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Nipple-Sparing Mastectomy: Reliability of sub-areolar sampling and frozen section in predicting occult nipple involvement in breast cancer patients

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ABSTRACT

Introduction: The oncological safety of nipple-areolar complex (NAC) preservation is a concern in the mastectomies performed for cancer indication. The detection of tumor cells during the intraoperative frozen section examination (IE) of sub-areolar/nipple tissue (SAT) leads to the removal of NAC, but frequently the final histology of the nipple is negative for malignancy. This study aims to evaluate the accuracy of SAT examination in predicting occult NAC involvement in case of Nipple-Sparing Mastectomy (NSM).
Methods: The study includes 76 NSM. We evaluated the concordance between histopathologic features of frozen and paraffin-embedded SAT sections. Moreover, we examined the "true margin" (TM), defined as the measurement of the distance between the tumor margin and the edge of the SAT. A margin >1 mm was considered negative.
Results: In 26/76 cases the IE of the SAT was positive. At the final histology, the NAC was negative in 57.7% of cases. The concordance between frozen and paraffin section examination of the SAT was 92.1%. The three false-positives were low-grade DCIS at the IE, and negative or DIN1a on permanent section. A negative TM seems to predict for a negative NAC (6/6).
Conclusions: The detection of a low-grade DCIS at the IE of the SAT may not be confirmed at the permanent section examination; we recommend caution in removing the NAC in these cases. The evaluation of the TM may improve the accuracy of SAT analysis in predicting occult NAC involvement; in our series, a TM wider than 1 mm correlates with a negative NAC.

Introduction

The Nipple-Sparing Mastectomy (NSM) technique is becoming a common surgical approach for both prophylactic and therapeutic indications, mostly because of its cosmetic outcome. However, the oncological safety of nipple-areolar complex (NAC) preservation is still a significant concern in mastectomies performed because of cancer. The current indication is that the NAC may be preserved without compromising oncologic safety as long as the sub-areolar/nipple tissue (SAT) specimen examination shows no evidence of malignancy on permanent sections [1]. In surgical practice, if the intraoperative frozen section examination (IE) of SAT, detects tumor cells, the NAC is removed by converting the NSM into a skin-sparing mastectomy (SSM).

The intraoperative pathologic assessment of the SAT is an important step. A good concordance between frozen section and permanent histology of SAT ranging from 84.6 to 95.4% [2–4] is reported in the literature. False negatives, ranging from 1 to 4.6% [5], lead to the need of second surgery. Furthermore, there is additional concern about the false positives, which lead to an unnecessary nipple removal.

Moreover, in many instances of true positive SAT as well, the final pathological report shows that the removed NAC contains no

Abbreviations and acronyms: NSM, nipple-sparing mastectomy; SSM, skin-sparing mastectomy; IE, intraoperative frozen section examination; SAT, sub-areolar/nipple tissue; NAC, nipple-areola complex; TM, true margin; IDS, invasive ductal carcinoma; DCIS, ductal carcinoma in situ; ILS, invasive lobular.

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malignancy [6,7]. This observation raises the question of the reliability of SAT analysis to predict nipple involvement and of the eventual overtreatment due to the poor concordance between the positivity of the SAT and the NAC.

This study aims to evaluate in breast cancer patients candidate to NSM **a)** the concordance between histopathologic features of the frozen and paraffin-embedded section of the SAT; **b)** the reliability of the pathological examination of the SAT in predicting the NAC tumor involvement.

Patients and methods

Patients selection

The study includes a series of 76 consecutive NSM performed for breast cancer at the Academic Breast Unit of the Umberto I Hospital, University of Turin, from January 2010 to December 2015. We obtained the data regarding patient history, surgical treatment, intraoperative and final histopathological report through the prospective institutional database. All the patients treated at our Institute sign a consent to the anonymous use of their clinical and instrumental information for scientific purposes.

Eligibility criteria for NSM

In our institution, we routinely measure the tumor-nipple distance by breast MRI. Patients with a radiological distance of less than 1 cm are not excluded a priori, but we inform them that, according to our data, the probability of preserving the NAC is low. We do not exclude patients based on tumor size, age or lymph node status. Conversely, we do not offer NSM to women with either clinical or radiological involvement of the NAC, as well as to women with locally advanced breast cancer with skin involvement, inflammatory breast cancer, and Paget's disease. Patients with marked ptosis or macromastia are excluded as well, for cosmetic and reconstructive reasons.

Surgical technique

Sub-areolar/nipple tissue is removed with the cold knife to avoid thermal injury to the specimen. The goal is to obtain a discoid specimen under the areola, about 1 cm thick containing the retro-areolar tissue and the ductal tissue just beneath the base of the nipple (Fig. 1). This specimen is oriented and sent for intraoperative frozen section examination. If the SAT contains malignancy at the frozen section, we remove the NAC, converting the nipple-sparing mastectomy into a skin-sparing mastectomy. SAT specimens containing precursor lesions such as lobular neoplasia (lobular carcinoma in situ and atypical lobular hyperplasia), atypical ductal hyperplasia, or epithelial atypia, are not considered positive. If a negative SAT at frozen section turns out to be positive at final examination, we recall the patient and remove the NAC.

Histopathological evaluation

The SAT specimen is sent for histopathological intraoperative frozen section examination. The specimen is oriented by the surgeon with the indication of the margin facing the NAC and the pathologist inks this margin. Subsequently, 1 to 4 cryostatic sections of 3–4 μm each are examined, depending on the size of the SAT specimen. Cryostatic sections are stained with Diff-Quick. The procedure requires about 15 min from the delivery of the sample to the intraoperative diagnosis. The bulk of the specimen is fixed in 10% buffered formalin, paraffin-embedded and evaluated as permanent histological sections of 4 μm each at 10 μm intervals, stained by hematoxylin and eosin and evaluated by a pathologist with specific expertise in breast pathology. When removed, the NAC is directly fixed in formalin, paraffin-embedded and entirely examined on permanent sections. In our procedure the NAC is divided into two halves in the sagittal direction, and each half is cut every 2–3 mm perpendicular to the first cut. All paraffin blocks are sectioned in slices of 4 μm each at 10 μm intervals.

For our study, the information about the frozen sections diagnosis was obtained from the original reports. All paraffin-embedded specimens of the positive SAT and the corresponding removed NAC have been re-analyzed. The following items were recorded regarding the **SAT:** size of the specimen, the histotype and size of the tumor focus, the pattern of growth and the “true margin” (TM), defined as the distance between the tumor edge and the margin of the SAT specimen facing the NAC. We ranked the true margin in three categories: a margin $\geq$1 mm was considered negative, a margin <1 mm, but no ink on tumor was considered intermediate, and a margin with ink on tumor was defined positive (Fig. 2). As to the **NAC**, the paraffin-embedded specimen was labeled positive for malignancy if cancer cells were detected either in the stroma of sub-areolar tissue or in the terminal ducts of the nipple. A NAC specimen containing precursor lesions such as atypical ductal or lobular hyperplasia or lobular carcinoma in situ was not considered positive.

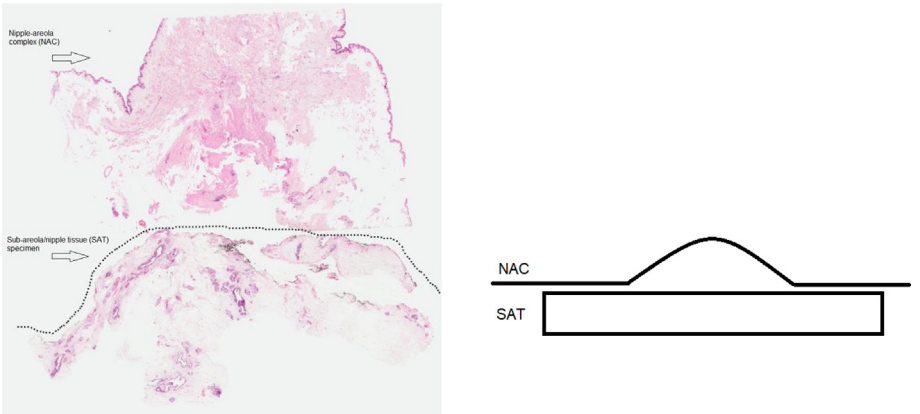

Fig. 1. Nipple-Areola complex (NAC) and Sub-areolar/nipple tissue (SAT). SAT: discoid specimen under the areola, about 1 cm thick containing retro-areolar tissue as well as the ductal tissue just beneath the base of the nipple.

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Statistical analysis

Continuous variables were evaluated by Student's t-test (2 sided); categorical variables were evaluated by the chi-square test or Fisher's exact test where appropriate. The multivariate analysis was performed using the logistic regression model. A value of $p < 0.05$ was considered statistically significant. Statistical analysis was performed using SPSS for Windows.

Results

The series includes 76 therapeutic NSM. Table 1 describes demographic information on patient's and primary tumor characteristics. The median age at the surgery was 49 years (min 37; max 83 years), the median tumor-NAC distance, measured by MRI was 36 mm (min 10; max 60 mm). The most common histotype in the primary tumor was invasive ductal carcinoma (59.2%), followed by ductal carcinoma in situ (22.4%), invasive lobular carcinoma (7.9%) and mixed or special histotypes (10.5%). Luminal B was the most frequent tumor subtype (62.5%).

In all cases, we performed intraoperative frozen section examination of the SAT. In 26/76 cases (34.2%) the frozen section of the SAT was positive for cancer, and in 25/26 cases the NAC was immediately removed. The SAT was more frequently positive in primary IDC. The histology of the positive SAT was 19 DCIS, 4 IDC, 2 ILC and 1 DCI + DCIS.

Overall only 10/76 patients (13.1%) had tumor in the NAC at conclusive pathology.

Concordance between the frozen and paraffin-embedded sections of the SAT

We compared the original report of the intraoperative SAT examination with the result of the paraffin-embedded post-operative specimen. Out of 76 frozen examinations of the SAT, 50 (65.8%) yielded negative results and 26 (34.2%) yielded positive results.

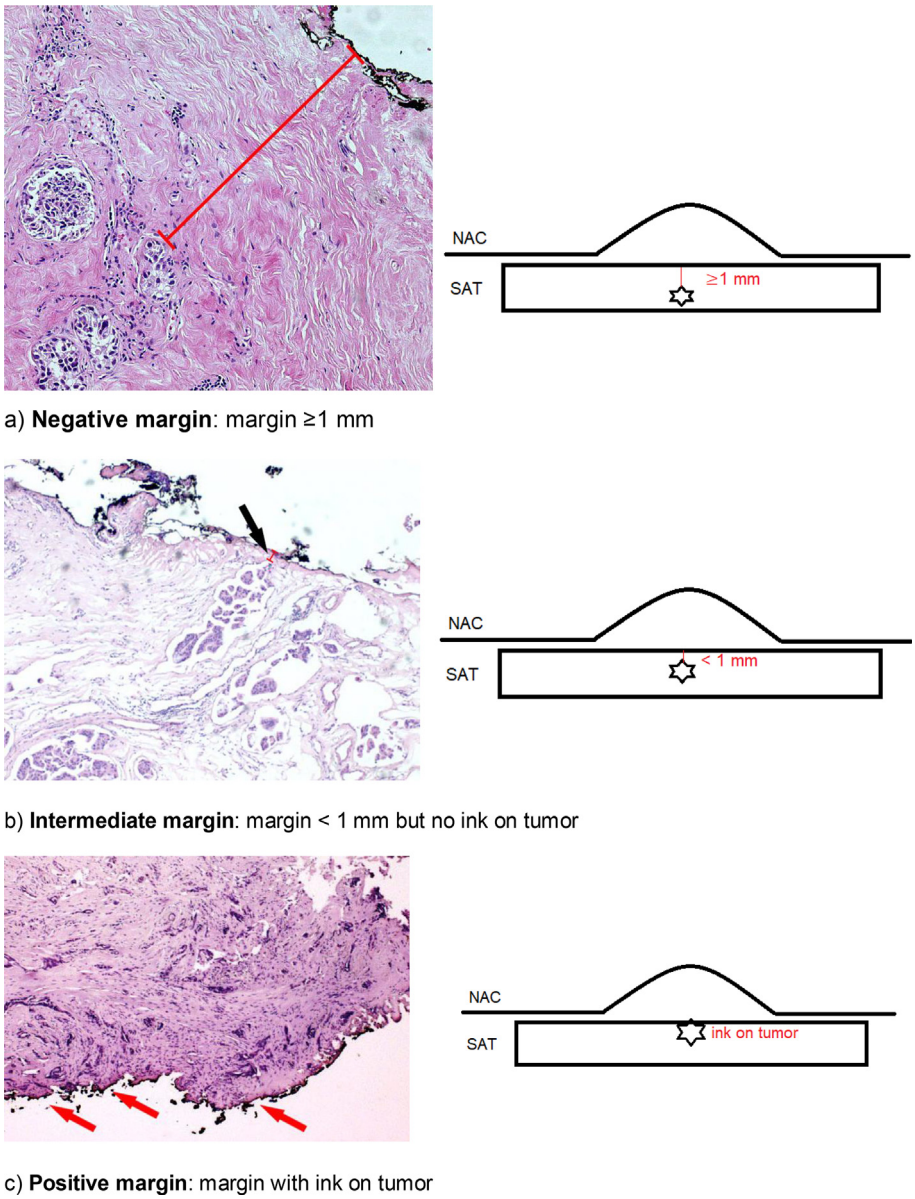

Fig. 2. True Margin (TM): distance between the tumor edge and the margin of the SAT facing the NAC in the sub-areolar tissue (SAT), ranked in three categories.

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Table 1
Demographic information related to patients and primary tumor characteristics.

Characteristics	Median (range) or %
Age *(years)*	49 (37; 83)
Size of largest tumor focus *(mm)*	22.5 (5–70)
Tumor-NAC distance *(mm)*[a]	36 (10–60)
Tumor pathology	
IDC	59.2%
ILC	7.9%
DCIS	22.4%
Other	10.5%
Grade for invasive tumors	
High	52.9%
Intermediate	43.2%
Low	3.9%
Grade for in situ tumors	
High	36.0%
Intermediate	32.0%
Low	32.0%
Lymphovascular invasion	
Present	86.2%
Absent	13.8%
Tumor subtype	
Luminal A	25.5%
Luminal B	62.5%
Her2 enriched	8%
Triple negative	4%
Lymph node status	
Positive (≥1)	51%
Negative	49%

[a] Tumor-NAC distance (mm): measured by MRI.

Three out of the 50 negative specimens at frozen section turned out to contain cancer cells at permanent section (false negative 6%); conversely, three out of the 26 positive specimens at frozen section turned out to be negative at permanent section (false positive 11.5%). The statistical analysis of the frozen examination showed a sensitivity (Se) of 88.4%, a specificity (Sp) of 94%, a positive predictive value (PPV) of 88.4%, a negative predictive value of 94%, and an Accuracy of 92.1% (Table 2).

The **3 false negative** SATs at the frozen section were: 1 case of high-grade DCIS and 2 cases of low-grade DCIS. The patients have been recalled: one patient had the NAC removed, the remaining two refused the procedure and did not receive postoperative radiation. So far, after three years of follow-up, they are free from recurrence.

The **3 false-positive** SATs were classified as low-grade DCIS at the frozen section, but at permanent section two specimen were negative and the last one was a DIN1a.

Accuracy of sub-areolar/nipple assessment in predicting occult NAC involvement

We have re-analyzed 22 out of the 26 intra-operative positive SATs with their corresponding removed NACs. One specimen has been excluded because the SAT had not been oriented during surgery, the remaining three excluded cases were the false positive SATs at the frozen section examination.

The median width of the SAT specimens was 30 mm (min 20; max 70 mm) and the median thickness was 9 mm (min 5; max 13 mm). The median size of the tumor focus within the SAT was 4 mm (min 1 mm; max 20 mm). In three cases the lesion was multifocal. The TM was negative in 6 cases (mean distance 3.6 mm), intermediate in 10 cases and positive in 6 specimens.

Overall, 26/76 women had the nipple removed due to the presence of a tumor in the SAT. After the permanent sections histological examination of the nipple-areola complex, 10/26 patients (42.3%) had tumor involvement of the NAC whereas the NAC was negative for cancer in 16/26 (57.7%) of the cases. The SAT examination shows a Sensitivity of 42.3% in predicting the NAC involvement.

We performed a **univariate analysis** to relate the NAC involvement to different histopathological parameters of the SAT. We evaluated the relation between several features of the SAT (thickness, type of malignancy, size and number of tumor foci, margin status) and the NAC status (Table 3). Results show that the specimen thickness and the pattern of tumor growth (in situ or invasive) were not statistically associated with the NAC status (p-value: 0.44 and 0.48, respectively). Instead, the size and the number of tumor foci on the SAT and the status of the SAT margin facing the NAC were associated with the NAC status. Multiple and large tumor foci on the SAT seem to predict for a positive NAC (p-value 0.07 and 0.02, respectively) as well as a positive ink margin predicts for a positive NAC (p-value 0.02). The **multivariate analysis** showed that the margin status of the SAT is the only factor independently associated with the NAC involvement: with no ink on tumor the risk of NAC involvement is less than 10% (OR: 0,09; p-value 0.049) Table 4.

We performed a sub-analysis focusing on the "true margin" ranked in three categories that resulted statistically associated with the NAC status (p-value 0.01). A negative TM is associated with a negative NAC in all cases (6/6). The NAC is positive in 5 out of 6 cases of positive TM (ink on tumor), the only case with negative NAC was a low-grade LCIS (LIN2) on the SAT. No correlation was found when the free margin is < 1 mm (5 negative and 5 positive NACs) Fig. 3.

Discussion

Despite the most common malignant finding of the primary tumor was invasive ductal carcinoma (59.2%), the 76% of the SAT

Table 2
Sub-areolar/nipple tissue (SAT) frozen-section results as compared to permanent section results (n = 76).[a–d]

SAT	Frozen-section Pos	Frozen-section Neg	Tot
Permanent-section Pos	23	3	26
Permanent-section Neg	3	47	50
Tot	26	50	76

[a] Sensitivity: 88.4%.
[b] Specificity: 94%.
[c] Positive predictive value: 88.4%.
[d] Negative predictive value: 94%.

Table 3
Correlation between Nipple-Areola Complex (NAC) involvement and histopathological parameters of the SAT (univariate analysis).

Sub areolar tissue	N	NAC involvement		p value
		No (%)	Yes (%)	
Mean SAT thickness (mm)		0.88	0.98	0.44
Tumor type				
in situ	16	8 (66.6)	8 (80)	0.48
invasive	6	4 (33.4)	2 (20)	
Focus size				
≤6 mm	16	11 (91.6)	5 (50)	0.029
>6 mm	6	1 (8.3)	5 (50)	
Multifocality				
Yes	5	1 (8.4)	4 (40)	0.07
No	17	11 (91.6)	6 (60)	
Margin facing the NAC				
Ink on tumor	6	1 (8.4)	5 (50)	0.029
No ink on tumor	16	11 (91.6)	5 (50)	
Ranked "True margin"				
Positive	6	1 (8.4)	5 (50)	0.014
≤1 mm	10	5 (41.4)	5 (50)	
Negative	6	6 (50)	0 (0)	

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Table 4
Multivariate analysis.

	OR	95% IC		p-value	Likelihood -2Log
SAT margin status					
Ink on tumor					
No ink on tumor	0.09	0.008	0.995	0.049	25.281

positive specimen was positive for DCIS. This data is also reported by other authors [6–8], possibly due to the natural tendency of cancer cells to spread along breast ducts.

In this series, 13.1% of the patients had a tumor involvement of the NAC; this observation is consistent with our previous series of total mastectomies (14%) [9]. In the literature, the rate of NAC involvement ranges from 0 to 58% [10] likely due to different pathological protocols for NAC evaluation and for clinical and pathological data collection. A recent series of 112 NSM reports a 27.7% of NAC involvement [7], a smaller study reports an occult nipple involvement rate of 11.7% [2]. In a large series including more than 2300 patients Weidong and Co found occult NAC involvement in 14.2% of the cases [11].

Concordance between the frozen and paraffin-embedded sections of the SAT

A good concordance between frozen section and permanent histology of SAT is generally reported in the literature. Two recent papers report respectively 94.1% [2] and 84.6% [3] of concordance between the two examinations. Another series of 158 NSM reports an accuracy of 95.4% with a sensitivity of 92.0%, a specificity of 96.2%, a positive predictive value of 82.1% and a negative predictive value of 98.4% [4]. Our results are consistent with the literature; we found an accuracy on 92% with a sensitivity of 88.4% and a specificity of 94% between the frozen and the paraffin section of the SAT. We had three false-negative and three false-positive findings between the two examinations.

False-negative frozen section results are generally uncommon, in the literature FN range from 1 to 4.6% [5]. The frozen section might miss small foci of DCIS; false negatives results may occur as a result of sampling, interpretation, and frozen artifactual errors. Luo et al. suggest to reduce the use of diathermy when obtaining the biopsy and to analyze more segments of biopsy specimens [3]. The need to re-operate these patients is a matter of discussion. Local-regional recurrence has been demonstrated to be low in these false-negative cases [12]. RongTang et al. [6] report the experience of seven nipples with positive margins managed with observation alone, also Amara et al. [8] present the experience of eight similar cases. To date, none of these patients has had a NAC recurrence, but follow-up is short (38 and 31 months respectively). In our experience, the only NAC removed in the following re-operation was negative and no recurrences occurred in the two patients whose nipple was not removed despite a positive SAT in the paraffin-embedded section. To draw any clinically relevant conclusion the number of patients and the follow-up need to be increased. To date, we can't suggest observation alone as standard practice; this strategy should only be considered in selected and informed patients.

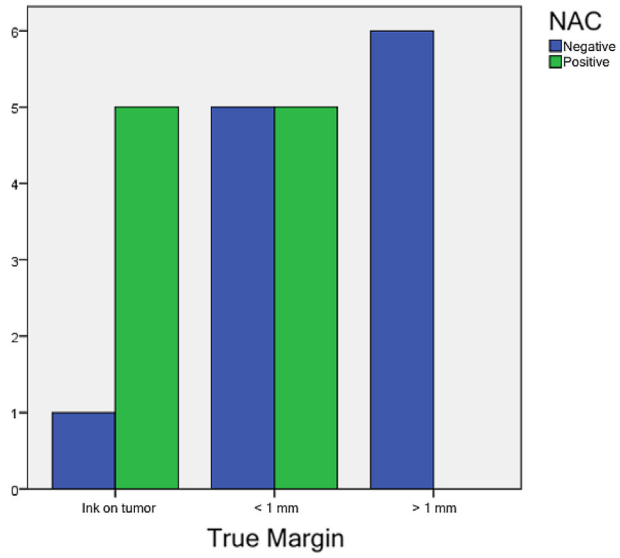

Fig. 3. Sub-analysis focusing on the "true margin" ranked in three categories. A negative TM (≥1 mm) is associated with a negative NAC in all cases (6/6). The NAC is positive in 5 out of 6 cases of positive TM (margin with ink on tumor). No correlation can be found when the free margin is <1 mm (5 negative and 5 positive NACs).

Another crucial issue is the **false-positive SAT**. Some positive specimen at the frozen examination subsequently turned out to be negative for malignancy on permanent section but in the meantime had lead to the immediate removal of a negative NAC. In our series we had three (11.5%) false positive SAT, that is higher than expected from the literature: a large series of NSM reports five false-positive (3.1%) findings [4]. Even if the small size of our sample may be a bias, interestingly, all of the false positive specimens had been diagnosed as **low-grade DCIS** at the frozen section. Possibly this was due either to the artifacts created by the freezing process that could simulate low-grade DCIS, which eventually is not found at the final examination, or to loss of tissue during frozen sections and subsequent permanent sections processing. Other authors recall this problem as well, with similar considerations [10]. Piato et all. observed that 3/5 false-positive cases corresponded to atypical ductal hyperplasia [4].

As the detection of a low-grade DCIS at the frozen section of the SAT could not be confirmed at the paraffin section examination, and the overdiagnosis of proliferative lesions can lead to the removal of a negative NAC, some pathologists advocate that proliferative lesions should not be diagnosed by frozen section [13,14]. We propose caution in removing the NAC during the same operation, in the case of detection of a low-grade DCIS at the frozen section of the SAT. If the malignancy is confirmed at the paraffin-embedded section, NAC resection could be performed at the stage of reconstruction (if a skin expander is implanted) or with a separate procedure (if an immediate reconstruction is performed).

Accuracy of sub-areolar/nipple assessment in predicting occult NAC involvement

In our series, we found a positive SAT in 34.2% of the NSMs performed on cancer patients, which is high compared with the results from several large series ranging from 6 to 10% [5,6] and notably higher than 2.7% positive specimen rate recently reported by Amara and colleagues [8]. Several factors likely account for our high positive specimen rate. For example, the thickness variability of the specimen among the studies is very important; in our series, the size and the median thickness of the SAT are consistent with the international recommendations previously described. It's reasonable that the rate of positivity is lower with a thinner specimen. Rong Tang reports a very low positive rate of 6,75% creating a sub-areolar/nipple duct margin specimen so thin not to allow an accurate orientation and its surface is entirely inked black [6]. Moreover, the frequency of a positive specimen may be influenced by the patient's selection criteria: we consider 1 cm at MRI the cut-off distance for offering this procedure while some authors offer

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NSM only to patients with a tumor-nipple distance of at least 2 cm, decreasing the likelihood of a positive SAT.

At the permanent sections of the removed NACs, the majority of the specimens (57.7%), had no evidence of malignancy; these data show a very low sensitivity of the SAT examination in predicting the tumor involvement of the NAC. Rong Tang et al. reports a similar rate: of the 39 nipple/NACs excised for positive margins specimen, 72% (28/39) had been found with no residual malignancy [6]. In a recent series published by Ponzone et al., the majority of excised NACs showed no residual tumor (54,5%), sensitivity and specificity of the pathologic assessment of the SAT are reported to be 46.5% and 100% respectively [7]. Similar results are reported in the previously cited article by Duarte, the sensitivity was 62.5%, PPV 21.4% and NPV was 94.7% when using paraffin histology. In another recent study, the majority of nipple/NAC excised for a positive margin had no residual malignancy: of the 17 nipples and NAC removed, only four (24%) had residual DCIS and none had residual invasive cancer. The remaining 13 specimens (76%) and no residual malignancy [5]. Several factors might account for the low rate of residual malignancy in the NAC removed for a positive SAT. Some authors hypothesize potentially that small foci of cancer in the NAC may be present but are not identified secondary to sampling error [15]. Other authors underline that the majority of positive SAT contained only a single and small focus of DCIS, therefore, if the bulk of disease in the SAT is small, this predicts a lower likelihood of residual disease in the excised NAC [5]. This has led to consideration of alternatives to NAC excision when small volume disease is seen in the SAT [6].

A different biopsy technique has been suggested using two separate sub-nipple biopsy samples dividing the outer (outer from the NAC) and the inner (just beneath the NAC) part of the subareolar tissue [16]. If the patient expressed preoperatively the desire to preserve the NAC, it could be preserved if the inner is negative for malignancy despite a positive outer result. This procedure could reduce the number of NAC excisions in motivated patients, but in our experience, we prefer to perform a single biopsy and let to the pathologic examination the definition of the width of the free margin toward the NAC.

The issue of the accuracy of sub-areolar/nipple assessment in predicting occult NAC involvement is still under discussion. However, we found no study which re-analyzed all positive SAT specimens and the corresponding removed NAC to identify the most accurate SAT factors to predict NAC status.

At the **univariate analysis** relating different SAT histopathologic factors to the NAC status, multiple (more than one focus) and larger ($\geq$6 mm) tumor foci on the SAT seem to predict for a positive NAC. The direct involvement of the margin of the SAT facing the NAC (ink on tumor) is statistically related to a positive NAC. In case of no ink on tumor, a TM wider than 1 mm correlates with a negative NAC. At the **multivariate analysis**, the margin status of the SAT remains the only factor independently associated with the NAC involvement. We think that the evaluation of the TM could improve the accuracy of frozen sub-areolar/nipple tissue analysis for predicting occult NAC involvement. In literature, few authors mention the margin analysis. Camp et al. state that, according to their current management algorithm, the excision of the nipple/NAC is warranted if any malignancy in the sub-areolar/nipple duct specimen is identified too close to the margin, without specifying any cut off [5]. Our series is too small to draw definitive clinical practice conclusions; however it seems that a negative margin in the SAT allows to preserve the NAC, but further research and larger numbers are needed.

Another crucial issue is the use of **postoperative radiation.** In the breast-conserving surgery, a margin with no ink on tumor is considered oncologically safe if postoperative breast radiation is performed. A possible issue is whether postoperative radiation may be an alternative to the NAC removal for the management of patients with a positive SAT. Petit and colleagues described the use of a single dose of intraoperative radiation (ELIOT) in more than 1000 patients to preserved NAC when small volume disease is seen in the SAT specimen. At 20-month median follow-up, they observed no NAC recurrences, even in the 160 patients with close or positive SAT specimen margins [17]. However, in a recently published update, by 50-month median follow-up, they report 11 NAC recurrences (1%) among 861 patients who underwent NSM with ELIOT [18]. This suggests that there is still a low risk of NAC recurrence following a single intraoperative dose of radiation targeted to the NAC, but no studies have directly compared the risk of NAC recurrence in patients who have received ELIOT versus those who have not [5]. As intraoperative radiation is not available in most centers, it couldn't be a widespread option. If adjuvant standard postmastectomy radiation is required, radiation without nipple excision may provide acceptable local control for a close nipple margin, but further researches are needed.

A limitation of this study is the small size of the sample, but at the best of our knowledge, no studies re-analyzed all positive SAT specimens and the corresponding removed NAC to identify the most accurate SAT factors to predict NAC status. This study can be seen as a “proof of concept”; we are working to validate our hypothesis on a more extensive and multicentric series of NSM performed for breast cancer following the same indications and criteria. If confirmed, this observation could help the surgeon to identify intraoperatively patients who may have the NAC preserved, even if cancer cells are detected in the SAT.

Conclusions

In conclusion, the reliability of the SAT evaluation in predicting nipple involvement is not yet fully established. We found a good concordance between frozen section and permanent histology of SAT, but in case of detection of a low-grade CDIS at the intraoperative examination, caution is recommended in removing the NAC based only on these results. Our data show a very low overall sensitivity of the SAT examination in predicting the tumor involvement of the NAC. In fact, the majority of the NACs removed because of the mere presence of tumor within the SAT had no evidence of malignancy. The evaluation of the True Margin, instead, could improve the accuracy of sub-areolar tissue histology for predicting NAC involvement. In our experience, the positive predictive value and the negative predictive value respectively are high both when there is ink on tumor and when the margin is clear for 1 mm or more. No prediction can be made when the clear margin is less than 1 mm.

Conflict of interest statement

No actual or potential conflict of interest including any financial, personal or other relationships has to be disclosed by the authors.

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