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Evaluation of Sentinel Lymph Node Intraoperative Touch Imprint Cytology in Breast Cancer Surgery

Cristiana Iacuzzo[1], Marina Troian[1], Laura Bonadio[1], Deborah Bonazza[3], Chiara Dobrinja[1]*, Gabriele Bellio[1], Serena Scomersi[1,2], Fabiola Giudici[2], Fabrizio Zanconati[2,3] and Marina Bortul[1,2]

[1]*Department of General Surgery, University Hospital of Trieste, Azienda Sanitaria Universitaria Integrata di Trieste, Trieste, Italy*
[2]*Breast Unit, Azienda Sanitaria Universitaria Integrata di Trieste, Azienda Sanitaria Universitaria Integrata di Trieste, Italy*
[3]*Department of Histopathology, University Hospital of Trieste, Azienda Sanitaria Universitaria Integrata di Trieste, Trieste, Italy*

Abstract

Background: One of the strongest prognostic factor for breast cancer is the regional lymph node status, which can be evaluated intraoperatively by sentinel lymph node biopsy. Many methods (e.g. frozen section, touch imprint cytology (TIC) and one-step nucleic-acid amplification) are available to detect metastatic cells in axillary lymph nodes. The aim of this study is to evaluate the feasibility of using TIC to detect metastatic cells in sentinel lymph nodes.

Methods: This is a retrospective single center cohort analysis conducted on prospectively recorded data. The study included all patients admitted to the Department of General Surgery of Trieste University Hospital for invasive clinically node-negative breast cancer who underwent sentinel lymph node assessment by means of TIC.

Results: Between January 2015 and December 2016, 343 patients (338 females and 5 males) underwent breast surgery and sentinel lymph node TIC. Patient's median age was 66 (33-92) years.

The sensitivity of TIC was 54% (95% C.I. 39% to 69%), whereas its specificity and accuracy were 99% (95% C.I. 98% to 100%) and 90%, respectively. The median time required to obtain the result was 20 (15-45) min. The overall cost per each TIC analysis was about 20.50€.

Conclusion: Touch imprint cytology appears to be a fast, cost-effective and reliable technique to intraoperatively detect breast cancer lymph node metastasis.

Introduction

Breast cancer is the most common malignancy in female and the second cause of death in women [1]. One of the strongest predictors of long-term prognosis in primary breast cancer is the regional lymph node status [2]. Sentinel lymph-node (SLN) biopsy is currently preferred over axillary dissection as the standard of care for axillary staging in early, clinically node-negative breast cancer [3]. The actual treatment reckons on axillary dissection in case of nodal macrometastases (i.e., 0.2-2.0 mm), whereas no further surgical treatment is required if no metastases are found [2-4]. The significance of occult micrometastases is still controversial, but recent studies reported that surgical treatment for occult micrometastases could be avoided since it does not appear to improve survival [5-7].

Several methods are available to intraoperatively detect metastatic cells in axillary lymph nodes. The most common ones include frozen section (FS), touch imprint cytology (TIC) [8] and one-step nucleic-acid amplification (OSNA) [9].

The aim of this study is to evaluate the feasibility of using TIC to detect metastatic cells in sentinel lymph nodes.

Materials and Methods

This is a retrospective single center cohort analysis conducted on prospectively recorded data extracted from the database of the Breast Unit of Trieste University Hospital, Italy. The database collected all patients affected by both benign and malignant breast pathologies since 2004.

Eligibility

This study included all patients with invasive clinically node-negative breast cancer who underwent SLN assessment by means of TIC at the Department of General Surgery, University Hospital of Trieste, between January 2015 and December 2016.

During the time-frame considered, 507 surgical procedures for breast cancer or ductal carcinoma *in situ* were performed, 119 of which presented synchronous nodal metastasis and required concurrent axillary dissection. Among the 388 tumors without lymph node metastases, 27 patients were not eligible for the study because they presented with ductal carcinoma *in situ*. In 10 cases, nodal sampling was performed because of failure to identify SLN and the patients were thus not included in this study.

Preoperative evaluation

Breast Imaging-Reporting and Data System (BI-RADS®) of the American College of Radiology was used to refer to breast composition categories and to assess categories for mammogram, ultrasound (US) and magnetic resonance imaging (MRI) [10].

All patients with suspected breast lesions at mammogram and breast ultrasound (US) underwent either US-guided fine-needle

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aspiration cytology (FNAC) or a stereotactic biopsy to obtain a definitive diagnosis.

Axillary US was always performed preoperatively to evaluate the nodal status. If abnormal lymph nodes were detected on imaging, a FNAC was carried out to confirm a possible nodal involvement.

All patients with certain diagnosis of invasive breast cancer were visited by a skilled breast surgeon.

Surgical treatment

All cases were preoperatively analyzed on a multidisciplinary meeting by the members of the Breast Unit of Trieste University Hospital, officially recognized by the European Society of Mastology (EUSOMA) in 2016.

Surgical treatment was decided according to: breast volume – tumor dimension ratio, molecular subtypes (when available), and patient preference. All patients underwent either breast conservative surgery (i.e., quadrantectomy) or mastectomy.

Patients without nodal involvement underwent SLN biopsy. Lymphoscintigraphy was performed 24 hours before surgery in all these patients and the radioactive axillary SLN was intraoperatively localized by means of a gamma probe. SLNs were analyzed during surgery using TIC to detect axillary metastasis. An axillary node dissection was carried out whenever macrometastases were detected by TIC in the SLN. All surgical procedures were performed by two expert breast surgeons.

Touch imprint cytology technique

The technique used to perform TIC analysis was the same as described elsewhere [11]. The SLN harvested by the surgeon was immediately sent to the cytopathologist, who was aware of the examination to perform. The SLN was then isolated from the surrounding fat and divided. One surface of the nodal section was used to prepare a cellular smear, scraping it with a slide and then smearing this material on to a second slide. The second slide was fixed in 95% alcohol, stained with hematoxylin and eosin and then used to detect possible metastatic cells. Each section of each SLN retrieved was used to prepare touch imprint slides (Figure 1).

Post-operative staging

Breast cancers stage was defined according to the American Joint Committee on Cancer (AJCC) [12]. Tumor type was recorded according to the World Health Organization (WHO) classification of breast tumors [13]. The histological grade was assessed using the Nottingham system [14].

Immunohistochemistry (IHC) was performed postoperatively in each surgical specimen. Estrogen and progesterone receptor (ER and PR) status, Human Epidermal growth factor Receptor 2 status (HER2), and cell proliferation activity in terms of Ki-67, were determined using IHC. ER and PR status were considered positive if at least 1% of cells were positive. HER2 was scored 3+ when there was a strong circumferential membranous staining in more than 30% of invasive carcinoma cells, 2+ when a moderate circumferential membranous staining in more than 10% of invasive carcinoma cells was recorded, 1+ when a weak and incomplete circumferential membranous staining was found in more than 10% of invasive carcinoma cells and 0 when no staining was registered. HER2 score of 0 and 1+ was considered negative, whereas HER2 score of 3+ was defined positive. Tumors scored as 2+ were considered equivocal and HER2 status was then
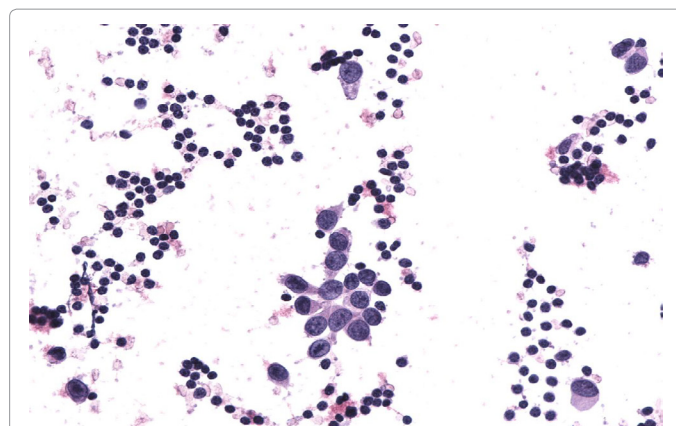

Figure 1: Touch imprint citology, x40 hematoxylin-eosin stain, of axillary lymph-node: single and clustered carcinomatous elements with glandular morphology on a background of lymphocytic elements.

determined using fluorescence *in situ* hybridization. Ki-67 values were measured as the percentage of positively stained malignant cells among the total number of tumour cells assessed. A Ki-67 cut-off point of 20% was defined to separate low from high proliferation grade. [15]. Tumors were classified according to the St. Gallen in 2013 guidelines [16].

Statistical analysis

Statistical analyses were performed using R software (version 3.0.3). Quantitative data are reported as mean ± standard deviation (SD) and median (range). Qualitative variables are expressed as number of patients and percentages. We evaluated the performance of TIC computing sensitivity (SN), specificity (SP), positive predictive value (PPV) and negative predictive value (NPV). Every diagnostic parameter (SN, SP, PPV, NPV) is reported with confidence interval (C.I.) at 95%.

Results

Between January 2015 and December 2016, 343 patients (338 (99%) females and 5 (1%) males) underwent breast surgery and SLN TIC for clinically node-negative invasive breast cancer.

Patient and tumor characteristics

Patient's median age was 66 (33-92 years). As reported in Table 1, most patients had low density breasts at mammograms (65% BiRADS A-B).

Eight patients had bilateral breast cancers and underwent concurrent bilateral surgical procedures. Overall, 249 patients had been submitted to conservative breast surgery, whereas 102 patients underwent mastectomy.

Tumors were more often ductal cancers, smaller than 2 cm and moderately differentiated. The two most frequent molecular subtypes according to St. Gallen classification were Luminal A and Luminal B HER2 Negative (45% and 35%, respectively).

SLN biopsies were negative in 80% of cases, whereas 12% presented macroscopic metastatic involvement and 8% had micrometastases. Axillary dissection was carried out in 44 cases, 75% of which performed during the same operation and 18% of patients requiring a second surgical procedure.

Touch imprint cytology results

In the 24 months considered for analysis, TIC was performed 351 times.

Variables	(N=351) (%)
Age	
Mean ± Standard Deviation	65 ± 12
Median (Range)	66 (33-92)
Side a	
Left	176 (51)
Right	159 (47)
Bilateral	8 (2)
Breast Surgery	
Conservative	249 (71)
Mastectomy	102 (29)
BiRADS a	
A-B	224 (65)
C-D	78 (23)
Not available	41 (12)
Tumor size	
<2cm (pT1)	265 (75)
>2cm (pT2+)	86 (25)
Invasive Cancers Histology Subtype	
Ductal	243 (69)
Lobular	56 (16)
Ductal-Lobular	10 (3)
Others (medullary, tubular, papillary…)	42 (12)
Grading	
G1	37 (10)
G2	221 (63)
G3	86 (25)
Not available	7 (2)
Node Status	
N0	279 (80)
N1mi	30 (8)
N1a-b	35 (10)
N2-3	7 (2)
Lymph Node Surgery	
Only Sentinel procedure	307 (87)
Axillary Dissection	44 (13)
Timing Axillary Dissection b	
In one session	33 (75)
Second surgery (supplementary)	8 (18)
Not available	3 (7)
Proliferative Index Ki67	
Ki-67 <20%	205 (58)
Ki-67 ≥ 20%	146 (42)
St. Gallen Molecular Subtypes	
Luminal A	157 (45)
Luminal B HER2 Negative	122 (35)
Luminal B HER2 Positive	17 (5)
HER2 Positive	9 (2)
Triple Negative	34 (10)
Not available	12 (3)

a = Patients (N=343)
b = Axillary dissection (N=44)

Table 1: Patients' and tumor's characteristics.

Touch imprint cytology correctly predicted the status of 315 SLN (90% accuracy; 276 true negative and 39 true positive).

The sensitivity of TIC in detecting breast cancer SLN metastases was 54% (95% C.I. 39% to 69%), whereas its specificity was 99% (95% C.I. 98% to 100%).

Positive and negative predictive values of TIC were 93% (95% C.I. 85% to 100%) and 89% (95% C.I. 86% to 93%), respectively.

Axillary dissection was required 6 times after primary surgery for false negative results, determining a reintervention rate of 2%. False negative TIC was found in other 27 patients, but they did not undergo axillary dissection because 23 cases presented with micrometastases and 4 cases were classified as N1a (sn) without lymph node capsule invasion.

On the contrary, 2 patients underwent unnecessary axillary dissection for false positive results. An axillary dissection was carried out also in 5 patients for positive TIC, which resulted in nodal micrometastases at definitive histopathologic examination.

The median time required by the pathologist to receive the specimen, perform the analysis and communicate the result to the surgeon was 20 (15-45) min.

The overall cost, including materials and resources, was about 20.50€ per each TIC analysis.

Discussion

Touch imprint cytology (TIC), frozen section (FS) analysis and one-step nucleic-acid amplification (OSNA) represent the most common techniques available to intraoperatively detect breast cancer axillary sentinel lymph node (SLN) metastasis [8,9]. However, recent studies have stated that TIC is not anymore, a useful technique to detect SLN metastasis in breast cancer [17,18].

In this study, we were able to correctly predict the status of 315 SLN out of 351 retrieved, obtaining an accuracy for the TIC technique of 90%. Although not so impressive, this result is comparable to others reported in literature [18].

Many studies have already shown that FS does not allow for accurate diagnosis in all cases, since its high specificity does not associate with high sensitivity [19,20]. OSNA is a relatively recent technique that detects mRNA expression of epithelial breast cancer marker cytokeratine 19 (CK19) in the biopsied lymph node. Amplification detectors have shown to own very high sensitivity, especially in finding micrometastasis and isolated tumor cells (ITC), but on the other hand are burdened by long preparing time (30-40 min on average) [20] and higher costs [21].

To date, indication to axillary lymph node dissection has been challenged by various studies limiting its routine completion. In a meta-analysis performed by Cserni et al. [22], the authors stated that the risk of non-SLN metastasis with a low-volume metastasis (i.e., micrometastasis and/or isolated tumor cells) is around 10% to 15%, depending on the method of detection of SLN involvement. According to Swenson et al. [23], SLN biopsy proved to be a safe and less invasive procedure than axillary dissection with an overall disease recurrence rate of 2.5% and an isolated node recurrence rate of 0.62%. Moreover, the results of the IBCSG 23-01 randomized controlled trial demonstrated no difference in disease free survival for patients with SLN micrometastasis undergoing either axillary dissection or biopsy alone [24].

Therefore, the abovementioned results have led the 2011 St. Gallen Consensus Conference to affirm that micrometastasis in a single SLN should not represent anymore an indication for axillary lymph node dissection, apart from the type of breast surgery given [25].

At the present time, FS is still a most popular technique for intraoperative SLN assessment [26]. However, the procedure is expensive, labor intensive, time-consuming and operator dependent. Besides, FS determines an irreversible tissue loss due to cryostat

processing, causing a more difficult and inaccurate detection of micro- and sometimes even macrometastatic involvement at routine histopathology [19]. As far as costs are concerned, FS is at least three times more expensive than TIC, considering that the cost of evaluating two SLNs using TIC is 131$ compared to 356$ for FS [27]. At our institution, the estimated cost per each SLN analyzed by TIC is 20.50€, lower than what reported by Kaminski et al. [27].

Molecular testing of SLN could ideally guarantee standardized, objective and rapid evaluation of the biopsied sample. However, the estimated cost of introducing an OSNA system in a hospital facility is 180.000$ per year. In addition, if routine CK19 immunohistochemical staining were to be performed on all SLN biopsies, this would add 50$ to each patient's costs [28].

Studies evaluating the time spent using OSNA system showed that it took about 33-45 min to obtain the results [28]. This timeframe is similar to the one spent to perform FS analysis and TIC [29]. In this study, the median time required to perform a complete TIC analysis was 20 min.

The reported sensitivity for OSNA in literature ranges between 88-90% and its specificity between 90-95%. However, studies comparing OSNA and FS or TIC demonstrated that the sensitivity in assessing macrometastases remained similar [30,31]. For this reason, since micrometastatic nodal involvement is not anymore an absolute indication for axillary dissection [25], the enthusiasm of the scientific community for the higher sensitivity of OSNA downscaled.

In our opinion, all these factors suggest a reconsideration of the routine use of TIC, which is a simple and fast method to set up a cytology specimen for examination. In addition, sectioning bigger SLN at 2-3 mm intervals, rather than simply bisecting them, showed to increase sensitivity without excessively extending the length of the examination [32]. In literature, TIC specificity reached 100% in most of cases, whereas its sensitivity ranges between 69% to 99%. What lowers TIC sensitivity is the detection of micrometastases, which go unnoticed in 84% of cases [17]. This means that false positive results, leading to unnecessary axillary dissection, are quite uncommon, whereas false negative rates are extremely variable, involving mostly micrometastasis. On the other hand, TIC is very accurate when only macrometastases are taken into consideration [32].

In literature, the major part of the studies evaluating TIC did not state the level of expertise of their pathologists, since it is well known that TIC is an operator-dependent technique. Therefore, its specificity and sensitivity inevitably increase proportionally to pathologist expertise, and vice versa.

In this study, the sensitivity was 54%, whereas the specificity was 99%. These results could be explained considering that, during the timeframe analyzed, TIC was frequently performed by young and less experienced pathologists. However, our results showed a good accuracy with more than acceptable positive and negative predictive values.

The main limits of this study were the small sample size, the short timeframe considered and the lack of dedicated cytopathologists. Conversely, the main strength of this study was the low reintervention rate registered. This could be explained by the fact that micrometastases were considered as positive nodal disease, although current guidelines are challenging this concept.

Further studies are required to evaluate the burden of micrometastases on TIC analysis and efforts should be directed into detecting potential risk factors influencing false positive and false negative rates.

Conclusion

In this study, TIC sensitivity was 54%, whereas its specificity was 99%. Although our sensitivity was low, the good specificity and the low rates of both reintervention and unnecessary axillary dissection make TIC a fast, cost-effective and reliable technique to detect lymph node metastasis intraoperatively.

Ethical Approval

All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For this type of study formal consent by the institutional research committee is not required in Italy.

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