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Comparison of Standard and Vacuum Specimen Mammography in the Detection of Margin Status in Conservative Surgery for Breast Cancer: a Cross-Sectional Diagnostic Study

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ABSTRACT

Background: One of the most important factors that increases breast cancer (BC) recurrence after Breast-conserving surgery (BCS) is the positivity of the margins, which is found in permanent histological exams. Intra-operative specimen mammography (SM) can reduce the rate of margin positivity and re-operation. Our aim was to examine whether vacuum SM (VSM) is more accurate than standard SM (SSM) in detecting the positivity of the margins.

Methods: For this cross-sectional study, in the operating room, excised specimens of 55 women with breast cancer who underwent lumpectomy were oriented by metallic staples and sutures for radiologic and histological assessment, respectively. In the radiology ward, SSM was first taken; then, the specimen was vacuum packed and VSM was performed. Afterwards, the specimen was sent for histopathological analysis as a gold standard for the assessment of surgical margins. Specimens' margins were classified according to the size of clear margins in millimeters as zero or >0; and ≤1 or >1.

Results: The mean age of all participants was 51.22 ± 10.58 years. Totally, 220 margins were assessed. According to classified margins (zero and 1 mm), for the detection of affected margins, the accuracy values of the VSM method were 90.52%, and 87.20% while these figures were 91.51% and 88.68% for SSM. There was substantial agreement between the two methods of detecting the affected margins (VSM and SSM), with Cohen's κ =0.66, 95% CI: 0.34-0.97, P-value <0.001). Finally, there was not a statistically significant difference in the proportion of detecting margin between SSM and VSM (McNemar test P-value =0.63).

Conclusion: Specimen mammography with an adequate orientation of the tissue is an accurate and practical method for immediate intraoperative examination of the margin status in BCS, and VSM is not superior to SSM in the detection of affected margins.

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INTRODUCTION

Breast-conserving surgery (BCS) is the most common operation performed for the treatment of breast cancer.[1] One of the most important surgical points that affects the rate of recurrence after BCS is

the status of the margins of resection.[1] A negative margin is a sine qua non of perfect cancer surgery, and the gold standard test that confirms the negative status of the margins is the permanent histological exam.[2] Nonetheless, this is carried out after the operation, and a positive margin necessitates a second surgery which involves stress and cost for the patient and the health system[3,4], and cannot always lead to clear margins without affecting the cosmetic appearance. The second operation might be prevented by the intra-operative histological assessment of the margins via frozen section examination (FS), but it necessitates the collaboration of an expert pathologist, increases the time of the surgery, needs specialized equipment, and is costly.

Another method for assessing margins is intra-operative radiologic assessment of the excised tissue. Specimen mammography (SM) can detect the lesion in most instances, and can roughly estimate the margin status. An affected or close margin seen in the SM directs the surgeon toward further resection and thus can reduce the rate of re-operation.[5] A drawback of the standard SM (SSM) is tissue overlap or folding of some parts of the specimen that occasionally occurs[6], which can distort the margins in the image and lead to unnecessary margin re-excision. Bau *et al.*[6] carried out a pilot study on 18 tumors to find out whether vacuum sealing of the specimen could improve the diagnostic performance of SM and found encouraging results.

Therefore, we performed this study with the objective of comparing the capacity of vacuum SM (VSM) and standard SM (SSM) in detecting the status of BCS margins in permanent histological exams.

METHODS

Settings

This study was undertaken from April 2021 to March 2023 in two hospitals (Arash Women's Hospital and the Cancer Institute) affiliated to TUMS.

Participants

Women attending the Breast Clinic of the study centers constituted the study population. Inclusion criteria consisted of pathologically-proven breast cancer, indication of BCS, presentation of the cancer as a mammographically-detectable breast mass or microcalcification, and consent to participate. Similarly, for patients who underwent neoadjuvant treatments, the presence of the mass or microcalcifications in a mammogram taken before surgery was necessary for inclusion in the study.

When the surgical technique did not involve excision of the skin above the tumor or the pectoralis fascia at the deep margin and when the mass was too large to be placed in the vacuum packing bag, the cases were excluded.

Study design and variables

At the point of entry into the study, an information collection form consisting of demographic, anthropometric, and reproductive details was filled out for all participants. In the operating room, the resected specimen was oriented on its superior, medial, inferior, and lateral margins using staples placed on sutured small sterile cardboards (as explained in our previous paper)[7] for radiologic assessments, and by sutures for the histological assessment (Figure 1a). The specimen was immediately sent to the radiology ward, where an SSM was taken (Figure 1b). The image was sent to the surgeon to go on with the operation, but in the radiology ward, the specimen was placed in a special bag and vacuum packed by a vacuum sealer (Figure 1c and 1d), and VSM was done (Figure 1e). Thereafter, the tissue was taken out of the pack, put in formalin, and sent to the pathology department. In order to have a double-blind assessment, the images were assessed later by a radiologist dedicated to women's radiology; while she was not aware of the histological assessment results, the anonymous images of SSM and VSM of different patients were presented in an accidental order. The radiologist verified and recorded the distance of the last margin from the border of the lesion in the four directions in the SSMs and VSMs. The pathologist, who specialized in women's disease pathology, measured and documented the four margins microscopically on the formalin-fixed paraffin-embedded specimens. She was not aware of the radiology results. Then, the results of the margin assessment in SSM and VSM were compared with histologic results.

Sample Size

A study by Bau *et al.*[6], reported a specificity of around 47% for SSM and 100% for VSM and a sensitivity of 67% for SSM and VSM. We considered a precision of 20% and estimated the prevalence of positive margin by pathology at around 10%. We calculated that 267 and 237 margins would be enough to estimate the diagnostic test value for SSM and VSM, respectively, using the online calculator available at: https://wnarifin.github.io/ssc/sssnsp.html. Therefore, the total sample was considered as 267 margins.

Statistical Analysis

For the statistical analysis, we used SPSS version 24 (IBM Corp. 2016. IBM SPSS Statistics for Windows, version 24.0. Armonk, NY: IBM Corp). The results of the categorical variables are demonstrated as numbers (and percentages), and the results of the continuous variables are stated as mean ± standard deviation (SD). Since the two tests (SSM

and VSM) have been performed on the same series of patients, McNemar test was used to compare the results of detecting the affected margins. We considered the results of the histological assessment as the gold standard method for the assessment of margin size and the detection of margin infection.

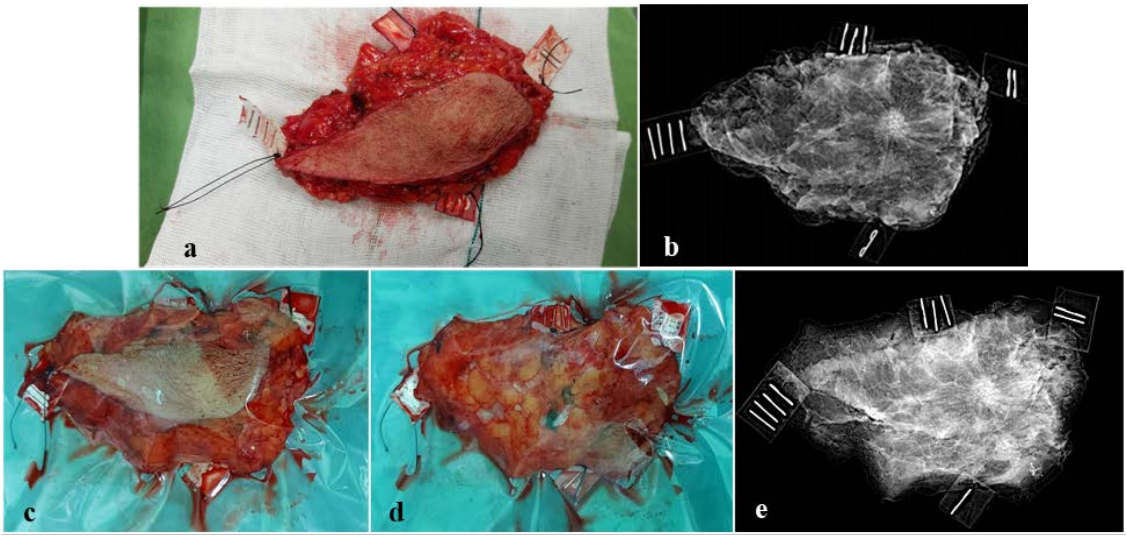

Figure 1. Specimen orientation, vacuum sealing, standard and vacuum specimen mammography of one patient. **a**. Orientation of specimen with staples for mammography, and with sutures for pathologic review. **b.** Standard specimen mammography. **c and d.** Vacuum sealing of the specimen ***b.*** anterior or superficial view, ***c.*** posterior or deep view. The light reflection is due to the shrinking of the bag after the air is completely evacuated. **e.** Vacuum specimen mammography.

For the assessment of the agreement between the size of the margins detected by the two intra-operative imaging modalities and pathology, we considered all the specimens' margins and classified them according to the size of clear margins in millimeters as zero or >0; and ≤1 or >1. Cohen's κ was run to determine if there was an agreement between the two methods (SSM and VSM) of detecting the affected margin. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of SSM and VSM for detecting the affected margins according to the two classifications of clear margins were calculated via an online calculator (https://www.medcalc.org/calc/diagnostic_test.php).

RESULTS

Overall, 55 patients were included in the study. The mean age of the participants was 51.22 ± 10.58 years (range 22-74). Regarding the menopausal status, 34 (61.8%) and 21 (38.2%) were post-and pre-menopause, respectively. The characteristics of the tumors are demonstrated in Table 1.

Considering the incomplete data (n=47), a total of 220 margins were assessed. Considering the missing data, the number of margins assessed by SSM was 212, by VSM, 211, and by histology, 220. The sensitivity, specificity, predictive values, and accuracies of SSM and VSM according to the two classified margins are shown in Table 2.

According to the classified margins (zero and 1 mm), for the detection of the affected margins, the

Table 1. Characteristics of all participants and their tumors

Variable (Number)	Type	Number	Percent
Tumor side (55)	Left	27	49.1
	Right	28	50.9
Exam findings (55)	Nothing	8	14.5
	Single mass	40	72.7
	Multiple masses	4	7.3
	Others	3	5.5
Surgery technique (55)	No guide-wire	29	52.7
	With guide-wire	26	47.3
Histology (55)	DCIS*	7	12.7
	IDC**	48	87.3
Grade (54)	1	14	25.9
	2	31	57.4
	3	9	16.7
ER (54)	Negative	6	11.1
	Positive	48	88.9
PR (54)	Negative	11	20.4
	Positive	43	79.6
HER2 (52)	Negative	40	76.9
	Positive	12	23.1
Ki67 % (51)		25.7 ± 16.34	
Lesion size in pathology in millimeter (53)		25.9 ± 9.6	

* Pure DCIS, ** +DCIS in 33 tumors. DCIS= Ductal carcinoma in-situ, ER= Estrogen receptor, IDC= Invasive ductal carcinoma, PR= progesterone receptor, Ki67% and Lesion size was reported in Mean ± Standard deviation.

accuracy values of the VSM method were 90.52%, and 87.20% while they were 91.51% and 88.68% for SSM. A McNemar test determined that there was not a statistically significant difference in the proportion of the margins detected by SSM and VSM (P-value =0.63). There was substantial agreement between the two methods of detecting the affected margins (VSM and SSM), with Cohen's κ =0.66, 95% CI: 0.34-0.97, P-value <0.001).

DISCUSSION

We carried out a study to find out whether VSM was superior to SSM for the intra-operative assessment of the margin status while conducting BCS. The present study found that both modalities had a substantial agreement with more than 85% accuracy considering different classifications of margins (zero and 1 mm). Positive margins of resection in a BCS for breast cancer increase the risk of recurrence. Therefore, a re-excision of the margins should take place. [1] When done through a second operation, especially if oncoplastic techniques had been used in the first operation, the margins are not oriented as they were originally. Consequently, wider excision might be required, cosmetic deformity is likely, a true negative margin may not be achieved, and even mastectomy may sometimes be needed. In addition, the adverse effects of a second anesthesia, the cost and equipment of the second procedure, the stress for the patient and physician, and the time spent throughout these processes are considerable. [3, 8-10]

While histological assessment of the margins during the operation is possible, FS of the tissue is very time-consuming and costly. The main shortcoming is that one or more points in each margin are examined, while the whole length of the resection border needs to be considered. [11, 12] Nevertheless, the accuracy is high as a sensitivity of 91.7% and a specificity of 77.8% have been stated in a meta-analysis. [13]

SM is easily feasible and lacks some of the disadvantages of FS. A clear advantage of SM is that the whole margin and the closest point to the lesion can be seen. Jin et al. [14] compared 84 cases of intra-operative FS with 182 cases of SSM. They found a significant correlation between radiological and histological margins and no difference in the rate of margin positivity and in the need for a second surgery between the two groups. However, the surgery took a significantly longer time with FS. Nevertheless, studies comparing SM with permanent histological assessment have shown a much lower sensitivity than studies evaluating FS. In a meta-analysis of 9 studies including a total of more than 500 patients, the accuracy of SM was around 70%. [11]

To perform SM, the specimen is placed and compressed between the mammography plates; the tissue needs to be fixed in place to prevent rotation and folding as far as possible. Several methods are used for this purpose; examples include fixing the four directions of the sample on a sheet by clips [15], putting the specimen in a dedicated container [16] or in a plastic bag while the surgeons take care of the precise location of the clips. [17] While these are acceptable options, vacuum packing the excised tissue as proposed by Bau et al. seems more helpful, as it not only fixes the specimen but also evacuates the air and compresses it. Bau et al. [6] found a concordance of 44% between the two modalities, and reported VSM to be much more specific (100% vs. 47%) and more accurate than SSM, while the sensitivities were similar (67%).

In our study, the concordance between the two modalities was very high (> 90%). We think that the dissimilarity of our results with that of Bau et al. [6] regarding the comparison of SSM and VSM is firstly due to our larger sample size, as they had carried out a pilot study on only 18 specimens, and secondly, due to the different techniques we used for the orientation of our specimens, which contributed to the adequate placement of the tissue between the mammography plates in SSM (Figure 2). Although we used this method because clips were not easily available during the project, the small cardboards sutured to the four directions (with one to four staples on them as markers of the four directions) [7] were used as small handles for the precise placement of the specimen between the plates, preventing any folding or rotation. Thus, the SSM was accurate enough and the vacuum packing did not improve the results. The importance of precise handling of the specimen for correct orientation in the image and accurate evaluation has been emphasized by other studies. [17, 18]

In the present study, the specificity, NPV, and accuracy of both methods were very high (around 90%). However, our figures regarding sensitivity and PPV are very low; the reason lies in the low number of positive margins in our study. This is probably because of the high rate of oncoplastic surgery in our centers, and also the fact that based on the experience of local surgeons, the need for a second operation is a great concern for patients in our country and is considered as an indicator of worse prognosis, which probably leads to slightly larger margins excised during the first surgery. This very low rate of margin positivity has been shown in another study in our country. [19]

Table 2. Sensitivity, specificity, predictive values and accuracies of standard and vacuum specimen mammography

	TP	FP	TN	FN	Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)	Accuracy (95%CI)
Margin 0 vs >0 mm									
SSM vs Path	1	4	193	14	6.67 (0.17 -31.95)	97.97 (94.88-99.44)	20.00 (0.51-71.64)	93.24 (88.91-96.25)	91.51 (86.91-94.89)
VSM vs Pathology	1	6	190	14	6.67 (0.17 -31.95)	96.94 (93.45-98.87)	14.29 (0.36-57.87)	93.14 (88.75-96.20)	90.52 (85.74-94.11)
Margin <=1 vs >1mm									
SSM vs Pathology	1	6	187	18	5.26 (0.13-26.03)	96.89 (93.36-98.85)	14.29 (0.36-57.87)	91.22 (86.48-97.71)	88.68 (83.63-92.61)
VSM vs Pathology	1	10	183	17	5.56 (0.14-27.29)	94.82 (90.68-97.49)	9.09 (0.23-41.28)	91.50 (86.74-94.97)	87.20 (81.93-91.40)

SSM= Standard specimen mammography, VSM= Vacuum specimen mammography, CI= Confidence interval, Con.= concordance, NPV= Negative predictive value, PPV= Positive predictive value.

Overall, the high accuracy detected by SSM and VSM in our study can confidently assure a safe pathologic margin during intra-operative assessment. In other words, the possibility for a margin detected as negative in SM for being positive in pathology is very low. Nevertheless, our study did not confirm a higher diagnostic yield for VSM compared to SSM.Nowadays, state-of-the-art techniques [10, 20] are being used that allow clear two - and three - dimensional imaging of the excised specimens during BCS, but they are expensive and only available in a limited number of surgical centers. Other techniques are being developed, including the injection of indocyanine green caught intraoperatively by near-infrared imaging[21], Raman spectroscopy[22], volumetric imaging[23], and other methods.[24] Investigators of these techniques claim that they are more accurate than the present methods, but further research is warranted to prove any real benefit. Meanwhile, SSM or VSM can be performed in any facility that possesses a mammography unit, and can effectively assist in margin assessment.

Our study had some limitations: first, we did not assess the superficial and deep margins in SMs. Assessment of these margins would need two sets of pictures by imaging the specimen again, but in the opposite (at right angles) direction for SSM and VSM; the latter would include opening the pack and vacuum sealing it again for the orthogonal projection. As this procedure would take some time (for two SSMs and two VSMs in different directions), we only included cases that comprised skin and pectoralis fascia resection in order to avoid the need to assess these two margins. The second limitation in our study was incomplete information in some samples and, as a result, sample size was reduced from the calculated one. It seems the sample size used was enough to reach the desired specificity which was 90 ± 10%; however, due to the small number of positive margins in our study, this sample size was not enough to reach a high sensitivity and it has given us a very low sensitivity with a wide range of confidence intervals. Therefore, further studies with larger sample sizes are needed to find an acceptable sensitivity.

CONCLUSION

Specimen mammography with adequate orientation of the tissue is an accurate and practical method for immediate intra-operative examination of the margin status in BCS for cancer, and vacuum packing does not produce a definite advantage over SSM. Neither SSM nor VSM can be a substitute for post-operative precise histological examination.

ETHICAL CONSIDERATIONS

This cross-sectional study was approved by the Ethics Committee of Tehran University of Medical Sciences (TUMS), Tehran, Iran; (Ethics code: IR.TUMS.SINAHOSPITAL.REC.1399.119) and was carried out in accordance with the ethical

principles of the Declaration of Helsinki. All participants signed an informed consent before entering the study.

CONFLICT OF INTEREST

There is no conflict of interest to declare.

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