

Sentinel lymph node biopsy with intraoperative touch imprint cytology (TIC) in breast cancer: experience of a mild-volume center

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SUMMARY: Sentinel lymph node biopsy with intraoperative touch imprint cytology (TIC) in breast cancer: experience of a mild-volume center.

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Although considered the gold standard in treatment of EBC, sentinel node biopsy still remains a debated issue. What to do in case of positive sentinel node and the need of intraoperative histological examination are the most topics under discussion. In this study we have retrospectively evaluate our case series of 359 sentinel node biopsy in the managing of breast cancer from January 2011 to December 2018, focusing on the TIC technique for performing intra-

operative examination. It results in 12,8% "FALSE NEGATIVE" rate, in which only 4,2% in macrometastases, with an overall sensitivity of 68,4% (macrometastases: 86%; micrometastases: 11%), overall specificity of 98,7% and an overall accuracy of 89,7%. The intraoperative examination of SLN allows to reduce delayed surgery procedures and greater therapeutic safety in case of mastectomy. The TIC method can be considered valid, simple and rapid in identifying macrometastases, also allowing to avoid under-staging. The low sensitivity for micrometastases is not a limit, considering that recent evidence has drastically reduced the indications for ALND in these cases. Further ongoing trials and the possible validation of NOMOGRAMMS and SCORE are necessary to identify low risk cases in which to definitively omit the ALND and/or even the SLNB itself.

KEY WORDS: Imprint cytology - Sentinel node biopsy - Breast cancer - Micrometastases.

Introduction

Sentinel Lymph Node Biopsy (SLNB), although today considered the Gold Standard in the treatment of EBC, remains a much-debated issue. Despite over the years most of the taboos have fallen (multicentricity, clinical stage, neoadjuvant CHT) (1-3), the topics still under discussion mainly concern what to do in case of positive LS and the need to carry out intraoperative histological examination (4-7). Moreover, some ongoing trials are evaluating the real need to "test the axilla" in selected cases

(SOUND, BOOG 2013-08) (8, 9). The aim of this study, in the light of these considerations, was to retrospectively evaluate the case report of a "medium volume centre", focusing on the method for performing intraoperative histological examination.

Patients and methods

From January 2011 to December 2018 451 patients, aged between 29-89 years, were treated for breast cancer. In 366 of them (81.1%) a SLNB was performed. In 7 cases (1.9%) sentinel node (SLN) was not detected, then a complete axillary lymph node dissection (ALND) was performed. The remaining 85 ALNDs (18.8%) were performed because of preoperative clinical suspicion of lymph node involvement (obtained by eco-guided FNAB, breast

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MRI and/or PET-TAC) or in the case of neoadjuvant CHT. The histological characteristics of the 359 BLS are reported in Table 1. The SN were analysed intra-operatively with the technique of “touch imprint cytology” (TIC): the lymph node is divided into two halves along its major axis, each is swiped on a slide, stained with Haematoxylin/Eosin (H/E) and then examined under a microscope. The lymph node is considered positive in the presence of epithelial cells with cytological atypia (10); in this case an ALND is performed. The two halves are then processed for final examination, cut and stained with H/E. In case of diagnostic doubt at the final stage, the procedure is carried out with immunohistochemical examination (IHC) for cytokeratin (CK). The lymph node is defined as “micro metastatic” when the tumour involvement is between 0.2 and 2 mm; “macro metastatic” if > 2 mm. In case of a positive response to the final examination, ALND is performed. From 2013 in the case of micro metastasis at TIC the decision whether to perform an ALND was postponed to the final histological examination, omitting ALND in case of micro-metastases respecting the evidence from IBCSG 23-10 Trial (4).

Results

Positive SLN was found in 79 of 359 SNLBs (22%) analysed with TIC, while negative in the remaining 280 cases (78.0%). Of the 79 positive cases,

TABLE 1 - PATHOLOGICAL CHARACTERISTICS OF THE 359 SLNB.

HISTOLOGIC SUBTYPES	N	%
DUCTAL	300	83,6
LOBULAR	37	10,3
MIXED (L/D)	9	2,45
MUCINOUS	3	0,81
HISTIOCYTOID	1	0,27
PAGET	1	0,27
HIGH GRADE DCIS	6	1,63
LCIS	2	0,54

5 were identified as micro metastatic. In particular, the 2 BLS diagnosed as TIC micro metastases, were then found to be macro metastases at definitive evaluation; in one of these, no subsequent ALND was performed for advanced age (81 aa); the other, instead, (prior to 2013) was treated with direct ALND and was found to be macro metastatic with 9 positive lymph nodes (N2+) at final evaluation; histologically it was a ductal type ER and PGR +. 1 case diagnosed as macro metastasis at TIC and directly treated with ALND, was found to be micro metastasis at final examination (FALSE POSITIVE). The other 3 micro metastases diagnosed with TIC and confirmed at definitive histological examination, did not undergo axillary clearing. Definitely, of the 79 cases identified at TIC as “Positive” (both macro metastasis and micro metastasis), 1 case can be defined as true “FALSE POSITIVE” (1.26%). Of the 280 cases defined as negative at TIC, 36 were positive in final examination, with a “FALSE NEGATIVE” rate of 12.8% (36/280). Of these, however, only 12 (4.2%) were macro metastases and were then treated with ALND, while 24 (8.4%) were instead micro metastases, 2 of which treated with completion lymph node dissection because of prior to 2013. Tables 2 and 3 show statistical data on diagnostic accuracy of the TIC method, differentiating sensitivity in identifying micro and macro metastases.

TABLE 2 - FORMULAS OF STATISTICAL PARAMETERS (TP: TRUE POSITIVE; TN: TRUE NEGATIVE; FP: FALSE POSITIVE; FN: FALSE NEGATIVE).

Sensitivity = TP/(TP + FN)
Specificity = TN/(TN + FP)
Overall Accuracy = (TP + TN) / (TP+FP+TN+FN)

TABLE 3 - STATISTICAL DATA OF TIC.

OVERALL SENSITIVITY	68,4 %
MACROMETASTASES	86%
MICROMETASTASES	11%
OVERALL SPECIFICITY	98,7%
OVERALL ACCURACY	89,7%

The average number of sentinel nodes was 2.3 (range 1-16). The extemporaneous TIC examination, however, was performed only on 1 or at most 2 lymph nodes that had shown a higher value at the detection with the Probe and/or coloured blue, in cases where the double tracer was used (radioactive + Methylene Blue). The other non-Sentinel lymph nodes (nSLN) collected during the BLS was assessed at final histologic examination. Of the negative ones, no positive nSLN were found in any case. In patients undergone ALND, 79 with positive TIC and 14 after the final examination, an average of 13.9 lymph nodes was removed (range 3-36). Finally, out of a total of 93 patients who underwent post-BLS ALND (immediate or delayed), 13/93 (13.9%) had metastatic lymph nodes >4 and then an additional adjuvant RT was performed (Table 4).

Discussion

SLNB is actually considered the method of choice for axilla staging in EBCs with clinically negative axilla. However, the strategy in case of positive SLN still remains controversial. The two most important randomized trials of the last 10 years (IBCSG 23-10 and ACOSOG Z0011) (4-6) have once again revolutionized the approach to the axilla with the aim of reducing as much as possible the number of patients candidates to ALND and the consequent risk of complications. While the IBCSG 23-10 study has been well received and it is now universally accepted to avoid ALND in cases with 1 or more micrometastatic SLNs regardless of “conditioning” factors (such as histological, BCS/mastectomy, etc.), the trial ACOSOG Z0011 still raises considerable controversy. This trial, in fact, has actually studied

patients with good prognostic factors (only BCS subsequently submitted to adjuvant RT, T1-2 tumours but $cT1>70\%$, ER and PGR+ in 83% of cases, low number of lobular types). Another limit is linked to the number of LS to be removed, which must always be >2 ; finally, this trial was closed early because of difficulties in recruiting patients: only 40% of patients were enrolled compared to the initial statistical design provided by the study.

Moreover, a survey published after the Z0011 trial evaluated the impact in clinical practice of the application of this trial in the USA (11). Table 5 summarises the results for which cases ALND was predominantly used in positive SLNB. However, one thing remains common to all the characteristics: a strong reduction in the use of intraoperative SLN examination.

Lombardi et al., on the other hand, in a case study of 1226 BLS reviewed retrospectively, verifying the results of the cases who met the Z0011 trial criteria, showed that intraoperative SLN examination still plays a decisive role in the choice of surgery because of not performing ALND in the case of positive SLN exposes to a risk of under-staging of 17%, as well as avoiding the risk and discomfort of delayed axillary surgery (12).

Our study, for homogeneity of clinical cases, rate of positive SLN at intraoperative and final examination, false negative rate at TIC technique, was in line with other international case studies. In particular, a low sensitivity in identifying “micrometastases” was shown, due to the limits of the TIC method in general, but also to the non-use of IHC analysis during the intraoperative examination. Despite this, however, this low sensitivity in detecting “micrometastases” (11%) is similar to other in which a TIC method is used (10, 14, 15).

TABLE 4 - LYMPH NODE ASSESSMENT ON ALND AFTER SLNB.

ALND	N° of lymph node removed	cases with N+>3
<i>Immediate: 79</i>		
<i>Delayed: 14</i>		
TOTAL: 93	13,9 (range 3-36)	13 (13,9%)

TABLE 5 - RESULTS OF POST-Z0011 SURVEY IN USA (CAUDLE AS, HUNT KK, TUCKER SL, HOFFMAN K, GAINER SM, LUCCI A, ET AL. AMERICAN COLLEGE OF SURGEONS ONCOLOGY GROUP (ACOSOG) Z0011: IMPACT ON SURGEON PRACTICE PATTERNS. ANN SURG ONCOL. 2012 OCT;19(10):3144E51) (11).

<i>ALND ONLY IN:</i>	
-	T > 2 cm
-	LOBULAR HISTOLOGY
-	FEWER SLNs
-	LARGER SLN METASTASIS SIZE
-	EXTRANODAL EXTENSION
-	HIGHER PROBABILITY OF POSITIVE non-SLNs

Petursson HI, et al. in a cohort of 1227 breast cancer patients evaluated the sensitivity of the TIC method, stratifying cases according to histological characteristics. The overall sensitivity of the method in identifying micrometastases was 29.1%, with a slight reduction in lobular tumours; however, the technique involved the intraoperative use of CK-IHC (10).

Regarding, instead, the ability to identify “macrometastases”, in our case study the sensitivity was 86%, significantly higher than that published by Petursson HI (78.6%) (10) and that published by Cox, in which the sensitivity for macrometastases, in a case study of 2137 cases of EBC (T1-2) was 69.3% (14). This difference is probably due to a higher percentage observed in our case study of cases with T>2cm (54/93; 58%), compared to Petursson (502/1227; 41%) and Cox, in which out of a total of 2137 EBC T1-2 only 533 were T2 (25%). A meta-analysis, published in 2005 and including 31 studies on the TIC method, showed an overall sensitivity of 63%, with a specific sensitivity of 81% for macrometastases and 22% for micrometastases (15).

From data of the literature, both in these and other cases in which intraoperative examination is performed with the Frozen Section (F/S) technique (8), there is relative evidence of greater difficulty in identifying intraoperatively a SLN metastasis in LOBULAR histotypes; even in our case study there is a statistically significant evidence that the rate of FALSE NEGATIVE in lobular histotypes (8.3%) is superior to that of TRUE POSITIVE (3,84%) with $p < 0,01$; for cases with ductal histology it was respectively 88% and 88,4% (Table 6).

Another consideration is the ability of intraoperative SLN examination to avoid a reintervention. In our case study, the overall accuracy of the TIC method was 89.7%, with a sensitivity in “macrometastases” of 86%. The reduced sensitivity for “micrometastases” (Table 3), on the other hand, does not have a particular meaning today, considering the evidence of recent years, in particular the IBCSG 23-01 Trial (4). In such cases, in fact, the probability that there are more than 3 metastatic LNs is very low. In our case study, the additional ALND performed immediately or after the final examination of the SLN showed 13.9% of cases with $N_{+>3}$ (Table 4), therefore susceptible to further adjuvant RT as widely indicated by clinical trials (ETCCTCG) (17, 18). Lombardi et al., moreover, have shown in a larger case study (1226 cases) that 17% of patients with intraoperative positive SLN had then >3 LNs positive after ALND and therefore submitted to further adjuvant RT (12).

Conclusions

Numerous Trials performed all over the world and international guidelines indicates SLNB as method of choice for axilla staging in EBCs The intraoperative examination of SLN allows to reduce delayed surgery

TABLE 6 - TP AND FP BASED ON PATHOLOGICAL CHARACTERISTICS IN METASTATIC SLN.

	Total	False negative	True positive	
LOBULAR	37/359 (10,3%)	3/36 (8,3%)	3/78 (3,84%)	($p < 0,01$)
DUCTAL	300/359 (83,6%)	32/36 (88,8%)	69/78 (88,4%)	n.s.

procedures, burdened by high morbidity, as well as allowing greater therapeutic safety in case of mastectomy (12, 13); the intraoperative examination carried out with the TIC method can be considered valid, simple and rapid in identifying macrometastases, also allowing to avoid under-staging (10, 14, 15). The low sensitivity for micrometastases is not a limit, consider-

ing that recent evidence has drastically reduced the indications for ALND in these cases (4, 19). Further ongoing trials (SOUND, SINODAR-ONE, BOOG 2013-08 (8, 9, 16) and the possible validation of NOMOGRAMMS and SCORE are necessary to identify low risk cases in which to definitively omit the ALND and/or even the SLNB itself.

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