observed when the patent blue dye was used or omitted (study 3). The sentinel lymph node was detected in all patients using NIR fluorescence before the blue dye could be observed.
Conclusion: These studies demonstrate the successful use of NIR fluorescence and ICG in sentinel lymph node mapping of breast cancer patients. The optimal parameters are a dose of 500 μM ICG that is not premixed with HSA, and the use of patent blue can be omitted.

5010 ORAL

Intraoperative Molecular Detection of Lymph Node Metastases and Micro-metastases − Results of the First UK Centre Using the One Step Nucleic Acid Amplification Assay

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Introduction: One step nucleic acid amplification (OSNA), a highly sensitive intraoperative assay of cytokeratin 19 mRNA, is used for the detection of sentinel lymph node (SLN) macro- and micro-metastases in breast cancer. We present our two year data following the introduction of OSNA in our unit.
Methods: Data was collected prospectively from 2008−10. All eligible patients were offered OSNA. Operations were performed by five consultant breast surgeons. On detection of micro-metastasis (+) and positive but inhibited metastases (i+), a level 1 axillary nodal clearance (ANC) and for a macro-metastasis (++), a level 3 ANC was performed.
Results: 471 patients had 999 SLN analysed, median age being 61. All except one were females. 34% (n = 161/471) had positive SLN who had further ANC. Of these, 48% (n = 78/161) had macro-metastases, 37% (n = 59/161) had micro-metastases and 15% (n = 24/161) had positive but inhibited results. 17% (10/59) of the patients with micro-metastases had positive non-SLN (NSLN), four (4/59, 6.8%) had four positive nodes (SLN+NSLN) thus receiving adjuvant radiotherapy. 8% (2/24) of those with positive but inhibited results and 39% (30/78) of those with macro-metastases had positive NSLN.
Conclusion: In our series, over a third of patients had OSNA positive SLN and underwent axillary surgery at the same operation. OSNA may potentially upstage patients with micro-metastases and long term studies are needed to determine the clinical relevance of molecular micro-metastatic disease.

5011 ORAL

Assessments of Proliferation in Breast Cancer

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Background: Proliferation rates of tumour cells provide prognostic and therapy predictive information. Mitotic index (MI), S-phase fraction (SPF) and ploidy assessed by flow cytometry and Ki-67/MIB-1 are commonly used assays of proliferation. Comparative studies of the assays are rare. Also, consensus of cut off values of Ki-67 in breast cancer is lacking.
Patients and Methods: Two different cohorts of patients were used to compare the correlation between assays. MI, SPF and 5-year follow-up data were explored for 1130 patients from the hospitals of Kalmar County (KC). In the second cohort MI, Ki-67 /MIB-1 and 3-year follow up data for 403 patients from the Sahlgrenska University Hospital (SU) were investigated. In further approximately 2000 cases the correlation between Ki-67 and MI is explored.
Results: In the KC cohort, tumours with MI 1 identified pts with the lowest 5 year distant recurrence rate, 4.3% and MI 3 those with the highest proportion of distant recurrence, 17%. Low SPF, diploid tumours had a similarly low 5 year distant recurrence rate as MI 1 tumours, 6%.
In the SU cohort MI and Ki-67 were both significantly correlated to early recurrence, $p < 0.001$. The optimal correlation between MI and Ki-67 was achieved when both were separated in 3 groups with cut off values for Ki-67 of 10 and 30%. Spearman $r = 0.69$, $p < 0.0001$.
Tumours with Ki-67 >30% had 22% distant recurrences within 3 years, those with Ki-67 10−30% recurred in 12% and tumours with Ki-67 <10% in only 1.4%. The proportion of early distant recurrences was almost identically distributed by MI score. Tumours with MI 3 had 21% recurrences, MI 2 11% and MI 1 only 1.6%.
Conclusion: Mitotic index is a solid instrument to identify tumours with inferior prognosis. When Ki-67 is stratified in 3 groups the assay performs equally well as the mitotic index. Low SPF, diploid tumours have approximately the same prognosis as tumours with low MI.

5012 ORAL

Prognostic Value of TWIST1 Expression in Breast Cancer Patients

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Background: Twist homolog 1, encoded by the *TWIST1* gene, is a transcription factor that promotes cancer development by inducing epithelial to mesenchymal transition (EMT), a key process of invasion and metastasis, in cancer cells through inhibition of E-cadherin expression amongst other genes. The purpose of the current study was to investigate whether *TWIST1* expression predicts the progression of disease in a large series of well-documented breast cancer patients with long term follow-up and to identify breast cancer genes and associated pathways co-expressed with *TWIST1*.
Materials and Methods: The mRNA expression level of *TWIST1* was analyzed by quantitative RT-PCR in 1,476 primary breast cancers. The expression level was dichotomized using the median value. Metastasis-free survival (MFS) was evaluated in all patients and, separately, in lymph node-negative patients (n = 802) who did not receive adjuvant systemic therapy, and were stratified into estrogen receptor (ER)-positive (n = 566) and ER-negative (n = 236) cohorts. MFS was evaluated using the Kaplan–Meier method and uni- and multivariate analysis was performed using the Cox proportional hazards method. Spearman correlation of *TWIST1* expression with other genes measured on Affymetrix chip was also analyzed. Plausible pathways containing genes showing significant positive and negative correlation with *TWIST1* expression were predicted using BioCarta and KEGG data basis.
Results: In all patients, a high expression level of *TWIST1* was associated with shorter MFS in both uni- and multivariate analysis (HR: 1.29, 95% CI: 1.12–1.49, $p = 0.001$ and HR: 1.33, 95% CI: 1.14–1.54, $p < 0.0001$, respectively). In lymph node-negative high *TWIST1* expression was associated with the luminal A breast cancer subtype and, both in uni- and multivariate analysis, with shorter MFS only in the ER-positive subgroup (HR: 1.37, 95% CI: 1.09–1.73, $p = 0.007$ and HR: 1.35, 95% CI: 1.07–1.71, $p = 0.012$, respectively). Pathway analysis indicated that *TWIST1* expression was correlated positively with genes involved in signal transduction and extracellular matrix and negatively with genes associated with cellular transport processes.
Conclusion: *TWIST1* is an independent prognostic factor for poor prognosis in breast cancer, particularly in lymph node-negative patients with ER-positive disease. Analysis of co-expressed genes suggests an involvement of the microenvironment in *TWIST's* adverse role during breast cancer progression.

5013 ORAL

FDG-PET/CT for Early Prediction of Response to Neoadjuvant Lapatinib, Trastuzumab, and Their Combination in HER2-positive Breast Cancer Patients: the Neo-ALTTO Study Results

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Background and Aim: The NeoALTTO study tested the efficacy of neoadjuvant lapatinib, trastuzumab, and their combination plus paclitaxel in women with HER2-positive primary breast cancer (BC). The primary objective of Neo-ALTTO − the rate of pathological complete response (pCR) − was achieved for the combination compared with either lapatinib or trastuzumab alone (51.3% vs. 29.5% vs. 24.7%, respectively; $p < 0.01$ for both) (Baselga et al. SABCS 2010). Positron emission tomography /computed tomography with F-18 fluordeoxiglucose (FDG-PET/CT) was performed in a subset of patients to assess the predictive value of FDG-PET/CT for pCR as a preplanned secondary endpoint.