Choosing a selection interval of 14 days yielded a type I error rate of about 27% at the common 5% nominal level. In other words there were significant results in I out of 4 cases, although there was nothing to detect according to the design of this simulation study. At a nominal level of 10% and 1% the false positive rates were 47% and 7.3%, respectively. The multiple testing problem became more evident allowing a minimum selection interval ranging from 7 to 14 days. The type I error rate increased to 63% at a nominal level of 5%. Even if we used the 'impressive' 1% nominal level, we got a significant result in 25% of the tests. The simulation study showed that for achieving an actual type I error rate (significance level) of approximately 5%, a P-value < 0.006 was necessary, using a selection interval of 14 days. This barrier dropped to a P-value < 0.001, when we used minimum selection intervals ranging from 7 days to 14 days.

Results: In view of the data described above and the works of Altman et al. (1994) it seems absolutely necessary to integrate the number of performed tests in the evaluation of a prognostic factor, which was (even if more tests have been performed) at least not mentioned by those authors who found a statistically significant benefit for menstrual cycle dependent timing of surgery on long-term outcome of breast cancer patients. Our results underline the necessity of cautious statistical interpretation when dealing with a cyclic covariate such as the menstrual cycle.

468 POSTER

A comparison of biochemical quantitative and immunohistochemical detection methods for the detection of the erbB2 oncoprotein in breast cancer tissue

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Aim: The management of breast cancer depends upon prognostic factors which allow separation of patients into high and low risk groups. Reviews of studies concerning the overexpression of the c-erbB2 oncogene in breast cancer tissue attribute different prognostic value to DFS and OST. Why so?

Material and Methods: The quantitatively and qualitatively analyzed grade of overexpression of the c-erbB2 oncoprotein has been evaluated in 101 breast cancer samples: biochemically via Western Blot analysis, immunohistochemically with the ABC on paraffin sections using two different monoclonal antibodies (CB 11 and 3B5). The quantification of the immunoblots was performed by densitometric measurement.

Results: With the CB-11 mAb, the biochemical detection showed an overexpression of the c-erbB2 oncoprotein in 34.6%, the immunohistochemical procedure in 27.7% of the cases. The 3B5 mAb has given a weak staining signal in the stroma of all examinated sections. Additinal 5 tumors with a strong staining signal in the tumour cells were found positive on 3B5 mAb, however showed to be negative in the biochemical procedure or by the use of CB-121 Ab in either method.

Conclusion: The difference in statements concerning the prognostic value of the c-erbB2 oncoprotein in breast cancer may in great parts be attributable to the different technical procedures used in the studies.

469 POSTER

Expression of cytosolic thymidine kinase in the proliferative breast carcinoma after primary chemotherapy: Therapeutic indication

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Thymidine kinase (TK) activity has been assessed in breast cancer after primary chemotherapy in relation to clinical and pathological response, proliferative activity and hormonal receptors. TK was assayed in the cytosol of 76 patients treated by induction chemotherapy: 26 AVCF/M, 32 NEM and 18 TNCF (Ecco 8, 95, S13, 53). After surgical resection of the remaining tumor, the enzyme activity was measured using a radioenzymatic method. High levels of TK (>30 U/mg protein) were found with 89% specificity and 64% sensitivity in residual invasive carcinoma. Conversely, TK activity was lower with presence of only *in situ* carcinoma or alterated cells residual after treatment. TK rate was positively correlated to the remaining tumor size ($p < 0.0001$) and aneuploidy ($p < 0.001$). TK was negatively correlated to the clinical complete response with a mean rate of 55 versus 92 U/mg protein for partial responses or no change ($p < 0.02$). Moreover, the mean TK activity measured for patients treated by TNCF, the most intense and effective regimen in breast cancer (51% of complete clinical and 30% of complete pathological responses), was lower. 57 *versus* 90 U/mg protein for the other two protocols ($p < 0.01$). TK was also increased in tumors with positive estrogen and progesterone receptors ($p < 0.001$ and $p < 0.04$).

In conclusion, after primary chemotherapy, TK expression was directly related to the residual active tumor amount (aneuploidy, invasion and size). We intend to increase our experience to examine more clearly if a high residual TK is rather a marker of residual proliferation capacity (i.e. resistance), or a biological factor linked to hormonal sensitivity.

470 POSTER

In vivo bromodeoxyuridine (BrdUrd) labeling index as a prognostic marker in human breast cancer

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Background: A practical prognostic index that works in 100 percent of cases is needed to measure cell proliferation in human breast cancers.

Methods: With informed consent, 133 women received 200 mg/M^2 BrdUrd preoperatively. IU4 antibody was used to measure the Labeling Index (LI) of DNA-incorporated BrdUrd in 2,000 cells. Ki-67 LI was determined with the MIB1 antibody (121 cases) and S-phase by flow cytometry (95 cases). Follow up was 2 to 8 years years. Patients were divided into groups above and below the median for each LI. Survival was compared between groups of women with each LI above and below the median with the Mantel-Cox test and univariate and multivariate analysis.

Results: Follow up was 100 percent. Women in the low BrdUrd LI group had significantly better disease free survival (DFS; $p = 0.0008$) and overall survival (OS; $p = 0.0004$). Ki-67 predicted a trend ($p = 0.06$) for better DFS and OS. Low S-phase predicted better OS but not DFS.

Conclusions: BrdUrd LI is a significant prognostic index which is superior to Ki-67 and S-Phase by flow cytometry.

471 POSTER

Tc-99 Tetrofosmin scintimammography in determining prognostic characteristics of breast cancer

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Purpose: To compare scintigraphic features of breast lesions imaged with scintimammography with tumour prognostic factors.

Materials & Methods: 36 consecutive patients with a clinically palpable abnormality requiring tissue diagnosis were examined with scintimammography prior to surgical intervention. Histological features were compared to scintigraphic characteristics.

Results: Out of the 36 cases evaluated 19 were benign and 16 were malignant. The average tumour to background ratio (TBR) in malignant cases was 2.1 (range = 1.4–4.0). There was a good correlation between size of tumour measured pathologically and by scintimammography ($r = 0.8$). There was no correlation between TBR and size of tumour or TBR and tumour grade. Patients with ER negative tumours tended to have a higher TBR. Scintimammography correctly categorised lymph node status in 10 out of the 13 patients who had axillary lymph node dissection. Unsuspected substernal lymph nodes were detected in a single patient.

Conclusion: Scintimammography may not only play a role in discriminating benign from malignant lesions but may also be useful in determining tumour prognostic factors in-vivo.

472 POSTER

HER-2/neu oncogene amplification in breast cancer

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The HER-2/neu oncogene is localized to chromosome 17q and shares significant homology with the epidermal growth factor receptor. HER-2/neu protein overexpression has been associated with poor prognosis in breast cancer.

Design: Formalin-fixed paraffin-embedded primary breast cancer tissues from 128 women (mean age 60 years) were tested for HER-2/neu gene amplification by automated (Ventana Gen II, Tucson, AZ) fluorescence in-situ hybridization (FISH) using the Oncor unique sequence probe (Oncor, Inc., Gaithersburg, MD). The tumors were also evaluated immunohistochemi-

cally for proliferation markers MIB1 (Ventana) and p34 CDC2 (Biogenex, San Ramon, CA). Patients were followed for a mean of 61 months (range 1–164 months).

Results: There were 63 (54%) node negative and 65 (56%) node positive cases. On univariate analysis MIB1 (p 0.002) and p34 CDC2 (p = 0.001) overexpression, HER-2/neu gene amplification and lymph node positive status ($p < 0.0001$) predicted disease related death.

HER-2/neu gene amplification correlated with lymph node metastasis (p = 0.001) and also predicted disease related death in lymph node negative patients (p = 0.029).

In multivariate analysis of combined lymph node negative and lymph node positive patients, HER-2/neu amplification (p = 0.04) and lymph node positive status independently predicted disease related death.

Conclusions: MIB1 and p34 CDC2 proliferation marker overexpression, HER-2/neu oncogene amplification and lymph node metastasis all predict disease related death in breast cancer, with HER-2/neu amplification and lymph node status independently predicting outcome. HER-2/neu amplification by FISH predicts disease related death in breast cancer independent of lymph node status.

473 POSTER

Prognostic factors in human breast cancer

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A variety of new prognostic factors for breast cancer have been advocated, however their potential for identifying the time of relapse or the patient survival are still uncertain. In order to estimate the prognostic impact of different parameters we evaluated breast infiltrating ductal cancer specimens from 150 patients with a maximum 15 and a mean 9.4 years follow up by means of flow cytometry DNA analysis (DNA Index and S-phase fraction), the immunohistochemical assessment of Her/neu, Cathepsin D expression and the evaluation of the AgNORs through argyrophilic method.

55% of cases proved aneuploid with DNA Index between 1.1 and 2.42, whereas 45% of cases proved diploid. SPF, obtained to diploid cases only, was higher than median value (8.4%) in 37% of cases while 52% of cases showed the AgNOR value higher than cutoff value (9.5). Her/neu overexpression was detected in 34% of the cases, whereas 38% proved Cathepsin D positive.

The data obtained in our study carried out by univariate analysis, confirm the prognostic value of the individual indexes. As a matter of fact, cancer patients with DNA aneuploid. High SPF, Her/neu overexpression, high Cathepsin D levels and AgNOR exceeding 9 present a shorter DFS. The DNA Index is a highly significant prognostic parameter ($rs = 0.56$, $p < 0.001$) and also is the only factor able of discriminating node negative patients. The multivariate Cox model shows that DNA Index is the most important prognostic fact (coeff. = 2.74). We conclude that the data obtained from DNA flow cytometry, associated with other parameters, can be of great importance for the decision at the level aggressiveness of adjuvant therapy for a individual patients.

474 POSTER

Axillary node involvement in T_1 breast cancer in pre-menopausal Chinese

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Purpose: Chinese Breast Cancer have some differences from that of western country, one of that is that half of the patients are ages between 35–50, The aim of the study is to assess the frequency of axillary rode involvement in T_1 breast cancer in young female, and to correlate the risk of nodal involvement in Chinese.

Methods: The study population consisted of 197 cases who were treated with modified radical mastectomy in our hospital from 1990 to 1995. All patients were T_1 lesion and age younger than 50. The lymph nodes all were classified to level I, II, III and Rotter's node. The number of dissected node were greater than 10. None of them has axillary recurrence in the follow up period. We analyzed patient's age, tumor size, axillary lymph node status, histologic grade, lymph vessel emboli, hormone receptor status, and P53 expression with immunochemical stain and flow cytometry.

Results: 1. T_1 breast cancer have a 27.92% risk of axillary LN involvement in premanupasl Chinese (24.14% for tumor 1 cm or smaller). 2. Patients younger than 50 years old are less likely to have positive lymph rode than older (P:0.038).

475 POSTER

The local recurrence after breast conserving surgery: The prognosis and the diagnosis

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Purpose: To study the relationship between local recurrence after breast conserving surgery and the distant metastases.

Material and Methods: Between January 1981 and December 1989, 409 patient with invasive breast were treated with conservative surgery and radiation therapy, with actuarial follow-up of 12 years. Life tables were computed by the actuarial method and the comparisons of the distribution of length of time to local recurrence and distant metastases were made with the summary χ^2 test. The follow-up programme conducted a clinical exploration mammogram, echography and cytological punctures under echographic control.

Results: The overall actuarial survival rate at 12 years was 86%, with a 12 years distant metastases free of 64% and 12 years actuarial breast recurrence free rate of 78%. The 10 years actuarial distant metastases free rate in patients without local recurrence was 82%, in the patients who developed a local recurrence the rate was only 42% ($p < 0.01$). The patients who developed local recurrence within 4 years of original diagnosis, 33% developed distant metastases, in contrast the patients who developed later breast relapses only 9.5% developed distant metastases ($p < 0.05$).

Conclusion: The local recurrence after breast conserving surgery is associated with a distant metastases. The early local recurrence is a predictor factor of distant metastases.

476 POSTER

Significance of soluble interleukin-2 receptors and natural killer cells in breast cancer

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Purpose: The aim of this study was to assess whether sIL-2r levels and/or percentage of natural killer (NK) cells were correlated with the pathologic stage and grade of tumors in breast cancer patients.

Methods: The study group consisted of 32 consecutive female patients undergoing surgery for breast cancer. The average age was 58 years. Sixteen patients underwent radical mastectomy, 13 had quadrantectomy with axially lymph node dissection and 3 had tumorectomy. Serum sIL-2r using the ELISA technique and NK cell count using flow citometry were determined prior to surgery. Tumor stage was recorde according the AJCC/UICC classification.

Results: Average sIL-2r levels was 1431 pg/ml. There was no significant correlation between sIL-2r levels and tumor stage or grade. However the sIL-2r were significantly higher in pts with ductal vs. those with lobular carcinoma and in pts with recurrent tumors of the same initial histotypo vs those who had primary and recurrent tumors of different histotypes. The NK cell count was significantly increased in in high grade tumor and there were inverse correlations between NK cell count and tumor size and the presence of lymph node metastasis. There was no correlation between sIL-2r levels and NK cell count.

Conclusion: These data show high sIL-2r levels may indicate breast tumor recurrence and tumor extension may be inversely correlated with NK cell count.

477 POSTER

Thymidylate synthetase levels in breast cancer: A predictor for early prognosis and outcome of adjuvant chemotherapy

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Purpose: Thymidylate synthetase (TS) plays an essential role in the synthesis of DNA. The levels of TS were concluded to be indicators for early prognosis and retrospectively the outcome of adjuvant chemotherapy in breast cancer.