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-