stomach were recorded. Slides of the stomach and breast were reviewed to determine the type of tumour and histological similarity. Cancer of the stomach was classified according to Lauren. All cases were stained with antibodies for cytokeratin 7, cytokeratin 20, E-cadherin, estrogen receptor and progesteron receptor and slides were stained in a Benchmark XT automatic stainer (Ventana).

Results: A total of 26 patients were retrieved from our files and in 18 patients slides of the breast and stomach could be reviewed and sufficient material was available for additional staining. Median age was 67 years (37−79 years). In 7 patients (37−71 years) the cancer of the stomach was shown to be metastatic disease. In 6 patients the gastric metastasis had a diffuse growth pattern and in 5 patients the breast cancer was of the invasive lobular type. Median time between diagnosis of breast cancer and gastric metastasis was 32 months (1–167 months). In 2 patients a diagnosis of gastric metastasis was made only after resection of the stomach. All gastric metastases were positive for hormone receptors.

Conclusion: Cancer of the stomach can be metastatic disease in breast cancer patients, even in those with a remote history of breast cancer. Proper clinical information and staining of the gastric biopsy are most helpful in avoiding misclassification.

306 Poster

Clinicopathological features of inflammatory versus non-inflammatory locally advanced non-metastatic breast cancer

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Background: Inflammatory breast cancer (IBC) is a rare but aggressive form of breast cancer. It is mainly a clinical diagnosis. The aim of this study was to compare IBC to clinically diagnosed non-inflammatory locally advanced non-metastatic breast cancer further (cLABC) with respect to clinicopathoIocial features.

Material and Methods: 108 patients were studied: 49 with IBC and 59 with cLABC. The following features were analysed: age at diagnosis, body mass index (BMI), axillary lymph node status (cN), oestrogen receptor status (ER), progesterone receptor status (PR), HER2 status, histological tumour grade and subtype. Short term disease-free and overall survival (DFS, OS) were also assessed in both groups.

Results: Compared with cLABC, IBC was less often PR positive (41.7% vs 66.1%, $p = 0.01$) and showed a trend to be more often HER2 positive (34.7% vs 19.3%, $p = 0.07$) The 3-year DFS was 63% and 77% respectively for IBC and cLABC ($p = 0.01$); these figures were 83% and 85% for OS ($p = 0.17$). No significant differences in age at diagnosis, ER, cN, BMI, histological tumor grade or subtype were detected.

Conclusion: Differences in PR, HER-2 and DFS confirm the distinctive biological nature of IBC and cLABC. Age at diagnosis, ER, cN, BMI, histological tumour grade and subtype show no difference and therefore these features might be more determined by or related to the locally advanced stage than to the inflammatory component itself.

307 Poster

HER-2/neu amplification detected by fluorescence in situ hybridization in touch imprint cytology in comparison with tissue sections

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Background: HER-2/neu status has been used in breast cancer as a prognostic and predictive factor to select patients for trastuzumab treatment. The analysis of HER-2 is usually performed on formalin-fixed paraffin tissue sections and testing with fluorescence in situ hybridization (FISH) is preferred. The objective of our study was to evaluate the reliability of HER-2/neu determination by FISH on touch imprints (TI) of breast core needle biopsies (BCNB) from primary breast cancer patients in comparison with the results obtained by FISH on the corresponding tissue sections (TS).

Material and Methods: The sections tissue of the BCNB were touched a lot of times to one slide, it was made another mirror slide and then, one of them was stained whit H&E to detect malignant cells and other was utilized for FISH: The slides of TI of the breast core needle biopsies and corresponding TS from breast cancer patients were eavluated for HER-2 gene amplification by determining the HER-2/CEP17 signal ratio in 20 tumor nuclei. If the ratio was <2.2, the specimen was considered to lack gene amplification; if the ratio was ⩾2.2, the specimen was considered to show HER-2 gene amplification. Chi square test was made.

Results: A total of 55 BCNB were examined and paired results by FISH cytology and FISH histology were available in 48 cases. Concordance was 83.33% (40/48). Eight cases didn't show concordance. It was not stastistically significant ($p > 0.05$) by chi square on both samples

Conclusion: We conclude that HER-2 gene analysis by FISH on TI is easily done and reliable tecnique. TI provided results earlier and quicker, were easier to score and were more accurate. However, the use of TI sacrifice the arquitectural tissue.

Thursday, 17 April 2008 12:30–14:30

POSTER SESSION

Pathology and biology

308 Poster Discussion

Detection of homologous recombination defects in biopsies of sporadic breast cancers

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Background: It has been suggested that up to 30% of sporadic breast cancers may have defective homologous recombination (HR), the only cellular mechanism that reliably repairs DNA double strand breaks. DNA-damaging drugs including alkylating agents can cause double strand breaks. Tumours with deregulating mutations in the key players of HR may be extremely sensitive to these and to a novel class of agents targeting DNA-repair: the poly (ADP-ribose) polymerase (PARP) inhibitors. The identification of such a subgroup of breast cancers before treatment could be of great clinical utility. This study was initiated to develop a test that can be incorporated in a routine clinical workup.

Materials and Methods: So far, 38 patients with HER2 negative tumours and scheduled for preoperative chemotherapy have been tested prospectively. We investigated the expression levels of BRCA1, FANCC, and FANCF by quantitative RT-PCR and amplification of the EMSY gene first by FISH and additionally by Multiplex Ligation-dependent Probe Amplification (MLPA). Triple-negative patients were additionally checked for BRCA1 germ line mutations by sequencing the BRCA1 gene locus.

Results: EMSY amplification assessment by FISH is technically challenging and is not an optimal choice for clinical routine. MLPA is a reliable alternative that can also detect amplification missed by FISH because of high background staining. Quantitative RT-PCR detected a number of tumours with a considerably lower expression of BRCA1 ($n = 5$) than the other ones ($n = 33$). Of those 5 tumours, four had a triple-negative phenotype, whereas the other one was a luminal tumour with a high expression of the estrogen receptor.

Conclusion: Amplification of the EMSY gene locus is a rather rare event. Detection by FISH may miss samples that can be detected by MLPA. The main changes in the investigated sporadic samples are low expression of the BRCA1 and FANCC protein. Gene expression arrays are available of these samples and an update and comparison of the applied techniques will be presented.

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TP53-mutated breast carcinomas are associated with specific array comparative hybridization (aCGH) patterns involving deletions of 3p, 4p, 4q and 5q

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The TP53 tumor suppressor protein acts as a major defense against cancer. Among its most distinctive features is the ability to elicit both apoptotic death and cell cycle arrest. TP53 plays a key role in mediating cell response to various stresses: one of these is DNA-repair. When *TP53*

is mutated the maintenance of DNA integrity may be reduced, resulting in copy number alterations (CNA) in the tumor. Aneuploidy is an important feature of cancer cells, and specific CNAs have shown to be of both prognostic and predictive value. Most of these alterations can be detected by use of array Comparative Genomic Hybridization (aCGH). The aim of this study was to investigate the CNAs in breast tumors, with a high resolution platform, in relation to *TP53* mutation status.

Tumor tissues from a series of 212 primary breast cancer cases were sequentially collected at Ullevål University Hospital in Oslo between 1990 and 1994. Tissues were sampled at the time of primary surgery and snap frozen. *TP53* mutation data from previous sequence analyses were available for 203 of the samples. We performed aCGH on 167 of these tumors. DNA was isolated using chloroform/phenol extraction, followed by ethanol precipitation. The aCGH-platform was the Agilent Human-Genome-CGH Microarray 244k. For detection of aberrations we used ACE (analysis of copy errors) and PCF (piecewise constant fit), both implemented in the CGH-explorer software. For visualization we used the software Nexus 2.0. Significance Analysis for Microarray (SAM) was performed using the R/BioConductor package "samr".

Many significant genetic alterations were found, with a large heterogeneity between the different tumors. The most frequently observed alterations were amplification of 1q, 8q, 16 p and 17q and deletion of 1p, 8p, 16q and 17p. When grouping the tumors by *TP53* mutation status we found a significant difference in the CNA patterns between *TP53* mutated vs. none-mutated tumors. Overall the mutated group had more aberrations than the wild type group, and interestingly the aberrations were not evenly distributed along the genome, suggesting that some chromosomal areas are more prone to instability or selected for in the presence of a mutated TP53 protein. The most frequent losses associated with *TP53* mutation status were regions on 3p, 4p, 4q, 5q and 8p, whereas significant gains were observed for 8q and 10p. A detailed structure of the CNAs and the involved genes will be presented in relation to type of mutations and to various clinical data.

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Comparison between circulating and disseminated tumor cells in breast cancer

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Background: The presence of disseminated tumor cells in bone marrow (BM) of breast cancer patients is an independent prognostic factor. The role of circulating tumor cells (CTC) in blood is not yet defined. Since BM aspiration is less accepted by patients compared to blood drawing, it would be highly desirable to replace bone marrow aspiration by blood analysis. Therefore, the purpose of the present study was:

1. to examine the presence of tumor cells in peripheral blood,
2. to evaluate how surgery affects the presence of CTC,
3. to assess the correlation between results in blood and in BM.

Materials and Methods: 314 blood samples from breast cancer patients were collected. 130 patients underwent blood sampling both pre- and postoperatively. All aspirates underwent immunomagnetic enrichment using AdnaTest BreastCancerSelect within 4 hours after blood withdrawal followed by RNA isolation and subsequent gene expression analysis by reverse transcription and Multiplex-PCR in separated tumor cells using AdnaTest BreastCancerDetect. Three breast cancer associated tumor markers and one control gene were amplified: GA733−2, Muc-1, Her-2 and β-actin (internal PCR control).

Furthermore bone marrow aspirates from 176 of these patients were analyzed by immunocytochemistry (pancytokeratin antibody A45-B/B3) using ACIS system (Chromavision) according to the ISHAGE evaluation criteria.

Results: 184 patients could be included in to this study. 10% of these patients had detectable tumor cells in the bloodstream. To assess the influence of surgery, 130 blood samples were analyzed both pre- and postoperatively. The positivity rates postoperatively were slightly higher but did not differ significantly (13% preoperatively and 17% postoperatively). It seems therefore that surgery or invasive procedures (core cut biopsy) do not influence positivity rates in the blood. The positivity rate in the bone marrow was 11%. The correlation between both compartments (blood and BM) in these patients was 80%.

Conclusions:

1. Circulating tumor cells can be detected in primary breast cancer.
2. Surgery does not influence significantly the tumor cell load in the blood stream.
3. Positivity rates obtained from both compartments (blood and BM) correlate highly. However, the prognostic significance of CTCs has to be further evaluated.

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Concordance between tissue microarray and whole tissue sections for ER expression and PgR and HER-2 status: a pilot study for the Trans-ATAC trial

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Background: The Tissue Microarray (TMA) format allows high-throughput molecular characterisation of tissue specimens with rapid linkage to clinical endpoints. However, assessment of the number of cores needed to represent the biomarker expression in whole tissue sections (WS) is a prerequisite of their valid use. Some studies suggest [1,2] that one core is adequate to represent the expression of ER, PgR and HER-2 in a WS, whilst others recommend at least two cores [3]. Therefore, we investigated whether ER, PgR and HER-2 expression in one TMA core was sufficient to represent the expression in WS in the Trans-ATAC trial population.

Materials and Methods: 4μm thick sections were cut from 7 of the Trans-ATAC TMAs and stained for ER (6F11, Novocastra 1:40), PgR (Novocastra 312, 1:100) and the HER-2 protein (DAKO Herceptest™ kit), with HER-2 2+ cases being analysed by FISH (PathVysion™, Abbott). A random selection of 30 tissue cores (total 210 cores) from each of the 7 TMAs was evaluated. Expression was considered positive for ER with a H-score >1, for PgR expression with >10% cells showing nuclear staining and for HER-2 protein expression if 3+ according to the DAKO Herceptest, or 2+ with HER-2:Chr17 gene ratio >2.

Results: Positive HER-2 expression (protein and gene amplification) was found in 8.1% of cores and WS. The concordance between the cores and WS HER-2 scores was 97.1%, (95% CI 94.8−99.4, $\kappa = 0.81$, 95% CI 0.75−0.86). For PgR expression, 67.2% of cores were positive vs 78.9% of WS. The concordance between the cores and WS for PgR expression was 82.2% (95% CI 76.9−87.5, $\kappa = 0.56$, 95% CI 0.49−0.63).ER positivity was found in 98.9% and 99.0% of the cores and WS respectively, with a concordance of 99.5% (95% CI 89.7–100). Considering the ER H-score as a continuous variable the correlation coefficient between one core and WS H-scores was $r = 0.78$, $r2 = 0.61$. The correlation between the mean H-score for ER (of the 3 TMA cores) versus the H-score of the WS was increased to $r = 0.83$, $r2 = 0.69$.

Conclusion: The disconcordance rate between TMAs and WS for HER-2 was only 3%, but this may be significant when considering the low prevalence of HER-2 expression in an ER positive cohort. The disconcordance of >10% for PgR is unacceptable and studies may require multiple cores or WS to assess this marker. There was a high concordance and correlation between the one core and WS for ER status, but quantitative assessment of ER on TMAs is substantially improved if three cores are taken.

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312 Poster Discussion

Molecular qRT-PCR grade index: a new tool for breast cancer (BC) patient grading improvement

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Background: We have recently shown that proliferation captured by the GGI is one of the most important prognostic indicators in BC and may encompass a significant portion of the predictive power of several previously published prognostic signatures in particular for ER+ disease. The aims of this study were 1) to convert this microarray index to an index using qRT-PCR and 2) to assess its prognostic and predictive value for tamoxifen response.

Methods: A qRT-PCR genomic grade index (PCR-GGI) was developed based on the expression of 4 genes selected from the GGI microarray signature and 4 reference genes. The accuracy and concordance with the original microarray-derived GGI was assessed using a BC set from which frozen, FFPE tissues and microarray data were available (N = 19). The evaluation of the prognostic value of the PCR-GGI was assessed using a