Conclusions: Low retention index in LPG means radioisotope accumulation to SN is impaired. Low retention index in n+ suggests that metastatic tumor cells may obstruct lymphatic pathway to SN. The results suggest that retention index in preoperative LPG can be useful factor for predicting sentinel lymph node metastasis.

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One-step nucleic-acid amplification (OSNA) for sentinel node intraoperative diagnosis: advantages from the classical procedures

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Background: Classical protocols for intraoperative diagnosis of sentinel node can be either histopathologic study of one or more sections of frozen tissue with or without inmunohistochemical technics or touch imprint cytology. In case of post-operative study the same histopathologic procedure is followed.

The one-step-nucleic-acid-amplification (OSNA) is a new procedure that detects mRNA of the Cytokeratin 19 and can be used for the intraoperative diagnosis of the sentinel node in breast cancer.

Material and Methods: We compare the results of histopathologic procedure in 478 sentinel nodes of breast cancer from the Ribera hospital with the OSNA results in 177 lymph sentinel nodes from the Lluís Alcanyís hospital.

The histopathlogic procedure consisted in the intraoperative study of pairs of frozen tissue sections, one of them stained with H/E and the other with Cytokeratin AE1/AE3 if the H/E result was negative.

The OSNA procedure consisted in the intraoperative homogenization of whole lymph node in a stabilizing solution of mRNA and next, it's amplification in a RD-100/® equipment.

Results: In the histopathological study, we found 116 metastatic cases (24.2%) where 15.6% were macro-metastasis, 5.8% micro-metastases and 2.7% ITC.

If we consider only the metastastic group, we found 64.6% macro-metastases, 24.1% micro-metastases and 11.2% ITC.

The mean time for the intraoperative procedure was 49min.

In the OSNA one, we found 18.1% metastatic cases, with 5.6% macro-metastases, 9.1% micro-metastases and 3.4% ITC. In the metastatic group, the macro-metastasis percentage was 31.2%, micro-metastatic percentage 50% and ITC 18.7%.

The mean of intraoperative time was 31min.

Conclusions:

1. The OSNA procedure diagnoses more micro-metastases and ITC than the classical histological procedure.
2. OSNA assay saves a mean of 18 minutes in the whole process.

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Predictors of non-sentinel node metastasis in patients with breast cancer after sentinel node micrometastasis

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Background: Sentinel lymph node biopsy (SLNB) is nowadays an overall accepted method for axillary lymph node mapping in patients with invasive breast cancer. The decision whether to proceed in case of negative regional lymph nodes or macrometastasis is clear. However in the case of sentinel lymph node micrometastasis the decision is still a subject of debate.

The aim of this retrospective study is to describe the significant factors correlated with involvement of non-sentinel lymph nodes (NSLN) in patients with sentinel lymph node micrometastasis.

Materials and Methods: We reviewed 226 patients in our institution who underwent a SLNB for invasive breast cancer from January 1999 to October 2009 with micrometastatic involvement in the sentinel lymph node. All patients underwent a completion axillary lymph node dissection (ALND).

Age of the patient, histopathological features of the primary tumor as well as the presence of involvement of non-sentinel lymph nodes were recorded.

Results: 31 cases (13.7%) showed involvement of the NSLN. In an univariate analysis, young age of the patient and grading of the primary tumor were associated with having positive NSLN findings. When all factors are included only grading of the primary tumor was a significant predictor of NSLN metastasis ($p = 0.027$).

Size of the tumor and vascular invasion were not significant associated with positive NSLN findings.

Table. Multivariate analysis[o]: association between lymph-nodes positivity and selected clinicopathological characteristics; ORs and 95% confidence intervals

Variable	Reference cat.	p-value	OR	95% CI
Age (groups)	<35 years	0.207	0.75	0.47–1.18
Er_Pr receptor status	negative	0.126	1.88	0.84–4.24
pT	1a,b,mic	0.291	0.71	0.38–1.34
Grading	1	**0.027**	**2.33**	**1.10–4.97**
Vascular invasion	positive	0.139	1.91	0.81–4.51
ErbB2	0,1,2	0.671	0.76	0.22–2.68
Ki67	⩽30	0.622	0.72	0.19–2.70
Focality	1	0.344	1.32	0.74–2.35
Histotype	1	0.515	1.15	0.76–1.73

Conclusion: Grading of the primary tumor is the most important independent predictor of NSLN metastasis in case of sentinel node micrometastasis. Although there is a trend towards omitting full ALND for sentinel node micrometastasis, in our opinion a full ALND is still recommended in case of high-grade primary breast cancer and certainly in young patients.

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One Step Nucleic Acid Amplification (OSNA) assay for molecular detection of sentinel lymph node metastases in early breast cancer classified according to molecular subtypes: an observational prospective study

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Background: The new molecular diagnostic tool OSNA has recently been validated and adopted in our Institute as routinary intraoperative test for metastases detection in sentinel lymph nodes (SLNs) of breast cancer (BC) patients. The aims of this study in a prospective series of early BC patients, were: (1) to evaluate the feasibility of intra-operative assessment of SLN using the OSNA system in our department; (2) to find out whether the performance of the OSNA method was comparable to post-operative histologic procedures; (3) to investigate the relationship between SLN status determined using OSNA method, and conventional bio-pathological factors taking into account the novel molecular BC classification: luminal A (LA), luminal B (LB), HER2 (HS), and triple-negative (TN); (4) to identify a subgroup of patients with positive SLN with higher risk of non-sentinel lymph nodes (NSLNs) metastatic involvement.

Materials and Methods: A prospective series of 416 consecutive SLNs from 327 BC patients was evaluated. The OSNA assay follows a short sample preparation step and subsequent rapid amplification of cytokeratin 19 (CK19) mRNA based on reverse transcription loop-mediated isothermal amplification. Each SLN was immediately divided into four slices. Two alternate slides were used for the OSNA assay. The remaining two slides were investigated by six-level histology. The results of these two methods were then compared. This series of BC patients were divided into four main subtypes taking in account the novel BC classification based on the immunohistochemistry phenotypic patterns identified by a few protein biomarkers. The relationship between SLN/NSLNs status and the molecular subtypes were analyzed by multiple correspondence analysis (MCA).

Results: The overall concordance of OSNA with histopathology was 95% with a specificity of 95% and sensitivity of 94%. The complex relationships among the bio-pathological variables analyzed by MCA showed that the metastatic involvement of NSLNs is associated with SLNs with a high copy numbers of CK19 mRNA (>5000) and HS subtype tumors.

Conclusions: This molecular assay can raise the standard of care for patient management as its accuracy is similar to that of standard postoperative histology with the advantage of being standardized, objective, and fast enough for intraoperative use. In our series of early BC patients with positive SLN the risk of NSLNs metastases was higher in the group of patients with HS subtype tumor.