O-56 Quality assurance issues in breast cancer pathology: a New Zealand teaching hospital experience

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This presentation looks at aspects of breast cancer pathology in the context of aiming to deliver a quality service in a public sector hospital in New Zealand. The quality assurance processes in place and the evidence for them will be discussed and reviewed. An in house HER2 testing audit will also be included and will be discussed with reference to Australasian data.

O-57 An image of cell permeability

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Water molecules are found in different environments in the body such as inside or outside the cells. When trapped inside they can only move within the cells, which make them appear to move slower than water molecules outside the cells. Therefore they have different diffusion coefficients and can be distinguished by a Nuclear Magnetic Resonance (NMR) diffusion measurement.
Our method can be used to determine the time it takes for a molecule to move from the inside of a cell to the outside and vice versa. This is called the exchange time. The exchange time is related to the permeability of the cell.
Our method is the only known non-invasive method to measure the exchange/permeability of molecules through the cell membrane on the timescale for an MRI experiment. The method offers a new way to achieve increased MRI contrast in systems where the studied molecule is not diffusing in the same way throughout the sample, i.e. where the exchange times between intra- and extra cellular compartments are different. It is reasonable to believe that the cell permeability can be correlated to different pathological conditions. Hence, it could be used as a diagnostic tool for a variety of diseases or disorders such as infarct, stroke and tumours.
Experiments have been preformed on breast cells and breast cancer cells. The results show that the method can be applied on the cells of primary interest, human breast cancer cells. Moreover we have identified a significant difference in permeability between normal and cancerous cells.
Future studies will be directed towards tissue samples and in vivo tests on rats. We will also explore the possibility to differentiate between cancer stem cells and cancer cells.

O-58 The UK MARIBS study of MRI breast screening: progress on genetic and density projects

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The MARIBS magnetic resonance imaging (MRI) study compared MRI with X-ray mammography for screening women at high risk of developing breast cancer due to a genetic disposition. This results led to revised NICE Guidelines for management of this group. Continuing studies are documenting the detailed genetic background and family history of the study cohort, and relating these to imaging features such as mammographic density, recall rates and diagnostic pathways. An audit of the family history pedigrees has been performed manually. 692 pedigrees have been collated and all but 18 have been assessed to evaluate study eligibility. There were 140 BRCA1, 70 BRCA2 and 15 TP53 positive or at 50% risk of carrying a mutation in an affected family. There were 442 with a family history of breast or breast and ovarian cancer, and 25 with Li-Fraumini syndrome. All of those from families with a known mutation met the eligibility criteria, 91% of family history and 48% of Li–Fraumini syndrome individuals were eligible. Currently 463 blood samples are undergoing whole gene sequencing. A new method of measuring breast density based on 3D MRI images has been developed. This has been piloted on 61 images from MARIBS and shown to correlate with visual scoring on a 21 point scale ($r = 0.84$, $p = 0.001$) of X-ray mammograms, and with an interactive computer analysis (CUMULUS) of the same mammograms ($r = 0.78$, $p < 0.0001$). This may provide a helpful method of refining breast cancer risk.

O-59 Identification of sub-classes of breast cancer through consensus derived from automated clustering methods

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Gene expression profiling has identified five biologically relevant breast cancer subtypes. A comprehensive definition of these subtypes using simple robust techniques, applicable to routine clinical use, remains to be determined.
We have applied different clustering techniques on protein expression to refine breast cancer subtype characterisation. Five algorithms were used for cluster analysis (Hierarchical, K-means, Partitioning Around Medoids, Adaptive Resonance Theory and Fuzzy C-means) on immunohistochemical scores of 25 proteins determined in primary operable invasive breast carcinoma ($n = 1,076$) prepared as tissue microarray. Conventional statistical techniques were used to derive characteristic biomarkers in each class. Associations between classes and clinical and pathological factors were examined.
A consensus of six distinct classes of breast cancer was determined between clustering techniques. Classes 1 ($n = 202$), 2 ($n = 153$) and 6 ($n = 80$) were characterised by high expression of luminal cytokeratins (ck7/8, ck18, ck19) and ER. Class 1 tumours expressed high levels of c-erbB-3/4, whereas Class 2 over-expressed BRCA1. Whilst Class 1 and 2 had high levels of PgR expression, Class 6 tumours had relatively low PgR expression. Class 3 tumours ($n = 77$) were characterised by c-erbB-2 over expression. Classes 4 ($n = 82$) and 5 ($n = 69$) showed basal cytokeratin expression (Ck14 and ck5/6) but were differentiated by p53. Kaplan-Meier survival analysis showed differences in survival of these groups where Classes 2 and 6 had the best overall survival, whilst Class 3 had the poorest.
In conclusion, we propose there are six biologically and clinically relevant breast cancer subtypes defined using a small panel of proteins determined using simple immunohistochemical techniques.

O-60 The role of primary stromal cell-derived chemokines in the breast tumour microenvironment

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Introduction: It is well established that within the breast tumour microenvironment, neoplastic epithelial cells coexist with stromal fibroblasts. Stromal cells secrete

a variety of chemokines which potentially mediate the reciprocal interactions between breast stromal and epithelial populations. The specific chemokines involved remain to be defined.
Aim: To identify factors secreted by tumour stromal cells and elucidate their potential role within the tumour microenvironment.
Methods: Breast tumour specimens harvested at surgery were separated into epithelial and stromal fractions for culture. Chemokines secreted by stromal populations were detected using Chemiarray™, ELISA and RQ-PCR. Transwell® inserts were used to assess migration of breast cancer epithelial cells (MDA-MB-231 and MCF-7) in response to primary stromal cells.
Results: Tumour stromal cells were shown to secrete a range of chemokines including GRO, IL-6, IL-8 and MCP-1. The level of MCP-1 secreted by tumour populations was significantly higher (mean 951±158 pg/ml) compared to normal stromal cells (mean 366±76 pg/ml). RQ-PCR analysis revealed increased MCP-1 gene expression in tumour relative to normal stromal cells ($p < 0.05$). There were significant increases in migration of both MDA-MB-231 and MCF-7 cells in response to factors secreted by tumour, but not normal stromal cells [range 2–10 fold increase]. Significant inhibition (20–70% reduction) of migration was observed in the presence of monoclonal antibodies to MCP-1.
Conclusion: Stromal cell derived MCP-1 stimulates epithelial cell migration and may play an important role in the breast tumour microenvironment. Increased understanding of the role played by stromal cells in breast cancer progression, may lead to the identification of novel therapeutic targets.

O-61 Reading the prognosis of the individual with breast cancer

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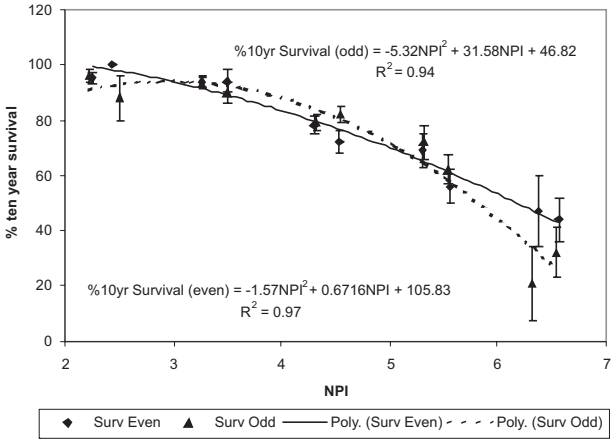
The Nottingham Prognostic Index (NPI) is well accepted and validated. The NPI predicts survival for 6 groups.
Aim: To obtain better survival estimates for the individual than is provided by placement in an NPI group.
Method & Results: Consecutive primary operable breast cancers treated at Nottingham City Hospital 1990–1999. Ten year % survivals plotted for 10 ranges of NPI from 2.0 to 6.9. There is an excellent inverse correlation between median NPI value for each range and survival at 10 years. Rank order is preserved with significant difference between neighbouring ranges.



To enable estimation of survival for all individual values of NPI, a curve fitting technique was applied to these results and gave the formula applied to the individual's NPI score: 10 year survival for the individual = $-3.0079 \times NPI^2 + 12.30 \times NPI + 83.84$. This gave an r^2 of 0.98.
Validity was demonstrated by dividing into odds and evens by case number, with good concordance shown between the two curves produced (Fig).
Example of improved prediction: Two patients with NPI's differing little, of 5.4 and 5.5 lie in different NPI groups (Moderate II and Poor); estimated breast cancer specific 10 year survivals of these groups are 75% and 53% respectively, a 22% difference. Calculations for the individuals from the formula for the individual NPI values gives their survivals as 63% and 60% respectively, only a 3% difference.

O-62 External validation in ONCOPOOL of updated survival according to the Nottingham Prognostic Index (NPI)

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From Nottingham City Hospital (NCH) data the NPI was described and validated in the early 1980s. Case survival has markedly improved and new survival figures for cancers treated in the 1990s NPI groups are presented in this meeting (n = 2235).
ONCOPOOL is a dataset of primary breast cancer assembled as an EC FP5 project in 12 European Breast Units. 6711 cases treated in the 1990s were available for this analysis.

	% Selected		10 Year BCS	
NPI Group	NCH	ONCOPOOL	NCH	ONCOPOOL
EPG	14	19	96±2	94±2
GPG	21	26	93±2	91±2
MPG I	28	27	81±4	84±2
MPG II	22	18	74±4	76±4
PPG	10	9	55±8	53±6
VPG	4	5	38±12	40±8
Overall			77	81±0.4

There are no significant differences in survival in any NPI group between the NCH set and ONCOPOOL nor do overall distributions to prognostic groups differ significantly.
ONCOPOOL gives an excellent intercentre and international validation of the new survival figures according to NPI of women treated to modern protocols.

O-63 Survival in East Anglia according to the Nottingham Prognostic Index

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The E Anglia (EA) (n = 6372), Nottingham City Hospital (N) (n = 2238) and Cambridge Breast Unit data (C) (n = 865) datasets are of women with primary invasive breast cancer aged $\leq$ 70, diam. <5 cm, treated 1998–2003 (EA & C) and 1990–99 (N). The EA set includes the C set.
Analysis according to Nottingham Prognostic Index (NPI) compares the figures in the three datasets. Figures shown in the table are actuarial survival for all causes of death (OS) at 84 months survival.