SLN positivity. The area under the ROC curve was 0.671 (range 0.629–0.714) as compared to 0.75 in the original population.

Conclusions: In a Dutch polpulation, the MSKCC nomogram to predict metastases of breast cancer in the sentinel node performed reasonably well. The nomogram could be used in high risk patients prone to complications of a SLN procedure.

330 Poster

GeneSearch Breast Lymph Node Assay for the diagnosis of sentinel lymph nodes of breast cancer − CBCSG-001a: China validation study

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Background: With the adoption sentinel lymph node (SLN) biopsy there is an increasing need for its rapid and accurate diagnosis. Intra-operative touch imprint cytology (TIC) and frozen section (FS) offered limited sensitivity. Post-operative histology (H&E) also risks missing metastases due to tissue sampling limitations and pathologist expertise. A real-time RT-PCR assay, GeneSearch™ Breast Lymph Node (BLN) Assay, may offer a standardized intra-operative way to evaluate larger portions of the SLN. CBCSG-001a was a prospective multicenter trial to validate the GeneSearch™ BLN Assay in China.

Methods: From February to June 2009, a total of 546 cases had been enrolled. SLNs were cut into alternating ~2 mm sections. One half of the sections were sampled for FS, TIC, and H&E. The other half was fully tested with the BLN assay after TIC. The assay detected the presence of cytokeratin-19 and mammaglobin to assess if metastases were in SLNs. Predetermined cutoffs were calibrated to detect metastases >0.2 mm only. Each run had internal and external controls to confirm a valid result.

Results: 479 cases with 1046 SLNs were available for analysis. The H&E histological positive rate was 26.7% (128/479) for cases and 17.2% (180/1046) for SLNs, respectively. The BLN assay showed high performance as compared to H&E pathology (Table). The BLN assay was superior to FS and TIC for the diagnosis of SLNs micrometastases, with sensitivity of 57.5%, 44.4%, and 40.0%, respectively.

The Cycle time was negatively correlated to the SLN metastases size and metastases type (negative, ITC, micro- and macro-metastases) both for CK-19 (k = −0.62, −0.73 respectively) and CK-19+MG (k = −0.59, −0.74 respectively).

Conclusions: Our results indicated that the BLN assay had high performance as compared to H&E, and it was superior to FS and TIC for the diagnosis of SLNs micrometastases. The Cycle time was negatively correlated to the SLN metastases size and metastases type. There was clinical potential of using the BLN assay for performance similar to H&E with the advantage of being an intra-operative, objective and standardized test that examined a larger portion on the nodal tissue.

GeneSearch™ BLN Assay: General results

Study	N	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
CBCSG-001a	479	88% (81−93)	93% (90−95)	82% (74−88)	95% (93−97)
US validation study	416	88% (80−93)	94% (91−97)	86% (79−91)	95% (92−97)

331 Poster

Histologic sections, immunohistochemistry and OSNA-CK19 in sentinel lymph node: a comparative study

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Background: Sentinel lymph node (SLN) biopsy is a prognostic procedure employed in breast cancer (BC) management. Although it has been used for many years ago, it remains being a non-standard method.

Many different techniques and protocols have been proposed in search of the most accurate evaluation of SLN.

We have selected a randomized group of nodes in which three different laboratory techniques were performed: HE stain in frozen sections (FS), immunohistochemistry (IHC) and a new molecular test based on a one step nucleic acid amplification (OSNA-CK19). Results have been compared.

Material and Methods: 55 cases of SLN dissection were randomly selected from the files of our hospital, including a 119 nodes altogether.

Fresh tissue was received for intra-operative assay and three parallel sections were performed. Two alternate pieces were reserved for OSNA and the others were cut and stained after being frozen.

OSNA-CK19 test consists in homogenization of tissue and amplification of CK19 cDNA by reverse transcriptase amplification assay. Amplificated product quantity was measured by turbidimetry; depending on the number of mRNA CK19 copies detected, results will be classified as *negative* (⩽250 copies), *micrometastases* (*micro*) (250–5000 copies) and *macrometastases (macro*) (>5000 copies).

Samples used in FS were later fixed and embedded in paraffin. Serial sections were performed at different levels, reserving two alternate sections for IHC study with cytokeratine (AE1AE3). Histopathological findings were classified as *negative*, if no malignant cells were found, *micro* (0.2−2 mm malignant cells clusters), *macro* (⩾2 mm) and *Isolated tumor cells* (ITC) (⩽0.2 mm).

Results: 4 *macro* were detected, 2 by OSNA-CK19, 2 different by IHC and none by FS. From 6 *micro*, 3 were detected by FS, 3 by IHC and all of them by OSNA. ICH assay showed ITC in 6 nodes, and none by the other two methods. OSNA system is not designed to identify ITC, although it could be considered as it those negative cases with 100–250 copies; however this consideration has not been attended in the present study.

Discussion: SLN biopsy is a prognostic method used in the BC management, which permits to avoid unnecessary lymphadenectomies, decreasing the morbidity associated to them.

Although it constitutes a routine method in most pathology labs, it is still a non-standard procedure; different techniques and actuation protocols have been proposed for the evaluation.

We have compared the accuracy of 3 different methods (FZ, IHC and OSNA), concluding that OSNA-CK19 has the highest sensibility for detection of nodal metastases (*macro* and *micro*): 90% OSNA sensibility, 69'2% IHC and 64'2% FS. These differences are more significant when we analyzed *micro* separately, with OSNA sensibility 33'3% higher than IHC and 40% more than FS.

Not significant differences were found between FS and IHC study in the SLN evaluation for *macro* and *micro*. However we have proved that IHC is more accurate than FS to detect ITC, finding without clinical significance.

332 Poster

Validation of nomogram in positive sentinel node

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Background: A positive sentinel node does not always indicate malignat disease beyond. Up to 40% of lymphadenectomies do not show metastases. Many efforts are being made to detect this cases in order to avoid unneccessary surgery to these patiens.

Material and Methods: 137 cases of metastatic sentinel node in breast cancer patients were studied; Three different nomograms were performed: Standford, Tenon, and Memorial Sloan Kettering Cancer Center. Area under the curve (ROC) was studied in order to analyze which was the best for this population.

Results: Medium age was 56.3 (SD = 13.1), Median age was 55 (R = 29−89). Medium tumor size was 19.7 (SD = 7.9) median tumor size was 20 (R = 5−43). ROC curves are expressed in table.

	ROC curve		
	Standford	Tenon	MSKCC
Area under the curve	0.66	0.73	0.71
p	0.002	0.044	0.049

Conclusions: Both Tenon and Memorial Sloan Kettering Cancer Center nomograms were acceptable in our population. Anyway, it is important to individualize patients to be choosen for the best treatment.