Conclusion: Mean substitution and multiple imputation performed equally well in dealing with missing data generated by TMA. Complete case analysis, the usual default method for statistical software, resulted in the least accurate and least precise estimates. Given the ease of implementing MS or MI, either approach should be preferred to CCA.

doi:10.1016/j.ejcsup.2010.06.020

O-20 RATES OF GROWTH OF BREAST CANCER

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Many estimates of growth rate have been published, mostly theoretical. Here 3 clinically based measurements have been used to produce curves of growth from inception (one cell) to 7 cm diameter for each grade.

1. Screening effect – In a trial of screening an excess of tumours were screen diagnosed by the end of the trial period. Time for excess to present was calculated as G3 3–6 months, G2 1–2 years and G1 5–6 years. The mean sizes by method of detection allowed volume doubling times to be calculated.
2. Occult time for inception to diagnosis – In young women there is an excess of G3's and few G1's. Assuming that inception by age is constant, graphs of actual detection rates at each age and of expected allows calculation of the times for which each grade is occult.
3. Time from primary treatment to death from breast cancer – Plotting times to death gives medians for G3 167 months, G2 98 and G1 50.
4. As tumours enlarge mitoses become concentrated in the outer 2 mm 'shell'.[1]
5. These observations allow curves of volume to be drawn for each grade from single cell inception from which cell doubling is assumed, giving a curve increasing logarithmically. From 10 mm diameter growth begins to slow logarithmically with cell doubling restricted to the outer shell.

Reference:

1. Connor AJM et al. *Breast* 1997;**6**:171–6.

doi:10.1016/j.ejcsup.2010.06.021

O-21 DETECTION AND QUANTIFICATION OF MicroRNAs IN LASER MICRODISSECTED FORMALIN-FIXED PARAFFIN EMBEDDED (FFPE) BREAST CANCER TISSUES

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MicroRNAs (miRNAs) are a class of endogenous non-coding RNAs that target protein coding mRNAs for cleavage or translational repression. Both profiling and functional studies demonstrate deranged miRNA expression in many human cancers including breast tumours. Research in this field is increasing and the potential of miRNAs for being used in clinical settings emphasises the need for sensitive detection techniques.

In this study, techniques for the analysis of miRNA expression in microdissected FFPE breast cancer tissues were developed and optimised. Full face sections from three invasive breast tumour samples and different microdissected areas (1000–10 million $l\mu^2$) and section thickness (10–20 $l\mu$) were analysed. Total RNA was extracted using commercially available RNA extraction kits (miRNeasy FFPE Kit, Qiagen; RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE, Ambion; PureLink FFPE RNA Isolation Kit, Invitrogen). Three miRNAs (miR21: highly expressed, miR-29c: intermediately expressed, and miR-127: low expression in breast cancer) extracted from both gross and microdissected invasive breast cancer tissues were quantified using real-time PCR.

The PureLink kit produced largest quantities of total RNA from FFPE breast tumours. All three miRNA (21, 29c and 127) were successfully detected by real-time PCR and levels of sensitivity were comparable between extraction methods. Our data showed that relative miRNA levels gradually decreased with diminishing amounts of microdissected tissue used but reliable miRNA quantification was obtained using at least 5 million $l\mu^2$ from 20 $l\mu$ thick FFPE breast tissue sections.

In contrast to previously published results, quantity of miRNA detected in breast tissue samples depends on the amount of tissue used, and cannot be performed reliably from one or a few cells.

doi:10.1016/j.ejcsup.2010.06.022

O-22 A NOVEL ARTIFICIAL NEURAL NETWORK BASED ALGORITHM TO ANALYSE THE INTERACTION PATTERNS EXISTING IN GENE MICROARRAYS: AN APPLICATION TO BREAST CANCER GENOMIC DATA

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Genomic array technologies, by allowing the assessment of the level of expression of thousands of genes simultaneously in the same experiment, have brought the hope to identify new biomarkers related to particular outcome in disease. The majority of the research conducted to date with gene microarrays has only been focusing on this biomarker discovery. However, these arrays hold the inherent information of gene co-expression patterns and only few groups have focused their attention toward deciphering such network of interactions and regulation.

We present here a novel approach based on Artificial Neural Network technology to further analyse the data extracted from gene array experiments. This approach has been applied to a well-known breast cancer dataset publicly available.[1] The results showed interesting patterns of interactions or gene regulation, and some of them could be confirmed by alternative methods,

or previously published work. The algorithm has been subsequently further tested on a second cohort[2] to assess the reproducibility of the approach.

References:

1. van't Veer LJ et al. Gene expression profiling predicts clinical outcome of breast cancer. *Nature* 2002;**415**:530–6.
2. van de Vijver MJ et al. A gene-expression signature as a predictor of survival in breast cancer. *N Engl J Med* 2002;**347**:1999–2009.

doi:10.1016/j.ejcsup.2010.06.023

O-23 IMPRINTED GENE METHYLATION IN BLOOD AND RISK OF BREAST CANCER

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Loss of imprinting is a common observation in tumours but it is not known whether this results from other pathologies or whether imprinting changes may predispose to disease. We set out to determine whether women newly diagnosed with breast cancer had altered imprinted gene methylation in non-tumour tissue (blood cells) compared to women without the disease.

Over 1000 women – newly diagnosed with breast cancer and disease free controls – were recruited at Aberdeen Breast Clinic. A sample of 92 controls and 92 cases were matched for age, weight, height, BMI and menopausal status. Multiple methylation sites were measured in two imprinted genes (IGF2 and PEG3) in blood DNA. Methylation was determined by pyrosequencing using a Qiagen PyroMark MD system after bisulphite conversion of DNA using Epitect Bisulfite kits (Qiagen, Crawley, UK). Analysis of variance was carried out using STATA 11MP (Stata Corp, College Station, USA).

The mean population methylation level was 47.6 (SD 2.5)% for PEG3 and 50.0 (SD 5.5)% for IGF2. Compared to controls, women diagnosed with breast cancer had significantly different levels of methylation in PEG3 ($p < 0.001$). IGF2 methylation was also different between groups but this was only approaching statistical significance ($p = 0.058$). Methylation was not related to menopausal status. These differences in normal tissue suggest that altered imprinted gene methylation may precede the development of the disease. They also point to early life programming as a possible cause of breast cancer.

This study was funded by Breast Cancer Campaign (Project 2008MayPR46).

doi:10.1016/j.ejcsup.2010.06.024

O-24 INVESTIGATION OF BRCA1 AND BRCA2 UNCLASSIFIED VARIANTS USING RNA STUDIES: EXPERIENCES AND INTERESTING CASES FROM THE WEST MIDLANDS REGIONAL GENETICS LABORATORY

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A very successful high throughput screening strategy for the BRCA1 and BRCA2 genes has now been established at the West Midlands Regional Genetics laboratory (WMRGL) since 2006. However having resolved the problems of large backlogs and long reporting times the challenge has now shifted to the prediction of the functional consequence of variants of unknown clinical significance which account for a significant proportion of reported sequence alterations in BRCA1 and BRCA2.

Laboratory methods to identify which of these sequence variants are pathogenic mutations would have utility for counseling and clinical decision making when identified in patients with a family history of breast cancer.

The WMRGL currently undertakes RNA investigations on unclassified sequence variants for several familial cancer disorders for both local and external referrals. To date we have undertaken analysis on 92 cases covering 13 different disorders including non-cancers. RNA investigations specifically for BRCA1 or BRCA2 variants have been performed on 25 samples. An overview of the service will be presented together with results from interesting cases highlighting the challenges faced in interpretation of this data.

doi:10.1016/j.ejcsup.2010.06.025

O-25 ER POSITIVE SCREEN DETECTED BREAST CANCERS (SDBC) DO NOT REQUIRE CHEMOTHERAPY

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Meta-analysis of symptomatic breast cancer trials advises chemotherapy to women <70 years of age at high risk of death (i.e. benefit of >1% survival benefit from treatment) but UK screen detected breast cancers (SDBC) (aged 50–65 years) have an overall 97.2% 5 year relative survival compared to 77.6% for symptomatic cancers. Guidelines recommend chemotherapy for all cancers >10 mm in size (i.e. 35% SDBC) whereas in 2001/2 only 23% SDBC in the UK received chemotherapy.

To determine which women benefit from chemotherapy, we analysed 1681 symptomatic and SDBC in Manchester. SDBC had a lower risk of relapse with 5 year cancer mortality for oestrogen receptor (ER) positive cancers being low in the Excellent (0%), Good (GPG) (1%) and Moderate Prognostic Group 1 (MPG1) (4%) but higher in the symptomatic GPG (4.1%) and MPG1 (15.5%) ($p \leqslant 0.001$). The ABS at BASO Audit 2001/2 indicates 5 year survival improvements in the Moderate Prognostic Group 2 (MPG2) and the Poor Prognostic Group (PPG) for SDBC since 1990.

ER positive SDBC had a <0.6% mortality annually whereas the symptomatic cancers had a 5% annual mortality in the first 5 years. ER positive SDBCs represent a group with a low risk of relapse, not requiring chemotherapy. Improvements in survival of SDBC relate to better treatment of ER negative and HER2