Original Article

Specimen Shrinkage and Its Influence on Margin Assessment in Breast Cancer

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OBJECTIVE: The determination of tumour-free margin in breast cancer is crucial in deciding subsequent patient management. To exemplify the phenomenon of margin contraction during specimen preparation for histopathological analysis, we quantified the shrinkage of breast specimens as a result of formalin fixation.

METHODS: Fifty consecutive mastectomy and wide excision specimens were prospectively appraised. The closest free margin and maximal tumour diameter of fresh, unprepared specimens were recorded. These measurements were compared with the corresponding parameters following tissue fixation.

RESULTS: Following formalin fixation, the mean closest free margin of the specimens was found to have decreased from 10.28 mm to 6.78 mm (34%). The reduction of the mean diameter of the tumour itself was less significant, from 41.74 mm to 39.88 mm (4.5%).

CONCLUSION: Breast specimens undergo shrinkage after histological fixation, losing more than a third of their original closest free margin, whilst the tumour itself does not shrink substantially. This phenomenon has vital implications in the accuracy of margin analysis and consequent decisions on further management, including re-operation and the institution of adjuvant radiotherapy. [*Asian J Surg* 2007;30(3):183–7]

Key Words: breast cancer, histological fixation, specimen shrinkage, tumour-free margin

Introduction

As with most malignancies, the assessment of tumour-free margin is vital in establishing the adequacy of surgery for patients with breast cancer.[1] Postoperative treatment options are significantly influenced by this single parameter. With the paradigm shift in breast oncology towards breast-conserving surgery,[2] the margin factor has assumed added importance. In general, patients with positive or involved surgical margins are offered additional surgery (either wider excision or mastectomy), patients with "close" margins are treated either with additional surgery or external beam radiotherapy, and patients with negative margins undergo postoperative radiotherapy.[2–4] Even after a total mastectomy, patients with close margins are usually advised to receive a directed boost of radiotherapy to the tumour bed.[3] Furthermore, margin status has emerged as the strongest predictor of local recurrence in breast-conserving surgery.[5,6]

It has been well established that following histological fixation, there is considerable specimen shrinkage which diminishes the eventual tumour-free margin, with tissues fixed in formaldehyde and embedded in paraffin wax shrinking by about 33%.[7] Despite a comprehensive MEDLINE search from 1960 to 2006, there is no literature report of the exact degree of shrinkage of breast cancer

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specimens. We therefore decided to prospectively quantify the differences in tumour-free margins due to the process of specimen fixation. The initiative for undertaking this detailed measurement was also incited by our experience with a cohort of patients who underwent breast-conserving surgery at our institution. These patients were initially reported to have either positive or close margins as defined by the Malaysian consensus,[3] but ensuing wider excision or mastectomy revealed no residual tumour. Though it is acknowledged that surgery induces tissue destruction and inflammation that may cause tumours within a few millimetres to be destroyed, we hypothesize that additional factors such as the process of specimen fixation may further consolidate and shrink breast tissue to a degree that is significant enough to influence subsequent treatment decisions.

Patients and methods

Between November 2003 and January 2005, all suitable patients undergoing mastectomy and wide local excision for breast cancer were recruited into this study. The study protocol was fully approved by the hospital's ethics committee, and the project was carried out in accordance with good clinical practice guidelines as endorsed by the Ministry of Health, Malaysia. Written informed consent was obtained from patients, and their anonymity was safeguarded throughout the study.

Exclusion criteria were T4 tumours and specimens from patients who had undergone prior downstaging chemotherapy. Only margins with extensive[8] tumour involvement were analysed; margins that were graded as focal, minimal or moderate extent of positivity[8] were omitted from analysis. Additional data recorded included patient age, tumour grade, stage of the disease, type of surgery, and final tumour histology.

Immediately after excision, the breast specimens were delivered fresh without immersion in formalin to the pathologist who then prepared them according to the accepted protocol.[9] Wide excision specimens were sectioned into 3–4 mm slices, whilst mastectomy specimens were cut longitudinally from their posterior aspect into slices approximately 2 cm thick. All margins as well as the maximal tumour diameters were then measured to the nearest millimetre. The pathologist was always alerted of an imminent specimen at the commencement of each breast operation; thus, the entire process of pre-fixation measurement was confined to within 10 minutes of its removal. Specimens were subsequently fully immersed in the prescribed volume of fixative, i.e. the ratio of 10% formalin to tissue approximated 10:1. The corresponding measurements were then recorded by the same pathologist the following morning, as is the standard practice at our laboratory.

The disparity of the closest free margins and the maximal tumour diameters were compared using paired *t* test analysis (Stata version 8.2; Stata Corp., College Station, TX, USA) and differences were considered significant when $p < 0.05$. The power of this study was calculated to exceed 90%.

Results

Fifty consecutive patients corresponding to 200 measurements were recorded. The median age was 57 years (range, 32–84 years). All tumours were confirmed to be infiltrating ductal carcinoma. Most (90%) of the specimens came from mastectomies, whilst the rest were from wide local excisions. All patients underwent at least a level II axillary clearance. Nodal involvement was present in more than half of the patients. The majority of tumours were grade 3 (44%), while grades 2 and 1 tumours constituted 42% and 14% of the specimens, respectively. Tumours were also relatively large, with 58% T2, 40% T3 and only 2% T1 tumours.

The distribution of data for pre- and post-fixation closest free margins is shown by the box plots in Figure 1. There was margin reduction post fixation as demonstrated by their respective median values, i.e. 7.0 mm before fixation compared to 3.5 mm post fixation. In contrast, the

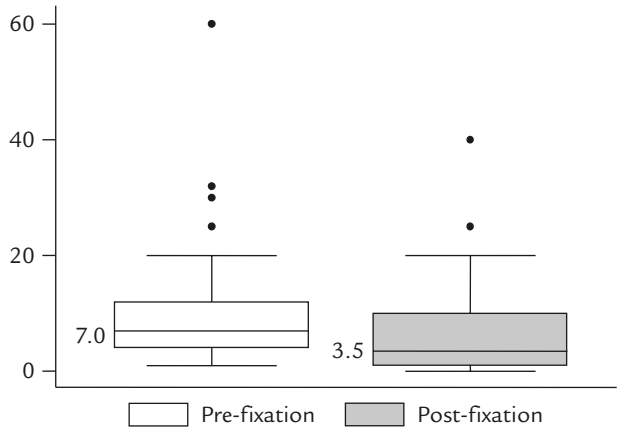

Figure 1. Pre- and post-fixation closest free margins (mm).

box plots representing tumour diameters both reveal median values of 35 mm (Figure 2).

As shown in the Table, the mean closest free margin before fixation was 10.28 ± 10.53 mm (range, 1–60 mm), whilst the mean margin after fixation was 6.78 ± 7.98 mm (range, 0–40 mm). This reduction was statistically significant ($p < 0.0001$). There was thus an average of 3.5 mm (34%) loss of margin following fixation. Conversely, the mean maximal tumour diameter of breast specimens before fixation was 41.74 ± 18.63 mm (range, 18–120 mm), whilst the mean diameter post fixation was 39.88 ± 17.65 mm (range, 10–95 mm). This difference of 1.86 mm (4.5%) was not, however, statistically significant ($p = 0.112$).

Every specimen showed margin shrinkage, which ranged from 6% to 93%. While mastectomy specimens demonstrated an average margin reduction of 33%, the mean shrinkage of wide excision margins was even more appreciable, at approximately 58%.

Discussion

With more breast-conserving surgery being performed for both *in situ* and invasive breast cancers, margin status is increasingly crucial in determining the outcome of therapy. Recent studies[10,11] have firmly established a significantly higher rate of local recurrence for breast cancers with positive microscopic margins compared to those with negative margins. The rate of residual tumour in specimens with positive margins ranged between 17% and 56%,[12,13] in contrast to no residual tumour in our earlier observation. Recurrence is also dependent on the degree of margin involvement, being higher in patients with extensive margin involvement than in those with focal or limited margin involvement.[14,15]

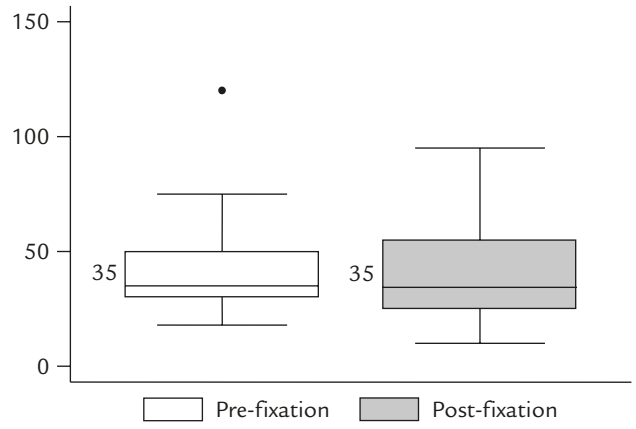

Figure 2. Pre- and post-fixation maximal tumour diameters (mm).

Yet there are many intricacies in the science that is margin evaluation. The most startling is the lack of universal agreement on margin definition. Despite the broad adoption of breast-conserving therapy, there exists significant variation in the perception of negative and close margins among pathologists and radiation oncologists.[15] For example, the US Joint Center for Radiation Therapy defines a close margin as < 1 mm and a negative margin as > 1 mm,[16] whilst the Radiotherapy and Oncology Consensus, Hospital Kuala Lumpur,[3] which formulates the Malaysian radiotherapy guidelines for breast cancer defines a close margin as between 5 and 10 mm and a negative margin as > 10 mm. Next, several technical difficulties have been acknowledged. Despite their posthaste delivery to the pathologist, some amount of specimen compression will be inevitable as specimens are not suspended in their transport container. From the pathologist's point of view, the large and complex surface of most breast specimens makes margin assessment imperfect.[17] Notwithstanding the success of breast conservation being heavily reliant on the quality of the pathological service, variation in margin assessment is known to exist among pathologists.[18] Finally, there is no clear consensus regarding the ideal margin after wide local excision,[15] even with the overwhelming evidence and existing guidelines for breast-conserving therapy.

Table. Measurements of the closest free margins and maximal tumour diameters in relation to specimen fixation

	Pre fixation	Post fixation	Mean difference
Mean closest free margin (mm)	10.28	6.78	3.5* 95% CI = 2.28–4.72
Mean maximal tumour diameter (mm)	41.74	39.88	1.86† 95% CI = 1.17–4.90

*$p < 0.0001$; †$p = 0.112$. CI = confidence interval.

The study cohort was recruited to reflect a cross section of our typical breast cancer patients, of which indigenous factors contribute to the preponderance of higher T stage and grade of the disease.[19] Thus, mastectomy specimens predominate even though the inference from this trial would probably best benefit patients undergoing wide local excisions. The effect of specimen fixation on mastectomy specimens was nonetheless explored on the premise that the outcome could influence the decision of whether to institute adjuvant treatment, namely postoperative radiotherapy, to the breast bed.

The period of specimen fixation is a balance between sufficient penetration of fixatives and prevention of secondary shrinkage and excessive hardening of tissue.[20] This study adhered to our standard laboratory protocol[9] of histological examination the following day, i.e. overnight fixation, although 6–8 hours may be sufficient. With the specimen entirely sliced, and standardized specimen-to-formalin ratio ensuring maximal exposure to the fixative, the proportion of margin shrinkage was deemed comparable irrespective of pre-fixation tissue volume.

The comparison of pre-fixation macroscopic margin to post-fixation microscopic margin in principle may be considered incompatible. However, this is quite possibly the most feasible and practical method of documenting and quantifying shrinkage due to specimen fixation, as attested by previous similar studies involving colorectal[21] and oesophageal[22] specimens. This analysis attempted to equate the two margins as accurately as possible by only considering margins with extent of positivity[8] graded "extensive", whilst lesser grades of margin involvement were disregarded. Moreover, particular attention was paid to closest margin, although all margins were recorded in view of its clinical significance in the determination of additional surgery or adjuvant therapy.

Nevertheless, specimen shrinkage in this study was striking and mainly in the form of tumour-free margin while the tumour itself did not shrink substantially. The explanation may be due to the degradation of lipid to their water-soluble derivatives by formaldehyde, thus effectively dehydrating the fat margin.[23] This conjecture could explain the absence of residual tumour in the re-excision specimens from our earlier observation, as well as elucidate the discrepancy between the specimen dimensions as measured by the pathologist and those perceived by the surgeon frequently encountered at our multidisciplinary breast conference. While colorectal[21] and oesophageal[22] specimens have been shown to lose at least half of their margins, it is believed that this is the first study to quantify the degree of breast specimen shrinkage as a result of formalin fixation. It may not be of that much concern in colorectal or oesophageal malignancies, but in breast, millimetres matter.

Specimen shrinkage may therefore cause the margins to appear spuriously involved with tumour and therefore classified as positive. The implications of this margin aberration are far reaching and can potentially alter the current guidelines on further surgery as well as adjuvant therapy. It may result in unnecessary surgery, either a wider excision or mastectomy after breast-conserving surgery for margins that are incorrectly classified as positive. A case in point: a margin of 5 mm in a fresh, unprepared specimen that undergoes shrinkage post fixation of the degree observed in this study would mean an average final reported margin of 1.7 mm. Some authorities would recommend wider excision or mastectomy based on this "close" surgical margin.[2–4] As far as the local guidelines are concerned, this qualifies as a major criterion for adjuvant radiotherapy.[3] Unnecessary expenditure aside, the inherent morbidity[24] and even mortality[25] associated with additional surgery or adjuvant therapy is hardly inconsequential.

In summary, the margin loss in breast cancer specimens due to tissue fixation is quite dramatic. In response to this phenomenon, several recommendations are proffered. Firstly, with such diverse interpretation,[15] a universal definition of margin is urgently required as a means of recommending re-excision and comparing treatment results. Next, techniques such as specimen suspension during transport and fixation could be initiated to mitigate specimen handling errors.

It must be emphasized that our current practice with regards to breast cancers with involved margins cannot as yet be changed, as we are unable to differentiate margins that are truly positive from those that are spuriously positive. Nevertheless, these preliminary findings have helped to establish a local benchmark for breast specimen shrinkage, apart from stimulating a new prospective randomized trial comparing the outcome of treatment based exclusively on pre- and post-fixation margins.

Acknowledgements

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