We have assessed HER2 status in a large well-characterised breast cancer series prepared as tissue microarray ($n = 1858$) using IHC (HercepTest, DakoCytomation) and chromogenic ISH (CISH; DuoCISH, DakoCytomation) in order to identify relationships with clinico-pathological variables and patient outcome. None of these cases have received anti-HER2 therapy.

There was excellent overall concordance between HercepTest negative (scores 0/1+) and positive (3+) with CISH positive/negative (defined as HER2/Chr17 copy number ratio of $\geqslant 2$; $p < 0.001$). Twelve percent of cases were identified as HER2 positive (those with 3+ HercepTest scores or 2+ with gene amplification). Of the 74 borderline HercepTest 2+ cases, 44 cases (59%) showed HER2 gene amplification. We identified that HercepTest 2+ non-amplified cases were not significantly different from those amplified 2+ or 3+ cases with respect to their clinical outcome (BCSS and DFS).

The overall concordance between HercepTest and CISH analysis for HER2 status was excellent. All HercepTest 2+ cases identified were observed to have poor outcomes similar to those HercepTest 3+ cases regardless of gene amplification status. In the current clinical environment, cases exhibiting IHC 2+ with non-amplified HER2 gene status are not offered targeted HER2 therapy but do exhibit aggressive clinical behavioural characteristics and therefore optimal treatment strategies for these patients need to be determined.

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O-18 PARP1 EXPRESSION IN HORMONE ESTROGEN RECEPTOR NEGATIVE BREAST CANCER: PREFERENTIAL EXPRESSION IN BASAL-LIKE AND HER2-POSITIVE TUMOURS

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Nuclear poly (ADP-ribose) polymerases (PARPs) are a family of global monitor of chromatin structure and DNA damage repair. The relative expression levels of PARP1 protein in estrogen receptor negative (ER–ve) BC remain unclear. Therefore the aim of this study was to investigate PARP1 protein expression in ER–ve BC with relevance to molecular subtypes and disease outcome.

Methods: PARP1 protein expression was assessed, using immunohistochemistry, in a well-characterised series of ER–ve primary operable invasive BC cases ($n = 251$) with long term clinical follow-up. Results were correlated with molecular and clinicopathological parameters and patients' outcome.

Results: PARP1 nuclear expression was classified as high or low using a cut-off of 80 H-score, determined using X-Tile bio-informatics software. One hundred and thirty five (53.8%) of the informative cases were classified showing high expression. Significant positive correlation was found between PARP1 expression and BRCA1 expression ($p = 0.002$) but no association was found with p53. High PARP1 expression was observed in both basal-like (HER2– and CK5/6 and or EGFR+) (60%) and HER2+ (64%) compared with triple negative (TN) non-basal BC classes (45%). PARP1 expression was significantly associated with BC specific survival ($p = 0.01$). When analysis was assessed by the molecular subtype, the association of PARP1 expression with improved survival was restricted to TN tumours.

Conclusion: We have observed a positive correlation between PARP1 protein expression and BRCA1 expression. Although high PARP1 expression is seen in ER–/HER+ and HER– tumours, its association with survival was only found in the ER–/HER2– subtype. Thus, its targeted inhibition may particularly benefit patients with TN BC.

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O-19 COMPARISON OF IMPUTATION METHODS FOR MISSING IMMUNOHISTOCHEMICAL MARKERS IN A STUDY OF BREAST CANCER PROGNOSIS

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Background: Tissue micro-arrays (TMA) are increasingly used to generate data for studies of tumour molecular phenotype however, TMA data are particularly prone to missingness. A variety of methods to deal with missing data are available; these have been extensively evaluated using simulated data, but there has been no empirical evaluations of these methods using real TMA data.

Methods: We pooled data from over 11,000 cases of invasive breast cancer from five studies that collected information on seven prognostic indicators including four molecular markers, together with survival time data. We compared the results of a multi-variate Cox regression using complete case analysis (CCA), mean substitution (MS) and multiple imputation (MI). We also performed an analysis in which missing data were simulated under different assumptions.

Results: Over half the cases had data on at least one of the seven variables and 10.5% had missing data on 4 or more. The hazard ratio estimates based on multiple imputation and mean substitution, were similar with similar standard errors. Hazard ratio estimates and confidence intervals (Table). The results from CCA were less precise (wider confidence limits). Accuracy of the estimates was based on the simulated data with CCA having the least accurate results.

Hazard ratios and confidence intervals using mean substitution and multiple imputation.

Method	ER status	PR status	HER2 status	BCL2 status
Multiple imputation – HR (CIs)	0.43 (.35–54)	0.39 (.30–.50)	1.25 (1.13–1.40)	0.85 (.76–.96)
Mean substitution – HR (CIs)	0.40 (.32–.49)	0.37 (.29–.46)	1.31 (1.17–1.46)	0.82 (.74–.92)

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.

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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.

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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.

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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,