correlation ($p = 0.04$) was observed between expression p65 and a low grade I and II of malignancy according to Bloom and Richardson. Estimation of p65 antigen may be usefull in the identification of precancerous changes and more differentiated ductal carcinoma of the breast. The results indicate the possibility for p65 to be helpfull in the screening examinations of women who have a high risk of cancer development.

**502** POSTER

### Prognosis of the typical medullary carcinoma of the breast

A. Stelmach, K. Herman, J. Mituś. *Cancer Centre, Kraków, Poland*

**Purpose:** Medullary carcinoma is very rare type of breast cancer. It's suspected, that prognosis in typical medullary cancer greatly differs from other NOS types.

**Methods:** A study was carried out based on 52 women with typical medullary carcinoma treated by radical surgery; 17 women with involved axillary lymph nodes were irradiated postoperatively.

**Results:** Twelve patients were stage I, 35 stage II and 5 stage III TNM. 10-year survivals in these groups were 83.3%, 85.7%, 80.0% respectively. Pathological tumour size (pT) where tumours were under 2 cm was found in 12 patients, tumours from 2.1 to 4 cm in 34 patients, and tumours bigger than 4 cm in 6 patients. 10-year survival in these groups were 83.3%, 85.3%, and 83.3% respectively. Disease-free 10-year survival was noted in 84.6% of the patients. The only independent prognostic factor was the microscopical status of axillary lymph nodes: 97.1% patients without metastases survived 10 years disease free, compared to 58.8% patients with metastases. There was no significant effect of other clinical and histopathological factors.

**Conclusion:** The criteria defined by Ridolfi et al. are as before basic for the diagnosis of typical medullary carcinoma of the breast.

**503** POSTER

### R2: Index of biological aggressive breast cancer

M. Cherubini, P. Baxa, M. Sustersich, G. Mustacchi. *University Hospital of Cattinara (Trieste); Istituto di Clinica Chirurgica, Italy*

**Purpose:** indicators of biological aggressive breast cancers are taken into consideration, through the deepening of R2 (macroscopic recurrence after breast cancer therapy) biological effects.

**Methods:** 160 patients operated for breast carcinoma are considered and a comparison is performed with a subgroup of 44 R2, examined in a follow-up (1–6 years after operation).

**Results:** in the subgroup the disease free interval is on average 55 months. Recurrence is more often noted after mastectomy and increased in patients operated at advanced Stages (IV 80%; III 40%; II 22%; I 7%) and may have been caused by neoplastic vascular or lymphatic embolization. A wide surgical excision defines: ER/PR ratio, which results significantly different (12.7) in R2, compared to Stage I, II (range 4.44–4.86; $p < 0.01$) and III (5.12; $p < 0.03$). In Stage I, ER+ (estrogen receptors) predominance in G1–2 (histologic grade) is observed; in R2, ER+ reduction in G2–3. In R2, ER+PR+ and ER−PR− decrease and ER+RP− increase. The average survival rate from R2 appearance is 50 mo.

**Conclusions:** This study clearly shows the features of R2 biological aggressive breast cancer, which may represent initial widespread disease and is more frequent when the Stage at the diagnosis is more advanced, when the cells are more indifferentiated, when the age is lower, often after total mastectomy and when an adequate radiotherapy may not have been followed. The cause may be vascular or lymphatic neoplastic microembolism, often with different hormonal receptor characteristics (ER/PR ratio, different receptor phenotypes).

**504** POSTER

### Receptor findings and menstrual status at radical operations due to breast carcinoma

M. Kocić, M. Inić, M. Juškić, J. Pralica, V. Nikolić, B. Marjanović. *Institute for Oncology and Radiology of Serbia, Department of Surgery, Belgrade, Yugoslavia*

**Purpose:** Examination of significance between receptor findings and menstrual status at radical operations due to breast carcinoma.

**Methods:** In the period from 1.1.1988 until 31.3.1989. on our Institute we operated 596 radical mastectomy in I and II stadium of breast carcinoma. In 566 cases we analysed state of the hormonal tumor receptor. From total number 257 patients (45.65%) were in pre-menopause, and 309 (54.35%) were in postmenopause. We assumed that menopause is starting in age of 55 years approximately, althea we have data for every single patient particularly. Status of receptors for ER and PGR were analysed in classical biochemical methods. Middle value is 51.42 and rang is from 1909–1960.

**Results:** From analysed 566 receptors status we have following results:

$$\text{ER+ } (\leq 10) = 219\ (36.74\%),\ \text{ER− } (>10) = 117\ (19.63\%). \quad (1)$$

In 43.96% status of ER receptors weren't examined (no data):

$$\text{PRG+ } (\leq 20) = 421\ (70.64\%),\ \text{PRG− } (>20) = 175\ (29.36\%). \quad (2)$$

**Conclusion:** We analyse hormonal dependence of tumor in regard of menstrual status, or age, which were taken as base, together with other prognostic factors, in order to choose kind of adjuvant therapies.

**505** POSTER

### Intratumoral beta-radiometry is an universal test of breast cancer (BC) activity

S.M. Portnoj, K.P. Lactionov, R.I. Gaboonia, V.P. Godin, S.V. Shiriaev. *N.N. Blokhin Cancer Research Centre RAMS, Moscow, Russia*

In 130 stage I–III BC patients before any treatment were investigated $^{32}P$ relative uptaking into primary tumor ($^{32}PRUT_1$). In 62 BC patients $^{32}P$ relative uptaking into residual tumor were detected after any treatment ($^{32}PRUT_2$). $^{32}PRUT$-detecting was performed intratumoraly by means of the needleform semiconductor beta-detector.

Low $^{32}PRUT_1$ level influenced on the actuarial disease free survival in BC stage I–II patients as a factor of good prognosis, and high and middle $^{32}PRUT_1$ levels – as a factor of poor one. 6-year disease free survival in first group was 90% and in second group – 50% ($p = 0.03338$).

In stage III BC patients after preoperative treatment $^{32}PRUT_2$ had significance as criterion of operability, 2-years actuarial survival without locoregional relapse in patients group with high $^{32}PRUT_2$ level was 31 ± 18%, and in patients group with low $^{32}PRUT_2$ level – 96+/8% ($p < 0.01$).

33 stage I–IV BC patients had been treated with part effect and stabilisation. $^{32}PRUT_2$ was detected in residual tumor and 6 months later patients were re-evaluated by the WHO criteria. Mean $^{32}PRUT_2$ in patients groups were: complete remission – 103 ± 13%, part effect – 269 ± 36%, stabilisation – 580 ± 260%, progression – 3138 ± 843% (all differences except "part effect" versus "stabilisation" are sufficient, $p < 0.01$).

So, level of the $^{32}PRUT$ has prognostic information in any clinical cases.

## Friday, 2 October 1998 16:00-18:00

**PARALLEL SESSION**

# Pathology

**509** INVITED

### Mammary mucin secretion: A modern revisitation and review

J.D. Davies. *Regional Breast Pathology Unit, University of Bristol, Southmead Hospital, Bristol BS10 5NB, UK*

Mucin secretion is well known in many forms of breast cancer. Despite surveys of benign breast tissue mucin conducted in Perugia 50 years ago, the findings have been largely ignored. In conjunction with the Mucin Research Group (head: Dr A T Corfield) at the University of Bristol an extensive re-examination of benign and malignant breast tissue has been undertaken using mRNA and immunohistochemical probes directed again the class-specific amino-acid tandem repeat domains. The eight currently sequenced distinct mucins (MUC1 to 7) display quite different patterns of expression. MUC1 (epithelial membrane antigen) shows increased expression in secretory breast and most carcinomas. MUC2 (and to a lesser degree MUC6) show enhanced expression in mucin-filled ducto-lobular units, including mucocoele-like lesions and mucinous carcinomas. MUC4 expression is limited to epithelium displaying secretory cytoplasmic vacuoles (including pregnancy, "adenomas of pregnancy", focal lactational change and a few, mostly better differentiated carcinomas). MUC7 is expressed in approximately 50% of breast carcinomas, and possibly showing positive correlation with tumour progression. MUC3, 5B, and 5AC were not detected in any functional or pathological breast conditions; conversely apocrine and

cystic hypersecretory (*sensu* Rosen) changes were devoid of expression of all the eight mucins sought. It is concluded that several mucins are secreted in benign conditions, unlike current conventional belief, and, that the patterns of mucin expression in them and carcinomas are not random. The biological functions of these mucins in the breast are mostly unknown, and potential prognostic or clinical management implications are as yet unexplored.

510 ORAL

## Loss of heterozygosity (LOH) in normal epithelial and myoepithelial cells from tissues adjacent to breast carcinoma

R.S. Lakhani[1], R. Chaggar[3], S. Davies[2], C. Odell[3], M. O'Hare[2].
[1]*Departments of Histopathology;* [2]*Breast Cancer Laboratories, LICR/UCL;* [3]*University College London Medical School & The Ludwig Institute for Cancer Research, Branch, UCL, UK*

**Purpose:** The multistep model for breast carcinogenesis suggests that invasive carcinoma arises via a series of intermediate (Ehyperplastic, and neoplastic stages. Using the method of loss of heterozygosity (LOH), we have previously demonstrated that genetic alterations identified at high frequency in invasive carcinoma are already present in in-situ carcinoma atypical hyperplasia and in non-atypical hyperplasia indicating that at least a proportion of these preinvasive lesions are clonal, neoplastic proliferations. LOH has also recently been demonstrated in apparently normal lobules adjacent to carcinomas. This has implications on the clonal nature of the normal lobule and the significance of LOH in carcinogenesis.

**Methods:** Using a microdissection technique and established methods to isolate and clone luminal and myoepithelial cells from breast specimens, we have investigated LOH independently in these two breast cell types. 7 microsatellite markers on chromosomes 3p, 11p, 13q, 16q, 17p and 17q were studied. Invasive carcinoma, ductal carcinoma in-situ and normal lobules were microdissected from paraffin embedded tissue in 3 cases. In two of these cases, 8–12 clones each of luminal epithelial and myoepithelial cells (total 40 clones) were also analysed. In one case, 12 clones of fibroblasts were also available.

**Results:** LOH was found in normal cells in 3/8 cases of breast cancer. In 2 cases, LOH was identified at the locus on chromosome 13q in the carcinoma as well as the adjacent (Enormal lobule or luminal and myoepithelial clones, with all samples exhibiting loss of the same allele. Loss of heterozygosity has not been identified in normal cells cloned from tissues away from the tumour. In 1/8 cases, LOH was identified in a single (Enormal, clone but this LOH was not seen in the adjacent tumour. One of 56 clones from 2 reduction mammoplasty specimens showed LOH at the locus on chromosome 13q.

**Conclusion:** The data confirm that LOH is present in normal lobules adjacent to carcinoma. The finding of LOH at the same locus independently in luminal and myoepithelial cells provides evidence for the presence of a common stem cell. Hence, genetic alterations predisposing to sporadic cancer probably occur very early in breast development.

511 ORAL

## Loss of heterozygosity (LOH) and allelic imbalance (AI) in apocrine metaplasia and apocrine adenosis of the breast

S. Selim, A. Ryan, C. Wells. *St. Bartholomew's Hospital, Department of Histopathology, 3rd Floor, West Wing, Royal Hospitals NH, St. Bartholomew's Hospital, West Smithfi, London EC1A 7BE, UK*

41 cases of apocrine metaplasia and 17 cases of apocrine change within sclerosing adenosis (apocrine adenosis) were analysed for LOH/AI at 8 loci reported to be involved in invasive and in situ breast cancer using a microdissection technique, polymorphic microsatellite markers and the polymerase chain reaction (PCR). Within apocrine metaplasia, examples of LOH and/or AI were identified in 2/28 (7.1%) of informative cases at 1p (MYCL1), 2/14 (14.3%) at 11q (INT2), 1/15 (6.7%) at 13q (D13S267), 3/22 (13.6%) at 16q (D16S539), 2/23 (8.7%) at 17p (TP53), 3/16 (18.8%) at 17q (D17S250) and 2/11 (18.2%) at 17q (D17S513). The frequency of abnormalities in apocrine adenosis was found to be higher in percentage terms with LOH/AI being detected in 3/12 (25%) informative cases at 1p (MYCL1), 2/7 (28.6%) at 11q (INT2), 1/3 (33.3%) at 13q (D13S267), 2/12 (16.7%) at 16q (D16S539) and 2/10 (20.0%) at 17q (D17S250). Neither LOH nor AI have been identified at 1p (D1S252), 17p (TP53) or 17q (D17S513) in apocrine adenosis. These findings indicate that a small percentage of apocrine metaplasia cases appear clonal and the finding of a higher percentage of abnormalities in apocrine adenosis suggests a possible progression of apocrine lesions to in-situ and invasive breast cancer.

512 ORAL

## An audit of grading and typing of invasive breast carcinoma on needlecore biopsy specimens

H.E. Denley, S.E. Pinder, C.W. Elston, I.O. Ellis. *Nottingham City Hospital Breast Team, UK*

**Purpose:** An audit of the assessment of pathological prognostic factors on breast needlecore biopsies (NCB) was carried out.

**Methods:** Over a 9 month period 191 malignant NCBs with follow-up excision specimens were received. Histological grade and tumour type were assessed by routine methods.

**Results:** There was excellent correlation between grade of invasive carcinomas on NCB and excision samples ($p < 0.0001$); 120 of the 173 cases with sufficient tissue for assessment were correctly classified. Scores for tubules, pleomorphism and mitotic counts were also individually highly significant (all $p < 0.0001$). For mitoses NCB tended to underestimate the overall scores (61 out of 67 cases) but the scores for tubules and pleomorphism were more randomly distributed. The accuracy of classification of type of invasive carcinoma was also high ($p < 0.0001$) with 126 of 173 being correctly identified.

**Conclusion:** NCB of the breast is recognised as a reliable test for the diagnosis of invasive breast carcinoma and can also accurately predict the grade and type. This may be clinically relevant in some situations.

513 ORAL

## The prediction of response to chemotherapy in invasive breast carcinoma by determination of histological grade

S.E. Pinder[1], S. Murray, I.O. Ellis[1], H. Trihia, C.W. Elston[1], R.D. Gelber, A. Goldhirsch, J. Lindtner, H. Cortés-Funes, E. Simoncini, M.J. Byrne, R. Golouh, C.M. Rudenstam, M. Castiglione-Gertsch. [1]*Histopathology Department, BA Gusterson; City Hospital NHS Trust, 1PB NG5 and International Breast Cancer Study Group (IBCSG), UK*

**Purpose:** We have examined the role of "Nottingham" histological grade of invasive breast cancer in predicting response to chemotherapy.

**Method:** Grade (HG) was examined in a group of 465 patients from the IBCSG randomised clinical trial of adjuvant chemotherapy (peri-operative or prolonged) (formerly Ludwig Trial V).

**Results:** HG predicted overall survival (OS) in both lymph node (LN) negative and LN positive breast cancer ($p = 0.045$ and $p < 0.001$ respectively). Hazard ratios of 1.651 ($p < 0.001$) and 1.437 ($p = 0.045$) respectively were seen for an increase of 1 grade in LN+ and LN− disease. In LN+ patients an increase by 1 grade gave a significant OS disadvantage regardless of whether prolonged or peri-operative chemotherapy was given. However, in LN− disease this survival disadvantage was seen only in patients receiving peri-operative chemotherapy. No observed difference in survival of LN− patients was seen according to whether peri-operative treatment was received or not, when grouped by HG. However LN+ patients with grade 3 tumours obtained a significant OS and DFS benefit from prolonged compared to peri-operative chemotherapy ($p = 0.016$ and $p = 0.013$ respectively); those with grade 1 or 2 tumours had comparable survivals for both treatment arms.

**Conclusion:** Histological grade predicts OS and can, in particular, identify a group of grade 3, LN+ patients who may benefit from chemotherapy.

514 ORAL

## Predictive factors of response to neo-adjuvant chemotherapy by immunohistochemistry (IHC)

G. Mac Grogan, L. Mauriac, M. Durand, F. Bonichon, A. Floquet. *Department of Medicine, 180, rue de Saint-Genes, F-33076 Bordeaux, France*

Neo-adjuvant chemotherapy is currently used for locally advanced and operable breast tumors not assessable for immediate conserving surgery due to their large size. The number of mastectomies is dramatically decreased and survival is identical to that obtained by mastectomy and medical adjuvant treatment. Conserving treatment rates are in correlation with several factors: type and intensity of neo-adjuvant chemotherapies, tumor size and characteristics. An immunohisto-chemichal study was performed on tumor samples from 128 patients enrolled in a randomized trial comparing mastectomy to neo-adjuvant chemotherapy (Ann Oncol 1991; 2: 347–54). Specific antibodies to p53, c-erbB-2 (Her-2/neu), Mib1 (antiKi-67), pS2, GSTpai, estrogen receptors (ER) and progesterone receptors (PR) were used to correlate these factors to tumor shrinkage during neo-adjuvant chemotherapy.