Tumours were classified as phenotype basal, luminal A, luminal B and phenotype HER2+.
Results: The mean serum values of methylated gene promoters significantly differed between breast cancer patients and healthy controls ($p = 0.0112$ for ESR1 and $p = 0.0047$ for 14-3-3σ). When their results were combined, it was found that hypermethylation of these two genes differentiated between breast cancer patients and healthy controls ($p < 0.0001$) with a sensitivity of 81% (95% CI: 72−88%) and specificity of 88% (95% CI: 78−94%). Presence of methylated ESR1 in serum of breast cancer pts was associated with ER-negative phenotype ($p = 0.0179$) and presence of methylated 14-3-3σ was associated with T3−4 stage (OMS) ($p < 0.05$) and nodal positive status ($p < 0.05$). We observed that methylated ERS1 was preferably associated with phenotype Basal Like and worse interval progression free and survival global though $p > 0.05$ and HER2+ subtype was correlation with significant more frequent methylation gene ($p < 0.05$). With a median follow up of 6 years, we found that patients with a significant decrease of sera methylated levels of both genes after surgery had better time to progression an overall survival respect patients without this observation
Conclusion: Our study identifies the presence of variations in global levels of methylation promoters genes in healthy controls and breast cancer with different phenotype classes and shows that these differences have clinical significance. In the future this panel of genes detected could be useful as markers for early detection of breast carcinoma and perhaps as a prognostic and predictor factor response to treatment. Although numerous issues remain to be resolved, the quantitative measurement of circulating methylated DNA is a promising tool for cancer risk assessment even this may be of significance in the assessment of targeted therapy resistance related to ER and HER2 status in breast cancer patients

PP83

Implementation and cost effectiveness of intra-operative qRT-PCR analysis of sentinel lymph nodes (SLN) in breast cancer

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Background: Accurate intra-operative SLN assessment enables axillary clearance to be completed immediately in node positive breast cancer patients.
Materials and Methods: An analysis of 254 consecutive patients with core biopsy proven breast cancer who underwent SLN biopsy in a single centre. 99mTc and Patent blue dye guided localisation with lymphoscintigraphy were used to identify SLN's which were harvested. The nodes were sectioned at 2 mm intervals and alternate slices were analysed using an FDA-approved and CE-marked commercial diagnostic RT-PCR assay (GeneSearchTM BLN Assay, Veridex, LLC) for mammaglobin (MG) and cytokeratin 19 (CK19). Remaining slices of node were sent for histological analysis, which included CK19 immunohistochemistry at multiple levels. Whilst the assay was being carried out, the surgeon performed the breast tumour resection. Operative time and hospital bed stay was analysed as part of a health economic evaluation.
Results: A total of 255 SLN in 250 patients including 5 with bilateral breast cancer were evaluated. There were no localization failures. The intra-operative assay showed positive SLN in 72 patients. There was 100% detection of macrometastases within sentinel nodes analysed by GeneSearchTM. Overall concordance between histological nodal status, including micrometastases, and GeneSearchTM analysis was 95% (Sensitivity 96%, Specificity 95%). The assay takes 40 minutes. A health economic evaluation suggests that this assay is cost neutral to the NHS, with substantial benefit
Conclusion: Intra-operative assessment of SLN in breast cancer, using an RT-PCR based assay is a safe, acceptable and accurate technique. This should allow a reduction in the frequency of delayed axillary clearance surgery with minimum unnecessary morbidity and no additional overall cost to the healthcare provider.

PP44

Development of a quantitative scoring algorithm for a Dual-Hapten, Dual-Color ISH assay (DDISH) to determine HER2 gene status

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Background: The HER2 gene, located on chromosome 17, is amplified in ~15−20% of patients diagnosed with invasive breast carcinoma. Determination of HER2 gene status is critical for selecting an appropriate therapeutic course of action, and several assays are commercially available for in situ hybridization-based quantification of the HER2 gene. However, essential data on developing scoring algorithms that maximize precision and efficiency are lacking. A new, fully automated assay (INFORM HER2 Dual ISH DNA Probe Assay) uses Red ISH to detect chromosome 17 and silver in situ hybridization (SISH) to detect the HER2 gene: the staining result is obtained in <12 hours and interpreted using standard brightfield microscopy. This study's goal was to develop a precise yet efficient quantitative scoring algorithm for the HER2 Dual ISH assay.
Materials and Methods: 24 specimens representing the dynamic range of HER2 status were stained with the Dual ISH assay: the cohort was spiked with borderline and low-level amplified cases. Raw counts from 100 nuclei/specimen were obtained and the HER2 status obtained from the 100 nuclei was considered "truth". Multi-stage Monte Carlo statistical methods were used to: (1) vary the distance from the cut-off value of 2.0 and (2) vary the number of nuclei counted. Three goals were to determine: (1) the number of nuclei needed to obtain a "stable" diagnosis; (2) the necessary "grey" zone window; and (3) the number of additional tumor nuclei that should be counted within that window.
Results: Quantification of as few as 10 nuclei resulted in a precise HER2 status diagnosis in cases outside of the 1.8−2.2 equivocal zone. Little statistical gain was achieved by expanding the window beyond the 1.8−2.2 range. Initially counting 20 nuclei for all cases, then counting 20 additional nuclei for equivocal cases resulted in a consistent classification rate of 96%.
Conclusion: The HER2 Dual ISH assay algorithm results in a stable and precise HER2 gene status determination. A combination of 20 nuclei chosen initially followed by an additional 20 nuclei for cases whose HER2/CHR17 ratio falls in the range of 1.8−2.2 is recommended. These data support the clinical need and significance for an equivocal zone within the 1.8−2.2 window.

PP58

Novel potential therapeutic targets for cholangiocarcinoma identified by array comparative hybridization

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Background: Cholangiocarcinoma (CC) is a devastating malignancy, with surgery presently offering the only chance of cure. Conventional chemotherapy is ineffective in CC, with a dismal 5-year survival. We evaluated DNA copy number alterations between intrahepatic (ICC), perihilar (PHCC), and extrahepatic (ECC) cholangiocarcinoma, to identify novel potential therapeutic targets.
Materials and Methods: 22 cases were analysed (7 ICC, 5 PHCC, 10 ECC) using DNA from FFPE archival specimens. Array CGH was performed using 1Mb BAC arrays. Spatial normalisation, circular binary segmentation, calling of gains and losses using the CGHcall method as well as secondary analyses were done within the R statistical language environment.
Results: This study found gains at 17p13.3-q21.33, 17q22-q25.3, and 22q11.1-q13.3 in all cases. Common gains were found in all cases of ICC and PHCC at 11q12.2-q13.4, 19p13.11-p13.3, and 19q13.11-q13.43. Alterations were least frequent among ECC, with no alteration being exclusively seen in all cases of ECC alone. Common regions of alteration detected among ICC and perihilar CC, but not ECC supports the hypothesis that carcinogenesis in these tumours is different, and may potentially require different targeted therapies. The clone covering c-erb-B2 at 17q12 was amplified in all cases of CC, potentially highlighting a role for monoclonal antibodies such as Trastuzumab, or small molecule tyrosine kinase inhibitors such as lapatinib. All cases of ICC and PHCC, and 77.7% ECC showed gain at 11q12.2-q13.4 containing VEGF-B (Vascular endothelial growth factor B) which is known to be overexpressed in ICC and PHCC and therefore may be an attractive target for treatment in these types of CC, possibly being less effective in ECC with this region affected slightly less frequently. This could be achieved using a global VEGF inhibitor such as Cediranib (AZD2171). EGFR is located at 7p11.2, and this region showed gain in 42.9% ICC and 20% PHCC, however no gain was detected in ECC. Alterations of EGFR in ICC and PHCC highlights a potential therapeutic target, for example with Getifinib and Erlotinib, or EGFR/ERB2 inhibitor lapatinib, for tailoring treatment in these patients. This will also enable the identification of patients that will not benefit from this treatment, such as those with ECC, and therefore not be subjected to negative side effects with no benefit.
Conclusion: In this study c-erb-B2, VEGF-B and EGFR have been identified by array CGH as attractive novel therapeutic targets in cholangiocarcinoma.