chemotherapy (n = 31), whereas no significant impact was seen in node-positive patients treated either by CMF chemotherapy (n = 49) (DFS: p = 0.605; OS: p = 0.934) or by adjuvant endocrine therapy (tamoxifen) alone (n = 106) (DFS: p = 0.735; OS: p = 0.275). While median DFS and OS in anthracycline-treated patients was >10 years if tumours showed high EFEMP1 expression, it was only 3.1 years (DFS) respectively 4.5 years (OS) in cases with low EFEMP1 expression.

Conclusions: The results point to a predictive value of EFEMP1 expression regarding anthracycline response in node positive patients, which needs to be further validated in larger collectives of homogeneously treated breast cancer patients. In view of clinically emerging angiogenesis inhibitors, identification and characterization of components of the angiogenic pathway as specific prognostic as well as predictive markers is of great relevance for the success of this treatment option. EFEMP1, with its anti-angiogenic properties, may serve here as an important molecular marker for defining an adequate tumour-biology oriented therapeutic strategy.

33 Poster Discussion

AlphaB-crystallin predicts poor breast cancer survival in basal-like tumors

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Background: Basal-like breast cancers are high grade tumors with poor prognosis (Perou et al. Nature 406:747−52, 2000; Nielsen et al. Clin Cancer Res 10: 5367−74, 2004). In gene expression profiling studies, α-basic (αB)-crystallin, a small heat shock protein with anti-apoptotic and oncogenic activity, is commonly expressed in basal-like tumors. We previously reported that tumors over-expressing this protein had a poor prognosis, and that approximately half of basal-like breast cancers express αB-crystallin (Moyano et al. J Clin Invest 116:261−70, 2006). In this study, we aim to validate the prognostic value of αB-crystallin in a regional population-based series of 4000 breast cancers, powered for subset analysis.

Materials: Tissue microarrays were constructed using 4046 invasive primary breast cancers referred to the British Columbia Cancer Agency from 1986 to 1992 with clinical outcome. Breast cancer subtypes were defined using the immunopanel of ER, PR, HER2, epidermal growth factor receptor (EGFR) and cytokeratin 5/6. Immunohistohemistry of αB-crystallin was scored as diffuse positive (⩾30% of cancer cells positive), focal positive (<30% of cancer cells positive), or negative. Univariate survival probabilities were estimated using Kaplan–Meier method. Multiple Cox regression analyses and likelihood ratio tests (LRT) were used to determine the independent prognostic significance of αB-crystallin.

Results: Among breast tumors interpretable for αB-crystallin, 11% (361/3285) of cases are positive. Consistent with the previous report, 55.4% (175/316) of basal-like tumors, defined as ER/PR/HER2 negative and (EGFR or cytokeratin 5/6) positive, express αB crystallin. αB-crystallin positive tumors are associated with 11% absolute decreased breast cancer survival [10-yr BCSS (95% CI) 75% (73−77) versus 64% (58−68)]. In the Cox regression model including lymphovascular invasion, tumor size, grade, nodal involvement, age at diagnosis and breast cancer subtypes, αB-crystallin remains as an independent poor prognostic marker with a hazard ratio of 1.310 (LRT p = 0.02113). Within the subset of basal tumors, αB-crystallin positive tumors are also independently associated with poorer breast cancer survival (Hazard Ratio 1.63, LRT p = 0.02).

Conclusion: αB-crystallin independently predicts poor survival in a large population based cohort and among basal-like tumors, suggesting its role as a novel biomarker that identifies a particularly aggressive subset of basal-like tumors.

34 Poster Discussion

Monoclonal antibodies specific for Phospho-4E-BP1 (Thr 70) and phospho-AKT (Ser 473) indicate prognosis in breast cancer

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Background: The activation of cell surface growth factor receptors initiates a cascade of signalling through overlapping signalling pathways via the phosphorylation of signalling proteins. Previous studies have suggested that the phosphorylation of 4E Binding Protein 1 (4E-BP1), Mitogen Activated Protein Kinase (MAPK) and v-akt murine thymoma viral oncogene (AKT) proteins has value as a prognostic indicator in breast cancer. However these studies have been limited by small patient groups and, in some cases, complicated by the interaction of systemic treatment on outcome.

Materials and Methods: Paraffin embedded invasive tumour samples from 430 patients who had received no adjuvant chemo or hormonal therapy were used to construct a Tissue MicroArray (TMA). Median follow up was 21 years. Tissue sections from TMAs were stained using monoclonal antibodies to ER, PgR, Ki67, phospho-AKT, Phospho-4E-BP1 and polyclonal HER2 and phospho-MAPK antibodies using standard immunohistochemistry methods. Nuclear markers (ER, PgR, Ki67 and Phospho-4E-BP1 (Thr 70) were scored with validated algorithms on an Ariol imaging system (Applied Imaging). All other markers (HER2, phospho-AKT (Ser 473) and phospho MAPK (Thr202/Tyr204)) were scored manually.

Results: ER and PgR histoscores were significantly positively correlated (p < 0.001). A significant inverse correlation observed between both ER and PgR histoscores and Ki67 (both p < 0.001). Analysis indicated a significant correlation between high phospho-4EBP1 staining and reduced recurrence free survival (p < 0.05). Conversely, high phospho-AKT staining was correlated with longer overall survival (p < 0.05).

Conclusions: We have found that the phosphorylation of 4E-BP1 and AKT proteins are prognostic for disease free and overall survival respectively in breast cancer patients in the absence of systemic therapy suggesting these are true prognostic markers in breast cancer. Further analysis of their significance in the context of other known markers of breast cancer prognosis will be performed and presented.

Wednesday, 16 April 2008 12:30–14:30

POSTER SESSION

Detection, diagnosis and imaging

35 Poster

Axillary study before surgery in patients with breast cancer

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Background: The axillary study with ultrasound and cytological puncture with fine needle aspiration of suspicious nodes are new diagnostic methos.

Material and Methods: We study 159 patients with axillary ultrasound and cytological puncture with fine needle aspiration (FNA) of suspicious nodes. Suspicious nodes were those with at least one of the following signs: long to short axis ratio less than 1.5, absence of hilius and cotical disrruption. If the results were compatible with metastases (positive) then we performed axillary lymphadenectomy, if it was found to be benign (negative) then we conducted sentinel node study.

Results: In the group of ultrasound positive plus ultrasound positive FNA positive was 54 patients (33.96%) when we conducted axillary lymphadenectomy, 13 patients (24%) were found to have one positive node, 7 patients (13%) two positive nodes, 9 patients (16%) three positive nodes, 25 patients (45%) more than three positive nodes.

The other group (ultrasound negative plus ultrasound negative plus FNA negative) was 105 patients (66.03%), the sentinel node study was: in 76 patients (72.38%) pNOi−, in 9 patients (8.57%) pNOi+, in 7 patients (6.66%) pN1mic, in 12 patients (11.42%) pN1a, in 1 patient (0.95%) pN2a.

Conclusion: The axillary study with ultrasound and FNA before surgery allows excluding a group of patients to make the sentinel node study.

36 Poster

Magnetic Resonance Imaging to predict pathological response in neoadjuvant chemotherapy for breast cancer

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Background: The rationale of neoadjuvant chemotherapy in patients with locally advanced breast cancer is to achieve down-staging of the tumour to enable breast conservative surgery. The objective of this study is to

prospectively compare magnetic resonance imaging (MRI) response with pathological response assessment in patients completing neoadjuvant chemotherapy. We evaluated whether MRI can be used as a tool for determining the type of surgery. We tried to identify subgroups based on initial core needle biopsy that would benefit from MRI to predict pathological response.

Materials and Methods: MRI was performed before, during and after neoadjuvant chemotherapy (4 cycles of doxorubicin plus cyclophosphamide followed by 4 cycles of docetaxel) in 55 women with locally advanced breast cancer ineligible for primary breast conservative surgery. We compared tumour size on MRI after completing chemotherapy with final histopathological findings. MRI response was correlated with the type of surgery performed (mastectomy versus breast conservative surgery).

Results: Based on MRI, there were 14 non-responders (NR), 20 patients with partial response (PR) and 16 patients with a complete response (CR). Diagnostic accuracy for assigning patients to the NR group was 84%. In 75% of patients with partial response MRI proved to be reliable in determining incomplete pathological response. In 50% of patients in the CR group, histology revealed residual tumour. Mastectomy in the NR group was performed in 100% of the cases, in 50% of the PR group and in 18% of the complete responders. When analysing for biological markers on initial biopsy (tumour type, hormonal receptor and neu oncogen status) no subgroup was identified to benefit from MRI in predicting pathologic response.

Conclusions: In the NR group, MRI response correlates well with pathologic findings. In our view, mastectomy is indicated in this group of patients. MRI overestimates complete response in half of the patients. Therefore surgery after neoadjuvant chemotherapy remains obligatory to determine complete pathological response.

37 Poster

Incidental breast lesions found on computer tomography – what is their significance?

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Background: The detection of incidental breast lesions on CT imaging is not uncommon and often results in referral for breast assessment. The aims of this study were twofold: to establish the rate of occult breast cancer detection by CT and to see if standardised analysis of lesion morphology and enhancement can predict malignancy.

Material and Methods: A retrospective review of 268 179 CTs at performed our institution between 19/03/1994 and 15/11/2007 yielded 392 radiology reports containing the keyword 'breast'. 56 of these patients had no current or previous history of breast cancer and were thus referred to the Cambridge Breast Unit (CBU) for standard triple assessment of the lesion seen on CT. The CT images were retrospectively reviewed by two experienced breast radiologists. Lesion morphology was analysed using the BI-RADS mammography template and enhancement characteristics using the MRI templates. The radiologists were blinded to the results and outcome of formal breast assessment.

Results: The overall malignancy rate (B5) was 30.3% (17 of 56 patients) with the most common malignancy being invasive ductal carcinoma (12 of 56 patients). 28 patients had an ultrasound guided biopsy and 28 did not need biopsy as they were deemed normal or benign following clinical and imaging (mammography and/or ultrasound) assessment. Positive predictive values (PPVs) for malignancy were obtained for each of the CT descriptive terms derived from BI-RADS.The term "spiculate" had a PPV for malignancy of 0.73 and "irregularity" a PPV of 0.57. Descriptors with low PPV for malignancy eg: "oval" (PPV 0.00), "round" (PPV 0.14) and "circumscribed" (PPV 0.00) were associated with benign lesions or normal breast tissue. The contrast enhancement characteristics of lesions will be described.

Conclusion: In our experience, occult breast cancer was found in 30% of patients referred for assessment following the detection of an incidental lesion on CT. Accurate analysis of such lesions using standardised descriptors may help in differentiating potentially benign from malignant lesions and therefore help clinicians in further management and appropriate referral.

38 Poster

Evaluation of a RT-PCR based routine screening tool for the detection of disseminated epithelial cells in the bone marrow of breast cancer patients

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Background: Detection of disseminated tumor cells in bone marrow of breast cancer patients is an independent prognostic factor. The gold standard for the detection of disseminated tumor cells (DTCs) is immunocytochemistry (ICC). The aim of our investigation was to evaluate a newly established RT-PCR based assay and compare both techniques with respect to their sensitivities on the basis of 405 bone marrow aspirates from BC patients. 331 of all samples were obtained at the time of surgery for primary breast cancer. 55 patients with no evidence of the disease underwent secondary BM biopsy and 19 presented with a relapse at the time of BM aspiration.

Materials and Methods: 405 bone marrow aspirates from breast cancer patients were processed with both methods.

Immunocytochemistry: After Ficoll enrichment of 10ml bone marrow, cytospins were prepared and stained using the A45-B/B3 primary antibody for pCK. Cytospins were analyzed using the ACIS system (Chromavision) according to the ISHAGE evaluation criteria.

RT-PCR: mRNA was extracted and purified using the mRNA isolation for blood and bone marrow kit (Roche® Molecular Biochemicals). RT-PCR was performed on the LightCycler® system, using the RNAMaster Hybridization Probes kit and custom primers and probes. Primers were selected to amplify a 380bp fragment of the CK19 gene.

Positive and negative controls were included with each batch of samples for both procedures.

Results: Altogether, in 48% (196 out of 405) of the aspirates, at least one method detected disseminated tumor cells. Highly significant correlation between ICC and RT-PCR results was observed ($p < 0.01$). However, in 111 patients discordant results were found. 57 BM aspirates were positive by RT-PCR but negative by ICC whereas 54 samples were positive only by ICC. The positivity rates of ICC and RT-PCR were 34% and 35%, respectively. The highest combined (obtained by at least one method) positivity rate was observed in the subgroup of patients with relapse of the disease at the time of BM aspiration (74%), followed by primary cancer patients (51%), the lowest in patients with no evidence of the disease (27%).

Conclusions: Immunocytochemistry is the current gold standard with well-known correlation to the prognosis. However, it is observer-dependent and labor intensive. RT-PCR is time-efficient and may increase the sensitivity but it lacks a standard protocol. We conclude that RT-PCR-based assays have a potential to improve diagnostics in this field.

39 Poster

Screening for metastatic disease in newly diagnosed breast cancer patients

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Background: Radiologic tests as bone scan (BS), chest radiography (CRX) and liver ultrasonography (LUS)) are commonly used as part of baseline staging of newly diagnosed breast cancers. However, in the absence of symptomatic disease, the usefulness of this routine diagnostic work-up is not evidence-based. So they may be overused.

The objective was to determine the yield of these radiologic tests in this population.

Materials and Methods: We evaluated 70 asymptomatic patients with newly diagnosed invasive breast cancer underwent an evaluation programme including CRX, LUS and BS. When metastatic disease was found, the suspicion was confirmed by other tests (bone X-ray, computerized tomography scan, magnetic resonance imaging) in order to identify true positive diagnoses.

Results: 10% of patients had pathologic tests. 4 out of 7 patients were correctly diagnosed by the initial staging investigations as having metastatic disease (true positive cases). BS detected true skeletal metastases in 4.2% of patients, CRX detected true lung metastases in 1.4% and in one in two suspicious CRX, two in two LUS and 0 out of 3 suspected BS were concluded false positive in terms of metastatic disease. Before imaging tests, all patients with BS evidence of metastases were previously classified as having stage III-B disease. The screening programme disclosed 5.7% of distant metastasis but due to false positive findings, 4.2% patients had to live with the psychological distress of suspected metastatic disease.

Conclusion: In newly invasive breast cancer patients without clinical signs of tumor spread baseline screening for metastases is not warranted because of low frequency of metastases and false positive findings.