**Friday, 22 March 2002** **16:30–18:00**

PROFFERED PAPERS

# Molecular markers I

**438** ORAL

## Identification of over-expressed polypeptides in breast cancer using proteomic technology

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**Introduction:** This project seeks to elucidate the differential expression of polypeptides in breast cancer. Tissue samples are obtained from public and private hoapitals in Hobart, Tasmania, and reagents and other materials provided by Bio-Rad and Sigma Laboratories.

**Method:** Patients diagnosed with breast cancer, and patients undergoing reduction mammoplasty are recruited to provide malignant and normal tissue for analysis, respectively. The study uses two dimensional gel electrophoresis to resolve complex protein mixtures into individual protein spots on a poly-acrylamide gel matrix. A biological map of all protein molecules that make up breast cancer and normal breast tissue is constructed using gel analysis software (PDQuest from Bio-Rad) and compared. Outstanding spots are excised and subjected to mass spectrometric analysis in collaboration with the Australian Proteome Analysis Facility (Macquarie University, Sydney).

**Results:** Preliminary results indicate the differential expression of 18 hydrophobic polypeptide spots of varying pI (Iso-electric point) and molecular weight. Of these, strongest signal intensity was obtained from spots with a pI range of 5.2-7.8 and molecular weight range of 24.4-55.1 kDa. These may represent proteins that are differentially expressed in breast cancer.

**Conclusion:** The identification and isolation of over-expressed polypeptides will enable the development of potential / therapeutic targets following exclusion of the currently known markers of the disease by antibody labelling.

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## The cytoplasmic localisation of p21WAF1/CIP1 in breast cancer associates with c-erbB2 expression.

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p21WAF1/CIP1 inhibits cyclin dependent kinases (CDKs) to influence cell cycle progression. Genotoxic activation of the p53 tumour suppressor upregulates p21WAF1/CIP1 to re-inforce cell cycle arrest and DNA repair. Nuclear localisation of p21WAF1/CIP1 underlies these tumour suppressor functions but recent evidence suggests that cytoplasmic p21WAF1/CIP1 in cancer tissues and cell lines may inhibit apoptosis1, and independently predict prognosis2. C-erbB2 expressing cell lines have been shown to mislocalise p21WAF1/CIP1 to the cytoplasm through phosphorylation of its nuclear localisation signal3. This study investigates the association between c-erbB2 expression and the subcellular distribution of p21WAF1/CIP1 in breast cancers using immunohistochemistry, and examines their relationship to prognosis.

**Methods:** 67 patients with unilateral, non-metastatic disease comprised 66% node-negative and 34% node-positive women. Median follow-up was 65 months. C-erbB2 expression was determined using the DAKO Herceptest and CB11 monoclonal antibody, and categorised as recommended into negative (0, 1+) and positive (2+, 3+). Nuclear and cytoplasmic p21WAF1/CIP1 were graded to produce an intensity distribution score2.

**Results:** C-erbB2 was expressed in 13/67 (19%) of tumours, and was associated with reduced OS (P = 0.03) and RFS (P = 0.004). C-erbB2 expressing tumours showed almost exclusive cytoplasmic p21WAF1/CIP1 staining (P = 0.005), compared to nuclear and cytoplasmic p21WAF1/CIP1 in c-erbB2 negative cancers, with no association between nuclear p21WAF1/CIP1 and c-erbB2 expression (P = 0.05).

C-erbB2 as a predictor of poor prognosis in breast cancer, may relate to its ability to influence the relocalisation of p21WAF1/CIP1from the nucleus to the cytoplasm, resulting in a loss of its tumour suppressor functions.

**References**

[1] Asada M et al. (1999) EMBO J 18(5): 1223-1234.
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## BRMS-1 expression in human breast carcinoma

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**Introduction:** A novel breast cancer metastasis suppressor gene (BRMS-1) has recently been identified on chromosome 11q13. This work has been carried out on breast cancer cell lines. In Vitro, Using MDA-MB-435 human metastatic breast cancer cell lines, mice injected with BRMS-1 transfectants develop fewer metastases compared to mice injected with parent MDA-MB-435 cells, indicating a role in cancer suppression. To date, this breast cancer metastasis suppressor gene has not been evaluated in human breast cancers. In our study we aimed to measure the expression of BRMS-1 in human breast cancer specimens, normal tissues, fibroadenomas and nodal metastases and correlated this with conventional pathological and biochemical prognostic indicators.

**Methods:** RT-PCR was performed using primers specific for BRMS-1, and GAPDH control. Optical densities were measured using the Eagle Eye densitometry and arbitrary units applied. A ratio of BRMS-1 to GAPDH was calculated and used in subsequent statistical analysis. Mann Whitney, Spearman Rank and Chi squared tests were used for analysis of non-parametric data.

**Results:** Normal breast tissue(n=6), percentage positivity was 100% and mean expression 0.25 units. Fibroadenoma (n=16), percentage positivity was 75%, mean expression 0.39 units. Primary Tumour(n=84) percentage positivity was 78.8% and mean expression was 0.43 units. Nodal Metastases(n=9), percentage positivity was 66.6% and mean expression 0.55 units. The difference in expression between tissue types was not statistically significant. There was no statistically significant correlation between BRMS-1 expression and tumour size, grade, histology, nodal status, or oestrogen and progesterone receptor status.

**Conclusion:** BRMS-1 has been reported to have a role in the suppression of metastases in a murine model. In women with breast cancer we have found no evidence to support this gene functioning as a suppressor of metastases.

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## Use of reverse transcription pcr to detect cytokeratin 19 expression in sentinel nodes of patients with breast cancer

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**Background:** While sentinel node biopsy is becoming increasingly accepted in the management of breast cancer, the optimum pathological handling of the sentinel node has not been established. Upstaging of sentinel nodes by serial sectioning and immunohistochemistry have been shown to increase the sensitivity of detection of nodal micrometastases. RT-PCR is potentially able to identify individual cells and thereby increase further the sensitivity of sentinel node detection. We have used RT-PCR on a series of sentinel nodes to look at the expression of the epithelial marker cytokeratin 19 (CK19) as a potential means of upstaging the nodes.

**Methods:** All patients had a solitary biopsy-proven invasive primary breast cancer and the sentinel nodes were detected in the standard way using a combination of radioactive colloid and blue dye. Once harvested, the nodes were bivalved and half the node analysed by routine paraffin histology. The other half was immediately frozen to -80c for subsequent RT-PCR analysis. RNA was extracted from the nodes using Qiagen RNeasy kits. Reverse transcription PCR was then carried out to detect the expression of CK19.

**Results:** 67 sentinel nodes were harvested from 54 consecutive patients. Paraffin histology was positive in 8 nodes from 6 patients. All of these nodes were CK19 positive by RT-PCR. A further 15 nodes from 14 patients were CK19 positive and so therefore 29% of patients with H&E negative sentinel nodes were upstaged by RT-PCR. In total 24% of all patients had a sentinel node positive only by RT-PCR and had no other nodes involved.

**Conclusion:** CK19 RT-PCR upstaged 29% of patients. In 24% of patients in this series this was the only positive nodal finding. The sensitivity of RT-PCR is clearly superior to routine paraffin histology. However, since RT-PCR may detect micrometastases of only a few cells the clinical significance of RT-PCR detected disease is currently unclear. Further evaluation of these nodes by immunohistochemical staining for CK19 may increase our understanding of the role RT-PCR may play in the upstaging of patients undergoing sentinel node biopsy.

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## Potential role of serum CYFRA 21-1 as a tumor marker for breast carcinoma

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**Rationale:** The use of tumor markers in the management of breast cancer has been under debate for some time. However, the American Society for Clinical Oncology (ASCO) stated in its clinical practice guidelines for the use of tumor markers in breast carcinoma that neither CA15.3 nor CEA is recommended for routine use because current literature data are insufficient.

The use of several other markers has been advocated to give additional information to the clinicians. Among these are the soluble products of cytokeratins 8, 18 and 19. Breast carcinoma cells have been demonstrated to express cytokeratin 19 fragments in primary and metastatic lesions. CYFRA21-1 is a monoclonal based assay measuring soluble fragments of cytokeratin 19. We studied the significance of CYFRA21-1 in patients with primary breast carcinoma radically resected and metastatic disease by a comparison with the serum CEA and CA15-3 titers tested in the same samples.

**Materials and Methods:** The serum samples of 123 patients with primary breast carcinoma (39 with metastatic disease) were provided for measurements of CYFRA21-1, CEA (carcinoembryonic antigen) and CA15.3. We observed the relationship between the stage of the disease and the serum levels of these three markers.

**Results:** The sensitivity of CYFRA 21-1 for patients with metastatic breast carcinoma was 56%, the specificity was 100% and the positive predictive value was 100%. The chi-square test was 14.9 for CEA with a p test of 0.0001, for CA15.3 chi-square was 26,5 with a p test $< 0.0001$, for CYFRA21.1 chi-quare was 53,9 with a p test $< 0.0001$.

**Conclusions:** Our results, even if on a small population, show that the seric CYFRA21-1 titers may be useful in monitoring patients with breast carcinoma. Our study is ongoing to confirm the role of this marker in breast carcinoma.

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## Chemical ovarian ablation upregulates HER-2/neu: serum results of a randomized trial comparing leuprorelin acetate to CMF for adjuvant therapy of nodal-positive breast cancer

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The expression of HER-2/neu is modulated by blockade of an estrogen-sensitive binding site at the promoter. We investigated whether chemical castration by adjuvant therapy of breast cancer leads to an upregulation of HER-2/neu using the extracellular domain of HER-2/neu in serum as surrogate marker. The hypothesis was investigated in a randomized, multicentre trial for nodal positive, hormone-receptor positive, pre- or perimenopausal patients (pts) who were treated with leuprorelin (11.25 mg every 3 months (mo) via s.c. injection over 2 years) or CMF chemotherapy (cyclophosphamide 500 mg/m2 days 1+8, methotrexate 40 mg/m2 d1, 5-FU 600 mg/m2 d1, q4w) for 6 cycles. Serum samples (80 pts in the leuprorelin arm, 53 pts in the CMF arm) were analyzed at baseline and at mo 3, 6, 12, 18 and 24 plus at 6 mo follow-up upon conclusion of leuprorelin therapy. The shed antigen of HER-2/neu was measured using a standardized ELISA assay with a cut-off for normal of 15 ng/ml. The classical tumor marker CA27.29 was evaluated in parallel at all time points. Results were correlated to suppression of estradiol as an indicator of chemical castration. Estradiol decreased promptly in both treatment groups and reached lower levels in the leuprorelin arm. Serum HER-2/neu levels increased significantly in comparison to baseline in both arms from 8 to 11 ng/ml (p=0.01) without any difference between the groups. The most significant increase occurred within the first 6 mo and remained at that level until mo 24. In only 2.5% (15/587) of HER-2/neu measurements, the HER-2/neu result was greater than 15 ng/ml, confirming the cut-off of normal irrespective of menopausal status. After 6 mo of follow-up, the HER-2/neu level started to decrease again in the leuprorelin arm, preflecting reversible castration and estradiol reconstitution. CA27.29 levels did not show the same pattern of gradual increase over the 24-mo period as HER-2/neu, indicating that HER-2/neu changes were of regulatory nature and did not reflect increasing residual disease. These data prove the upregulation of HER-2/neu as measured by the serum antigen right at the time when postmenopause is chemically induced. The results fit with data showing that HER-2/neu (measured by immunohistochemistry on the primary tissue) is significantly upregulated during the bleeding phase of the menstruation cycle (Menard et al., 1999). We must consider that tissue results may be misleading for later choice of trastuzumab therapy.

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## Higher plasma vascular endothelial growth factor levels correlate with menopause, over-expression of p53, and recurrence of breast cancer

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**Purpose:** Vascular endothelial growth factor (VEGF) is one of the important factors involved in this angiogenesis. Many studies have reported that the expression of VEGF in breast cancer is an unfavorable prognostic factor. However, there are few studies that have analyzed blood VEGF levels, and most of them used serum containing VEGF. It is necessary to measure plasma VEGF levels in order to evaluate the level of tumor-derived VEGF. In this study, we measured plasma VEGF levels in various breast diseases and evaluated the clinical significance of its levels.

**Methods:** We analyzed 15 patients with benign breast disease, 187 patients with primary breast cancer, 32 patients with no postoperative recurrence, and 56 patients with recurrence, all of whom were treated in the Kumamoto City Hospital, Department of Surgery, between November 1999 and May 2001. Plasma VEGF levels were measured by an ELISA method with a detection sensitivity of 15.6 pg/ml or higher.

**Results:** The mean plasma VEGF level of 25.7 pg/ml in patients with primary breast cancer was significantly higher than that of 16.0 pg/ml in patients with benign disease. In breast cancer patients, VEGF levels were clearly low in those with no postoperative recurrence, high in those with recurrence, and still higher in patients with distant metastasis. In patients with primary breast cancer, VEGF levels were high in postmenopausal patients, patients of advanced age, and p53-positive patients. In regard to postoperative prognosis, postmenopausal patients with high levels of VEGF had a significantly low disease-free survival rate, although the follow-up period was short. In terms of survival rates, relapsing patients with high levels of VEGF clearly had an unfavorable prognosis.

**Conclusion:** Plasma VEGF levels were higher in malignant than in benign breast disease, and were also high in patients with recurrence or distant metastasis. These findings suggest that plasma VEGF levels reflect the extent or metastasis of malignant lesions more accurately. In patients with primary breast cancer, plasma VEGF levels were high in patients with p53 overexpression and in postmenopausal patients, and the prognosis was associated with VEGF levels only in postmenopausal patients. These results suggest that VEGF is involved in angiogenesis in postmenopausal rather than in premenopausal patients.

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## Loss of ar expression in BRCA1 mutated breast tumours

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The world-wide incidence of breast cancer is about one million new cases per year. About 5-10% of breast cancers are hereditary; a genetically and clinically heterogeneous disease in which several susceptibility genes, including BRCA1&2, have been identified. BRCA1 is involved in a broad range of cellular activities that include transcriptional control and DNA repair. Recent studies showed that BRCA1 might function as a transcriptional coactivator.

We have used a high density membrane based array (containing 588 cDNA's) for screening of RNA isolated from frozen breast tumour samples of BRCA1 and sporadic tumours. With the algorithms and software described by Eisen we were able to distinguish subgroups of sporadic cases and a group consisting mainly of BRCA1-mutated breast tumours. This analysis also revealed a cluster that differentially expresses genes related to cell substrate formation, adhesion, migration and cell organisation in BRCA1-mutated tumours compared to sporadic breast tumours. Interestingly FGF-8, formally known as androgen induce growth factor, was not expressed in BRCA1 mutated tumours but highly expressed in all but three sporadic tumours. This factor has been described to be regulated by androgens. This prompted us to study the androgen receptor (AR) expression in BRCA1 and -2 and sporadic tumours.

Paraffin embedded tumours of women with verified Dutch founder BRCA1 mutations (n=40, median age of patients was 40 years[range 25-59 years]at time of surgery) or BRCA2 mutations (n=13, median age of the patients was 42 years [range 31-85 years] at time of surgery) were sectioned. The 4 micron sections were analysed for ER,PR,P53 and androgen receptor (AR) expression using commercially available antibodies and standard immunohistochemical techniques.

As expected ER expression was observed in 10 out of 39 tumours, PR in 5 out of 39 and P53 overexpression in 27 out of 40 BRCA 1 mutated tumours. Intriguingly AR was only expressed in 5 out of 40 BRCA1 mutated tumours, with mutations mainly located at the C-terminus. In contrast, the AR was expressed in 75% of control sporadic breast tumours. In BRCA2 mutated tumours these factors were expressed in half of the tumours studied. Thus the set of aberrantly expressed genes characteristic for BRCA1 mutated tumours can be expanded with another steroid hormone receptor, i.e. the loss van androgen receptor expression.

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## Effect of continuous combined HRT on apoptotic cell death of human breast cancer cells in vitro

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**Introduction:** Women treated previously for breast cancer should not use unopposed low dosages of estrogens, however epidemiological, laboratory and clinical studies suggest that certain combinations of estrogens and progestogens are more likely to be benefical than harmful.

In vitro and in vivo studies showed that unopposed estrogens induces proliferation while progestogens promote apoptosis in human breast epithelial and breast cancer cells.

The balance between programmed cell death (apoptosis) and cell proliferation determines tumor growth rate and any alteration between the two factors may be a key element for the uncontrolled expansion of malignant tumors.

**Material and Methods:** 17$\beta$-estradiol and dihydrodydrogesterone, the major metabolite of dydrogesterone, were administered in concentrations of 10 -6 M for 4, 24, 48 and 144 hours in vitro to MCF-7 cells, an estrogen receptor positive human breast cancer cell line. Apoptosis was determined by the Nicoletti technique and performed in duplicate. Activation of endonucleases results in the breakdown of DNA. The DNA double strands were labelled with Propidium Iodide (PI) and measured using fluorescence. Decreased fluorescence is the result of leakage of DNA fragments from the nuclei of the cells.

Proliferation was determined by measuring the expression of Mucine 1 (Muc 1) and cyclin D1, both overexpressed in malignant cells, using the quantitative RT-PCR technique. Ki67, a nuclear antigen related to proliferative activity, was measured by flowcytometry.

**Results:** The incubation of the continuous combined preparation of 17$\beta$-estradiol and dydrogesterone demonstrated after 144 hours a significant reduction of proliferation and increase of apoptosis of human breast cancer cells (MCF-7) in vitro.

**Conclusions:** Continuous combined HRT with 17$\beta$-estradiol and dydrogesterone might reduce relapses of breast cancer and is also the treatment of choice in hysterectomized women. To days error (HRT and breastcancer), is tomorrows treatment.

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## Insulin-like growth factor and its receptor MRNA expression in breast cancer

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**Background:** Insulin-like growth factor 1 (IGF-1) is thought to be one of the non-hormonal survival signals for breast cancer with its effects mediated by the IGF-1 tyrosine kinase receptor. Increased circulating levels of IGF-1 are associated with an increased risk of breast cancer. This study investigated the relationship between the expression of IGF-1 and IGF-1 receptor (IGF-1 R) mRNA levels in breast cancer specimens and adjacent non-cancerous tissues (ANCT).

**Methods:** The specimen tissues (25 tumours and 23 ANCT) were homogenised; RNA extracted and converted to cDNA. mRNA levels were measured using a Taqman RT-PCR machine.

The relative levels of mRNA expression were calculated compared to the level of expression of a housekeeping gene (ribosomal mRNA). The association between the mRNA in cancerous and ANCT was calculated by a Mann -Whitney U test. Spearman's rank correlation was used to examine the relationship between mRNA expression in tumour samples and patients' age, tumour size, histopathological grade, oestrogen and progesterone receptor status, lymph node status, lympho-vascular invasion and prognosis.Results

The median values of relative expression of IGF-1 mRNA were 0.27 (0.08-0.61) and 0.33 (0.11-0.71) in tumour and ANCT respectively. The IGF-1 receptor values were 0.16 (0.06-0.37) in tumour specimens and 0.15 (0.00-0.61) in ANCT.

The p-values for IGF-1 and IGF-1 R were 0.347 and 0.627 respectively showing no relationship between tumour and ANCT.

No correlation was found between IGF-1 and IGF-1R mRNA expression in tumour specimens and the clinicopathological parameters assessed (p-values from 0.1-0.966)

**Conclusion:** The mRNA expression of IGF-1 and IGF-1R seems to be upregulated in both tumour and ANCT suggesting that IGF-1 plays a role in mammary carcinogenesis via autocrine and paracrine modes of action. In established breast cancer, the levels of IGF-1 and IGF-1R mRNA expression does not seem to be associated with standard prognostic parameters.

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## mRNA expression of cyclo-oxygenase-2(cox-2) and vascular endothelial growth factor(VEGF)in human breast cancer.

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**Background:** There is a growing body of evidence that cyclo-oxygenase 2 (COX-2) plays an important role in carcinogenesis and angiogenesis of human tumours.

**Objectives:the** present study aims to compare COX-2 expression in human breast cancer and adjacent non-cancerous tissue (ANCT), and to identify any correlation between COX-2 and VEGF expression.

**Methods:** Total cellular RNA was extracted from frozen breast tissue samples according to standard methodology. The mRNA copy numbers for COX-2 and vascular endothelial growth factor 189 (VEGF-189) were determined in 40 infiltrating carcinomas and 40 matched ANCT specimens using quantitative RT-PCR and TaqMan methodology.

**Results:** The COX-2 mRNA copy number per $\mu$g of RNA was 2 fold higher in ANCT compared with the cancerous tissue (p=0.01). Median mRNA copy number was $5.44 \times 10^6$ for ANCT and $2.30 \times 10^6$) for tumour (ANCT range: $1 \times 10^6$ - $4.12 \times 10^7$; tumour range: $1.29 \times 10^5$ - $1.07 \times 10^7$).

There was a significant correlation between COX-2 and VEGF-189 mRNA copy numbers in the cancer specimens (correlation coefficient = 0.5528, p = 0.0076).

**Conclusions:** COX-2 is over-expressed in both human breast cancer and ANCT. The level of Cox-2 expression is significantly higher in the matched ANCT, which suggests that paracrine effects may be important in the action of COX-2. Furthermore, our results indicate that in human breast cancers COX-2 over-expression is linked to VEGF-189 over-expression, and therefore tumour angiogenesis.

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## Effect of surgery in serum platelet endothelial cell adhesion molecule-1 (sPECAM-1) levels in breast cancer (BC) patients

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sPECAM-1, is an early and sensitive marker for tumor-induced angiogenesis and metastasis. Few studies have evaluated the role of sPECAM-1 in BC progression with inconsisted results. We examined the levels of sPECAM-1 in BC patients (pts) pre- and post-surgery and compared them with various prognostic parameters. Serum levels of sPECAM-1 were measured by ELISA in 18 lymph node negative (Group A); 17 lymph node positive (Group B); 8 pretreated (Group C) BC pts and in 20 pts with benign breast lesions (Group D) before and 6 days after surgery. Pts receptor status histological grade and stage were also considered. Our results are presented on Table.

| | | sPECAM-1 Concentration (ng/ml) | | [sPECAM-1] decreased | |
|---|---|---|---|---|---|
| Groups | n | Pro Surgery (Pr.S) | Post Surgery (P.S) | n | % P.S |
| | | Mean ± SD | Mean ± SD | | |
| A* | 18 | 16.78 ± 7.18 | 11.76 ± 4.80 | 13 | 72.2 |
| B | 17 | 16.27 ± 7.50 | 13.29 ± 8.11 | 12 | 70.58 |
| C | 8 | 13.65 ± 5.55 | 11.56 ± 5.57 | 6 | 75 |
| D | 20 | 15.06 ± 4.81 | | | |

*Presurgical* sPECAM-1 levels do not provide valuable diagnostic information. No correlation was found with tumor size, nodal status, histologic grade or receptor status. The ranges of sPECAM-1 levels of different groups of BC pts partially overlapped with a significant fraction of pts with benign lesions. *Surgery* decreases the mean levels of sPECAM-1 in all groups but this decrease is statistically significant only in negative lymph node BC pts (*p = 0.00619). Further more in each group there were a few cases with increased sPECAM-1 levels. The differences of pro- and post surgery sPECAM-1 levels might have prognostic value but this cannot be resolved by this study.

The authors like to thank Mrs. G. Barlagianni & S. Foka for their valuable assistance.

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## Usefulness of bone metabolic markers in breast cancer with bone metastasis (BM)

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The purpose of this study was to evaluate the meaning and clinical value of bone metabolic markers in BM from breast cancer. We investigated serum carboxyterminal telopeptide of type I collagen (ICTP, ng/ml), tartrate resistant acid phosphatase (TRACP, IU/l) and urinary type I collagen cross-linked N-telopeptides (NTx, nM BCE/mmol Cr) as potential makers. These bone markers were evaluated in 233 breast cancer patients; 145 patients without known metastasis (Group A), 44 patients with metastasis at sites other than bone (Group B), 34 patients with BM (Group C). The mean value of every marker in Group C was significantly increased than that in Group A and B, there was no difference between Group A and B in any markers. Group C consisted of the patients with varying degrees of BM and variation in their treatments. The patients in Group C were divided into two groups: BM+ from patients with one to three BM sites (n= 23), BM++ from patients with more than four BM sits (n=11). The mean values of TRACP in Group A, BM+ and BM++ were 8.0 ± 1.6, 9.6 ± 2.5 and 12.9 ± 4.8 (mean ± SD), respectively. There was a significant difference only in TRACP between Group A and BM+ ($p<0.01$). TRACP were elevated in proportion to the number of BM. What is more, the mean value of TRACP in patients with first recurrence as BM without treatment (n=14) was significantly increased than that of Group A ($p<0.01$). In the treated patients of Group C, the mean values of every marker with responder and stable disease of BM (n=5) were significantly lower than those with progression (n=11).

We concluded that bone metabolic markers were specifically increased in BM of breast cancer and TRACP was especially useful makers in diagnosis and evaluation with treatment of BM.

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## Syk expression in human breast cancer

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**Background:** Syk (Splenic Tyrosine Kinase) is a non cellular receptor protein kinase involved in cell proliferation, differentiation and phagocytosis. It has been studied in T and B lymphocytes, NK cells and platelets but there have been very few studies about its role in breast cancer with conflicting results. This study aims to investigate the hypothesis that Syk expression is down-regulated in breast cancer compared with ANCT and the association between its expression and clinicopathological parameters.

**Materials and methods:** mRNA was extracted from breast cancer specimens (n=25) and adjacent non-cancerous tissues (ANCT) (n=23). Relative Syk to ribosomal RNA expression was determined by RT-PCR and Taqman methodology. Mann-Whitney U test was used to examine the association between Syk expression in cancer and ANCT. Spearman's rank correlation test was used to examine the association between Syk expression in tumours and patients' age, tumour size, tumour grade, estrogen and progesterone receptor status, lymph node metastasis, vascular invasion and clinical outcome.

**Results:** The median for the relative value of Syk expression was 0.17 and 0.18 (range: 0.12 - 0.56 and 0.0 - 1.77) for tumours and ANCT respectively. There was no significant association between Syk expression in cancers and ANCT (p= 0.598) nor between Syk expression in tumours and patients' age, tumour size, tumour grade, estrogen and progesterone receptor status, lymph node metastasis, vascular invasion or prognosis.

**Conclusions:** This study shows that Syk mRNA expression does not seem to vary between breast tumour tissues and ANCT. Furthermore, we observed no significant association between Syk expression and clinicopathological parameters.

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## Analysis of env MMTV-like sequences expression in T-cells and possibility for early human mammary carcinoma diagnostics

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Earlier we have found that expression of env MMTV-homologous sequences in T-lymphocytes is highly specific for human mammary carcinoma (MC). It occurres in 75.2% of MC patients. By analogy with mice, the expression in human T-cells might to be important in transmission and dissemination of a factor involved in carcinogenesis.

To clear a possible way of induction of the expression we use T-cell cultures from 11 healthy men and women and analysed cDNA transcripts by RT-PCR using primers for gp52 coding area of env MMTV gene. All the samples, except one from women, were negative by RT-PCR. However, PCR-products were revealed in 7 from 11 samples after 5-aza-cytidine treatment of T-cell cultures. The gp52-related human antigenes have been found by indirect immunofluorescence assay using anti gp52 serum in the same samples treated by 5-azaC. So, these sequence expression is activated by promoter demethylation. Normally the expression in donor T-cells is supressed. The occurrence of the sequence expression in one female donor before demethylation by 5-azaC is indicative of MC risk. A preliminary sequencing of 280bp RT-PCR product shows its significant homology to env MMTV. This and specific 650bp PCR-product, obtained earlier, seems to be perspective for MC screening. The data reported support mouse model of MC in human. It allows to use PCR/PT-PCR of T-cell RNA/DNA together with indirect immunofluorescence method for MC risk diagnostics.

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## Apoptosis and disease course in Indian breast cancer

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Several studies have documented a possible role for programmed cell death (PCD) or apoptosis in the development, progression and response to treatment of malignant tumors. To assess the significance of PCD in breast cancer for apoptosis, apoptotic regulatory proteins p53 and bcl-2, presence of mutant p53 and expression of Ki 67, we analysed 168 patients reported at Regional Cancer Centre, Trivandrum. The control group was normal breast

issue taken from excised benign breast lesions. A growth index was created by evaluating the total tumor proliferating compartment and extent of cell loss by both apoptosis and necrosis. All patients had stage 1 to 3 disease, primary surgery with or without adjuvant chemotherapy or radiotherapy and were on tamoxifen. Significant correlation (correlation coefficient r =0.49) was observed between high apoptotic index and poorly differentiated tumors with high Ki 67 and p53 immunoreactivity. Increased apoptotic index (r=0.23, p =0.03) was associated with larger tumor size, positive lymph nodes and advanced TNM stage at presentation. A balanced growth rate was observed in control breast tissue while malignant tumors often had a positive growth rate. Low apoptotic index (p =0.01), bcl-2 expression (p= 0.0000), well differentiated tumors (p = 0.0000) and tumors of small size (p = 0.002) were significant predictors for better prognosis in terms of both disease free and overall survival. Presence of mutant p53 was associated with poor prognostic factors such as estrogen receptor negativity and lymph node positivity and increased proliferation rates. In summary, these results imply that rapidly proliferating tumors appear to have a high 'cell turn over state'in which there may be increase chance of apoptosis amongst the proliferating cells.

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## Cytokeratin profile of invasive ductal and lobular breast cancers

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**Introduction:** Cytokeratins are tissue specific intermediate filaments present in epithelial cells. Breast tissue contains basal and luminal epithelial cells. Invasive breast cancers arising from basal epithelial cells are thought to be more aggressive. The aim of this study was to correlate the cytokeratin profile with histological grade of cancer.

**Materials & Methods:** Paraffin embedded tissue sections from 33 patients with invasive breast cancer were immunostained for basal (cytokeratin 14) and luminal epithelium (cytokeratin 8) specific keratins with monoclonal antibodies LL002 and NCL CK8 respectively by the indirect immunoperoxidase technique.

**Results:** 82% of invasive ductal cancers and 67% of lobular cancers expressed cytokeratin 8 while only 14% of ductal and none of lobular cancers expressed cytokeratin 14. There was no correlation between cytokeratin 8 expression and histological grade of the tumour, however, cytokeratin 14 was expressed only by poorly differentiated ductal cancers.

**Conclusion:** We conclude that majority of breast cancers arise from luminal epithelial cells. Differential cytokeratin expression is seen with increasing histological dedifferentiation.

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## Expression of cyclins D1, p21 and p53 oncoprotein: correlation with mammographic appearance in non-palpable and palpable T1N0 breast carcinomas

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**Purpose:** Study of cyclins D1, p21 and p53 oncoprotein expression in non-palpable and palpable T1N0 breast carcinomas and evaluation of possible correlation with mammographic appearance.

**Material and Methods:** Thirty nine T1N0 and 33 non-palpable patients with breast carcinomas were retrospectively evaluated. Twenty seven out of 39 T1N0 breast carcinomas showed mammographic density and 12 of them had density and malignant microcalcifications as mammographic appearance. Mammography of the non palpable breast carcinomas revealed density in 9/33, malignant microcalcifications in 15/33 and density with malignant microcalcifications in 9/33 carcinomas. In all of them immunohistochemistry was conducted.

**Results:** In 7 out of 12 (60%) T1N0 carcinomas with mammographic density with malignant microcalcifications positive immunoreactivity for cyclin D1 was found. In 7 out of 9 (77%) non-palpable breast carcinomas with positive immunoreactivity for cyclin D1 mammography showed malignant microcalcifications or density and malignant microcalcifications. Cyclin p21 immunoreactivity was found in 6/33 (18%) non-palpable carcinomas and all of them showed malignant microcalcifications as mammographic appearance. In T1N0 carcinomas p21 expression was detected in 6/39 (15%) patients while no correlation was found with mammographic features. In 14/33 (42%) non-palpable carcinomas p53 overexpression was detected. Eight out of these 14 patients (57%) showed density or density and malignant microcalcifications in mammography. In T1N0 carcinomas p53 overexpression was detected in 12/39 (31%) carcinomas and no correlation was found with mammographic appearance.

**Conclusion:** Cyclin D1 expression in non-palpable and T1N0 breast carcinomas is correlated with mammographic density or density with malignant microcalcifications. In non palpable breast carcinomas p21 expression and p53 overexpression is correlated with mammographic density or density with malignant microcalcifications.