 (34.3%)
II	73 (52.1%)
IIIA	10 (7.1%)
Tumor Histology	
LCIS	1 (0.7%)
DCIS	8 (5.9%)
IDC	117 (86%)
ILC	10 (7.4%)
Tumor size	
T1	50(39.1%)
T2	68(53.1%)
T3	10 (7.8%)
Axillary surgery	
SLNB	96
ALND	62
Grade	
I	6 (5.4%)
II	78 (69.6%)
III	28 (25%)
ER/PR	
Positive	104 (87.4%)
Negative	15 (12.6%)
HER2	
Positive	47 (43.5%)
Negative	61 (56.5%)
Breast surgery	
Mastectomy	29 (21.8%)
Lumpectomy	104 (78.2%)

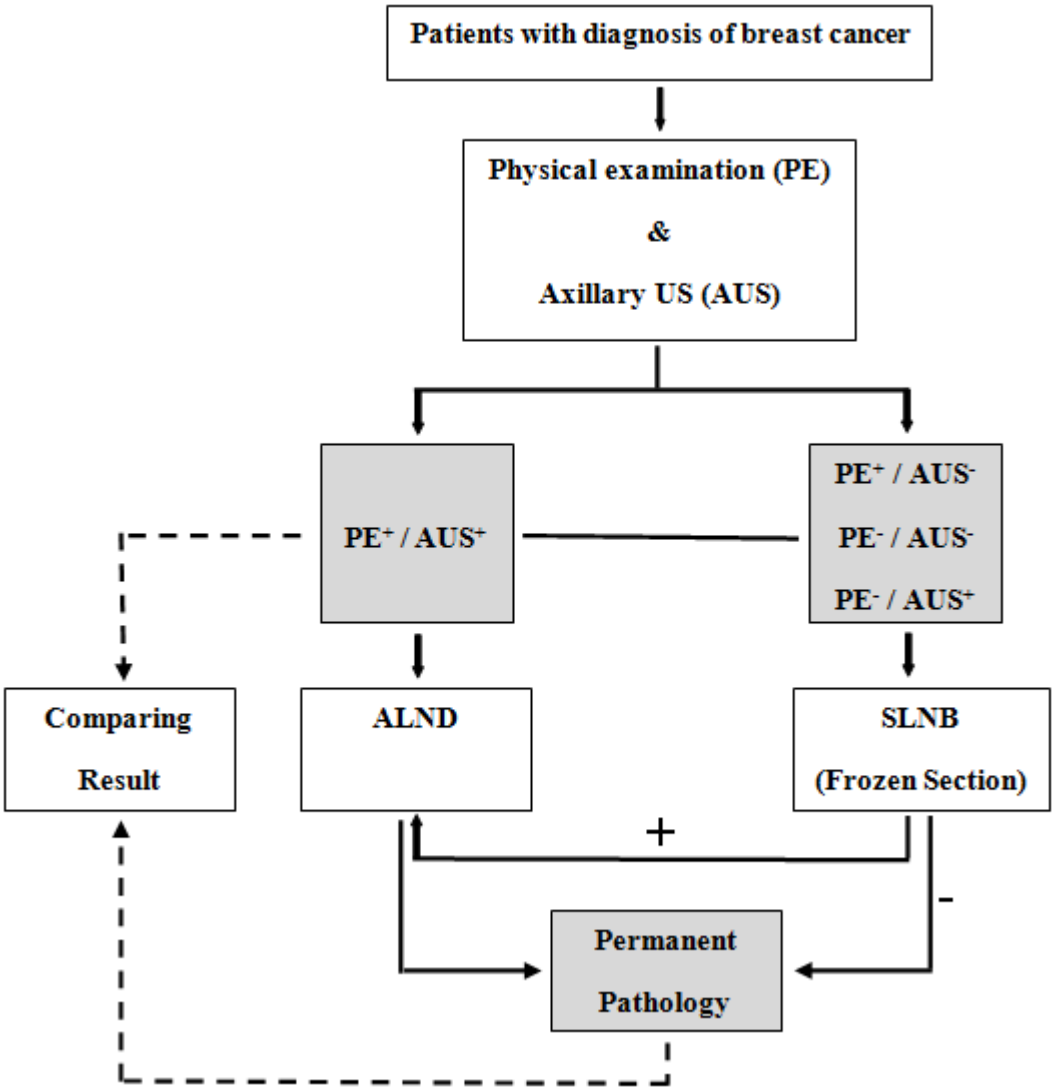
Table 2. The absolute and relative distribution of the diagnoses made by the AUS, PE and the combination of them in comparison with the pathology results

Pathology Results		Positive	Negative	Total
Test Result				
AUS	**Positive**	30 (21.4%)	10 (7.1%)	40 (28.6%)
	Negative	24 (17.1%)	76 (54.3%)	100 (71.4%)
	Total	54 (38.6%)	86 (61.4%)	140 (100%)
PE	**Positive**	17 (12.1%)	2 (1.4%)	19 (13.6%)
	Negative	37 (26.4%)	84 (60%)	121 (86.4%)
	Total (%)	54 (38.6%)	86 (61.4%)	140 (100%)
Combination of PE and AUS	**Positive**	38 (27.1%)	12 (8.6%)	50 (35.7%)
	Negative	16 (11.5%)	74 (52.8%)	90 (64.3%)
	Total (%)	54 (38.6%)	86 (61.4%)	140 (100%)

Table 3. A comparison of the diagnostic accuracy of AUS and PE in preoperative LN evaluation

Test	PE	95% CI	AUS	95% CI	AUS +PE	95% CI
Sensitivity	0.31	0.19 – 0.44	0.56	0.42 – 0.69	0.70	0.56 – 0.82
Specificity	0.98	0.94 - 1	0.88	0.82 – 0.95	0.86	0.77 – 0.93
Positive Predictive Value	0.89	0.76 - 1	0.75	0.61 – 0.88	0.76	0.65 – 0.85
Negative Predictive Value	0.69	0.61 – 0.78	0.76	0.68 – 0.84	0.82	0.75 – 0.88
Positive Likelihood Ratio	13.53	3.26 – 56.29	4.78	2.5 – 8.97	5.04	2.9 – 8.76
Negative Likelihood Ratio	0.7	0.58 – 0.84	0.5	0.37 – 0.68	0.34	0.23 – 0.52
Accuracy	0.72	0.64 – 0.79	0.76	0.68 – 0.83	0.80	0.72 – 0.86

Figure 1: The diagram of the patients' management



OPEN PEER REVIEW

Revision (2)

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According to corrections that have been made, this manuscript is publishable.
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Thank you for submitting your manuscript to IJCM. Please define all abbreviations, when they are used for the first time both in the abstract and manuscript body, such as PE, FNA, CNB in the abstract and LN, H&E in the manuscript body. In addition, all abbreviations used in each table should be defined below that table. Furthermore, please add the ORCID ID of the first and corresponding authors.
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OPEN PEER REVIEW

Revision (3)

Reply to Reviewers

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Dear Reviewers,

Dear chief editor

Thanks for your valuable comments about this manuscript. In following you will find our response to comments and the corrected manuscript with highlighted subjects.

Kind Regards

The Accuracy of Preoperative Axillary Ultrasound in the Diagnosis of Lymph Node Involvement at Motamed Cancer Institute, Iran

Abstract

Background: Axillary lymph node status plays an important role onthe local and systemic treatment of breast cancer patients. Preoperative Axillary Ultrasound is routinely used for evaluation of axillary lymph nodes as a non-invasive method.

Objectives: The purpose of this study was to evaluate the sensitivity and specificity of axillary Altrasound (AUS)in comparison with pathology in a referral breast center.

Methods: During a cross-sectionalstudy all breast cancer patients presenting to the imaging department between September 2015 and August 2016 were evaluated for axillary lymph node (LN)status by Ultrasound. After comparing these results with pathology of LNs, the sensitivity, specificity and accuracy of the Ultrasound in diagnosis of axillary lymph node involvement were measured.

Results: Overall 140 patients were enrolled in the study. The mean age of patients were 48.87 (±10.46) with age range between 25-81 years. The sensitivity, specificity and accuracy of the Ultrasound were reported as 56%, 88% and 76%respectively.The highest sensitivity rate was related to AUS+ Physical examination (PE) with 70%. The best specificity was for AUS, about 88% and the accuracy of AUS+PE was the highest about 80%.

Conclusions: The sensitivity of the AUS in the diagnosis of axillary involvement in our center was moderate and the specificity was good. The combination of physical examination and Ultrasound

could improve the sensitivity in comparing to each one alone. If both are suspicious, axillary dissection could be considered when FNA (Fine Needle Aspiration) or CNB (Core Needle Biopsy)of the lymph node is not available.

Keywords: Breast cancer,Axilla, Lymph node,Ultrasonography

Background

Axillary staging is one of the most important prognostic factors in breast cancer that plays asignificant role in local and systemic treatment planning. The traditional method for axillary staging is Axillary Lymph Node Dissection (ALND).Nonetheless, there is some controversy about the effect of axillary dissection on the survival of breast cancer patients. In a randomized trial with a long follow-up period, Fisher et al. showedthat removing occult positive nodes at the time of the initial surgery has no significant survivaladvantage for patients who have undergone ALND(1).With this background, Sentinel Lymph Node Biopsy (SLNB) was selected as the standard methodfor staging of the axilla in patients with clinically negative lymph node.SLNB is safe and accurate alternative to ALND with less morbidity, however about7% false negative rate has been reported for this procedure, and in these casesaxillary lymph node dissection may be omitted although it is necessary(2).

Although Physical Examination is the first step in evaluation of axillary LNs, most studies have reported a low sensitivity (8.3%-41%), specifity of 94.8% and a Negative Predictive Value (NPV) of 46.6% for this method(3, 4). Detecting whether apalpable lymph node is inflammatory, reactive or metastatic is difficult. The rate of false positiveresults is about 53% in this method(5).
Axillary Ultrasound (AUS) is a non-invasive, inexpensive and available method that is routinelyused in breast cancer patients to determine the status of the LNs. Several studies have shown thatAUS with or without Fine-NeedleAspiration Cytology (FNAC) is a suitable method fordetermining metastatic LNs with acceptable sensitivity and specificity.
According to studies, the diagnostic specificity of lymph node sampling under Ultrasound guidance for Core Needle Biopsy (CNB) or Fine Needle Aspiration (FNA) might be as high as 100%(5).

The sensitivity of these methods is reported as 65% to 70% (6), and if positive, axillary dissection can be performed directly for the patient. These practices cannot be performed in all imaging centers and are highly dependent on the radiologists' skill and available facilities.

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Methods

Patients

During a retrospectivecross-sectional study, through a nonprobability convenience sampling, all patients referred to the Motamed Cancer Institute (MCI) between Sep 2015 and August 2016 with a pathologic diagnosis of breast cancer were included.The Ultrasound report and pathological diagnosis of the axillary LNs were recorded. The inclusion criteria consisted of breast carcinoma, stage 0, I, II and IIIA who had not received neoadjuvant chemotherapy.

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Physical exam and Axillary Ultrasound

All patients underwent axillary palpation at Motamed Cancer Instituteby two breast surgeons with more than ten years of experience in breast surgery.Positive PE is defined as clinically suspicious LN, it means a palpable firm or hard LN.The criteria for suspicious LNs by AUS were observing at least one of these morphological changes, including Non-round shape, Hypoechogenicity, Cortical thickness>2.5 mm, Obliteration of the hilum and Lobulation. Axillary Ultrasounds were performed by two radiologists who passed breast imaging course.

Axillary surgery and Histological exam

Axillary surgeries including SLNB or ALND were done based on the criteria, it means that ALND was selected in subjects with highly suspicious LN during palpation or AUS or in

situations of positive SLNB. Before SLNB, lymphoscintigraphy was performed with a TC-99m with or without blue dye by sub-dermal, peri-areolar injection. Intraoperative detection of SLNs were carried out using a Gamma probe and hot LNs as well as the blue-colored LNs were excised and evaluated by frozen section.The SLNs were evaluated using H&E (Haematoxylin and Eosin) staining. A metastasis ≥2 mm was taken to indicate macrometastasis and <2 mm was taken as micrometastasis. Axillary Lymph Node Dissection (ALND) was not performed in the cases with micrometastasis. The results were analyzed by comparing the PE and AUS results of LNs by permanent pathology. (Figure 1)

Statistical Analysis

True Positive (TP),True Negative (TN), False Positive (FP) and False Negative (FN) of Physical Examination, AUS and a combination of them were identified.The sensitivity [TP/ (TP + FN)],specificity [TN/ (TN + FP)], positive predictive value (PPV) [TP/ (TP + FP)] and negativepredictive value (NPV) [TN/ (TN + FN)] were also calculated.The accuracy, Positive and Negative LikelihoodRatios (PPV, NPV) of AUS, PE and combination of them were calculated through their specific formula. Statistical analysis was carried out by SPSS version 20, using the significance level of 0.05 for P-value.The study was approved by the Ethics Committee of Motamed Cancer Institute (MCI) with code numberIR.ACECR.IBCRC.REC.1394.5.

Results

A total of 140 patients were eligible for participation in the study. The mean age of the patientswas 48.87 years (±10.46) and their median age was 48 years (range: 25-81). The mean BMI (Body Mass Index)was 27.55 (±4.55) and 67.8% had a BMI ≥25 (range:16-40).

The clinical and pathological characteristics of the subjects are presented in Table 1.

Among 62 patients who underwent ALND, 18 patients had positive sentinel node and 44 patients had suspicious LN in physical exam. The flowchart of the management plan is showed in figure1.

Table 2 represents summary of the AUS, PE and combination of them compared to the pathology results. According to the table, the highest true positive was for combination of AUS and PE and the best true negative was related to PE.

Table 3 shows the comparison of Sensitivity, Specificity, PPV, NPV, Positive Likelihood Ratio and Negative Likelihood Ratio of AUS, PE and AUS+PE. The highest sensitivity rate was related to AUS+PE with 70%. The best specificity was for AUS, about 88% and the accuracy of AUS+PE was the highest, 80%.

The accuracy of AUS was compared between the patients with BMI<25 and ≥ 25. Thesensitivity of AUS was 60% for the patients with BMI<25 and 52.9% for the patients who were overweight; the specificity was 83.3% and 86.48%, respectively. (Not shown in tables)

Among 53 patients with pathologically involved LNs, 36 (66.6%) had 1-2 (low-burden) and 18 (33.4%) had three or more (high-burden) involved LNs. Twentyfour patients had falsenegative results by AUS, including six patients in the high-burden group (25%) and the rest (75%) in the low-burden group.

Discussion

The sensitivity of AUS in the diagnosis of axillary involvement was moderate (56%), its specificity was good (88%) and the accuracy was obtained 76% when lymph node morphology was used. The sensitivity increased up to 70% and the sensitivity decreased (86%) when a classification of combined physical examination and AUS was usedin comparison to each of them alone.

Evaluation of axillary LN in breast cancer is crucial in determining the plan of treatment, including type of breast and axillary surgery, immediate reconstruction and the choice of systemic therapy. Insuspicious situations, the patient may become a candidate for neoadjuvant chemotherapy, or directly undergone ALND without SLNB. If this probability is negligible, the patient becomes a candidate for SLNB and should be prepared in advance for the procedure (6).

What is the best method for determining the axillary LN status before surgery? Various studies have considered this challenge. Feng et al, showed that the sensitivity and specificity of axillary palpation are 32% and 95.5% respectively(3). Other studies have reported a sensitivity of 8-35.5% and a specificity of 93-98.8% (7-9). The results of the present study showed a sensitivity and specificity of 31% and 98% for PE, which is consistent with previous findings. The false positive rate for PE was 1.4% in this study, which is different from the results obtained by Specht et al., who estimated the rate as 23% in very suspicious cases and up to 53% in relatively suspicious cases. They reported overallfalse negative rate of 25.7% for PE(5).

Imaging techniques can improve the diagnostic accuracy of physical examinations. AxillaryUltrasound is one of the most common methods that is inexpensive and available, however largely dependent on radiologists' experience. The sensitivity and specificity of Ultrasound were reported as 63.8% and 73.6% by Gurleyik et al., 45% and 85% by Jackson et al.,

54.3% and 100% by Gipponni et al., 58.6% and 89.4% by Feng et al., and 72% and 79% by Omranipouret al.(3, 10-12).

The Ultrasonic criteria for LN involvement may be related to size or morphology. Previous studies which have used morphological criteria, reported AUS as an acceptable method for detecting involved LN. Alvarez et al reported the sensitivity and specificity of the Ultrasound to differentiate between benign and suspicious LNs as 54.7-92.3%and 80.4-97.1% respectively. In our study, the sensitivity was 56% and specificity was 88%, which is consistent with the results reported by Alvarez et al.

The only similar study in Iran was performed by Omranipour et al., who reported a sensitivityand specificity of 72% and 79% for AUS. In this study the specificity of AUS was lower than ours. Like them, in some studies only patients with normal physical examinations were included(10-12), while in the present study, the patients had both positive and negative examinations.

One of the most important reasons for the low sensitivity rate of the AUS in this study may be the selection ofearly-stage patients. Micrometastasis to the LNs is possible in these patients and may not lead toclear changes in morphology or tumor size in Ultrasound.

The combination of PE and AUS improved sensitivity up to 70%, but the specificity did not change significantly (86% instead of 88%). In the case of positive PE and AUS, the probability of axillary involvement was76% (38/50). The false negative rate of the combination test was 8.6% and these patients could be directly candidates for axillary dissection if FNA/ CNB of LN is not accessible.

In 2011 with the publication of the results of the Z0011 trials, a new type of categorization was made for the first time which showed that patients with 1-2 sentinel lymph nodes with specific criteria (breast conserving surgery, postoperative radiotherapy and positive hormone receptors) should not undergo axillary dissection. In the present study, the false negative AUS was reported in 24 patients, including six cases who had more than three (high-burden) and 18 who had 1-2 (low-burden) involvement. On the other words, in 75% of those who had false negative axilla, axillary involvement was low–burden. It means that negative AUS predicts negative or low burden nodal involvement and these patients rarely have high-burden axillary involvement.Farshid et al. showed that, of those who had a false negative AUS, LN involvement

was low-burden in 86.8% (13).Jackson et alin another study showed the false negative rate of AUS in detecting≥3 nodal involvement is 4% (12).

In most studies, when FNA is added to the AUS, sensitivity is reduced, but specificity is increased. Gipponi et al. (10) reported sensitivity as 44.1% and specificity as 100%, and they concluded that AUS alone might be suggested for early stage breast cancer. FNA- positive findings are more reliant when there is a plan for Neoadjuvant chemotherapy or ALND.Gurleyik et al. reported a 100% PPV for AUS+FNA (11).

Regarding the specificity of Ultrasound and false negative results, it is impossible to eliminate axillary surgery based on negative Ultrasound results until the results of Sound and INSEMA trials are released. Exclusion of axillary surgery for early-stage breast cancer may be possible in near future.

Although axillary palpation was performed by expert breast surgeons and axillary Ultrasound by breast radiologists,we did notconsider the difference between clinicians' experience on the results and the lack of the reliability assessment could be a limitation of this study.

In conclusion, the axillary Ultrasound is a moderately sensitivewith good specificity for diagnosis of lymph node involvement. The combination of physical examination and Ultrasound could improve the sensitivity in comparing to each one alone. If both the physical examination and AUS are suspicious, axillary dissection could be considered when FNA or CNB of the lymph node is not available. Patients with a negative axillary Ultrasound and even the false negative cases often have low-burden LN metastases and better prognosis even without ALND.

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Ethical considerations: The study was confirmed by Ethics Committee of Motamed Cancer Institute, ACECR. No IR.ACECR.IBCRC.REC.1394.5

Conflict of Interests: There was no conflict of interests for any of the authors

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Table 1. The clinical and pathological characteristics of the patients.

Variable	Number (%)
Stage	
0 (LCIS, DCIS)	9 (6.4%)
I	48 (34.3%)
II	73 (52.1%)
IIIA	10 (7.1%)
Tumor Histology	
LCIS	1 (0.7%)
DCIS	8 (5.9%)
IDC	117 (86%)
ILC	10 (7.4%)
Tumor size	
T1	50(39.1%)
T2	68(53.1%)
T3	10 (7.8%)
Axillary surgery	
SLNB	96
ALND	62
Grade	
I	6 (5.4%)
II	78 (69.6%)
III	28 (25%)
ER/PR	
Positive	104 (87.4%)
Negative	15 (12.6%)
HER2	
Positive	47 (43.5%)
Negative	61 (56.5%)
Breast surgery	
Mastectomy	29 (21.8%)
Lumpectomy	104 (78.2%)

LCIS (Lobular Carcinoma In Situ), DCIS (Ductal carcinoma in situ), IDC (Invasive ductal carcinoma), ILC (Invasive lobular carcinoma), ER (Estrogen receptor), PR (Progesterone receptor), HER2 (Human epidermal growth factor receptor 2).

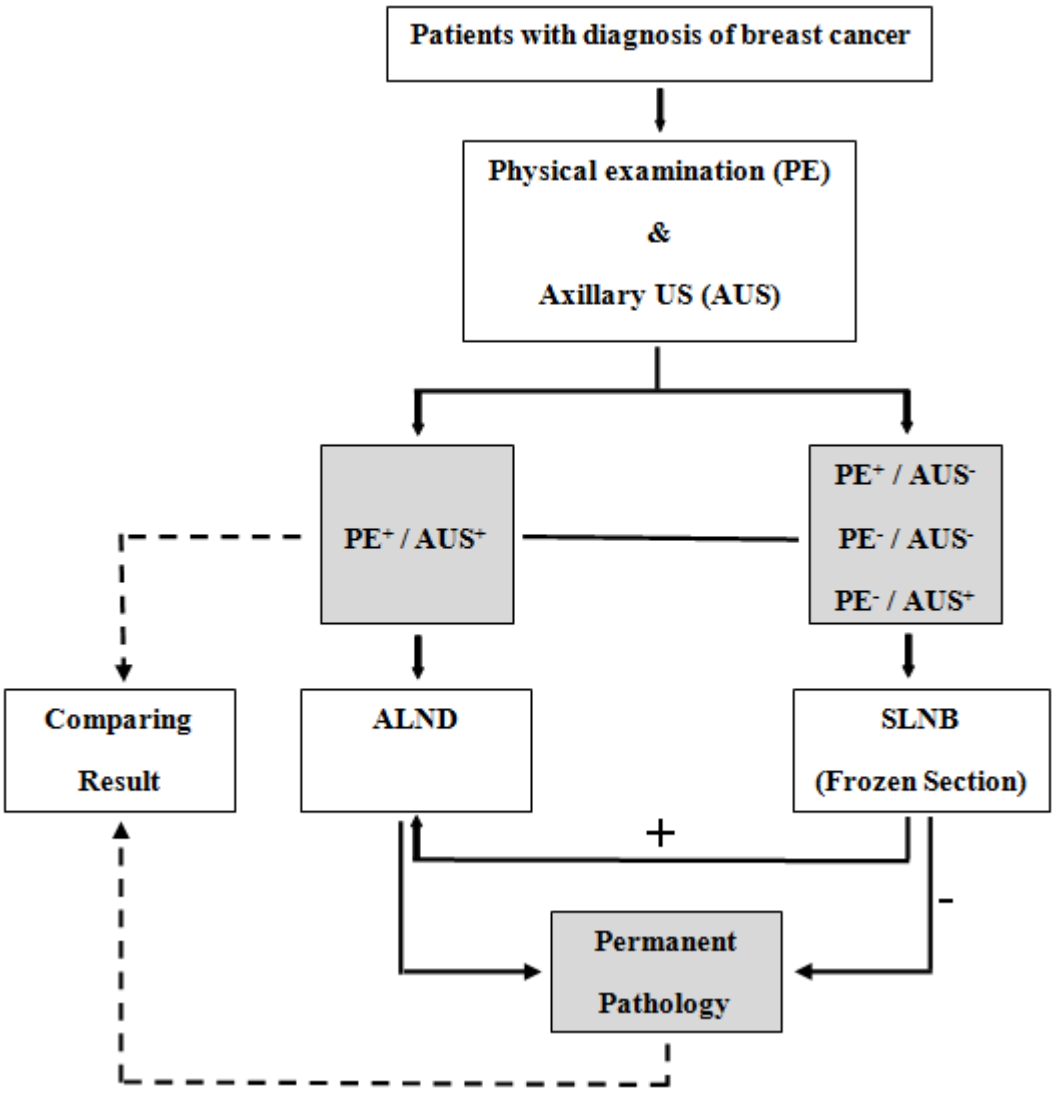
Table 2. The absolute and relative distribution of the diagnoses made by the AUS, PE and the combination of them in comparison with the pathology results

Pathology Results		Positive	Negative	Total
Test Result				
AUS	**Positive**	30 (21.4%)	10 (7.1%)	40 (28.6%)
	Negative	24 (17.1%)	76 (54.3%)	100 (71.4%)
	Total	54 (38.6%)	86 (61.4%)	140 (100%)
PE	**Positive**	17 (12.1%)	2 (1.4%)	19 (13.6%)
	Negative	37 (26.4%)	84 (60%)	121 (86.4%)
	Total (%)	54 (38.6%)	86 (61.4%)	140 (100%)
Combination of PE and AUS	**Positive**	38 (27.1%)	12 (8.6%)	50 (35.7%)
	Negative	16 (11.5%)	74 (52.8%)	90 (64.3%)
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OPEN PEER REVIEW

Revision (3)

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Reply to Reviewers

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You can read the authors' responses to the reviewers' comments in the next page.

Dear Reviewers

Thanks to your notes

I have defined and abbreviations in the manuscript and below the tables. Please inform me about other essential editions.

Sincerely,

Asiie Olfatbakhsh, MD, The corresponding Author

02-30-2020

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Revision (4)

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OPEN PEER REVIEW

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Since the aim of this study was only to evaluate the sensitivity andspecificity of AUS, without FNA or CNB, so those patients who had been subjected to these invasive procedures were excluded. Regarding to the previous studies(7)that reported the sensitivity and specificity of AUS about 78% and 79% respectively, $\alpha<0.05$ with assumption of Positive Likelihood Ratio (PLR)>2.5, the predicted sample size was at least 100 patients. Informed consents were obtained routinelyfrom patients for using data with considering their privacy. This study was accepted in scientific committee and received the ethics committee approval. The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki)

Physical exam and Axillary Ultrasound

All patients underwent axillary palpation at Motamed Cancer Instituteby two breast surgeons with more than ten years of experience in breast surgery.Positive PE is defined as clinically suspicious LN, it means a palpable firm or hard LN.The criteria for suspicious LNs by AUS were observing at least one of these morphological changes, including Non-round shape, Hypoechogenicity, Cortical thickness>2.5 mm, Obliteration of the hilum and Lobulation. Axillary Ultrasounds were performed by two radiologists who passed breast imaging course.

Axillary surgery and Histological exam

Axillary surgeries including SLNB or ALND were done based on the criteria, it means that ALND was selected in subjects with highly suspicious LN during palpation or AUS or in

situations of positive SLNB. Before SLNB, lymphoscintigraphy was performed with a TC-99m with or without blue dye by sub-dermal, peri-areolar injection. Intraoperative detection of SLNs were carried out using a Gamma probe and hot LNs as well as the blue-colored LNs were excised and evaluated by frozen section.The SLNs were evaluated using H&E (Hematoxylin and Eosin) staining. A metastasis ≥2 mm was taken to indicate macrometastasis and <2 mm was taken as micrometastasis. Axillary Lymph Node Dissection (ALND) was not performed in the cases with micrometastasis. The results were analyzed by comparing the PE and AUS results of LNs by permanent pathology. (Figure 1)

Statistical Analysis

True Positive (TP),True Negative (TN), False Positive (FP) and False Negative (FN) of Physical Examination, AUS and a combination of them were identified.The sensitivity [TP/ (TP + FN)],specificity [TN/ (TN + FP)], positive predictive value (PPV) [TP/ (TP + FP)] and negativepredictive value (NPV) [TN/ (TN + FN)] were also calculated.The accuracy, Positive and Negative LikelihoodRatios (PLR, NLR) of AUS, PE and combination of them were calculated through their specific formula. Statistical analysis was carried out by SPSS version 20, using the significance level of 0.05 for P-value.The study was approved by the Ethics Committee of Motamed Cancer Institute (MCI) with code numberIR.ACECR.IBCRC.REC.1394.5.

Results

A total of 140 patients were eligible for participation in the study. The mean age of the patientswas 48.87 years (±10.46) and their median age was 48 years (range: 25-81). The mean BMI (Body Mass Index)was 27.55 (±4.55) and 67.8% had a BMI ≥25 (range:16-40).

The clinical and pathological characteristics of the subjects are presented in Table 1.

Among 62 patients who underwent ALND, 18 patients had positive sentinel node and 44 patients had suspicious LN in physical exam. The flowchart of the management plan is showed in figure1.

Table 2 represents summary of the AUS, PE and combination of them compared to the pathology results. According to the table, the highest true positive was for combination of AUS and PE and the best true negative was related to PE.

Table 3 shows the comparison of Sensitivity, Specificity, PPV, NPV, Positive Likelihood Ratio and Negative Likelihood Ratio of AUS, PE and AUS+PE. The highest sensitivity rate was related to AUS+PE with 70%. The best specificity was for AUS, about 88% and the accuracy of AUS+PE was the highest, 80%.

The accuracy of AUS was compared between the patients with BMI<25 and ≥ 25. Thesensitivity of AUS was 60% for the patients with BMI<25 and 52.9% for the patients who were overweight; the specificity was 83.3% and 86.48%, respectively. (Not shown in tables)

Among 53 patients with pathologically involved LNs, 36 (66.6%) had 1-2 (low-burden) and 18 (33.4%) had three or more (high-burden) involved LNs. Twentyfour patients had falsenegative results by AUS, including six patients in the high-burden group (25%) and the rest (75%) in the low-burden group.

Discussion

The sensitivity of AUS in the diagnosis of axillary involvement was moderate (56%), its specificity was good (88%) and the accuracy was obtained 76% when lymph node morphology was used. The sensitivity increased up to 70% and the sensitivity decreased (86%) when a classification of combined physical examination and AUS was usedin comparison to each of them alone.

Evaluation of axillary LN in breast cancer is crucial in determining the plan of treatment, including type of breast and axillary surgery, immediate reconstruction and the choice of systemic therapy. Insuspicious situations, the patient may become a candidate for neoadjuvant chemotherapy, or directly undergone ALND without SLNB. If this probability is negligible, the patient becomes a candidate for SLNB and should be prepared in advance for the procedure (6).

What is the best method for determining the axillary LN status before surgery? Various studies have considered this challenge. Feng et al, showed that the sensitivity and specificity of axillary palpation are 32% and 95.5% respectively(3). Other studies have reported a sensitivity of 8-35.5% and a specificity of 93-98.8% (7-9). The results of the present study showed a sensitivity and specificity of 31% and 98% for PE, which is consistent with previous findings. The false positive rate for PE was 1.4% in this study, which is different from the results obtained by Specht et al., who estimated the rate as 23% in very suspicious cases and up to 53% in relatively suspicious cases. They reported overallfalse negative rate of 25.7% for PE(5).

Imaging techniques can improve the diagnostic accuracy of physical examinations. AxillaryUltrasound is one of the most common methods that is inexpensive and available, however largely dependent on radiologists' experience. The sensitivity and specificity of Ultrasound were reported as 63.8% and 73.6% by Gurleyik et al., 45% and 85% by Jackson et al.,

54.3% and 100% by Gipponni et al., 58.6% and 89.4% by Feng et al., and 72% and 79% by Omranipouret al.(3, 10-12).

The Ultrasonic criteria for LN involvement may be related to size or morphology. Previous studies which have used morphological criteria, reported AUS as an acceptable method for detecting involved LN. Alvarez et al reported the sensitivity and specificity of the Ultrasound to differentiate between benign and suspicious LNs as 54.7-92.3%and 80.4-97.1% respectively. In our study, the sensitivity was 56% and specificity was 88%, which is consistent with the results reported by Alvarez et al.

The only similar study in Iran was performed by Omranipour et al., who reported a sensitivityand specificity of 72% and 79% for AUS. In this study the specificity of AUS was lower than ours. Like them, in some studies only patients with normal physical examinations were included(10-12), while in the present study, the patients had both positive and negative examinations.

One of the most important reasons for the low sensitivity rate of the AUS in this study may be the selection ofearly-stage patients. Micrometastasis to the LNs is possible in these patients and may not lead toclear changes in morphology or tumor size in Ultrasound.

The combination of PE and AUS improved sensitivity up to 70%, but the specificity did not change significantly (86% instead of 88%). In the case of positive PE and AUS, the probability of axillary involvement was76% (38/50). The false negative rate of the combination test was 8.6% and these patients could be directly candidates for axillary dissection if FNA/ CNB of LN is not accessible.

In 2011 with the publication of the results of the Z0011 trials, a new type of categorization was made for the first time which showed that patients with 1-2 sentinel lymph nodes with specific criteria (breast conserving surgery, postoperative radiotherapy and positive hormone receptors) should not undergo axillary dissection. In the present study, the false negative AUS was reported in 24 patients, including six cases who had more than three (high-burden) and 18 who had 1-2 (low-burden) involvement. On the other words, in 75% of those who had false negative axilla, axillary involvement was low–burden. It means that negative AUS predicts negative or low burden nodal involvement and these patients rarely have high-burden axillary involvement.Farshid et al. showed that, of those who had a false negative AUS, LN involvement

was low-burden in 86.8% (13).Jackson et alin another study showed the false negative rate of AUS in detecting≥3 nodal involvement is 4% (12).

In most studies, when FNA is added to the AUS, sensitivity is reduced, but specificity is increased. Gipponi et al. (10) reported sensitivity as 44.1% and specificity as 100%, and they concluded that AUS alone might be suggested for early stage breast cancer. FNA- positive findings are more reliant when there is a plan for Neoadjuvant chemotherapy or ALND.Gurleyik et al. reported a 100% PPV for AUS+FNA (11).

Regarding the specificity of Ultrasound and false negative results, it is impossible to eliminate axillary surgery based on negative Ultrasound results until the results of Sound and INSEMA trials are released. Exclusion of axillary surgery for early-stage breast cancer may be possible in near future.

Although axillary palpation was performed by expert breast surgeons and axillary Ultrasoundby breast radiologists,we did notconsider the difference between clinicians' experience on the results and the lack of the reliability assessment could be a limitation of this study.

In conclusion, the axillary Ultrasoundis a moderately sensitivewith good specificity for diagnosis of lymph node involvement. The combination of physical examination and Ultrasound could improve the sensitivity in comparing to each one alone. If both the physical examination and AUS are suspicious, axillary dissection could be considered when FNA or CNB of the lymph node is not available. Patients with a negative axillary Ultrasound and even the false negative cases often have low-burden LN metastases and better prognosis even without ALND.

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Conflict of Interests: There was no conflict of interests for any of the authors

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Table 1. The clinical and pathological characteristics of the patients.

Variable	Number (%)
Stage	
0 (LCIS, DCIS)	9 (6.4%)
I	48 (34.3%)
II	73 (52.1%)
IIIA	10 (7.1%)
Tumor Histology	
LCIS	1 (0.7%)
DCIS	8 (5.9%)
IDC	117 (86%)
ILC	10 (7.4%)
Tumor size	
T1	50(39.1%)
T2	68(53.1%)
T3	10 (7.8%)
Axillary surgery	
SLNB	96
ALND	62
Grade	
I	6 (5.4%)
II	78 (69.6%)
III	28 (25%)
ER/PR	
Positive	104 (87.4%)
Negative	15 (12.6%)
HER2	
Positive	47 (43.5%)
Negative	61 (56.5%)
Breast surgery	
Mastectomy	29 (21.8%)
Lumpectomy	104 (78.2%)

LCIS (Lobular Carcinoma In Situ), DCIS (Ductal carcinoma in situ), IDC (Invasive ductal carcinoma), ILC (Invasive lobular carcinoma), ER (Estrogen receptor), PR (Progesterone receptor), HER2 (Human epidermal growth factor receptor 2), SLNB (Sentinel Lymph Node Biopsy), ALND (Axillary Lymph Node Dissection)

Table 2. The absolute and relative distribution of the diagnoses made by the AUS, PE and the combination of them in comparison with the pathology results

Pathology Results		Positive	Negative	Total
Test Result				
AUS	**Positive**	30 (21.4%)	10 (7.1%)	40 (28.6%)
	Negative	24 (17.1%)	76 (54.3%)	100 (71.4%)
	Total	54 (38.6%)	86 (61.4%)	140 (100%)
PE	**Positive**	17 (12.1%)	2 (1.4%)	19 (13.6%)
	Negative	37 (26.4%)	84 (60%)	121 (86.4%)
	Total (%)	54 (38.6%)	86 (61.4%)	140 (100%)
Combination of PE and AUS	**Positive**	38 (27.1%)	12 (8.6%)	50 (35.7%)
	Negative	16 (11.5%)	74 (52.8%)	90 (64.3%)
	Total (%)	54 (38.6%)	86 (61.4%)	140 (100%)

AUS (Axillary Ultrasound), PE (Physical Examination).

Table 3. A comparison of the diagnostic accuracy of AUS and PE in preoperative LN evaluation

PE (Physical Examination), AUS (Axillary Ultrasound).

Test	PE	95% CI	AUS	95% CI	AUS +PE	95% CI
Sensitivity	0.31	0.19 – 0.44	0.56	0.42 – 0.69	0.70	0.56 – 0.82
Specificity	0.98	0.94 - 1	0.88	0.82 – 0.95	0.86	0.77 – 0.93
Positive Predictive Value	0.89	0.76 - 1	0.75	0.61 – 0.88	0.76	0.65 – 0.85
Negative Predictive Value	0.69	0.61 – 0.78	0.76	0.68 – 0.84	0.82	0.75 – 0.88
Positive Likelihood Ratio	13.53	3.26 – 56.29	4.78	2.5 – 8.97	5.04	2.9 – 8.76
Negative Likelihood Ratio	0.7	0.58 – 0.84	0.5	0.37 – 0.68	0.34	0.23 – 0.52
Accuracy	0.72	0.64 – 0.79	0.76	0.68 – 0.83	0.80	0.72 – 0.86

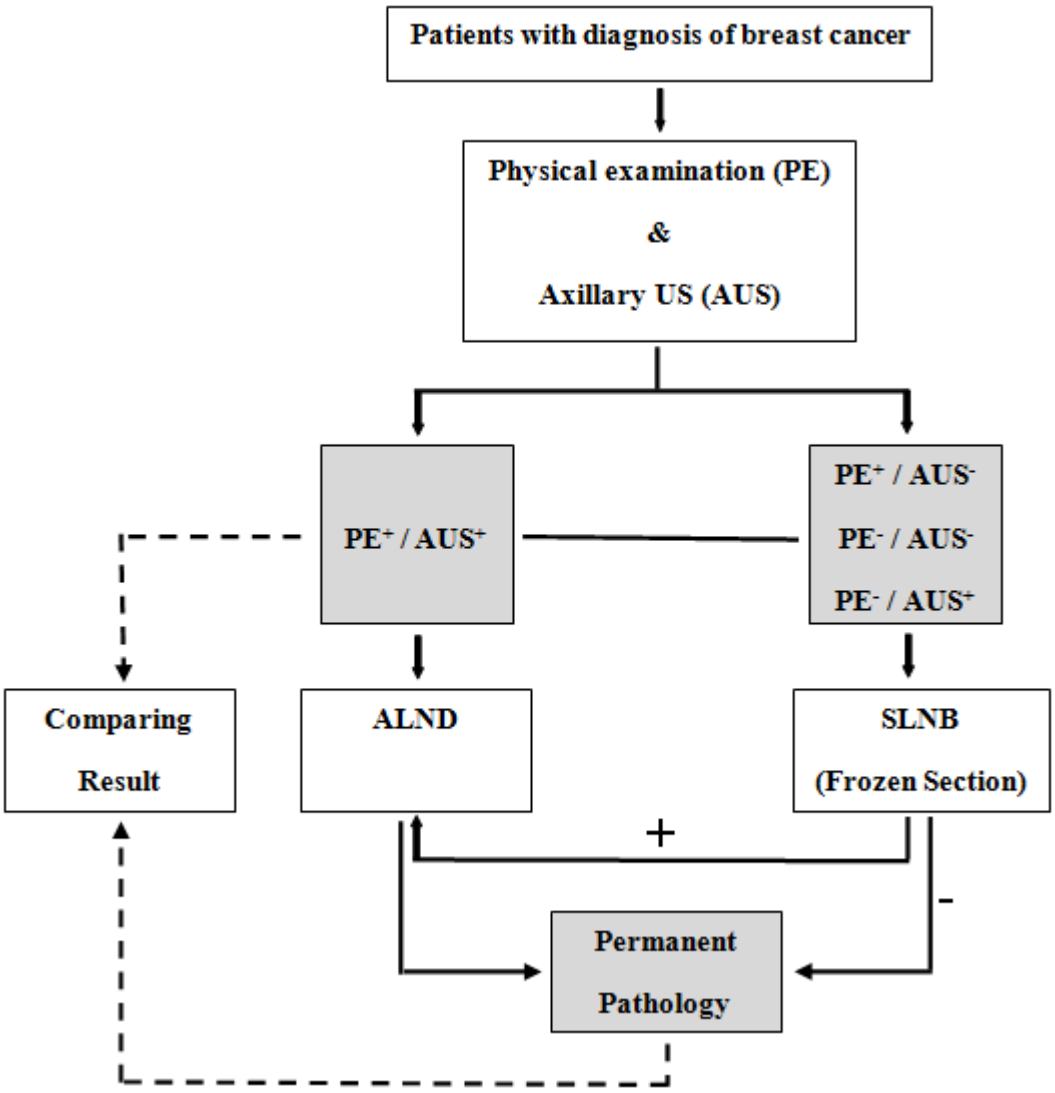


Figure 1: The diagram of the patients' management

OPEN PEER REVIEW

Revision (5)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: Reviewer | **Revision** (5) 13 Mar 2020

Dear AE,
The topic could be an interesting one however there are some problems in the manuscript that should be considered for revising.
Firstly, please use more recent sources.
Secondly please revise final conclusion about ALND.
Finally, The manuscript should be edited by a native translator.
Kind Regards

REFEREE: Associate Editor | **Revision** (5) 20 Mar 2020

Dear EIC
Based on the authors' answers to the reviewers' comments, the reviewer(s) have recommended publication.
Kind regards.

REFEREE: EIC | **Revision** (5) 26 Mar 2020

Dear Author,
The document is reviewed carefully by the reviewers. According to their comments and your revisions as requested, this manuscript is accepted.
Thanks.