Differences between both groups in histologic grade and IMN irradiation have statistical significance, whereas tumor localization, histology, metastatic axillary nodes, adjuvant chemotheraphy or hormonotheraphy, DFS and OS are not statiscally significant. Possible statistically significant differences can be hidden because of a small positive IMN sample.

Conclusions: Metastatic IMN in breast cancer is rare and can be influenced by some factors: breast tumor location, size or histopathological grade. Sampling the IMN can be necessary to obtain complete staging. Without IMN surgical approach, 22% of our patients would have been under-staged and 80% under-treated. IMN affection means a higher breast cancer stage and usually involves an aggressive treatment, what could increase DFS and OS. More studies are needed to increase the number of patients and confirm our data.

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Influence of the type of breast cancer surgery on the upper limb function

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Background: Sentinel lymph node biopsy (SLNB) is widely accepted as an excellent method in the management of early breast cancer in patients with clinically negative axillary lymph nodes. Because SLNB requires less invasive surgery to the axilla than axillary lymph node dissection (ALND), it was assumed to result in reduced shoulder mobility. The aim of this study was to evaluate the relationship between the type of breast cancer surgery and early postoperative arm mobility.

Materials and Methods: The study group consisted of 361 consecutive patients, aged 28 to 86 years (median age 57) with breast cancer, operated upon in the Department of Breast Surgery and evaluated in the Department of Rehabilitation Medicine afterward, between February 2005 and April 2009. Out of the 361 patients, 169 (46.8% − group 1) underwent breast conserving therapy with SLNB, 78 (21.6% − group 2) simple mastectomy with SLNB, 54 (15.0% − group 3) breast conserving therapy with ALND and 60 (16.6% − group 4) modified radical mastectomy. Upper limb function was evaluated one day before surgery and 3 days, 1 month, and 3 months after surgery. In this study assessment consisted of shoulder flexion and shoulder abduction.

Results: After 3 days, a significant impairment of shoulder flexion and abduction was measured and patients in the ALND group (group 3 and 4) had more limitations in shoulder mobility than patients in the SLNB group (group1 and 2) [shoulder flexion: 95.2 ± 32.7 degree (ALND group) vs. 118.9 ± 36.6 degree (SLNB group), $p < 0.001$, shoulder abduction: 87.2 ± 32.4 degree (ALND group) vs. 112.9 ± 42.1 degree (SLNB group), $p < 0.001$]. After 1 month, ALND patients had more limitations in shoulder mobility than SLNB patients [shoulder flexion: 142.2 ± 24.6 degree (ALND group) vs. 161.8 ± 21.3 degree (SLNB group), $p < 0.001$, shoulder abduction: 136.6 ± 35.3 degree (ALND group) vs. 159.4 ± 30.2 degree (SLNB group), $p < 0.001$]. After 3 months, shoulder mobility was not different between the ALND group (n = 44) and the SLNB group (n = 84).

Conclusions: In terms of upper limb functional status, the benefits of SLNB over ALND was observed at the early postoperative time. However, long-term effects have to be confirmed by further, larger series.

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Preliminary experiences of sentinel lymph node biopsy for early breast cancer by a new camera system simultaneously capturing color and near-infrared fluorescence

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Background: Sentinel lymph node biopsy (SLNB) for early breast cancer has already become a standard of care, alternating axillary lymph nodes dissection. The most common methods for identifying sentinel lymph node (SLN) are a blue dye and/or radioisotope (RI) method. However, a dye method requires special surgical training to maintain high identification rate of SLN and an RI method requires a radiation control area. A new method, based on the near-infrared (NIR) fluorescence of indocyanine green (ICG) dye, might be considered to be a new alternative to resolve these problems of conventional dye and RI methods. By providing direct visual images of lymphatic flow over skin, it makes a dye method easier and more accurate. We developed a new camera system for simultaneous capturing of color and NIR fluorescence to visualize ICG-enhanced structures against a background of vivid tissue color. We named our new camera system Hyper Eye Medical System (HEMS).

Materials and Methods: A combination of custom-made optical filters for attenuation of visible light and enhancement of NIR fluorescence was mounted on an ultra-high sensitive color CCD image sensor. A light source

for excitation of ICG dye was made with an array of light emitting diodes (LED) at 780 nm.

Between April 2007 and August 2009, we performed SLNB in 113 patients with histologically confirmed breast cancer, tumor size ⩽3 cm and clinically node negative, using HEMS. After ICG dye (1.25 mg/body) was subdermally injected in the subareolar site, the subcutaneous lymphatic drainage pathway was observed on the color monitor and the site of skin incision was decided. Simultaneously, usual combination methods of indigo carmine dye and RI with 99m-Tc Sn colloid were performed in all patients.

Results: In all of 113 patients, SLNs were identified by NIR fluorescence. This result was equal to those of simultaneously performed dye and RI methods. Moreover, the images, acquired using HMES, can be used as an intra-operative navigator.

Conclusion: Fluorescence method using HMES, which can simultaneously capture color and NIR fluorescence, is expected to be a new alternative to conventional dye and/or RI methods.

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Blue dye alone in sentinel node biopsy is a safe and accurate technique in selected early breast cancer − a single institution experience

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Aim: To assess the safety and efficacy of sentinel node biopsy in selected cases of early breast cancer in a single institution using the blue dye method only.

Materials and Methods: Histologically proven invasive breast cancer with an a priori predictive lymph node positivity of 20% were offered sentinel lymph node biopsy (SLNB) using a blue dye only method. 220 consecutive patients from April 2002 till April 2007 with early breast cancer who fit the criteria were prospectively entered into this study. SLNB using blue dye alone was performed in all the patients. Those with any invasive cancer in the SLNB proceeded to have an Axillary Lymph Node Dissection (ALND). This includes those positive on frozen section as well as on definitive histology, including immunohistochemistry. Those in which no SLNB were found proceeded to have an ALND.

Results: Most studies utilizing a dual method of SLNB report axillary recurrence after negative SLNB at 1% or less after a median follow up of 2−3years. With a conservative estimate of recurrence in this study of 4%, with an a=0.05 and b=0.9, the sample size necessary would be 154. We thus anticipate that 200 patients would be needed for a difference in the blue dye alone SLNB compared to the dual method, if any, is to be apparent.

In our experience, we had an identification rate of 94% (207/220). Of those identified, 21% (45/207) was positive for invasive cancer on frozen section. A further 8 SLND were positive only on final histology. With a median follow up of 49.5 months (range 7−74 months), there were no axillary recurrences and 2 patients with breast only local recurrence. 1 patient subsequently developed metastatic bone disease 49 months after initial treatment and is still alive. One patient had a subsequent diagnosis of leukemia and died due to leukemia related causes.

Conclusion: Blue dye only technique is safe, and in experienced hands has comparable results to published combined techniques.

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Validation of the MSKCC nomogram to predict sentinel lymph node metastases in a Dutch breast cancer population

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Background: The Memorial Sloan-Kettering Cancer Center (MSKCC) developed a nomogram to predict the likelihood of sentinel lymph node (SLN) metastases in breast cancer patients. The nomogram was validated on a Dutch population.

Methods: Data of 716 breast cancer patients who underwent a successful SLN biopsy were collected. Positive SLN metastases were found in 204 patients. Frozen section, serial haematoxylin and eosin (HE) and immunohistochemistry (IHC) were performed routinely on each sentinel node. A ROC curve was drawn and the area under the curve was calculated to assess the discriminative power of the nomogram.

Results: Tumour size (p = 0.000), multifocality (p = 0.038) and lymphovascular invasion (p = 0.000) were identified as independent predictors of

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.

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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[TM] 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[TM] 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[TM] 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.