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Intraoperative Imprint Cytological Assessment of the Subareolar Tissue of the Nipple Areola Complex (NAC)

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A B S T R A C T

One of the criteria of selection for skin sparing mastectomy (SSM) with nipple areola complex (NAC) preservation is to exclude the neoplastic involvement of subareolar tissue (NAC base) in order to minimize the possibility of local recurrence. The most common way to assess the possible neoplastic involvement is intraoperative frozen section of the NAC base tissue. Because of its limitations, particularly the false negative results due to unsampling, we tried to use intraoperative imprint cytology for more thorough intraoperative assessment. The aim was to compare intraoperative imprint findings with the definitive histology of the NAC base, to evaluate diagnostic accuracy of this method and possibility to substitute frozen section for intraoperative assessment of NAC base. A prospective clinical study was conducted of 208 consecutive female patients who underwent open biopsy because of carcinoma. Intraoperative imprints were taken from the excised subareolar tissue which was then routinely processed for definitive histology. Imprint findings designated positive, negative, suspicious or atypia, were compared with definitive histological findings. Our results with 7.5% false negative rate, 9.8% false positive rate, sensitivity of 50% and specificity of 87.58% argue that imprint cytology might not be sufficient as an exclusive method for the intraoperative assessment of the NAC base though it should be used routinely in conjunction with frozen section examination.

Key words: *intraoperative imprint cytology, NAC base, nipple areola complex preservation*

Introduction

Considering the psychological impact of disfigurement on well being of patients after surgical breast cancer treatment, alternative surgical procedures like skin sparing mastectomy (SMM), that are oncologically safe and allow breast reconstruction, have been promoted to improve the overall quality of life for women[1–4]. The feeling of mutilation in mastectomies is enhanced because of the routine removal of the nipple based on the presumed risk of occult cancer involvement[3]. Studies have shown that nipple involvement varies from 5.6% to 58% depending on the size of the primary breast tumor, location, multicentricity, lymph node positivity and the presence of an extensive intraductal component[5,6]. Because a nipple or nipple areola complex (NAC) is a hallmark of breast identity, the reconstruction or even the preservation of NAC becomes an integral part of breast reconstructive surgery[1,4]. The results of secondary NAC reconstructive procedures seem to be less satisfactory than NAC preservation itself[7,8].

The main limiting factor of the NAC preservation is the possibility of local recurrence in the subareolar tissue. Though the criteria of selection for skin sparing mastectomy (SSM) with NAC preservation have not been defined, most authors agree that in order to minimize the possibility of local recurrence, criteria should be clin-

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ically normal nipple, adequate areola-tumor distance (TND), T1/T2 tumors (<5 cm tumor size) and negative frozen section of the subareolar tissue[1,3–6,9].

TND and tumor size could be assessed preoperatively using mammograms and ultrasound together with the assessment of axillary lymph nodes, followed by ultrasound-guided fine needle biopsy of the enlarged nodes[1]. Histology of the subareolar tissue is crucial for detection of occult invasive or in situ carcinoma or lymphovascular invasion as these lesions are impossible or difficult to diagnose preoperatively with standard mammography or ultrasonography. Some authors even suggest preoperative needle core biopsy of retroareolar tissue or the use of MRI to exclude cancer involvement[10,11].

The most common way for histological assessment of NAC base is intraoperative frozen sections. Because of its limitations there is a possibility of false positive or negative results[1,6,12]. False positive results at intraoperative biopsy indicate NAC removal while false-negative results at intraoperative biopsy require additional removal of the NAC a few days after the initial procedure[1]. Beside misinterpretation as a cause of these false results, most of false negative results are caused by unsampling of intraoperative specimen[13]. To reduce the possibility of false-negative results and consequent secondary NAC removals we tried to perform more thorough sampling of the specimen. Keeping in mind that intraoperative biopsy is time limited procedure unsuitable for taking serial sections, we have applied intraoperative imprint cytology as simple, tissue sparing method suitable for rapid and complete sampling of the specimen[14–17]. The aim was to compare intraoperative imprint findings with the definitive histology results of NAC base, to evaluate diagnostic accuracy of this method and possibility to substitute frozen sections for intraoperative assessment of NAC base.

Material and Methods

A prospective clinical study was conducted of 208 consecutive female patients who underwent open biopsy because of carcinoma regardless of tumor size, localization, multicentricity, bilateral disease and preoperatively noted axillary lymph-node status or intended type of surgery. Informed consent was obtained from all of the patients included in the study.

For each patient excised tumor with surrounding tissue marked with three sutures was sent for intraoperative biopsy. Intraoperatively obtained data were tumor size, surgical margins and tumor histology assessed on frozen section. Separately, tissue 1 cm in depth beneath NAC was excised (omega incision)[18] and sent for intraoperative imprint cytology and definitive histology. NAC base tissue was marked in the same manner as tumor; with double short suture for »nipple-facing« side, single long and single short suture for lateral and medial side. NAC base tissue was handled separately from tumor specimen to avoid possible contamination with tumor cells through instruments or working area. Imprints were taken from the »tumor-facing« surface of the base, the »nipple-facing« surface of the base (double short suture) and from two sides marked with single long and short suture in order to morphologically analyze the whole surface of the NAC base. One imprint was also taken from a vertical section, usually through the middle of the NAC base, same as it would have been done for frozen section. Imprints were air dried and quickly stained with Hemacolor (Merck KGaA, Darmstadt, Germany). Intraoperative imprint findings were designated inadequate, positive when tumor cells had been found, negative, suspicious of malignancy and atypical, meaning probably benign.

After imprint had been taken, vertical (bread-loaf like) sectioning of the base at 2mm intervals for definite histology was done. The sections of NAC base together with the sections of tumor, surrounding breast tissue and lymph nodes (sentinel lymph node biopsy or axillary dissection) were routinely formalin fixed, paraffin embedded and hematoxylin and eosin stained. Standard definitive histology report of tumor, NAC base and surrounding breast tissue included the exact tumor size, histological type (according to WHO 2003 breast tumor classification), grading (Elston Bloom Richardson for invasive and Van Nuys system for DCIS), lymphovascular invasion, immunohistochemically assessed hormone and Her2 status, surgical margins and histological axillary lymph-node status. Intraoperative imprint cytology and definitive histology findings of the NAC base were compared.

Results

Among 208 patients in this study there were 6 cases of bilateral tumor that made a total of 214 analyzed cases. There were 169 cases of ductal invasive carcinoma, 17 cases of lobular invasive carcinoma, 7 cases of microinvasive ductal carcinoma and 15 cases of ductal in situ carcinoma (seven high grade, three medium grade and five low grade cases). There were six cases of various benign tumors and changes preoperatively mistaken for malignancies. In all these cases retroareolar tissue was sent for intraoperative imprints.

Results of intraoperative imprints and definitive histological findings of NAC base are summarized in Table 1. Among 214 histological findings of NAC bases there were 18 (8.4%) invasive carcinomas (11 ductal and 7 lobular), 17 (7.9%) ductal in situ carcinomas (DCIS), 7 (3.3%) lobular in situ carcinomas (LCIS), and 14 (6.5%) cases of atypical ductal hyperplasia (ADH). Lymphovascular invasion was found in 4 (1.9%) specimens. Normal breast tissue or benign changes were found in 154 (72%) cases.

Among DCIS cases majority was of high grade with or without commedo necrosis (11 cases), while five were low grade and only one was medium grade. High grade DCIS involvement of NAC base was found in four cases of multicentric high grade DCIS with commedo necrosis, in three cases of multicentric microinvasive carcinoma and in four cases of ductal invasive carcinoma. LCIS involvement of NAC base was found in six invasive lobular carci-

TABLE 1
COMPARISON OF INTRAOPERATIVE IMPRINT CYTOLOGICAL FINDINGS WITH DEFINITIVE HISTOLOGICAL FINDINGS OF NAC BASE

Histology	Cytology					Total for histology
	Positive imprint	Suspicious	Atypical	Negative imprint	Inadequate	
Invasive carcinoma	9		2	6	1	18
LVI	1			3		4
DCIS high grade	11					11
DCIS medium grade	1					1
DCIS low grade	0	1		4		5
LCIS	0	2	1	3	1	7
ADH	5			7	2	14
Normal tissue or benign changes	16	17	6	110	5	154
Total for cytology	43	20	9	133	9	214

LVI – lymphovascular invasion, DCIS – ductal carcinoma in situ, LCIS – lobular carcinoma in situ, ADH – atypical ductal hyperplasia

nomas and in one ductal invasive carcinoma. Altogether 60 (28%) NAC bases had pathohistological findings of various malignant potential, among them 33 (15.4%) were doubtless malignancies; high grade DCIS, invasive carcinomas and lymphovascular invasion.

Cytological analysis has shown that 9 (4.2%) out of 214 imprints were inadequate to analyze, 43 (20.1%) cases were reported positive for malignant cells, 133 (62.1%) were negative, 20 (9.4%) cases were suspicious of malignancy and 9 (4.2%) were reported atypical. Compared to histology only 50% (9 out of 18) of all NAC bases with invasive carcinoma were detected on imprints, six were false negative and two were reported as atypical. Only one out of four NAC bases with lymphovascular invasion was detected on imprint, the rest were false negative. All 11 cases of high grade DCIS were detected on imprint cytology, same as the only case of medium grade DCIS. Among neoplastic changes with low malignant potential, imprint results were less sensitive; among five cases of low grade DCIS and seven cases of LCIS none was detected as positive. One case of DCIS and three cases of LCIS were reported as atypical or suspicious. Most confusing imprint results were for ADH; five out of twelve adequate NAC bases with ADH were reported as positive and 7 as negative. There were 20 (9.5%) suspicious imprints, 85% of them were histologically normal breast tissue or with benign changes, while the rest of them (15%) were low grade DCIS and LCIS in histology. Atypical imprints account for 4.2% (nine cases) of all imprints and again the majority of them (67%) was normal or had benign changes in histology.

To simplify comparison between histology and imprints, histological findings were grouped as »positive« (21% of cases) if invasive or in situ carcinomas were found, irrespective of nuclear grade and »negative« (78.5% of cases) if ADH, benign changes or normal tissue were found. Despite of growing evidences that ADH represents true neoplastic proliferation, it was designated negative because its biology and management differs from the rest of intraductal carcinomas[19–21]. These compared results revealed 16 (7.5%) false negative and 21 (9.8%) false positive imprints. The mean sensitivity was 50% and specificity 87.58%.

Discussion and Conclusion

The incidence of NAC involvement with malignant process reported in the literature varies from 5.6% to 58%. In this study the incidence was 21.5% similar to the incidence observed in a study by Vlajčić et al.[1] that had been conducted several years ago in our institution. The highest incidence of NAC involvement (76.5%) was observed in lobular carcinoma followed by multicentric high grade DCIS (57.1%) and multicentric microinvasive carcinoma (42.9%), while in ductal invasive carcinoma the NAC involvement incidence was 21.3%. Though results are in concordance with well known biology of these carcinomas, one should be aware that other parameters that influence NAC base involvement like tumor size, multicentricity, location and tumor-areola distance have not been analyzed in this study.

Although the percentage of positive imprints (20.1%) in this study is similar to the percentage of positive histological findings, when results of these two methods are compared, major discrepancies emerge. Among 43 NAC bases with positive intraoperative imprint cytology, in 16 cases only benign changes or normal tissue has been found in definitive histology. Among invasive carcinomas found in NAC base in definitive histology 50% were recognized on imprints irrespective of their nuclear grade or histology (36.4% of ductal invasive carcinomas found in NAC base had been recognized in imprints and 73% of them were grade 3 compared to 71.4% of lobular invasive carcinoma recognized in imprints with 80% of them being grade 2). The least sensitivity was observed for low grade malignancies. Among 5 cases of low grade DCIS and 7 cases of LCIS in this study, none was recognized as neoplastic proliferation. The best concordance was observed in extensive malignancies with obvious high grade nuclear features; all high grade DCIS cases

were recognized as malignant on imprints. Most confusing imprint results were for ADH, where 5 out of 12 were reported as positive with no suspicious or atypical cases. Considering that morphological changes in ADH are even more subtle than in low grade DCIS or LCIS and that none of DCIS or LCIS has been detected on imprints, those 5 positive results are a bit puzzling. False negative rate (7.5%) of imprints observed in this study is a bit higher than false negative rate Vlajčić has observed at intraoperative histology[1]. While there were no false positive results in his study, we have more false positive results (9.8%) then false negative at intraoperative imprints. In a contest of NAC preservation, false positive results bear more unpleasant consequences that could not be corrected easily. While negative results at intraoperative biopsy require additional removal of the NAC after the initial procedure false positive results indicate unjustified NAC removal with less satisfactory results. Another problem arises with reporting suspicious or atypical findings. Should suspicious findings or atypia in imprint cytology indicate the NAC removal? According to our results the most of suspicious (17 out of 20 cases) and atypical (6 out of 9 cases) imprints had benign changes or normal histology and to our opinion it would be justified to postpone NAC removal until definitive histology if other criteria for NAC preservation are fulfilled.

According to minimum standards proposed for cytology within the UK Breast Screening Program, the false negative rate should be less than 5% and false positive less than 1%, suspicious rate that includes suspicious and atypical cases, less than 20% and further on, sensitivity and specificity should be more than 60%[22]. Our results with 7.5% false negative rate, 9.8% false positive rate and sensitivity of 50% argue against imprint cytology as an only method for the intraoperative assessment of the NAC base.

The accuracy of cytology reporting depends on several factors including diagnostic skill of the cytopathologist as well as the skill of the person who performs imprints and the nature of the lesion[22]. An adequate amount of material is an obvious prerequisite for a reliable diagnosis and it has to be stressed that we were dealing with occult lesions. It is possible that many of false negative imprints actually failed to »pick up« a detectible amount of neoplastic cells, like in cases of lymphovascular invasion which is a very subtle change even in histology. On the other hand, false negative and positive results may reflect inability to recognize cytological features of malignancy[22,23]. Several authors stress that nuclear morphology, signs of invasion, degree of dissociation, myoepithelial cells, calcium deposits, and necrosis could distinguish between the diagnostic categories. Masood et al. devised a scoring system based on the assessment of cellular arrangement, cellular pleomorphism, anisonucleosis, myoepithelial cells, nucleoli, and chromatin clumping and achieved very good results when compared with histology with a diagnostic accuracy of 89%[24]. For low grade malignancies characterized by cellular monotony, cytology is at a disadvantage compared to histopathology, as also observed in our study, because of the specific diagnostic architectural details that could not be assessed in cytology[23].

Comparison of cytological with frozen section techniques showed different results; sometimes better accuracy had been achieved with frozen sections, sometimes with cytological techniques, but most authors agree the diagnostic accuracy is higher when intraoperative cytology is performed along with frozen section[14–17]. Additional advantages like tissue sparing, possibility of complete sampling and rapidity make imprint cytology eligible method for intraoperative examination[17,25]. The accuracy of intraoperative imprint cytology for the assessment of NAC-base was unsatisfactory in our study. A correlation with histology was not so good suggesting that it could not substitute for intraoperative frozen section. One of the reasons for unsatisfactory accuracy could be erroneous sampling of the NAC base for imprint cytology. Taking imprints mainly from the surface of the base proved to be insufficient for the assessment of occult lesions. Instead, our suggestion is to take imprints from both sides of vertical (bread-loaf like) sections of the base done for definite histology. Thus more complete sampling could be done and comparison with histology could be facilitated and more reliable. Further on, revision of imprint findings together with the revision of histology findings is needed for the assessment of morphological criteria for a specific diagnosis.

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INTRAOPERATIVNA PROCJENA TKIVA BAZE MAMILE PRIMJENOM CITOLOŠKIH OTISAKA

S A Ž E T A K

Kako bi se prilikom mastektomije mogla sačuvati koža dojke zajedno s bradavicom, potrebno je isključiti eventualni okultni neoplastični proces u retroareolarnom tkivu radi spriječavanja lokalnog recidiva. Obično se ta histološka procjena radi intraoperativno na zaleđenim rezovima retroareolarnog tkiva tzv baze mamile. Jedno od ograničenja ove metode je nemogućnost pregleda većeg broja uzoraka, prvenstveno zbog vremena, zbog čega se javljaju lažno negativni rezultati. Intraoperativnom primjenom citoloških otisaka pokušali smo u istom vremenu povećati pregledani uzorak. Cilj je bio usporediti nalaze intraoperativnih citoloških otisaka baze mamile s definitivnim patohistološkim nalazima, kako bi procjenili vrijednost ove metode za intraoperativni pregled baze mamile i da li može zamijeniti zaleđene rezove pri intraoperativnom pregledu. U ovoj prospektivnoj studiji pregledano je retroareolarno tkivo u 208 konsekutivnih intraoperativnih biopsija carcinoma dojke. Nakon intraoperativnog uzimanja otisaka retroareolarno tkivo se u cijelosti preuzelo, uobičajeno obradilo i uklopilo za trajne parafinske rezove. Citološki nalazi otisaka, označeni kao pozitivni, negativni, suspektni i atipični, zatim su uspoređeni s patohistološkim nalazima pregledanih parafinskih rezova baza mamila. Usporedbom nađeno je 7,5% lažno negativnih citoloških nalaza, 9,8%lažno pozitivnih nalaza, pri čemu je senzitivnost bila 50% a specifičnost 87,58%. Iako prema ovim rezultatima izgleda da citološki otisci nisu dostatni za intraoperativnu procjenu baze mamile smatramo da ih treba koristiti kao rutinsku, korisnu nadopunu zaleđenim rezovima.