(2) diffuse stromal infiltration with mononuclear cells and (3) sparse (<25%) necrosis. The tumors are further characterized by high histological grade (96% gr. III) and by predominance of estrogen receptor negativity (67%). Both factors normally indicate poor prognosis. However, this group of tumors has a significantly better overall survival and recurrence-free survival compared with a control group of infiltrating ductal carcinomas (IDC). Classical risk factors for breast cancer have a significantly different distribution and only minor prognostic importance in the group of MC compared with the control group of IDC.

According to the proposed definition, MC is biologically unique and the results indicate that the risk factors presently used for selecting breast cancer patients for systemic adjuvant treatment probably should be modified in MC.

651 ORAL

APOPTOSIS ACCOUNTS FOR THE NECROSIS SEEN IN DCIS

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The biology of DCIS is poorly understood and treatment highly controversial. Apoptosis, a genetically triggered death program has been described in the normal breast, possibly as a regulator of tissue homeostasis. To evaluate the presence of apoptosis in DCIS, we studied 25 cases of DCIS accessioned in 1993 and 1994. 10 patients presented with comedo DCIS, 9 with cribriform DCIS with intraductal necrosis, and 6 with cribriform or micropapillary DCIS without intraductal necrosis. All cases were stained for presence of apoptosis using the terminal transferase assay (TUNEL) staining free DNA ends. In all 19 cases with necrosis, TUNEL stain was positive. The 6 DCIS cases that lacked intraductal necrosis displayed no intraductal apoptosis by TUNEL assay. p53 analysis suggests that this apoptosis is independent of p53. For 2 additional cases with synchronous invasive cancer and DCIS with intraductal apoptosis, not included in this series, apoptosis was restricted to the DCIS tumor.

652 ORAL

THE RELATION OF EXTENSIVE INTRADUCTAL CARCINOMA COMPONENT (EIC) WITH PROGNOSIS AND TREATMENT RESULTS OF PATIENTS (PTS) WITH PRIMARY BREAST CANCER

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Within the context of a large retrospective study on a broad scale of prognostic factors we studied the clinical significance of EIC. In a series of 1064 primary infiltrating ductal breast carcinomas, 133 tumors appeared to contain EIC (>25% of the tumor being DCIS) as described in the pathology report. In general no significant differences in stage were found between the groups with and without EIC. Of the 133 pts with EIC 48 underwent breast conserving therapy (BCT) and 85 modified mastectomy (MM Patey). Of the 931 pts without EIC 462 underwent BCT and 469 MM Patey. After a follow-up of 7 years the 85 pts with EIC treated with MM Patey showed a better disease-free survival (DFS) than the 48 patients treated with BCT (difference 17.5%; $P = 0.07$). Within the whole group of 510 pts treated with BCT the 48 pts with EIC tended to show a worse prognosis as compared with pts without EIC (difference 9.5%; n.s.). The opposite was observed in the group of 554 pts treated with MM Patey, indicating that the 85 pts with EIC had a clearly better DFS (difference 27.9%; $P < 0.01$). With respect to overall survival only pts treated with MM Patey showed a better overall survival for pts with EIC as compared to pts without EIC (difference 31.5%; $P < 0.01$).

In conclusion: the results of this retrospective study suggest that modified mastectomy might be a safer treatment modality in patients with EIC (this study is supported by the Dutch Cancer Society, project DDHK 92-04).

653 ORAL

CHANGES IN PROLIFERATION IN PRIMARY BREAST CANCERS DURING CHEMOENDOCRINE THERAPY

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The proliferation markers Ki67 and S-phase fraction (SPF) are important biological variables in determining the course of malignant disease. Changes in these variables may provide additional prognostic information.

We have studied changes in Ki67 (measured by immunocytochemistry using the Mib 1 antibody) and SPF (by flow cytometry) on samples obtained by FNA from patients with early breast cancer.

In a control group of 25 patients repeat FNAs were taken 2 weeks apart, with no intervening treatment, in order to determine the normal variation. For Ki67 the median % +ve for the first sample was 3.0% (range 0–23%) and the second was 4.0% (range 0–23%). For SPF the median for the first sample was 7.8% (range 1.5–21.8%) and for the second 10.3% (range 0.8–22.5%). This demonstrates (i) the good reproducibility of the technique and (ii) that FNA itself does not affect subsequent measurement of proliferation in the same tumour. In 24 patients repeat FNA was performed at 10 or 21 days after chemoendocrine therapy (CET) with Mitozantrone, Methotrexate and tamoxifen. Pre-CET the median Ki67 was 12.9% (range 1–37.7%) and post-CET 5.5% (range 0–14.7%), $P < 0.05$. Pre-CET the median SPF was 4.1% (range 0.9–27.7%) and the post-CET 3.2% (range 0.4–19.2%), P = NS.

These changes in Ki67 may be used as an intermediate marker of response to evaluate the effectiveness of different therapeutic agents in groups of patients. For individual patients change in relation to response to therapy needs to be evaluated with more patients. Additional quantitative measurement of apoptosis might enhance the biological and clinical significance of these measurements.

654 POSTER

HORMONAL-METABOLIC STATUS IN SMOKING AND NON-SMOKING BREAST CANCER (BC) PATIENTS WITH NORMAL (N) AND EXCESSIVE (E) BODY MASS: POSSIBLE PROGNOSTIC SIGNIFICANCE

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Survival is decreased in smokers and obese patients with BC. We compared indices of hormonal-metabolic status in 118 pts with BC, 35 of whom smoked and 43 have had Quetelet index $\gg$32. PreMP BC pts with E demonstrated greater than postMP pts increase in body fat content, LBM, waist/hip ratio, blood glucose and free cortisol excretion in relation to these values in pts with N. In postMP obese pts increase in reactive insulinemia and triglyceridemia was expressed more than in corresponding group of preMP pts. Smoking increased waist/hip ratio and decreased ER content in tumor tissue in greater degree in pre- and postMP pts with N than with E. The lowest level of FSH and LH and highest of estradiol in blood was discovered in smokers with E. Thus, different hormonal-metabolic mechanisms can mediate devastating effect of body mass excess and smoking on prognosis in pre- and postMP type of BC.

655 POSTER

COMPUTER ANALYSIS OF BREAST CANCER CELL POPULATIONS IN ER AND PR POSITIVE AND NEGATIVE TUMOURS

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The ER and PR status are known to be one of the most significant prognostic factors for selection of adjuvant hormone and/or chemotherapy. It is regarded as the indicator of the malignancy of the tumour cell population. Therefore the cell population characteristics in tumors with positive or negative ER and PR is of utmost importance, allowing morphological prediction of possible malignancy of the process and prognosis of the survival of the cancer patients.

We have investigated routine stained cytological imprint slides from 34 breast cancer specimens obtained during operation. In the slide we

analysed 100 cells, each of them according to 32 cell features and introduced into database along with the ER and PR receptor status in tumour tissue.

The results of the cell population computer analysis have shown distinct differences in cell population structure between ER+ and ER− tumours (percentage of cells with certain chromatin structure, presence of well defined nucleoli associated chromatin etc.) as well as between PR+ and PR− tumours. There were also cell population differences among various ER± PR± status combinations in tumours.

656 POSTER

TAMOXIFEN ADJUVANT HORMONAL TREATMENT ACCORDING TO PROGNOSTIC FACTORS. SHORT TERM RESULTS OF A SERIES OF 695 T1 T2/N0 N1 BREAST CARCINOMAS

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From 1982 to 1990, 695 women less than 75 years, without any previous or synchronous carcinoma, suffering from an invasive breast cancer classified as T1 T2/N0 N1/M0 according to clinical TNM staging, were enrolled in this study. 82.4% underwent a breast conservative procedure and 17.2% a modified radical mastectomy; all the patients received a post-operative irradiation. Histological axillary lymph node status, Scarff-Bloom grade and/or cytological grade, estradiol receptor content, were used to define three groups of patients. 416 women were N−/grade I II/ER+ (group I), 110 were N−/grade III/ER+ (group II), 169 were N+ $\leqslant$ 3/grade I II/ER+ (group III); patients from groups II and III received tamoxifen (20 mg per day for 2 years) due to grade III or N+ $\leqslant$ 3, considered as poor prognostic factors. With a median follow-up of 35 months (1–138) the overall survival of the three groups was respectively 95%, 96%, 96% (logrank NS) and the disease free survival 86%, 93%, 90% (logrank NS); the actuarial local regional remission rate was 94%, 97%, 99% ($P = 0.07$). Such results need to be updated, but show the ability of tamoxifen to tailor the short term survival thanks to prognostic factors.

657 POSTER

TISSUE EXPRESSION AND SERUM LEVELS OF HER-2/*NEU* IN PATIENTS WITH BREAST CANCER

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We have analyzed serum levels of soluble HER-2/*neu* by using an enzyme linked immunosorbent assay in 42 patients prior to any therapy and put it into relation to the overexpression and amplification of HER-2/*neu* in the primary tumor after surgical excision and to data obtained by pathohistological staging. In addition, we have investigated the sera of 62 patients with stage IV breast cancer. We have further compared the possible prognostic value of serum HER-2/*neu* to two other known serological tumor markers CEA and CA15-3 in both patient groups.

We have observed an elevated serum HER-2/*neu* level (>20 U/l) in 6/42 (14.2%) preoperatively patients, out of whom those with HER-2/*neu* tissue expression/amplification showed elevated serum levels in 42.8%. In contrast, 8.5% of patients without HER-2/neu expression/amplification in the primary tumor presented with elevated serum levels. There was a significant difference in soluble HER-2/*neu* serum concentrations between patients with tumors of different size ($P < 0.0001$) and various degrees of axillary lymph node involvement ($P < 0.0001$), thus reflecting a close correlation of tumor load with serum concentrations of soluble HER-2/*neu*. In patients with stage IV disease, 27 out of 62 (43.5%) had elevated soluble HER-2/*neu* serum levels. A highly significant correlation of serum concentrations of HER-2/*neu* with CA 15-3 ($P < 0.002$) was observed.

We conclude that the measurement of serum HER-2/*neu* levels at diagnosis defines a small subgroup of breast cancer patients with a relatively advanced stage of disease. Its strong correlation with tumor load in patients with stage II disease and the high prevalence in patients with stage IV disease make it a promising tool for the assessment of disease activity and biologic behaviour in breast cancer.

658 POSTER

PROGNOSTIC FACTORS IN BREAST CANCER LESS THAN 3 CM WITHOUT HISTOLOGIC LYMPH NODE INVOLVEMENT

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Axillary lymph node involvement is the most important prognostic indicator for breast cancer. Nevertheless, nearly 25% of patients with histologic negative lymph node will develop distant metastasis. Between 1974 and 1985, 201 patients with breast cancer, less than 3 cm, without axillary lymph node involvement were followed in the "Centre René Gauducheau". The aim of our study was to analyse on this population which clinical and histological parameters were correlated with local recurrence or distant metastasis. We realise a multivariate analysis using the Cox model. We showed that hormonal status, age and multifocality were significantly and independently correlated with local recurrences, and that pathologic grading (SBR),tumor localization in the breast were prognostic factors of distant metastasis.

Finally we found that the nuclear grading (MSBR) 4 or 5 permit to determine in the population SBR II, a sub-group of women associated with a high risk of metastatic evolution, in which it will be probably necessary to discuss systemic adjuvant therapy to prevent distant metastasis.

659 POSTER

SOLUBLE CD44 STANDARD AND V6 IN SERUM OF BREAST CANCER PATIENTS: AN INDICATOR FOR THERAPY RESPONSE

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CD44 splice variants which contain sequences encoded by exon v6 (CD44 v6) seem to be involved in the process of tumor growth and metastasis in some human tumors. Besides cell surface expression, CD44 v6 was also identified in soluble form (sCD44) in the serum. To evaluate the prognostic potential of CD44 serum levels for clinical progression we determined the concentration of CD44 v6 and CD44 standard in sera of 88 breast cancer patients. Sera of tumorectomized, non-metastatic breast cancer patients contained sCD44 v6 and sCD44 std similar to those of healthy blood donors. In contrast, the mean values for sCD44 v6 and std of sera from metastatic breast cancer patients were significantly higher than those of patients with non-disseminated tumor disease. We found no correlation of sCD44 v6 and sCD44 std serum levels to age, tumor grading, disease-free interval, hormone receptor levels and location of metastases (visceral vs. non-visceral) in breast cancer patients. However, sCD44 v6 concentrations correlated with the number of metastatic sites, while those of sCD44 std form did not. The lack of correlation of v6 expression to lactate dehydrogenase (LDH) levels, a marker of tumor load, indicates that high v6 levels reflect rather the metastatic potential of tumor cells than tumor burden. Furthermore, v6 expression correlated with responsiveness to hormone- or chemotherapy: 93% of patients with low v6 serum levels ($\leqslant$386 ng/ml) responded to therapy, while only 33% of the overexpressing patients responded. Therefore determination of sCD44 v6 serum concentrations may provide a valuable prognostic marker for responsiveness to drug treatment of breast cancer.

660 POSTER

FOLLOW-UP ROUTINES FOR EARLY STAGE BREAST CANCER PATIENTS IN ISRAEL

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Controversy continues to exist in the literature concerning the necessity and intensity of routine follow-up tests in breast cancer patients after primary treatment. In order to assess the current status of care in Israel, we sent a questionnaire to all 18 cancer clinics requesting details of tests ordered during the first five years after diagnosis. For each cancer clinic a score was calculated according to the number and frequency of tests. The economic cost of all the procedures was then calculated. We found a wide disparity of routines with the score ranging from 12 to 89 (median 52). The cost similarly reflected up to a six fold difference between the most and least expensive center. Since intensive follow-up of early stage breast cancer patients does not appear to improve survival, the policy should be reconsidered.