Materials and Methods: Archival formalin-fixed, paraffin embedded tissue materials from 40 cases of invasive breast cancer were randomly selected and revealed from the archives of our department. The breast cancer specimen had been processed according to standard laboratory protocols. Tumors had been classified and staged according to the WHO criteria. Immunohistochemic staining with D2−40, according to protocol of the manufacturer DAKO® was used to selectively mark lymphatic vessels. Statistical analysis was performed using the chi-square test, calculated by Statgraphics Plus®.

Results: The possibility for peri-tumor lymphatic evaluation depended significantly on the amount of peri-tumor tissues analyzed χ^2=14.48; p = 0. 0007 (n = 40). In general, the presence of LVI correlated with axillary lymph node (LN) status χ^2=6.37; p = 0. 0116 (n = 40). When analised in more than 3 mm of peri-tumor tissues, LVI matched significantly better with axillary LN status than when analyzed in peri-tumor tissues less than 3 mm wide χ^2=8.65 p = 0.0343; (n = 35). D2−40 played decisive role in the differentiation of LVI from post-fixation tumor tissue shrinkage.

Discussion: We believe that underestimation of lymphatic invasion in breast cancer can be reduced by evaluation of at least 3 mm of peri-tumor tissues. Overestimation can be practically avoided by the application of specific endothelial marker. The application of large section technique may be the most appropriate approach for LVI evaluation in breast cancer.

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Turnaround-time reduction of pathological examination

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Background: Reduction of waiting time is very important for patient service because short turnaround time (TAT) of pathological examination allows for rapid response including medical decision-making by clinicians that may increase patient satisfaction. Pathological examination of breast specimens tends to take longer TAT because breast tissue contains abundant fat and its diagnosis routinely requires results of IHC staining. To shorten the TAT of breast cancer cases, improvements of routine work and rescheduling of procedures have been performed in our hospital.

Material and Methods: 202 breast cancer specimens, performed surgical resection (preservation resection or mastectomy) from 2007 November to 2008 May, were typically examined according to the protocol as follows. Specimens were fixed with formalin injection immediately after tissue removal. On the second day, tissue specimens were cut for pathological samples, and samples for IHC (ER, PgR, HER2) were selected at the same time. The whole of the cancer areas including intraductal spread was cut for pathological examination. After cutting sections, fixation and delipidation were accelerated by supersonic wave and thermostat bath. On the third day, pathological reports with results of IHC and digital images (demarcated cancer area with separate colors accordingly invasive or non-invasive lesions) were submitted to clinicians through electronic-pathology-data-record system (Dr. Helper, FUJITSU, Japan). In our pathology section, there are two full-time pathologists and six technicians.

Results: Average number of slides was 36.2/case (excluded LN samples). In 202 cases, TATs (after-operation days, excluding holidays) were consumed to complete each diagnosis as follows, 2 days for 78 (38.6%); 3 days for 89 (44.0%); 4 days for 25 (12.4%); 5 or more days for 10 (5.0%) cases. Less than 20% of all cases required 4 or more days mainly because of necessity of additional samples and staining.

Conclusions: In more than 80% of the surgical cases, final pathological reports including result of IHC and digital images were completed within three days after operation. To compare with previous period before practice of this protocol, average TAT of breast cancer cases was obviously shortened. These procedures do not need special equipment or extra manpower and thus this protocol for breast cancer specimens is available in other laboratories.

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Characteristics of invasive micropapillary carcinoma of the breast: Is it related to the triple negative breast cancer?

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Background: Invasive micropapillary carcinoma (IMPC) of the breast is a rare and associated with high incidence of lymph node metastasis and poor outcome. The aims of this study were to provide a comprehensive analysis of clinicopathologic and mmunohistochemical characteristics of IMPC and to elucidate the differences between IMPC and invasive ductal carcinoma (IDC).

Methods: Sixty-one patients of IMPC were identified by retrospective review of database from Jan 2004 to December 2008. 221 patients were randomly selected among the IDC patients who received operation during the same period. Two groups were compared with uni- and multi-variate analysis.

Results: We observed significant differences in mean number of metastatic lymph nodes (6.1 vs. 1.9, $p = 0.001$), positivity of lymph node (70.5% vs. 45.2%, $p < 0.001$), and presence of lymphatic vessel invasion (75.4% vs. 34.8%, $p < 0.001$) between IMPC and IDC patients. Although it has been known that triple negative breast cancer (TNBC) have lymphotropic tendency in their early T stage, 11.8% (26/221) of IDC and 3.3% (2/61) of IMPC patients were TNBC in this study ($p = 0.050$). In multivariate analysis, IMPC histology showed no correlation with disease-free survival (DFS) and the lymphatic vessel invasion was a significant predictor of DFS.

Conclusions: The results of this study confirm that IMPC is unique subtype of breast cancer that is commonly accompanied by axillary lymph node metastasis and shows poor outcome, although it rarely presents the pattern of TNBC. Lymphatic vessel invasion rather than histology of IMPC seems to be more closely related to DFS.

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Basal-phenotypes in breast carcinoma − morphological, immunohistochemical and clinical analysis

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Background: The basal-like breast cancer sub-type represents probably a new treatment challenge for oncologists. The aim of the study was to analyse morphological, immunohistochemical and clinical features in breast invasive carcinomas which diagnosed as basal-like type of invasive ductal carcinoma (BLIDC) from needle core biopsies (NCB).

Methods: We reviewed 21 (11.7%) breast carcinomas :20 primary ones (T1-T4) and one after 7 years recurrence carcinoma (r T1) for hematoxylin-eosin slides, immunohistochemistry for ER, PR, HER-2, CK 5/6, CK17, CK 18, p53, *BRCA1* testing, clinical information. Morphological aspects as architectural features (sheets, nests, tubular formation, ribbon-like formation), intensity of lymphocytic infiltration, necrosis, fibrous and/or hyalinised stroma, nuclear pleomorphism, mitoses has been studied. Semiquantitive evaluation of basal cytokeratine positive cancer cells has been performed. Overexpression of p53 cases has been examined.

Results: BRCA1 cases of BLIDC (3/21) were triple negative and just a case overexpressed p53. All sporadic BLIDC (18/21) were ER, PR negative but 3 cases were 3+ scored and 4 cases 2+ scored for HER-2. Overexpression of p53 was noted in 11/18 carcinomas. At least one basal cytokeratin CK 5/6 or CK17 was positive in all cases. 5−30% cancer cells expressed CK 5/6 in 53% BLIDC and there were more than 30% positive cells in 29% BLIDC. CK 17 was expressed in 10−30% neoplastic cells in 36% BLIDC and more than 30% positive cancer cells were noted in 56% cases. CK 18 was positive in all BRCA1 cases and 75% sporadic cases carcinoma cells.

Conclusions: CK 17 is recommended as first basal marker in NCB for immunohistochemical confirmation of basal-like type when amount of cancer cells is small.

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The prognostic factors of breast cancer related with axillary lymph node metastasis in T1 breast cancer

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Background: Axillary lymph node metastasis can occur even in breast cancer sized less than 2 cm. There are several prognostic factors related to the lymph node metastasis in breast cancer. We aimed to investigate the clinicopathologic factors that affect the node metastasis in T1 breast cancer. **Methods:** We reviewed the medical records and pathologic reports of all breast cancer patients who had undergone surgical procedure from 2001 to 2006. Among these patients, we retrieved 230 T1 breast cancer patients divided them into two groups according to the presence or absence

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.

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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.