

STUDY ABOUT (PRESENCE OF METASTASIS) IN SENTINEL LYMPH NODE BY ONE-STEP NUCLEIC ACID AMPLIFICATION (OSNA) ASSAY

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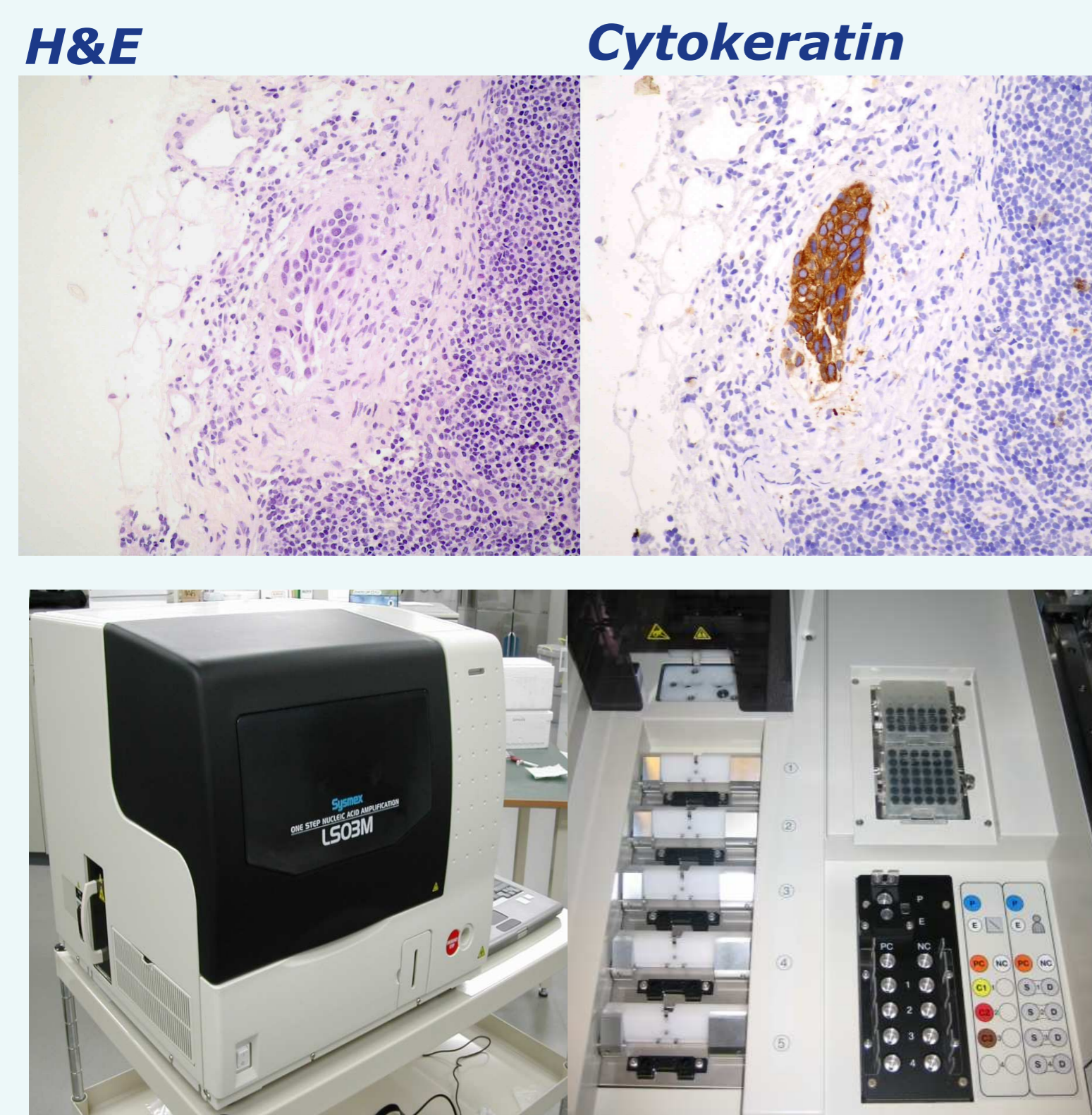


Background

- * Sentinel lymph node biopsy has largely replaced axillary lymph node dissection as the standard method of staging of breast cancer patients.
- * The conventional study of the sentinel lymph node (SLN) is performed by frozen sections or cytology (touch-imprints), followed by a subsequent study (H&E and IHC for cytokeratins).
- * Preoperative sensitivity is low (false negatives between 5%-50%), with consequent reintervention of patients for axillary dissection.
- * In the OSNA (one step nucleic acid amplification) method the whole node is analysed, and the results for the detection of metastasis and size are obtained between 30-40minutes.
- * The OSNA method is based on homogenisation of lymph node followed by real-time amplification and quantification of cytokeratin 19 (CK19).

Material and methods

- Comparative study of the presence of metastasis in SLNs was performed by frozen section (64 patients, 131 lymph nodes) and OSNA assay (57 patients, 114 lymph nodes) for a period of 6 months each at the Hospital de Navarra.
- The conventional study after the preoperative study was performed in 4 sections stained with H&E and 10 sections stained with keratin AE1-AE3.



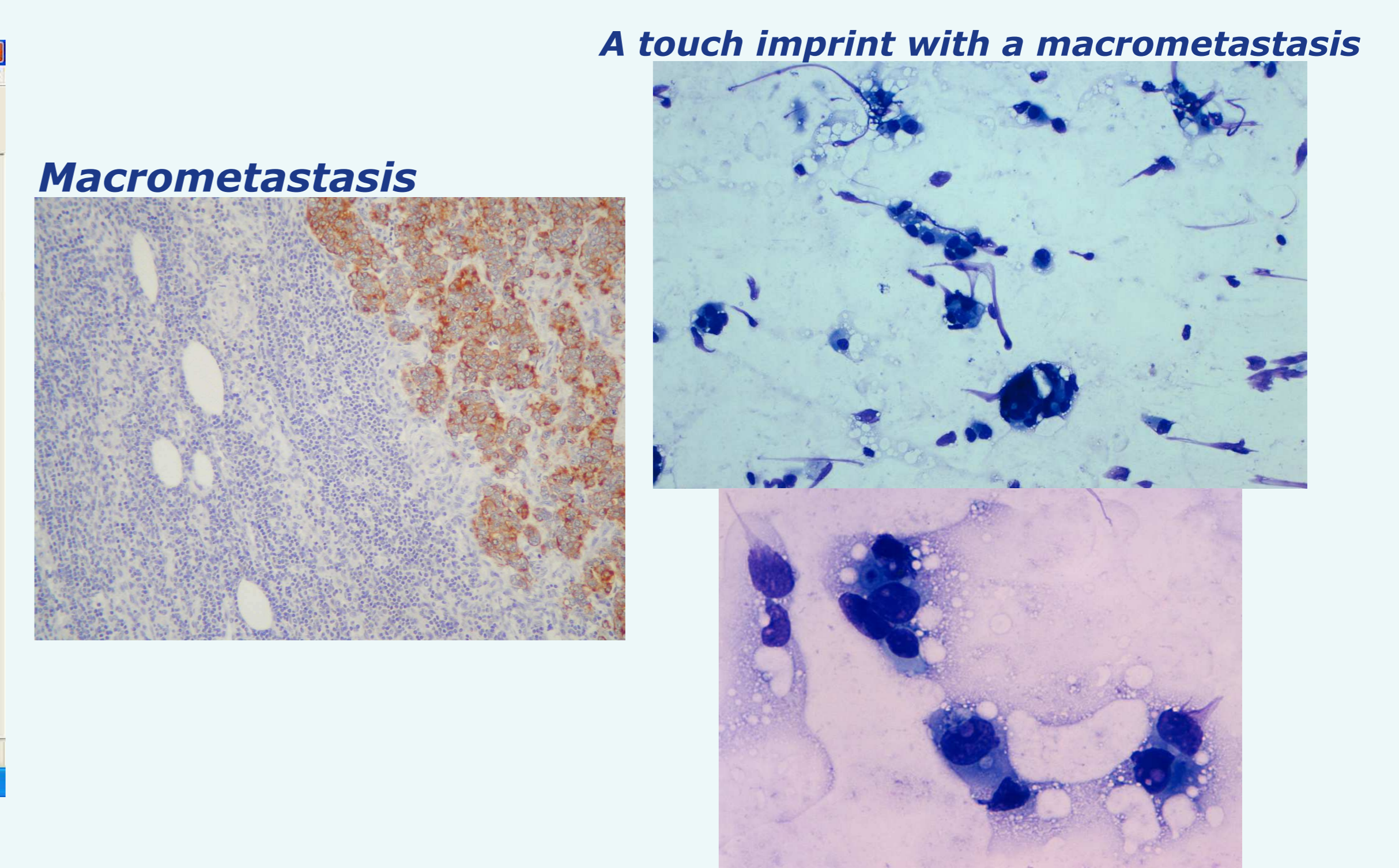
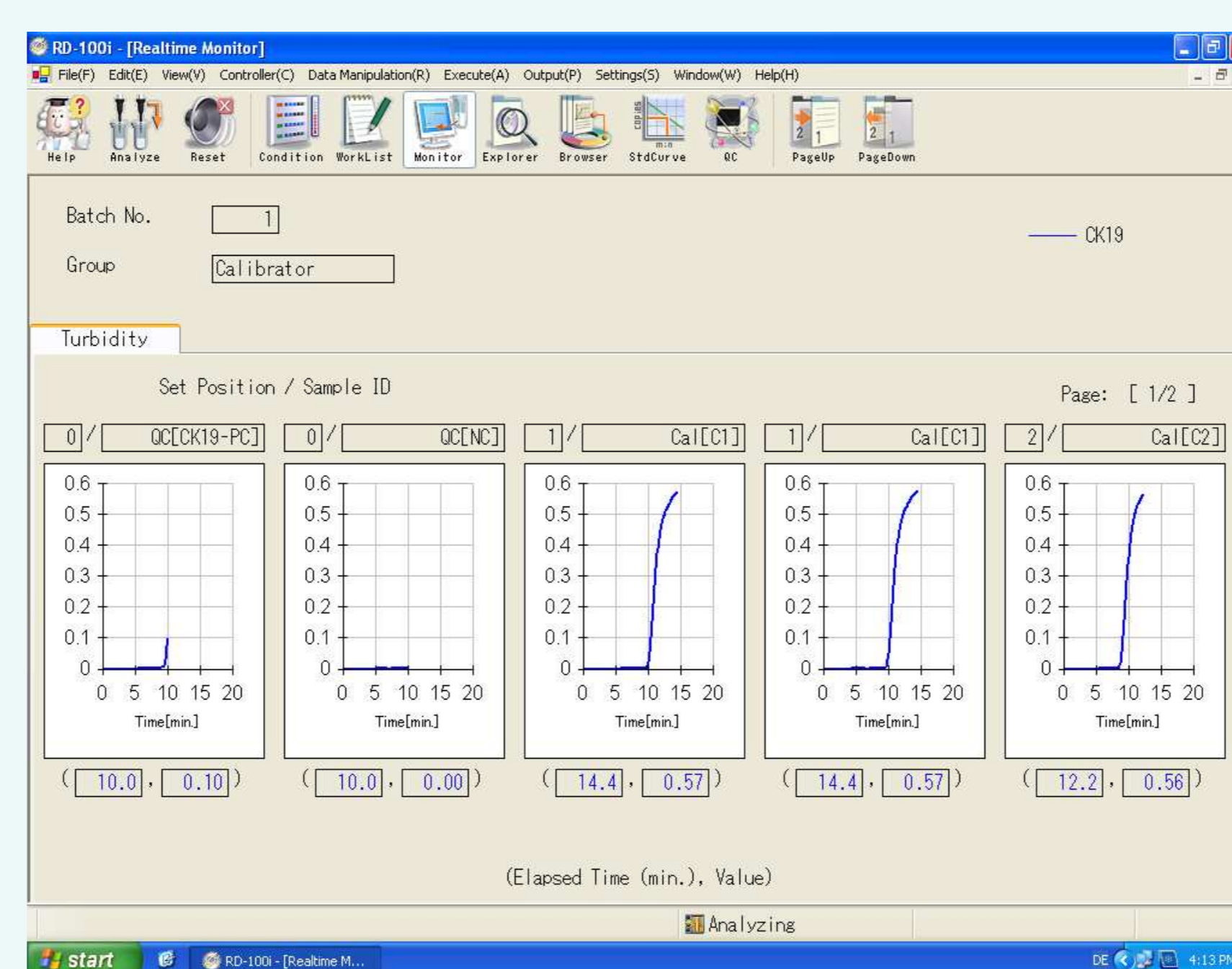
In the OSNA method:

- A touch imprint is obtained from the lymph node.
- The lymph node is homogenized with 4 ml of a lysis buffer solution and centrifuged.
- Two microliters of the supernatant are used for automated amplification of CK19 mRNA via reverse transcription loop-mediated isothermal amplification.
- The degree of amplification is detected via a by-product of reaction, pyrophosphate.
- The resulting change in turbidity is correlated to CK19mRNA copy number/ μ l.
- A standard positive control sample and a negative control sample are used for calibration in every assay.
- The results of the assay are expressed as the number of CK19 nRNA copies/ μ l.
 - The lymph node is classified as negative when the result is $< 2,5 \times 10^2$ copies.
 - The lymph node is classified as positive when the result is $> \text{ or } = 2,5 \times 10^2$ copies.

Results

- We found 11 patients (17%) with metastases in the SLNs analysed by the conventional method, and 4 out of these were found in preoperative (6.25%) by frozen section and 7 (10.9%) in the subsequent conventional study (H&E, CK). Hence, these 7 patients were false-negative cases.
 - Among the cases detected in the subsequent study 4 were micrometastasis and 3 were macrometastasis.
- The OSNA assay detected 17 (29.8%) metastasis.
 - The distribution according to the size of the metastasis of these metastasis was 9 micrometastasis and 8 macrometastasis.

Sample	Result
$5000 \leq \text{copies}$	(++) macrometastasis
$250 \leq \text{copies} < 5000$	(+) micrometastasis
$100 \leq \text{copies} < 250$	(-) L Isolated tumor cells (ITC)
Copies < 100	(-) Negative



Conclusions

- OSNA assay has a great sensitivity of detection (95%, Tamaki et al, 2009) solving some problems encountered with other molecular methods in the study of SLN.
- This method is conducted in a short period of time (30-40 minutes) and provides a quantitative result of the size of the metastasis (macro, micro and ITC).
- Regarding the conventional histological study consisting of frozen section and postoperative histological examination has resulted in a percent of 10.9% of false-negative cases.
- Conversely, the OSNA method is based on the complete use of the lymph node favouring the detection of occult metastasis not detected by other methods.
- OSNA provides a definitive result during per-operative management.
- The use of OSNA may spare a patient from the complications of a second surgical intervention.
- Therefore, the reintervention for axillary dissection in false negative cases is clearly avoided.

References

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