Factors contributing to suboptimal tumour preservation in breast carcinoma specimens at a tertiary health care setting in Sri Lanka

H.D. Wijesinghe [1,2], R.P.J.C. Nandasena[1], J. Fernando[2], M.D.S. Lokuhetty[1]
1 Department of Pathology, Faculty of Medicine, University of Colombo, Sri Lanka
2 Department of Pathology, National Hospital of Sri Lanka

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Introduction

Suboptimal tumour preservation (STP) in breast carcinoma specimens (BCS) precludes accurate diagnosis and evaluation of prognostic indicators. Our objective was to study factors contributing to STP in BCS specimens received at the laboratories of the National Hospital of Sri Lanka (NHSL) and Faculty of Medicine, Colombo (FMC).

Materials and methods

One hundred and fifty consecutive BCS received over eighteen months were reviewed. Factors associated with STP were analysed with SPSS 16.0 using independent-t-test for continuous variables and chi-square test for categorical variables.

Results

21 (14%) of BCS showed STP. STP correlated positively with delay in specimen receipt to the laboratory of ≥1 day (p=0.002) and increased distance from tumour to surgical resection margin (p=0.037). There was no correlation with type of surgery (wide local excision vs. mastectomy) (p=1.000), tumour size (p=0.698) or delayed specimen dissection at the laboratory of >1 day (p=0.191). Immunohistochemistry (IHC) was performed on 15/21 BCS with STP and showed positive results for oestrogen (14/15 - 93.3%), progesterone (13/15 – 86.7%) and HER2 (4/15 – 26.7%) receptors.

Conclusion

A significant proportion of BCS in the present study showed STP. A delay in delivery of the specimen to the laboratory of ≥1 day and deep seating of the tumour within the specimen was significantly associated with STP. Close coordination between surgical and pathological teams to ensure delivery of formalin-fixed BCS to the laboratory on the day of surgery is recommended.

Correspondence: H.D. Wijesinghe
E-mail: harshima@hotmail.com
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Introduction

Breast carcinoma is the commonest malignancy among women worldwide [1] including Sri Lanka [2]. The prognosis and treatment of this malignancy is determined by the assessment of many factors including tumour size, histological subtype, tumour grade, lymphovascular invasion, lymph node status, hormonal receptor and human epidermal growth factor 2 (HER2;cerbB2) receptor status [3].

Histological subtyping of invasive carcinoma and tumour grading provide a simple, inexpensive method for assessing patient prognosis [4-6]. Oestrogen receptor (ER) and progesterone receptor (PR) expression are positive prognostic markers of outcome and strong predictive markers of response to hormone-based therapies such as tamoxifen [7-11]. HER2 over-expression has been shown to be a poor prognostic factor in breast carcinoma [12,13] and predicts response to targeted therapy such as trastuzumab [14].

The assessment of tumour type, grade, lymph node status, hormone and HER2 receptor status is by histological examination for which adequately preserved tumour tissue is essential [5,15,16]. According to The Royal College of Pathologists of the United Kingdom (RCPath UK), adequate fixation is important to preserve mitotic figures and good tissue morphology which affect the assessment of tumour type and tumour grade, lymphovascular invasion, diagnosis of some difficult intra-ductal proliferations and retention of proteins such as ER, PR and HER2 receptors [5].

Breast carcinoma specimens (BCS) are a common specimen received in histopathology laboratories throughout Sri Lanka. Currently these specimens are not delivered immediately to the laboratory in its' fresh state due to logistic reasons operating at a local level. In this context, suboptimal tumour preservation (STP) is a common problem faced by Sri Lankan pathologists in their routine practice. STP precludes accurate assessment of tumour (subtype, grade, lymphovascular invasion) and could result in false negative hormone receptor measurement [5], HER2 status and Ki67 proliferation index [5,16,17]. This is of clinical significance as invalid results could significantly change the therapeutic management of a patient with potentially negative effects on the outcome.

The study objective was to assess the rate of STP and evaluate

major factors contributing to STP in breast carcinoma specimens received at the laboratories of the National Hospital of Sri Lanka (NHSL) and the Faculty of Medicine, Colombo (FMC).

Materials and methods

One hundred and fifty consecutive cases of mastectomy and wide local excision specimens of women undergoing primary surgery for breast carcinoma at the National Hospital of Sri Lanka were reviewed retrospectively. The samples were received in formalin by the laboratories of NHSL and FMC over a period of eighteen months. All haematoxylin and eosin stained tumour sections of the above cases were reviewed for STP. Tumour showing extensive autolysis (≥80%) resulting in inability to subtype, grade and assess lymphovascular invasion were categorized as showing STP (Figure 1). Tumours with areas of better preserved tumour (>20%) that allowed subtyping and grading were not categorized as STP. Results of ER, PR or HER2 immune assays of sub-optimally preserved tumours were reviewed. Information on date of surgery, date of specimen receipt to the laboratory, date of specimen dissection, type of specimen (mastectomy vs. wide local excision), macroscopic size of tumour and distance from tumour to the closest and deep resection margins, was retrieved from the laboratory records. On receipt to the laboratory, mastectomy specimens are sliced from the deep resection margin. Therefore for uniformity the distance from the deep resection margin was used in the analysis for mastectomy specimens. In the case of wide local excision specimens, the distance from the closest resection margin was considered in the analysis. The delay in specimen receipt to the laboratory was determined from the date of the surgery and the date of specimen receipt to the laboratory. The delay in taking the specimen for dissection was determined from the date of specimen receipt to the laboratory and the date of specimen cut up. The factors associated with STP were evaluated with independent-t-test and chi-square-test, applied using SPSS 16.0.

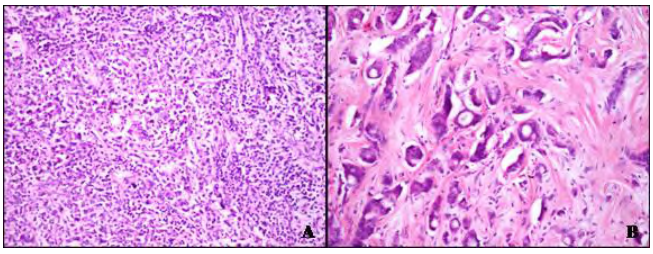

Figure 1. Comparison of a tumour showing suboptimal preservation (A) with a tumour showing adequate preservation (B). In (A), poor preservation of tumour morphology prevents the assessment of histological subtype, tubule formation and mitotic count, thereby precluding accurate Nottingham grading and assessment of lymphovascular invasion.

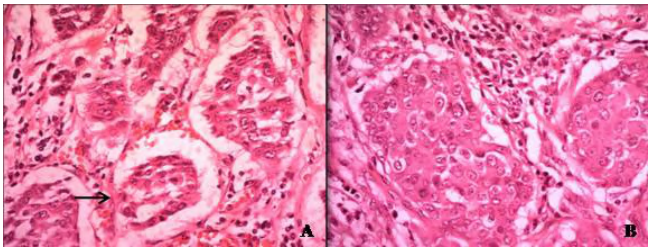

Figure 2. Tumour retraction that occurs with suboptimal tumour preservation can mimic lymphovascular invasion. A) A tumour showing suboptimal tumour preservation resulting in retraction artefact of the tumour which mimics lymphovascular invasion. B) Better preserved areas of the same tumour.

Results

Of the 150 cases, 131 (87.3%) were mastectomies and 19 (12.7%) were wide local excision specimens. 21 (14%) of these showed STP. 19 (19/131 – 14.5%) mastectomy specimens and 2 (2/19 – 10.5%) wide local excision specimens showed STP. STP did not correlate with the type of surgery (i.e. wide local excision vs. mastectomy (p=1.000) (Table 1).

None of the 39 breast carcinomas received to the laboratory on the day of surgery showed STP. 21 of the 111 (18.9%) specimens received on the day after surgery showed STP. STP correlated positively with a delay in specimen receipt to the laboratory of ≥1 day (p=0.002) (Table 1). 18 (85.7%) of the 21 tumours that showed STP had been taken for specimen dissection on the day following specimen receipt, whereas in 2 (9.5%) there was a delay of two days. STP did not correlate with a delay in specimen dissection of >1 day (p=0.191) (Table 1).

The majority of breast carcinomas were of tumour stage T2 (2-5cm), with a mean value of 3.14 cm. The tumours that showed STP had a mean tumour size of 2.81 cm, whilst those that showed adequate preservation had a mean size of 3.19 cm. There was no relationship between the tumour size and the presence of STP (p=0.698) (Table 1). The mean distance from tumour to the deep/closest resection margin was significantly greater in specimens that showed STP (2.66 cm vs. 1.37 cm, p=0.037). Only 2 (9.5%) of the 21 tumours with STP were located within 1cm from the deep/closest resection margin (Table 1).

ER, PR and HER2 had been requested in 19 (90.5%) of the 21 tumours showing STP. Of these, results of 15 cases (71.4%) performed at the National immunohistochemistry laboratory at the NHSL, were available. The immunohistochemistry results of 125 of the tumours showing adequate preservation were available. The tumours showing STP showed a high rate of ER and PR positivity in comparison to the tumours showing adequate preservation (Table 2). The difference in HER2 over expression was not statistically significant.

Factors affecting tumour preservation		Preservation of tumour		Independent T-test/ Fischer Exact test
		Suboptimal preservation (SP)	Adequate preservation (AP)	
Type of surgery	Wide local Excision	2 (9.5%)	17(13.2%)	P=1.000
	Mastectomy	19 (90.5%)	112 (86.8%)	
Delay of specimen receipt to laboratory	No delay	**0 (0%)**	**39 (30.2%)**	**P=0.002***
	≥1 day	**21 (100%)**	**90 (69.8%)**	
Delay of specimen dissection in laboratory	No delay	18 (85.7%)	89 (69.0%)	P=0.191
	≥1 day	3(14.3%)	40 (31.0%)	
Distance of tumour from DRM	Mean distance	**2.66cm**	**1.37cm**	**P=0.037***
Tumour size	Tumour size	2.81cm	3.19cm	P=0.698

Table 1. Impact of variables (type of surgery, delay of specimen receipt and dissection, distance from deep resection margin to tumour and tumour size) on tumour preservation.
(* statically significant)

	Suboptimal tumour preservation (STP)	Adequate preservation (AP)	P value
Oestrogen receptor	14/15 (93.3%)	79/125 (61.2%)	P=0.019
Progesterone Receptor	13/15 (86.7%)	66/125 (51.2%)	P=0.011
HER 2	4/15 (26.7%)	15/125 (11.6%)	P=0.053

Table 2. Comparison of Immunohistochemistry results in tumours showing STP and adequate preservation.

Discussion

In this study, STP was not seen in any of the specimens received to the laboratory on the day of surgery. A delay of one or more days in specimen receipt to the laboratory was associated with an increased risk of STP. Only 26% (39/150) of the specimens studied had been received on the day of surgery despite breast surgery being an elective surgery where surgeries are performed on working days when full theatre and laboratory staff are available to coordinate specimen transport to the laboratory. Therefore, with better coordination between the surgical and pathology teams it should be possible to deliver BCS to the laboratory on the day of surgery.

Other studies carried out worldwide too have shown delays to be associated with STP. A retrospective study on 5007 BCS carried out in the USA found that specimens obtained late in the week and weekends (i.e. Friday to Sunday) were more likely to be ER/PR negative than specimens obtained on other weekdays (i.e. Monday to Thursday) [18]. They hypothesized that delays in dissection of specimens received at the end of the week, resulted in poor fixation of tumour. The delay in specimen transport to the laboratory and delay occurring within the laboratory were not studied separately in this study and since this was a retrospective study involving multiple centres it was not known if the BCS had been sliced prior to fixation.

The RCPath UK recommends that BCSs should be sent immediately to the pathology laboratory, ideally in their fresh state [5]. This enables specimen examination on receipt, surgical margin inking, immediate tumour incision and slicing of the breast into 1cm thin slices prior to placing it in the fixative. They recommend that if it is not possible to send the fresh specimen immediately to the laboratory, the surgeon should, by arrangement with the pathologist, make a controlled single or cruciate pair of incisions into the lesion (from the deep resection margin side in the case of mastectomy). This will preserve the integrity of key margins while allowing the benefit of rapid fixation. The specimen should then be immediately placed in a fixative. RCPath UK believes that the benefits of rapid fixation in general outweigh the desire to preserve the specimen intact prior to examination by the pathologist [5].

In our setting, specimens are received in the laboratory in formalin fixative. Therefore if a specimen is going to be sent fresh to the laboratory it is extremely important to keep the laboratory staff and the pathologist informed of the expected time of its arrival since it is not routine practice in the local setting. In cases where a delay is anticipated (lack of theatre staff to transport specimen, surgery performed on a holiday or weekend, etc.) it may be advisable for the surgeon, in consultation with the relevant pathologist to ink the deep resection margin of mastectomy specimens with India ink and fix the ink with acetic acid (a common household vinegar could be used). A single cut should then be made on the specimen, on the deep resection margin side, extending from the superior border to the inferior. This would aid deep penetration of formalin and allow proper fixation of the tumour. This is of great importance in mastectomy specimens especially when the tumour is deeply located within the specimen. However, in the case of wide local excision specimens, the emphasis should be to send the intact specimen to the laboratory on the same day of surgery with oriented surgical margins. In wide local excision specimens it is best to leave it to the pathologist to assess each surgical margin of the specimen by painting them in different colours at cut up.

A delay in specimen dissection in the laboratory was not associated with STP in this study. This is probably due to the routine practice of specimen slicing on the same day of receipt to the laboratory after painting the surgical margin/s with ink. The formalin fixative penetrates tissue at a rate of 1mm/hour [19,20]. Therefore even when the specimen is placed immediately in a formalin fixative, fixation of the tumour will depend on how soon the formalin reaches the tumour. This in turn is determined by the quantity and quality of formalin,

tumour size, depth at which the tumour is located and the temperature [19,20]. Slicing the specimen on the day of receipt to the laboratory by the pathologist brings the tumour into direct contact with formalin and therefore aids in fixation of the tumour. This is most important in mastectomy specimens for which formalin penetration can be particularly poor. Although STP was commoner in mastectomy specimens in comparison to wide local excision specimens, this difference did not reach statistical significance in our study sample. One of the two wide local excision specimens that showed STP was large (11x8x1.5cm), approaching the size of a mastectomy specimen. Deep seated tumours were more likely to show STP, probably due to the delay in formalin penetration of the tumour. Studies comparing immunohistochemical marker positivity in core biopsy and excision specimens have found higher rates of ER/PR positivity in core biopsy specimens in comparison to excision specimens which has been attributed to a delay in exposure of the center of a surgical specimen to formalin [21].

Immunohistochemical (IHC) analysis of ER, PR and HER2 should be performed in all newly diagnosed cases of breast carcinoma [15,16]. IHC analysis had been performed at the National immunohistochemical laboratory in 15 (15/21) tumours showing STP in this study. It had not been arranged in 2 cases (2/21) which showed STP, as possibly the reporting pathologist felt that the severity of poor preservation precluded IHC analysis. In the remaining cases (4/21) the tissue blocks had been issued to the patient on clinicians request for IHC assay outside the study setting. High rates of ER and PR positivity (14/15 and 13/15 respectively) were present in the tumours showing STP that were analysed. This suggests that even in cases with STP the benefit should be granted to the patient and IHC analysis should be performed on the material available. However the results should be interpreted with caution, especially with HER2. It has been documented that variation in the length of tissue fixation can result in differences in the intensity of staining for HER2 in tumour cells [22]. Positive cell membrane staining is taken in to account for HER2, unlike ER/PR which are nuclear stains. Cellular discohesion occurring in STP may result in misinterpretation of cell membrane staining of HER2. STP may also result in unnecessary waste of resources for in-situ hybridization in HER2 equivocal cases. Missing a HER2 positive tumour as a result of STP may also result in patients not receiving anti HER2 therapy.

In this study we assessed only the overall positivity or negativity for hormone receptors. We did not assess the score for ER/PR positive tumours studied. In a study conducted under experimental conditions on the effects of delayed fixation on breast biomarkers, the scores for ER positivity and PR positivity started to decline at a 2 hour and 1 hour delay respectively [23] which resulted in a change in prognosis of the tumours from relatively good (with score of 6 or 7) to intermediate (with score of 4 or 5).

Since this study was performed retrospectively, the quantity and the quality of formalin used could not be evaluated. However as all the specimens were received in 10% formal saline at room temperature prepared by the histopathology laboratory of the NHSL and supplied to all operating theatres of the NHSL, it could be assumed that the quality of formalin did not vary significantly. All specimens were also received in similar containers of appropriate size (small plastic buckets with lids). Other factors that could affect tissue fixation such as the size of the specimen, cold ischaemia time, duration of fixation and the pH [17,18] were not studied.

In conclusion, there was a high rate of STP (14%) of BCS in this study. Delivery of formalin-fixed BCS to the laboratory on the same day of surgery contributed most to prevent STP. STP did not appear to correlate with delayed specimen dissection in the laboratory. Close coordination between surgical and pathological teams to ensure delivery of formalin-fixed BCS to the laboratory on the same day of surgery is recommended in our setting since this enables immediate specimen slicing, ensuring adequate formalin penetration to deep-seated tumours. In cases of mastectomy where an unavoidable delay is anticipated, alternate measures such as the surgeon placing a single cut through the tumour should be implemented following discussions between the surgical and pathological teams. In view of the high rates of positivity for immunohistochemical markers seen in the tumours showing STP, it is recommended that IHC is performed even in such situations.

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