Predictive factors associated with involved margins in breast cancer treated with neoadjuvant chemotherapy followed by breast-conserving therapy

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Running title: involved margins in breast-conserving therapy after neoadjuvant chemotherapy

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The authors have no conflict of interest to declare.

Abstract

Introduction: This study sought to identify predictive factors of involved surgical margins in breast-conserving surgery (BCS) after neoadjuvant chemotherapy (NAC) to help guide the surgical procedure.

Materials and Methods: Retrospective study of patients who had BCS after NAC between January 2008 and December 2013. Outcome measure: tumor-involved margin, defined by tumor cells on ink for invasive cancer and tumor-free margin < 2mm for DCIS.

Results: Ninety-seven patients were included. The median age of patients was 46 years old [28-71]. The initial average tumor size was 47.8 mm [+/- 18.6]. Twelve patients (12.4%) had involved tumor margins on final histology after BCS and NAC. According to the multivariate model including only preoperative variables of positive margins, initial ultrasound tumor size ≤ 27 mm (p = 0.045) and low SBR grade (p = 0.009) were independently associated with tumor-involved margins. According to the multivariate model including pre- and postoperative variables of positive margins, ductal carcinoma *in situ* was also independently associated with tumor-involved margins (p=0.021).

Conclusion: Initial ultrasound tumor size ≤ 27 mm and low SBR grade were independently associated with tumor-involved margins. These preoperative data were very helpful to guide the surgical procedure in breast cancer.

Keywords: neoadjuvant chemotherapy, breast-conserving surgery

Introduction

Breast-Conserving Therapy (BCT), including lumpectomy and sentinel lymph node surgery followed by radiation therapy, is the treatment of choice for early-stage breast cancer, i.e. tumor sizes smaller than 3 cm [1]. For larger tumor sizes, mastectomy is the gold standard. Oncoplastic surgery can change this dogma, combining the principle of cancer resection with plastic surgery. It can allow Breast-Conserving Surgery (BCS) in larger tumors but this is not always feasible. Since the 1980's, neoadjuvant chemotherapy (NAC) has been developed to reduce tumor size. It can allow BCS in patients who otherwise would have required mastectomy. Randomized trials have shown the same efficiency for adjuvant and neoadjuvant chemotherapy in terms of survival [2-5]. Likewise, several trials have shown similar overall survival between mastectomy after NAC and BCT after NAC [1, 6-9]. After neoadjuvant chemotherapy, BCS is performed in 17% to 85% of cases [10] depending on the histological subtype. BCT allows oncologic treatment to be undertaken with an acceptable cosmetic outcome. This is easier for women to accept as it is less of a psychological trauma [11, 12]. However, some studies have shown specific factors to be associated with BCT failure, such as initial clinical tumor size > 50 mm, tumor size after NAC > 30 mm [1, 13], multifocality and lobular histology [1, 14, 15].

In BCS, involved margins are correlated with high locoregional relapse (LRR), increasing rates by 2 or 3 times [16-22]. Recent trials show narrow margins to be sufficient. Studies or meta-analysis have shown the same LRR rates for margins of 1 mm and 5 mm [18] [23, 24], and a report from the annual Meeting of the American Society of Breast Surgeons [25] concluded that "no ink on tumor" is an adequate surgical margin for invasive breast cancer. On the other hand, to achieve a good cosmetic outcome, most of the healthy tissue should be preserved. For BCS, the surgical dilemma is between tumor-free margins for optimum oncologic results and no tumor-free tissue removal for optimum cosmetic results.

Few studies so far have investigated predictive margin status in BCT after NAC. For successful BCS with optimum cosmetic results, the surgeon has to carefully plan the procedure and determine whether a small or large amount of breast tissue should be removed. This study sought to identify predictive factors of involved surgical margins in BCS after NAC to help guide the surgical procedure.

Patients and Methods

Patients and study design

Between January 2008 and December 2013, we conducted a retrospective study of patients who had BCS after NAC in Eugène Marquis breast cancer center.

The patients were selected using the center's software. Inclusion criteria were BCS after NAC. Exclusion criteria were radical mastectomy after NAC, multifocal tumor and metastatic disease.

Neoadjuvant chemotherapy, surgical procedure and pathologic assessment

Six cycles of anthracycline- and taxol-based neoadjuvant chemotherapy were administered to all patients. Trastuzumab was also given for HER2-positive tumors. Wires or marker clips were placed before the first chemotherapy cycle. Breast tumor response after chemotherapy was assessed by clinical examination, mammography, ultrasonography and MRI. RECIST criteria [26] were used to assess tumor response according to which mastectomy or BCT was chosen. Surgery was performed 4 weeks after completion of chemotherapy.

In the case of no clinical or radiological tumor signal after chemotherapy, a radiological examination was performed the day before the procedure using the hook wire left attached to the clip or wire marker. Four experienced breast surgeons performed BCT with axillary lymphadenectomy. The operative specimen was localized in the 3 spatial planes by the surgeon and referred to the pathologist who dried it with absorbent paper. A numbered glass slide was applied to each surface of interest (superior, inferior, anterior-posterior, lateral) after gently pressing the specimen. The slides were room-dried and stained with toluidine blue prior to interpretation. The results reported to the surgeon were expressed as: (a) acellular slides corresponding to healthy tissue (shown by a lack of normal epithelial cell desquamation); (b) presence of benign cells (macrophages, columnar or apocrine metaplasia cells); or (c) suspect positive slide in the presence of malignant cells. The precise site of the

lesions was deduced from their location on the slide with anatomical mapping to the specimen to guide resection. In the case of resection, also guided by the surgeon, the cytological procedure was reproduced until standardization of the imprint cytology technique was achieved.

Histological examination was performed after fixing and embedding in paraffin. The specimen was inked with different colors (1 color per specimen surface), then sectioned into 3-mm slices in the frontal plane to enable us to best analyze the 4 surfaces of interest. The inked resection margins were analyzed in 3-mm slices perpendicular to the lumpectomy bed.

Data collection

Relevant data was collected. This included personal data such as age, weight, cup size, menopausal status, medical and family history, method of diagnosis (clinical or imaging and biopsy), tumor size and node status determined by clinical examination and imaging before and after NAC. Data on hormone status including ER/RP and HER2 status were also collected, as well as antigen Ki-67. Tumors were classified as defined by the 2011 St Gallen consensus [27] (luminal A: ER+, RP+, HER2 negative, Ki-67<14%; luminal B: ER+, RP+, HER2positive or HER2negative, Ki-67>14%; basal like: ER-, RP-, HER2-; HER2 positive: ER-, RP-, HER2 positive). Scarff-Bloom and Richardson (SBR) grading on biopsy before NAC was also recorded. For surgical specimens (post NAC), ductal carcinoma *in situ* (DCIS) or invasive residual tumor, node assessment and margin status were recorded. The number of procedures and patient status (survival and recurrence diagnosis) at last follow-up were also recorded.

Outcome measure

The outcome measure was tumor involvement on margin assessment. Tumor-involved margins were defined by tumor cells on ink for invasive cancer and tumor-free margins < 2mm for DCIS.

Statistical analysis

In the univariate analysis, continuous variables were compared using the Wilcoxon test, and qualitative variables were compared with Fisher's exact test or the Chi-square test. For continuous variables, cutoff was determined by a standard value in the literature or the ROC curve (value with best sensitivity and specificity). Finally, a logistic regression model was used to identify the risk factors associated with tumor-involved margins in a multivariate analysis. The variables found to have a p-value < 0.1 in the univariate analysis were used in the model. For all the analyses, $p < 0.05$ was considered to be statistically significant.

Results

Population characteristics and tumor response after NAC

Ninety-seven patients were included in the study (Figure 1). The median age of patients was 46 years old [28-71] and the body mass index (BMI) median was 24 [20-47]. For eighty patients (82.5%) the lesion was diagnosed by palpation. The initial average tumor size was 47.8 mm [+/- 18.6]. Forty percent of the tumors were localized in the supero-external breast quadrant. Patient and tumor characteristics are provided in Table 1.

After neoadjuvant chemotherapy and before breast-conserving surgery, clinical and radiological tumor assessment showed a normal clinical breast examination in 59 patients (60%) and a complete response in 22% of patients assessed by mammography and 27.3% of patients assessed by MRI. On MRI evaluation, the scores for complete response and partial tumor response were 27.3% and 48% of patients respectively (data on 25 cases) according to RECIST criteria.

According to the pathologic assessment of the surgical specimens, a pCR was observed in 27 patients (27.8%). For a further 4 patients, no invasive residue was found, but persistent DCIS was observed.

Predictive factors for positive margins

Twelve patients (12.4%) had no tumor-free surgical margins on final histology. The method of diagnosis (screening or clinical), method of pre-surgical identification and surgical techniques (small or large lumpectomy, round block procedures) were not significantly associated with positive margins. Preoperative data associated with margin status are shown in Tables 2 and 3. Postoperative data are shown in Table 4. The result of the multivariate analysis using pre- and post-operative data is shown in Table 5.

We also developed a multivariate model including only preoperative variables (predictors) of positive margins: echo T0, SBR grade, and continuous Ki-67 content. The data independently associated with positive margins were the initial ultrasound tumor size ($T \leq 27$ mm) ($p = 0.0456$) and a low-grade *vs* high-grade SBR ($p = 0.0097$).

Surgical re-excision

Sixteen patients, i.e. 16.5% of the study population, had one or two re-excision procedures. Fifteen patients (15.4%) had two operative procedures (initial BCT and re-excision) and one patient had three operative procedures (initial BCT, second BCT and a final mastectomy). For patients with two procedures: 12 patients out of 15 had repeat surgery due to positive surgical margins (11 patients had a second BCT and one patient had a mastectomy) and 3 patients out of 15 due to poor cosmetic results, abscess after conservative surgery or at the patient's request after learning of her BRCA1 mutation. Finally, there were 5 patients with secondary mastectomies (5.1%), but only 2 out of 5 for tumor-involved margins.

Survival

The average follow-up was 2.84 years [+/- 1.12]. The rate of recurrence-free survival was 99% at 2 years and 85% at 5 years. The 2-year and 5-year overall survival rates were 95% and 77%, respectively (Figure 2).

Discussion

This study identified three factors independently correlated with positive margins after neoadjuvant chemotherapy: preoperative sonography size, low SBR grade and DCIS on pathologic surgical specimen analysis. Two factors relate to preoperative data and one to postoperative data. To our knowledge, ours was the first study to show criteria independently correlated with margin status in cases of NAC with BCT. One of the aims of NAC is to allow conservative surgery for cosmetic reasons. Narrow margins are important to maintain breast appearance while removing the whole tumor. However, if there are involved margins, additional breast surgery is necessary and it is correlated with worse cosmetic results, psychological trauma, high costs and subsequent radiation therapy [28]. Surgeons need to know whether to remove more tissue or perform radical mastectomy in order to avoid a second surgical procedure.

Chemotherapy is more effective against tumors with high cell division potential. Several trials have shown that a high grade (SBR 3) is associated with better pCR rates [29-32]. Conversely, low-grade tumor size decreases less after NAC, which increases the risk of positive margins in the case of BCT, as shown in our study. Forty percent of tumors in our study have an initial US tumor size < 27 mm but this represents 87.5% of tumors with positive margins. There are no other studies published with which to compare our results. However, large tumors (independently of histological subtype) seem to decrease to multifocal and patch-like lesions, and this increases the risk of involved margins [33]. On the other hand, luminal tumors are initially smaller, and trials have shown that NAC is less efficient [34]. Therefore, the residual tumor may be large, increasing the risk of positive margins. In our study, the luminal subtype seemed more associated with positive margins (58 vs 34%, p=0.13) and with an initial tumor size < 27 mm (55 vs 35%, p=0.16). This finding could explain why “smaller” tumors are associated with positive margins. Many studies have shown

that DCIS is a risk factor for positive margins in BCT as a first treatment step. We showed that it was also a risk factor in BCT after NAC. The presence of DCIS is generally not considered in the case of NAC as studies tend to focus on pCR. Future studies could be performed to assess DCIS preoperatively. It is noteworthy that preoperative MRI is not efficient for detecting DCIS [36, 37] and DCIS is not palpable.

One limitation of this study is its retrospective nature. Therefore, given the inherent bias of the study design and the absence of other data confirming our results, we stress that our data should be interpreted with caution and be confirmed by prospective multi-institutional studies. In fact, in the absence of level I evidence the evaluation of prospective series, as they evolve, is crucial. Furthermore, the low number of included patients and the low number of patients with involved margins were also others weakness of present study. Nevertheless, our results are consistent with other studies. Our pCR rate is 27.8%, in agreement with the rate observed in the Beriwal [10, 38] and Komekana trials (24-25%) [10]. After NAC, the BCT rate varies between 17 and 85% [10], which is consistent with the 60% in our study. The re-excision rate was 16.5% in our study, consistent with the literature [20] which showed a rate of between 12.4% [15] and 18% [38]. Finally, our secondary mastectomy rate was 5.1% in line with previous published data which showed 9% [39]. Thus, our findings could be used in practice due to the lack of other available data on this key aspect of margin status after NAC and BCS.

Future prospective evaluation of the use of predictive criteria of tumor-involved margins as a way of reducing re-excision rates is required. Besides, preoperative detection of DCIS could increase negative margin rates, using wider BCT. As seen previously, MRI is the gold standard for detecting residual tumors after NCT but it failed to detect DCIS [40, 41]. In his meta-analysis, Marinovich shows that MRI is better for detecting pCR when the finding is "no invasive residue" as opposed to “no invasive ductal tumor + DCIS” [42]. Sardenelli’s trial shows that the DCIS detection rate is 35% in mammography, 46% in MRI and 54% in clinical

examination [43]. The use of a second biopsy after NAC, in order to detect DCIS, could improve treatment. The completion of a second series of biopsies after NAC is not currently offered. The main pitfall of performing a biopsy to detect DCIS is that false-negative results may be obtained due to the site being missed. However, if the presence of DCIS is identified by biopsy, it increases the risk of extensive component DCIS [44, 45], [46]. Detection prior to surgery may allow the surgeon to perform wide surgery to achieve tumor-free margins. To determine whether implementation of this second set of biopsies would improve surgical outcome, a prospective evaluation should be undertaken.

Conclusion

Our study showed that 3 factors: initial US tumor size < 27 mm, SBR grade 1-2 and presence of DCIS after NAC independently correlated with positive margins. To our knowledge, there is no other study reporting on margin status in the context of NAC plus BCT. These criteria could help surgeons to optimize BCT and reduce second surgery rates. A prospective randomized trial assessing the benefit of a surgical biopsy after neoadjuvant chemotherapy should be performed to investigate the presence or absence of DCIS and its correlation with involved margins.

Conflict of interest: The authors have no conflict of interest to declare.

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Figure 1- Inclusion flow chart and number of second surgery procedures

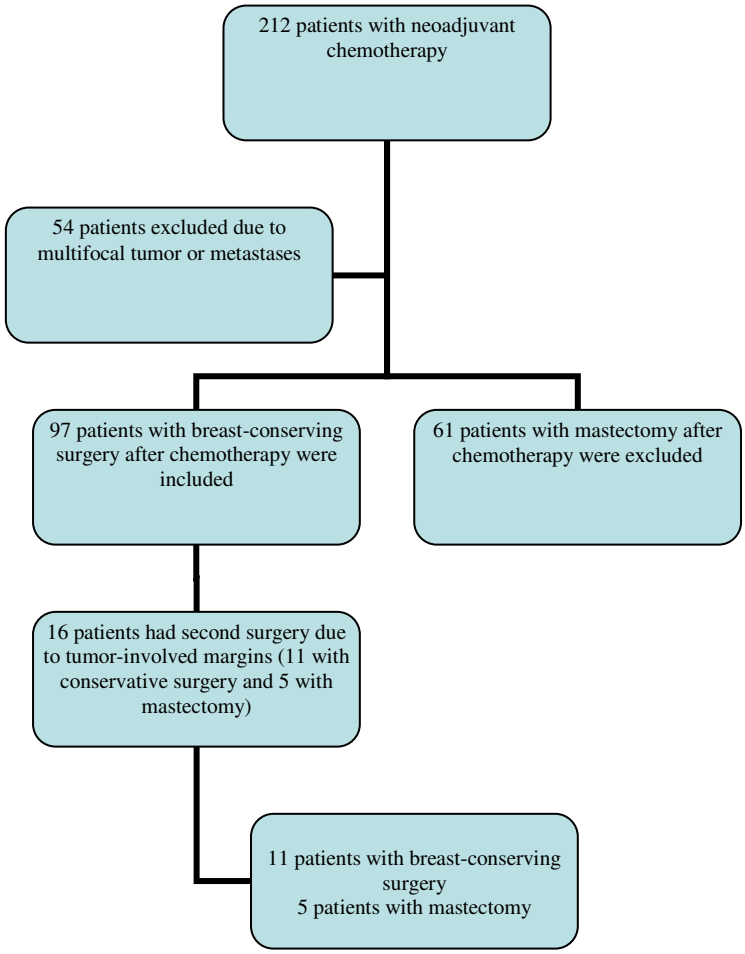

Figure 2- overall survival curve

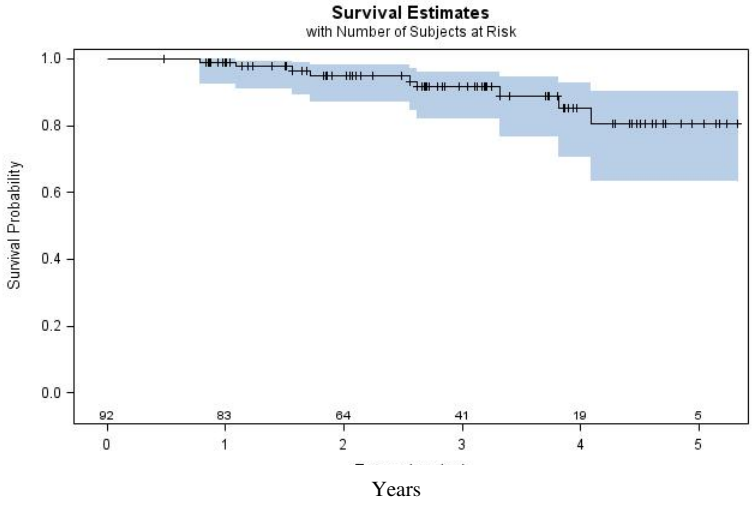

Table 1- Patient and tumor characteristics

Variable	N/NB (%)
Menopausal status	
Yes	68/97 (70.1)
Hormonal therapy	
Yes	06/97 (06.2)
Personal history of breast disease	
Benign	18/97 (18.6)
Malignant	02/97 (02.0)
Family history	
First degree	15/97 (15.5)
≥ Second degree	26/97 (26.8)
Histological type	
Invasive Ductal Carcinoma	87/97 (89.7)
Invasive Ductal Carcinoma plus DCIS	06/97 (06.2)
Invasive Lobular Carcinoma	04/97 (04.1)
Initial tumor stage	
T1	01/97 (1.0)
T2	48/97 (49.5)
T3	42/97 (43.3)
T4	06/97 (06.2)
Initial clinical axillary node status	
N0	42/97 (43.3)
N1	55/97 (55.7)

DCIS: Ductal Carcinoma *in situ*

Table 2- Preoperative clinical data and radiological findings associated with tumor-involved margins

Variable	N0/total (%)	Unadjusted OR [95% CI]	p value
Initial size			
≤ 45mm	04/53 (7.5%)	1	
> 45mm	05/34(14.7%)	2.72 [0.66 - 13.20]	0.13
Size after NAC			
≤11 mm	09/71 (12.7%)	1	
>11 mm	03/26 (11.5%)	0.9 [0.14 - 4.04]	1.00
Initial US size			
≤ 27mm	07/32 (21.9%)	1	
> 27mm	01/48 (2.1%)	0.08 [0 - 0.06]	**0.0059**
US size after NAC			
≤ 18mm	07/55 (12.7%)	1	
>18mm	05/34 (14.7%)	1.18 [0.27 - 4.79]	1.00
Initial MRI size			
≤ 36mm	02/25 (08%)	1	
>36mm	03/11 (27.3%)	4.31 [0.4 - 57.70]	0.15
		0.99 [0.94 - 1.05]	0.97

US: Ultrasound; NAC: Neoadjuvant chemotherapy

Table 3- Preoperative histological data associated with tumor-involved margins

Variable	N0/total(%)	Unadjusted OR [95% CI]	P value
Histology			
Invasive ductal carcinoma	03/12 (25%)	1	
other	09/85 (10.6%)	0.35 [0.07 - 2.44]	0.17
Tumor grade (SBR)			
Grade 3	04/68 (05.9%)	1	
Grade 1-2	08/29 (27.6%)	6.1 [1.43 - 29.89]	**0.05**
Ki-67			
≥14	11/86 (14.3%)	1	
[0-14]	01/07 (12.8%)	1,14 [0.02 - 0.87]	1.00
ER status			
No	04/53 (07.5%)	1	
Yes	08/44 (18.2%)	2.72 [0.66 - 13.20]	0.13
PR status			
No	06/63 (09.5%)	1	
Yes	06/34 (17.6%)	2.04 [0.49 - 8.32]	0.33
HER2 status			
No	09/70 (12.9%)	1	
Yes	03/27 (11.1%)	0.85 [0.14 - 3.80]	1.00
Luminal A			
No	10/89 (11.2%)	1	
Yes	02/08 (25.0%)	2.63 [0.23 - 17.40]	0.26
Luminal B			
No	06/60 (10.3%)	1	
Yes	06/37 (16.2%)	1.74 [0.42 - 7.10]	0.53
HER2+			
No	11/83 (13.2%)	1	
Yes	01/14 (07.1%)	0.5 [0.01 - 4.08]	1.00
Triple negative subtype			
No	09/58 (15.5%)	1	
Yes	03/39 (07.7%)	0.45 [0.07 - 2.00]	0.35

SBR: Scarff Bloom and Richardson; ER: Estrogen receptor: PR: Progesterone receptor

Table 4- Postoperative data associated with tumor-involved margins

Variable	N0/total	Unadjusted OR [95% CI]	P value
Invasive ductal carcinoma			
≤21mm	04/77 (0.5.2%)	1	**0.003**
≥21mm	08/21 (40.0%)	12.2 [2.65-61.8]	
DCIS			
No	03/58 (05.2%)	1	**0.023**
Yes	08/38 (21.1%)	4.89 [1.05-30.21]	
Lymphovascular invasion			
No	06/83 (07.2%)	1	**0.0017**
Yes	06/14 (42.9%)	9.63 [1.98-44.87]	
Necrosis			
No	08/80 (10.0%)	1	0.37
Yes	03/15 (20.0%)	2.025 [0.33-11.13]	
Axillary node positivity			
≥3	05/15 (33.3%)	1	**0.0193**
[0-3]	07/81 (08.6%)	0.19 [0.04-0.93]	

DCIS: Ductal Carcinoma *in situ*

Table 5- Multivariate analysis of data associated with tumor-involved margins based on pre- and post-operative data

Variable	Adjusted OR [95% CI]	P value
Preoperative US		
≤27mm	1	
>27mm	0.06 [0.01-0.79]	**0.0319**
Tumor grade (SBR)		
3	1	
2-1	20.61 [1.77-239.3]	**0.0156**
DCIS		
No	1	
Yes	18.86 [1.54-231.32]	**0.0216**

US: Ultrasound; SBR: Scarff Bloom and Richardson; DCIS: Ductal Carcinoma *in situ*