of lymph node metastasis. We analyzed prognostic factors in each group such as age, histologic grade, nuclear grade, lymphovascular invasion (LVI), estrogen and progesterone receptor status, HER-2/neu expression, Ki67-labelling index, bcl-2 expression, extensive ductal component (EIC), DCIS, and comedonecrosis.

Results: The node negative (T1N0) group included 157 cases and the remaining 73 cases were allocated to the node positive (T1N1−3) group. In the univariate analysis, lymphovascular invasion ($p = 0.000$), histologic grade ($p = 0.012$), HER-2/neu ($p = 0.012$) and bcl-2 ($p = 0.025$) were the statistically meaningful prognostic factors that were related to the node metastasis in T1 breast cancer. But in the multivariate analysis, LVI ($p = 0.000$), bcl-2 ($p = 0.048$), and HER-2/neu ($p = 0.031$) were statistically significant factors related to the node metastasis in T1 breast cancer.

Conclusions: The presence of LVI, increased bcl-2 expression, and HER-2/neu overexpression were related to the increased incidence of ALNM in T1 breast cancer. LVI was the most predictable factor of ALNM.

394 Poster

Is there any negative impact on histologic assessment of breast masses and sentinel nodes marked with blue dye?

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Introduction: Blue dye is widely used in breast surgery nowadays. In sentinel node biopsy, combined method with radiolabeled material and patent blue dye is the most accepted method.

Methylene blue dye is also used as a safe and cost effective martial for marking non palpable breast masses before surgery and it's use in sentinel node biopsy has been reported to be effective and accurate for sentinel node identification in some studies. But there is debate about possible adverse effect of blue dye on histology and immunohistochemistry evaluation in tissues that are colored with blue dye. So we studied this effect in non palpable breast masses that were marked with methylene blue dye before surgery and sentinel nodes that were detected by blue dye or combination method in our center.

Materials and Method: Pathology slides of 56 masses from 49 patients that methylene blue dye was used as marking method before surgery for them were considered for effect of methylene blue dye on permanent pathology of breast masses and 28 sentinel nodes that were assessed by frozen section were considered for effect of patent blue dye on frozen section assessment.

Two pathologists reviewed slides separately and reported if there was any adverse effect on slide that interfered with assessment. They also reviewed Imunohistochemistry samples and reported probable difficulties.

Results: From 56 masses that were assessed, 4 of masses were malignant one of them insitue ductal carcinoma, 3 atypical ductal hyperplasia, 2 sclerosing adenosis, 10 fibrocystic change, 25 fibroadenomas (3 of them mied type and one with phyloid features), 2 tubular adenomas, one epithelialized liomyoma, 2 intraductal papillomas, one foreign body granuloma, 2 tubular adenomas and 5 epithelial hyperplasia without atypia. Both pathologists did not find any adverse effect due to blue dye in histologic assessment of breast tissue or mass in these 56 excisional biopsies.

From 28 lymph nodes that were sent as sentinel node biopsy, 12 were positive for tumoral involvement in frozen section that 2 of them were micro metastasis. All of these lymph nodes were proved to be metastatic in permanent section. In one case that the frozen section did not found any metastatic tumoral cells in lymph node, tumoral cells were found in permanent section and it was not due to dye interference but because of size of tumor nest that was small.

Conclusion: Injection of blue dye (patent blue or methylene blue) do not have adverse effect on pathology and immunohistochemistry assessment and it can be used for marking non-palpable breast masses and also sentinel node biopsy in breast cancer patients even when frozen section is going to be done for them.

Thursday, 25 March 2010 18:15–19:15

POSTER SESSION

Pathology and biological markers in breast cancer

395 Poster discussion

Valid PCR quantification of mRNA from 16 year old formalin-fixed, paraffin-embedded breast cancer tissue: a methodological study comparing manually trimmed sections and whole tissue sections

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Background: Archival formalin-fixed, paraffin-embedded tissue (FFPE) constitutes a biobank of tumors of all sizes, often linked to clinical studies of great statistical power and long follow-up time. Gene expression analysis on RNA from FFPE has been considered impractical due to the extracted mRNA being fragmented and chemically modified. Furthermore, the technique has been time consuming and characterized by a low grade of automation.

In addition, FFPE often contains an admixture of normal tissue, premalignant changes and invasive cancer. This has called into question the specificity and interpretation of results from analysis of the total amount of collected RNA.

Material and Methods: Two FFPE blocks from each of 21 breast carcinomas, diagnosed 15−17 year ago, were chosen. From each block a whole slide section and a manually trimmed, tumor enriched section (discarding surrounding non-invasive tissue) were prepared. mRNA was isolated with a silica bead-based, fully automated technique developed by Siemens (Siemens Healthcare Diagnostics, Deerfield, IL; not commercially available) including an integrated xylene/ethanol-free deparaffinization step. Tumor content defined as invasive carcinoma with interposed stroma was estimated stereologically from Hematoxylin-Eosin stains. Eluates were analyzed with kinetic RT-PCR for 1 housekeeping gene RPL37A and 3 target genes (ESR1, PGR and HER2). Raw data (C_T values) for target genes were normalized to RPL37A, and relative expression levels calculated and compared to immunohistochemical data.

Results: RNA was successfully extracted from all sections, and gene expression reliably quantified for the three target genes. Agreement between whole slide and trimmed sections were optimal, indicating that expression levels for ESR1, PGR and HER2 are not strongly influenced by contamination from surrounding tissue. Concordance between RNA- and protein expression was excellent for ESR1 and HER2, making it possible to define RNA tresholds, distinguishing between positive and negative samples.

Conclusions:

- Isolation and quantification of ESR1, PGR and HER2 mRNA from >15-year-old FFPE with kinetic RT-PCR are feasible and reproducible using the automated technology by Siemens, and do not require prior trimming of the tissue.
- High level of concordance between the quantitative RNA expression level and the semi-quantitative protein level for ESR1 and HER2.
- Quantitative expression analysis using kinetic RT-PCR in routinely processed FFPE is feasible and could be adapted in diagnostic testing.

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Role of miR-143 regulating DNA methyltransferases 3A in breast cancer

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Background: MicroRNAs (miRNAs) are 19−25-nucleotides regulatory non-protein-coding RNA molecules that regulate the expressions of a wide variety of genes including some involved in cancer development. In particular, decreased expression of miR-143 has been reported in various human cancers including colorectal cancer and B-cell lymphomas. The aim of this study was to elucidate the role of miR-143 dysregulation in breast cancer.