riareolar area. First surgical step is to largely prepare glandular tissue, detaching breast from the skin, and separate breast from the muscular fascia.At this point half breast is taken out from the periareolar incision and we decide the resection extent according with tumor size. Before starting breast reshaping, axillary dissection has to be performed. This periareolar approach allows us to perform a complete three levels axillary dissection without adding an incision in the axilla. Than we have to reshape the breast according with the superior or inferior pedicle technique depending by which part of the breast we have resected).Nipple-areola complex now can be settled in a new position, its central vascular support is save and it is adjusted to the central skin incision avoiding any deformities.Contralateral mastopexy by the same technique is performed.In this way we obtain two symmetrical breasts rounder, no more ptosic and more projected with only a periareolar scar

Conclusions: (1) Periareolar approach allows large glandular resection even larger than a traditional quadrantectomy.

(2) It allows a complete axillary lymphophoadenectomy

(3) It allows a good breast simmetry with a good ptosis

(4) No nipple-areolar complex ischemic failure has been recorded

(5) No local relapse are still now recorded (median follo-up 12 month ranging 24-2 mt)

(6) All patients are satisfied of the result

Thursday, 21 March 2002 16:30–18:00

PROFFERED PAPERS

Predictive and prognostic factors

256 ORAL

Detection of persisting isolated tumor cells in the bone marrow as possible surrogate marker for the failure of systemic treatment of breast cancer

W. Janni, B. Strobl, B. Rack, D. Rjosk, Ch. Kentenich, Ch. Schindlbeck, W. Sigg, S. Braun, K. Pantel, H. Sommer. *Dept. ObGyn, Ludwig-Maximilians-University, and Dept. ObGyn, Technical University, Munich; Frauenklinik Hamburg-Eppendorf, Germany*

Background: To date, there is no surogate marker available, to evaluate the therapeutic efficacy of adjuvant treatment in individual breast cancer patients before subsequent distant relapse. Detecting the persistence of disseminated tumor cells in the bone marrow (BM) may help to identify patients with increased risk for recurrence after the completion of primary treatment.

Method: We analyzed bone marrow aspirates of 156 patients without evidence of recurrence at the time of primary diagnosis and a median interval of 19.8 months thereafter. Carcinoma cells were detected using a standardized immunoassay with monoclonal antibody A45-B/B3 directed against cytokeratin (CK).

Results: At the time of primary diagnosis, 44 of 156 patients (28%) had a positive BM finding, while 37 (24%) had a positive BM finding at the time of the second BM analysis. Among those patients with an initially negative BM finding, 19 patients (12%) had a positive BM finding at the second aspiration, while 18 patients (11%) were BM-positive in both examinations. Of the 44 patients with ITC at the time of primary diagnosis, 26 patients (59%) received adjuvant chemotherapy, 7 patients (16%) received endocrine therapy and 11 (25%) patients had no systemic treatment at all. 55% of the patients without systemic therapy (n = 6) converted to a negative BM status at time of follow-up examination, while 60% of the patients, with endocrine (n = 4) or cytostatic (n = 16) therapy became negative (P = 0.79). Patients with a negative BM status at the time of follow-up examination (n = 119) had a significantly better overall survival than patients with a positive BM status at the time of the second BM aspiration (n = 37), both by univariate analysis (P = 0.0014. Log-rank) and multivariate analysis (P = 0.003, Cox Regression).

Conclusion: Indpendently of systemic therapy, a considerable number of patients remain BM-positive suggesting failure of therapy and risk of subsequent development of distant disease.

257 ORAL

Clinical response after two cycles is superior to HER2, Ki-67, p53, and bcl-2 in independently predicting a pathological complete response after preoperative chemotherapy in patients with operable carcinoma of the breast

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Purpose: To investigate the predictive value of clinical and biological markers for a pathological complete remission (pCR) after a preoperative dose-dense regimen of doxorubicin and docetaxel (AT), with or without tamoxifen, in primary operable breast cancer.

Patients and Methods: Patients with a histologically confirmed diagnosis of previously untreated, operable, and measurable primary breast cancer (T2-3(≥3 cm)N0-2M0) were treated in a prospectively randomized trial with four cycles of biweekly AT chemotherapy, with or without tamoxifen, prior to surgery. Clinical and pathological parameters (menopausal status, clinical tumor size and nodal status, grade, and clinical response after two cycles) and a panel of biomarkers (estrogen and progesterone receptors, Ki-67, HER2, p53, bcl-2, all detected by immunohistochemistry) were correlated with the detection of a pCR.

Results: A pCR was observed in 9.7% in 248 randomized patients and in 8.6% in the subset of 197 patients with available tumor tissue. Clinically negative axillary lymph nodes, poor tumor differentiation, clinically complete or partial response after two cycles, negative estrogen receptor status, negative progesterone receptor status, high percentage of Ki-67 positive cells, and loss of bcl-2 were significantly predictive of a pCR in a univariate logistic regression model, whereas in a multivariate analysis only the clinical response after two cycles provided significantly independent information. Backward stepwise logistic regression revealed a response after two cycles, with progesterone receptor status and lymph-node status as significant predictors. Patients with a low percentage of cells stained positive for Ki-67 showed a better response when treated with tamoxifen, whereas patients with a high percentage of Ki-67 positive cells benefited more when treated without tamoxifen. Tumors over expressing HER2 showed a similar response to that in HER2-negative patients when treated without tamoxifen, but when HER2-positive tumors were treated with tamoxifen, no pCR was observed.

Conclusions: Reliable prediction of a pathological complete response after preoperative chemotherapy is not possible with clinical and biological factors routinely determined before start of treatment. The response after two cycles of chemotherapy is so far the strongest independent predictor, and can be used to save patients from further ineffective and toxic chemotherapy.

258 ORAL

TP53 mutation and/or overexpression of the HER2 receptor are strong indicators of poor prognosis in both node-negative and node-positive early breast cancer

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Purpose: Mutation in TP53 and overexpression of the HER2 receptor have been described to have prognostic importance for the outcome of breast cancer. The present study was performed to evaluate if TP53 mutation, HER2 expression, or a combination of these would be feasible prognostic markers in the routine diagnostic evaluation of early breast cancer, and especially in node negative patients.

Materials and Methods: Tumour material were obtained from women with sporadic early breast cancer. TP53 gene mutations in exon 2-11 were identified using DGGE and characterized by sequencing (Clin Cancer Res. 2000; 6:3923). Tumours were counted as HER2 overexpressing when a strong staining of the entire membrane (using the c-erbB-2 antibody from DAKO) was observed in more than 10% of the tumour cells ('3+' in the HercepTest guidelines). All patients were treated according to the Danish Breast Cancer Cooperative Groups guidelines for the DBCG 89 protocols.

Results: The study included 456 patients, 222 node-negative and 236 node-positive. TP53 mutation was found in 24%, HER2 overexpression in

18%, and 6% had both alterations. TP53 mutation and HER2 overexpression was associated with parameters related to tumour aggressiveness (positive lymph nodes, negative receptor status, high degree of anaplasia etc). Univariate analysis showed that disease-specific survival was correlated to tumour size, nodal status, degree of anaplasia, estrogen receptor status, TP53 mutation and HER2 overexpression. A similar pattern was observed for overall survival. When analysed according to nodal status, TP53 mutation and HER2 overexpression significantly correlated with poor survival probability in each of the subgroups. A Cox proportional hazard analysis including all 456 patients demonstrated that positive nodal status (1-3 positive nodes: relative risk (RR) 2.0, 95% CI: 1.2-3.2, and >3 positive nodes: RR 4.2, 2.9-6.5), grade 3 anaplasia (RR 2.1, 1.1-4.0), TP53 mutation (RR 1.9, 1.3-2.7), and HER2 overexpression (RR 2.3, 1.6-3.5) were the only parameters which had independent poor influence on reduced disease-specific survival. The same pattern was observed for overall survival. TP53 and HER2 retained their independent poor influence on survival when analysed according to nodal status.

Conclusion: TP53 mutation and/or HER2 overexpression are very strong markers for the prediction of disease-specific and overall survival in early breast cancer, irrespective of nodal status.

259 ORAL

Lympho-vascular invasion (LVI) and prognosis in invasive breast cancer

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The prognostic power of micrometastases in lymph nodes (LN) is currently under re-evaluation. Another marker of early lymphatic spread is lympho-vascular invasion (LVI). This was assessed on haematoxylin and eosin-stained sections at the periphery of 3931 primary operable invasive breast cancers in women presenting to the Nottingham Breast Unit between 1973 and 1998. Definite vascular invasion was categorised as positive and probable or absent vascular invasion as negative. Lymph node status was determined by an axillary sampling surgical procedure and each node carefully examined histologically. Long term follow-up and tumour and patient characteristics were recorded. Of the total cases, 75% showed no LVI and 25% were classed as LVI+. Forty-three percent of patients with LN+ disease showed LVI compared with only 17% of 2309 LN- cancers ($p<0.001$). In lymph node negative breast cancer the 10 year overall survival was 67% in LVI+ disease and 79% for LVI* tumours ($p<0.001$). Multivariate analysis in LN* cancers with histological grade, invasive tumour size and LVI entered, showed that LVI retained independent prognostic significance. Beta co-efficients for grade, size and LVI were 6.9, 10.0 and 3.1 respectively. In conclusion, LVI appears promising in adding prognostic discrimination in lymph node negative invasive breast cancer.

260 ORAL

Prognostic value of different proteolitic factors in primary breast cancer

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Introduction: Tumor biological factors involved in tumor invasion and metastasis have been recognized as strong novel prognostic factors in breast cancer (BC). We therefore evaluated prognostic value of proteolitic factors, such as cathepsins D and L (Caths D and L), u-PA, PAI-1, PAI-2 and u-PAR, for disease-free survival (DFS) and compared it with that of established prognostic factors such as nodal status (NS), tumor size and type, hormone receptor status (HRS), grading (G), vascular (V)/ lymphatic (L) invasion and menopausal status in our BC patients.

Material and Methods: The prognostic value of Caths D and L and u-PA, PAI-1, PAI-2, u-PAR, was assesed in a series of 715 operable BC patients, who underwent their primary therapy between 1996 and 2000. Most patients received some kind of adjuvant systemic treatment. Median patient age was 58 years. All proteolitic factors were determined prospectively in breast cancer tumor tissue extracts. u-PA, PAI-1, PAI-2, u-PAR and Cath L were determined by commercially available ELISAs, Cath D was determined by ELSA.

Results: At the median follow-up of 37 months disease reccured in 151/715 (21%) patients. In univariate analysis for DFS, among proteolitic factors Cath L (<5.35pmol/mg vs.≥5.35pmol/mg, p=0.0067), PAI-1 (<17ng/mg vs.≥17ng/mg, p< 0.0001) and u-PAR (<2.71ng/mg vs.≥2.71ng/mg, p=0.044) were significant prognostic factors in addition to NS, tumor size, HRS, G and V/L invasion. u-PA (<7.85 ng/mg vs.≥7.85 ng/mg, p=0.079), PAI-2 (<14.8 ng/mg vs.≥14.8 ng/mg, p=0.076) and Cath D (<50.3 pmol/mg vs.≥50.3 pmol/mg, p=0.21) had no significant prognostic impact. In multivariate analysis, among proteolitic factors only PAI-1 (p=0.0001) retained a statistically significant value, together with NS (p<0.0001) and HRS (p=0.0001). Cath L had a borderline prognostic significance (p=0.069) for DFS.

Conclusion: Among proteases, PAI-1 and Cath L seem to be the most important prognostic factors in BC patients and deserve further research, especially their combinations.

261 ORAL

Immunohistochemical features of early breast cancer in young women; a translational research project using high-throughput tissue microarray technology

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Young age (i.e. <35–40 years) is an independent prognostic factor for both poor local control and poor overall survival in early breast cancer. Therefore, patients younger than 35 years of age at time of diagnosis currently receive systemic cytotoxic therapy irrespective of tumor stage or grade. We hypothesised that the disadvantageous effect of young age in early breast cancer must be due to tumor-biological defects. Therefore we conducted a translational research project using a high throughput tissue micro-array to study the prognostic impact of several tumor markers in young breast cancer patients.

Methods: Paraffin embedded tumor blocks were collected for 550 early breast cancer patients younger than or equal to 40 years at time of diagnosis. All patients participated in prospective randomised trials conducted by the EORTC Breast Cancer Group and the EORTC Radiotherapy Group.

From every tumor block, three tissue core biopsies were taken and brought into one "recipient" paraffin block. Subsequently, sections from these recipient blocks will be stained and analysed.

In this subset of patients we first plan to study the estrogen receptor, p53, Her2, Ki-67, and PS6K, in order to assess the relation of these markers to prognosis.

The results of this project will be presented at the conference.

262 POSTER

Value of detection of bone marrow (BM) micrometastases by quantitative real-time RT-PCR in primary operable breast cancer(BC)

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Introduction: A sensitive and quantitative assay to detect micrometastases in BC patients (pts.) using real-time quantitative RT-PCR (ABI Prism 7700, Taqman ñ) identifying transcripts of the cytokeratin-19 (CK19) gene was previously developed. We detected significantly elevated numbers of CK19+ cells in BM and peripheral blood samples of BC patients. We investigated the value of the presence of CK 19 positivity as a surrogate marker for BM micrometastases in primary operable BC patients.

Methods: We analysed BM samples of 131 pts. with primary operable BC {pathological stage I(47 pts.), stage II(75 pts.), stage III(9 pts.)}. These samples were collected immediately before surgery. cDNA of BM samples from 38 patients with haematological malignancies in complete clinical and molecular remission were used as control population.

Results: We detected a median of 24 (95%CI [11; 43], IQR=50.5) and 267 (95%CI [200; 371], IQR=559.5) CK19 positive cells/5x106 leukocytes in the BM of the control group and the BC patients respectively (Mann-Whitney p < 0.00001). Using the upper limit of the 95% CI of the control group as cut-off, 79.4% of the patients were considered as positive. In stage I, II, and III patients, we detected CK 19 positivity in 83, 76, and 89% of samples respectively. After a median follow-up of 33 months (range 15-44), 11/131 patients developed metastases with a median time to disease progression of 20 months (range of 11-41). 8/11 pts. were CK19 positive at diagnosis. 6/11 patients died (4 pts. CK19+). The relationship between

CK 19+ cells in BM and classical prognostic factors showed no significant correlation with pathological tumour size, nodal involvement, stage, differentiation grade and receptor status. At this early interim analysis, there was no correlation between BM positivity and DFS or overall survival.

Overview: In primary BC patients, we detected high numbers of CK 19+ cells in BM compared to the control population. We found no significant correlation between presence of CK 19+ cells in BM and the classical prognostic factors in this population. To further estimate the value of the presence of BM micrometastases, a longer follow-up is needed.

263 POSTER

Are early breast cancers in patients from deprived areas less favourable?

C. Sharp[1], C. Wilson[1], W. Angerson[3], E. Mallon[2], J. Doughty[1], W. George[1]. *[1] Western Infirmary, University Department of Surgery, Glasgow, Scotland; [2] Western Infirmary, Department of Pathology, Glasgow, Scotland; [3] Glasgow Royal Infirmary, Glasgow, Scotland*

Introduction: It is well recognised that patients from deprived areas with breast cancer have a poorer outcome, however the reasons for this are unclear. Some studies have found that patients from deprived areas are more likely to have oestrogen receptor negative tumours where as others have found no relation between deprivation and pathological prognostic factors and suggest poorer outcome may be due to late presentation or poorer host responses.

Method: Data was collected prospectively from five hospitals in Glasgow from October 1995 until March 2001. Patients who had tumours of 10mm or less excised over this time period were identified and separated into three groups - affluent, intermediate and deprived according to the Carstairs index of deprivation at the time of diagnosis. The influence of deprivation on ER status, histological grade, axillary node status and the presence of lymphovascular invasion were examined.

Results: A total of 608 patients were included in the study. 135 affluent, 293 intermediate and 180 deprived. We found that patients in the deprived group were significantly more likely to have higher grade tumours with 11.1% of affluent, 15.4% of intermediate and 21.7% of deprived patients having grade 3 tumours (p= 0.032). There was a trend for the more deprived patients to have a higher incidence of ER negative tumours with 11.3%, 17.9% and 19.3% of patients in the affluent, intermediate and deprived groups having a negative ER status respectively. This however was not significant (p= 0.073). More patients in the deprived group had lymphovascular invasion present, 13.9% compared to 10.6% and 10.4% in the intermediate and affluent groups (p= 0.3). There was no relationship between node positivity and deprivation.

Conclusion: We have found that early breast cancers in deprived patients are less favourable with significantly more patients having high grade tumours. They also had an increased incidence of ER negativity and lymphovascular invasion. This may explain why patients from deprived areas have a poorer outcome.

264 POSTER

Predictive value of serum HER2/neu ECD during trastuzumab-based therapy in patients with breast cancer

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Background: The Her-2/neu extracellular domain (ECD) is a 97 to 115kD oncoprotein cleaved from the cellular membrane of Her-2/neu overexpressing breast cancer cells. Previously we have reported that Her-2/neu ECD is increased in the sera of patients with Her-2/neu overexpressing breast cancer. Consequently, we were interested to elucidate whether serum levels of Her-2/neu ECD parallel the clinical course of disease in patients with Her-2/neu overexpressing breast cancer undergoing anti-Her-2/neu antibody (Trastuzumab, Herceptin®) based immunotherapy.

Patients and Methods: After obtaining patients' written informed consent a total of 3122 sera were obtained from 75 patients immediately before each administration of trastuzumab based (chemo)immunotherapy and analysed by a commercially available ELISA kit (Oncogene Science/Bayer Diagnostics). In a first step, we compared Her-2/neu ECD levels before initiation of treatment with those at the time of clinical restaging examinations. Subsequently, we analysed Her-2/neu ECD levels throughout the course of treatment to determine at which point kinetics of Her-2/neu ECD could adequately predict response to treatment

Results: Whereas baseline levels of Her-2/neu ECD did not predict response to trastuzumab based therapy, the ratio of Her-2/neu ECD levels before initiation of treatment and at the time of restaging examinations, respectively, was significantly higher ($p<0.01$) in patients responsive to treatment. This difference in kinetics of serum Her-2/neu ECD between respondents and progressing patients was already significant after the first infusion of trastuzumab.

Conclusions: Monitoring of Her-2/neu ECD during Her-2/neu antibody based therapy for breast cancer represents a valuable tool for early prediction of response and thus optimization of trastuzumab based treatment and resource allocation.

265 POSTER

The proliferation marker MIB1 correlates less well with S-phase fraction but rather with mutant p53, poor Elston grade, high cyclin E expression and worse prognosis in human primary breast cancer

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Introduction: Proliferation rate measured as S-phase fraction (SPF) with flow-cytometry is considered of prognostic value for breast cancer patients. The SPF method has been described to have clinical utility.

Aim: We investigated possible correlations between the proliferation marker MIB1 with SPF, established and new biologic prognostic markers and outcome in primary breast cancer.

Patients and Methods: Of 315 consecutive patients with operable breast cancer, diagnosed between 1987 and 1989, 309 had paraffin embedded material available for determination of MIB1. The median age was 63 years, the median-follow-up time was extended to 122 month. MIB1 index, c-erbB2 status, and Cyclin E were determined by immunohistochemistry (IHC). p53 mutations were determined by cDNA based sequencing of the entire gene, estrogen- and progesterone receptor status by immunocytochemistry, VEGF by an ELISA and SPF by flow cytometry.

Results: In the 50 first cases was MIB1 index determined manually and with a grid graticula based method. A high correlation was seen between these methods ($p < 0.001$; $r = 0.89$), and for the following samples was only the graticule method used. SFP was inconclusive in 18 patients. High MIB1 index was statistically significant correlated with high SPF ($p = 0.0029$; $r = 0.17$), aneuploidy ($p < 0.0001$; $r = 0.26$), but more markedly to mutant p53 ($p < 0.0001$; $r = 0.49$), poor histologic grade by Elston ($p < 0.0001$; $r = 0.50$), high Cyclin E ($p < 0.0001$; $r = 0.48$), high VEGF expression ($p < 0.0001$; $r = 0.30$), and negative ER ($p < 0.0001$; $r = 0.37$). Associations were also seen to PgR negativity, over expression of c-erbB2, larger tumor size, and lymph node metastasis. Univariate ananlysis showed high MIB1 index as statistically significantly associated with shorter relapse-free survival (RFS) ($p = 0.0003$), overall survival (OS) ($p = 0.0003$), and breast cancer corrected survival (BCCS) ($p = 0.0056$). SPF was only statistically significantly correlated to OS ($p = 0.0121$), but not for RFS ($p = 0.7655$) or BCCS (0.1913).

Conlusions: High MIB1 index is associated with high SPF and aneuploidy. A higher degree of correlation was seen to other prognostic markers as, mutant p53, poor histologic grade, high Cyclin E and VEGF expresson, and negative steroid receptors. MIB1 was possible to determine in a higher proportion of patients and was stronger correlated with survival than SPF. The results indicate that MIB1 index may be a useful and applicable prognostic factor for breast cancer patients.

266 POSTER

Tubular carcinoma of the breast: is the long term survival insured?

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Introduction: Pure tubular invasive carcinoma (TC) of the breast (Grade I and more than 90% tubular formation) is thought to have favourable prognosis in treated patients observed over short and intermediate periods of 5-10 years. There is, however, little data relating to more extended follow up. This abstract retrospectively reviews the long term (20-25 years) survival in patients with pure tubular carcinoma to investigate whether this lesion does confer a better survival compared with the control group of other commoner grade I invasive ductal carcinomas of no specified type, (NST).

Materials and Methods: Case records and computerised data from 1975 - 2000 were obtained for all grade I invasive ductal carcinomas (NST and TC) treated at Guy's Hospital London. There were 73 patients with pure TC and 420 grade I (NST). The mean follow up was 9.7 years. These cohorts were of similar age (TC median 52 years (35-83); G1 (NST) median 55.1 years (27-88). All patients had local surgery for the primary tumour as well as a complete axillary dissection. Kaplan-Meier survival curves were obtained with regard to lymph node status and histology and statistical analysis was carried out using the Log Rank method.

Findings: There was no correlation between age (p=0.102), nodal status (p=0.205) and histological type within the pure TC group long term survival was significantly better in patients without lymph node metastasis as might be expected (P = 0.0412). However, no significant statistical differences in long-term survival of patients with TC and those with G1 (NST) were identified (P = 0.487). Moreover the similarity in overall survival remained after sub-division into node negative (p= 0.2.3) and node positive (p=0.70) groups.

Conclusion: Although pure tubular carcinoma has been thought to be a favourable sub-type of breast cancer, it is concluded that its overall long term survival is no better than that of the commoner grade I invasive carcinoma of non-specific type. Therefore tubular carcinoma should continue to be regarded as potentially life threatening in the longer term and accordingly should not merit an attenuated treatment or follow up protocol.

267 POSTER

Overexpression of HER2/neu and epidermal growth factor receptor in node-negative T1-3 breast cancer: an appraisal of the prognostic value and their impact on the selection of patients for systemic adjuvant therapy

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In a series of 286 female breast carcinomas with pathological node negative and clinical T1-T3 tumor, we evaluated the usefulness of four biological markers, overexpression of HER2/neu, epidermal growth factor receptor (EGFR), estrogen receptor (ER) and progesterone receptor (PgR) as the possible prognostic factor including clinicopathological variables such as menopausal status, tumor size and histological type. The overexpression of HER2/neu protein was determined by immunohistochemically and classified into three groups (negative, weak positive and strong positive groups). The overexpression of EGFR was determined by competitive ligand binding assay. A total of 97 tumors (33.9%) were HER2/neu positive (weak positive: 51, strong positive: 46), 58/153 tumors (37.9%) were EGFR positive (> 1 fmol/mg protein), 160/267 tumors (59.9%) were ER positive and 106/260 (40.8%) were PgR positive. There was no link between ER status, PgR status, menopausal status, tumor size and HER2/neu expression. A significant inverse relationship was found between ER and EGFR ($p<0.0001$). There was a significant linear relationship between tumor size and EGFR expression ($p<0.0001$). After follow-up period (median 50.6 months), a total of 21 recurrences (6 locoregional only) and 22 deaths were recorded. By univariate analysis, menopausal status and overexpression of both HER2/neu and EGFR were significantly related to disease-free interval (log-rank, premenopause versus post-menopause: p=0.003, HER2/neu/EGFR both positive versus both negative: p=0.014). HER2/neu, ER and PgR were significant predictor to overall survival (log-rank, HER2/neu positive versus strong positive: p=0.0134, ER: p=0.035, PgR: p=0.025). By multivariate analysis, HER2/neu expression was only predictor of overall survival (Cox multivariate analysis, p=0.0385). The results indicate that, in node-negative T1-3 breast cancers, assessment of HER2/neu and EGFR provide newly predictive and prognostic information and the selection of patients for systemic adjuvant therapy additional to the well established prognostic factors.

268 POSTER

Comparison of clinical, radiological and pathological assessment of response to neoadjuvant chemotherapy for primary breast cancer

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Clinical and radiological (mammography and/or ultrasound) assessment based on UICC criteria is used to evaluate response to neoadjuvant chemotherapy in primary breast cancer. More recently pathological assessment of post-treatment resected specimens has been advocated. Study compares clinical, radiological and pathological assessments of response to neoadjuvant chemotherapy.

72 patients received 6 cycles of neoadjuvant treatment using FEC (54), AC (10), MMM (5) or CMF (3) chemotherapy. All patients were reassessed both clinically and radiologically (by ultrasound (35), mammography (14) or both modalities (23)) and categorised into complete (CR), partial (PR) or non-responders (NR). Pathological response in the surgical specimen was defined as follows: CR - no residual tumour; PR - histological tumour response; NR - no tumour response.

Response (percent) categorised by various methods of assessment:

	Clinical	Mammography	Ultrasound	Pathological
CR	23 (32)	5 (14)	3 (5)	6 (8)
PR	35 (49)	20 (54)	41 (71)	25 (35)
NR	14 (19)	12 (32)	14 (24)	41 (57)
n	72	37	58	72

Mammographic and ultrasound categorisation of response was identical in 22 of the 23 patients assessed by both modalities. All 23 clinical complete responders achieved a radiological response (CR or PR) with the exception of one patient with stable disease both mammographically and pathologically. However, pathological response varied widely amongst clinical complete responders: 5 CR, 10 PR and 8 NR. Of the 41 patients (57%) with pathological NR, the majority were judged to have responded by other methods: clinical examination 10 NR, 23 PR, 8 CR; radiological assessment 16 NR, 22 PR, 3 CR.

Conclusion: This study highlights the inadequacies of clinical assessment of response to neoadjuvant chemotherapy. A substantial proportion of patients with complete response, either clinically or radiologically, had stable disease by pathological examination. The clinical significance of these results may be revealed by correlation with long-term survival data.

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Assessment of concordance of prognostic indicators between breast core needle biopsies and excision specimens

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Introduction: In patients with symptomatic breast lumps, surgical intervention, neoadjuvant treatment and primary medical therapy are often based on core biopsy (CB) results. The concordance of biological characteristics between CB and the surgical excision specimen (SES) has not yet been reported conclusively. In this study we report the concordance rate of tumour grade, Cerb-B2, Bcl-2 and oestrogen receptor (ER) status between CB and SES.

Method: We analysed 161 consecutive symptomatic breast cancer patients in whom there had been no therapeutic intervention between obtaining the CB and SES. The histological grade as well as Cerb-B2, Bcl-2 and ER status were confirmed with standard histological and immunocytochemistry method, respectively.

Results are presented in the table. The histological grade of the SES was upgraded in 30.3% and downgraded in 4.1% of cases. The ER status of the CB changed from poor to rich in 1.9% and from rich to poor in 4.5% of SES results. Cerb-B2 staining of the CB went from both negative to positive and positive to negative in 4.7% of SES results. The Bcl-2 status of the CB altered from negative to positive in 6.0% and from positive to negative in 6.0% of SES cases.

	Histological Grade *n = 122*	ER Staining *n = 154*	C-erb B2 Staining *n = 148*	Bcl-2 Staining *n = 149*
CB and SES Concordance (%)	65.6	93.5	90.5	87.9

Conclusion: Our results show that there is good concordance of ER, CerbB2 and Bcl2 status between CB and SES. CB is, therefore, a useful way to identify these biological characteristics of a breast tumour which can then reliably be used to plan and administer neoadjuvant treatments prior to surgery. The concordance of histological grade between CB and SES is much lower and implies that surgical treatment based on grade alone must be planned with caution.

270 POSTER

Assessment of HER2/neu status in core needle biopsy of 215 patients with primary breast cancer

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Purpose: Her2-neu overexpression in breast cancer patients is associated with worse prognosis and resistance or sensitivity to specific treatment. Core needle biopsy is an easy and cost-effective method to obtain pretherapeutically information about tumorbiology and cancer specific markers. The aim of our study was to investigate the accuracy of her2-neu assessment in core needle biopsy tissue.

Methods: Her2-neu status was evaluated by immunhistochemistry (HercepTest®) of formalin-fixed, paraffin embedded biopsy tissue and surgical removed tissue in 215 patients, 72pat. (33,5%) received preoperative chemotherapy, 143pat. (64,5%) had no preoperative treatment. Intensity of staining was scored according to the guidelines of HercepTest® (neg, 1+, 2++, 3+++). We calculated the accuracy, sensitivity, specifity and Kappa coeffizient of her2-neu results and correlated strong positive tumors versus negative, 1+ and 2++; separate analysis of patients with or without preoperative treatment was performed.

Results: Her2-neu overexpression was found in core needle biopsies of 26pat.(18.2%) in the preoperative untreated group and in 14pat.(22%) in the preoperative chemotherapy group. Concordant results of her2-neu determination in needle biopsy and the final pathological specimen was found in 132pat. (92%) without preoperative treatment and in 56pat.(87%) with preoperative chemotherapy. The sensitivity of the method was 69%, specifity 97%, accuracy 92% and simple Kappa coeffizient was 0.72 in the preoperative untreated group. The preoperative chemotherapy group did show slightly worse results - the sensitivity was 71%, specifity 92%, accuracy 87% and simple Kappa coeffizient was 0.63, respectively

Conclusion: The assessment of her2-neu status by core needle biopsy in breast cancer is feasible and preoperative treatment with trastuzumab can be based on these results.

271 POSTER

Predictive value of PAI-1 and PAI-2 in breast cancer

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Introduction: Besides searching for new prognostic factors there is an increasing interest to find possible predictive factors in breast cancer. Prognostic value of proteolytic factors has already been recognized, however their predictive value has not been established yet.

Aim: To assess the predictive value of PAI-1 and PAI-2 (plasminogen activator inhibitor 1 and 2) for response to systemic therapy in metastatic breast cancer patients.

Patients and Methods: The study included patients treated with chemo (CMF or anthracycline-based) therapy (ChT) or hormonal (tamoxifen or aromatase inhibitors) therapy (HT) for metastatic breast cancer in whom PAI-1 and PAI-2 levels in primary tumor extracts had been measured using ELISAs (American Diagnostica Inc.; CT). High and low values of PAI-1 as well as PAI-2 were dichotomized using median value for PAI-1 and optimized cut off value for PAI-2. Responders were considered those who achieved complete or partial response by WHO criteria, for hormonal therapy, stable disease for more than six months was also classified as response. Differences in response were calculated by chi-square test.

Results: The response rate to ChT was 37/69 (54%) and to HT 21/45 (47%). There were no statistical differences in response rate to ChT nor HT according to PAI-1 levels (both, $p>0.05$). The response rate to ChT was found significantly higher in a subset of patients with low PAI-2 levels (33/55; RR=60%) compared to high PAI-2 levels (4/14; RR=29%), $p< 0.05$. The response to HT was also found to be better in patients with low PAI-2 levels (15/31; RR=48%) compared to high PAI-2 levels (4/12; RR=33%), however the difference did not reach the level of statistical significance (p=0.373).

Conclusion: According to our results based on a small number of patients PAI-2 could be a predictive marker for response to ChT and HT in breast cancer patients.

272 POSTER

PAI-1 is independant prognostic factor in the elderly breast cancer patients

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Introduction: Serine proteases as urokinase-type plasminogen activator (uPA), and its main inhibitor (PAI-1) has been confirmed as independant prognostic factors in breast cancer patients that could be helpful in tailoring adjuvant treatment. Because of comorbidity in the elderly breast cancer patients the decision of adjuvant therapy is usually difficult and additional prognostic factors are needed.

Aim: To find out whether uPA and PAI-1 are important prognostic factors in the breast cancer patients over 70 years.

Material and Methods: 112 operable breast cancer patients, median age of 73 years, were included into our analysis. After surgery most of them were treated with adjuvant hormonal therapy (HT) (86/112; 76%). uPA and PAI-1 were determinated in tumor tissue extracts by using ELISAs metod (American Diagnostica Inc., CT).

Results: After a median follow up of 35 months 19% of the patients relapsed. Univariante analysis determinated PAI-1 (p=0.0068), hormonal receptors (p=0.0017), nodal status (p=0.0070) and grade (p=0,0018) as statistically significant prognostic factors for DFS. In contrast uPA, tumor size, and histological type failed to be of prognostic value. In multivariante analysis only PAI 1 (p=0.0055; RR=5.8) and nodal status (p=0.0160; RR=3.9) showed the independant prognostic value.

Conclusions: PAI-1 was found to be the most important prognostic factor in our group of elderly breast cancer patients and should be considered in adjuvant treatment decision. Because uPA was not found as independant prognostic factor and most of the patients recived adjuvant HT we assume that uPA could be a predictive factor for response to HT and its predictive value should be further investigated.

273 POSTER

Young age and prognosis in invasive breast cancer

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Young age has been claimed to be a poor prognostic factor in breast cancer patients. This was addressed in a series of 3715 invasive primary operable invasive breast carcinomas in women aged 70 or less. Patients presented to the Nottingham Breast Unit between 1973 and 1998. Follow-up data was available, as well as patient and tumou characteristics including known lymph node stage, histological grade and tumour size. Age was categorised for the purposes of this analysis as: 35 or less, 36 to 49 or 50-70 years.

In univariate analysis a significant association between age and death was seen only when patients were identified as alive, dead from breast cancer or dead of other causes ($p<0.001$); older women were more likely to have died from causes other than breast cancer. The 10-year breast cancer specific survival however was 64%, 69% and 68% respectively for patients aged 35 or less, 36-49 or 50-70 ($p = 0.425$). There was a significant difference in tumour characteristics by patient age, including histological grade ($p<0.001$), vascular invasion ($p<0.001$) and tumour size ($p = 0.004$). Younger women in particular tended to have more grade 3 tumours with definite vascular invasion. As a result more women 35 years or less fell into the

Poor Prognostic Group compared to patients aged 36-49 and 50-70 (29%, 16% and 15% respectively). When stratified according to the Nottingham Prognostic Index (NPI), however, there were no differences in survival in any prognostic group according to age.

In conclusion, age is not an independent prognostic factor in invasive breast cancer but women aged 35 or less have poorer prognosis tumours.

274 POSTER

Patients with elevated serum HER2/neu levels demonstrate increased response to herceptin therapy

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Introduction: Breast cancer patients with increased expression of HER-2/neu have poor prognosis. Patients who are HE-2/neu tissue positive are eligible for Herceptin therapy at recurrence of disease. The objective of this study was to evaluate the ability of serum HER-2/neu to predict patients' response to Herceptin therapy.

Method: A total of 39 patients who were tissue positive (IHC 3+ or 2+ using Dako's Herceptest) at diagnosis were treated with Herceptin at recurrence. Serum samples were drawn before the initiation of drug therapy. Serum HER-2/neu was measured on the Bayer Immuno 1 system. Objective clinical assessments were recorded according to the WHO criteria to demonstrate Herceptin response. The calculated response rate (RR) was determined as Responders/Responders + Progressors. Patients with stable disease were excluded from the analysis.

Results: For all patients tested, 25 responded to therapy, whereas 14 showed disease progression (RR=64.1%). For 28 serum positive patients, 21 showed response to Herceptin (RR=75%), whereas only 4 out of 11 patients with normal Her-2/neu values responded to therapy (RR=36.4%). These patients were further stratified into IHC 3+ and 2+ groups. For 23 IHC 3+ patients, 13 out of 18 serum positive patients responded to Herceptin (RR=72.2%) compared to only 3 out of 5 patients who were serum negative (RR=60%). For the IHC 2+ group, 4 out of 5 serum positive patients showed response (RR=80%) while only 1 out of 4 responded in the serum negative group (RR=25%)

Conclusions: These findings indicate that serum HER-2/neu as compared to IHC may better predict patients' response to Herceptin therapy in recurrent breast cancer.

275 POSTER

Tumour VEGF levels in relation to efficacy of systemic therapy for specific sites of breast cancer metastases

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Introduction: Recently we showed that a high primary tumour level of vascular endothelial growth factor (VEGF) is related with a poor outcome on first-line tamoxifen (n = 618) and CMF/FAC chemotherapy (n = 227) in patients with advanced breast cancer (*Cancer Res 61: 5407–5414, 2001*). In the present study we evaluated the predictive value of VEGF in these patients stratified by type of distant metastasis.

Methods: The 845 patients were divided in groups regarding their dominant site of relapse (19 brain, 118 liver, 208 lung, 335 bone, and 165 others, mainly loco-regional relapses). VEGF levels were measured by ELISA (*Int J Biol Markers 15:184-191, 2000*). Endpoints were the rate of response, progression-free survival (PFS) and post-relapse overall survival (PR-OS), for both first-line tamoxifen and chemotherapy.

Results: For patients treated with tamoxifen, high VEGF levels predicted a poor response (odds ratio [OR]: 0.52; $p < 0.01$) and a short PFS (relative hazard rate [RHR]: 1.30; $p = 0.03$) for bone metastases. For those patients with lung metastases high VEGF levels were associated with a short PFS (RHR: 1.50; $p = 0.01$) and PR-OS (RHR: 1.64; $p < 0.01$), VEGF predicted a shorter PR-OS (RHR: 3.42; $p = 0.002$) for patients with liver metastases. In patients with liver treated with chemotherapy, high VEGF levels were related with a poor rate of response (OR: 0.34; $p = 0.03$) and a short PR-OS (RHR: 1.68; $p = 0.05$).

Conclusion: The primary tumour level of VEGF might be helpful to select the type of systemic therapy for patients with advanced breast cancer.

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276 POSTER

Is weight of breast associated with hormone receptor expression in postmenopausal invasive breast cancer?

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Objective: In postmenopausal women, oestrogens are produced from aromatisation of C19 steroids in stromal adipose cells of peripheral and breast fat. Enzymes in breast cancer tissue modulate intratumoral levels of oestrone, oestradiol and oestrone sulfate. We examined how the endogenous oestrogenic environment is associated with steroid hormone receptor expression. We hypothesized that the oestrogen driven PR is more likely to be present in breast cancers from obese patients, in those with large size breasts and large tumours. We also expected an ER+ breast cancer to be of larger size in an oestrogen-rich environment compared with an oestrogen-poor situation.

Methods: Retrospectively, we examined the effect of weight of mastectomy and tumour size on ER and PR-expression in 139 postmenopausal women with an invasive breast cancer. Age and tumour differentiation were also taken into account. In a subgroup of 82 of these 139 women, ER and PR expression were also examined in relation with the same set of variables plus the woman's BMI. Hormone steroid receptors were analysed with IHC using the H-score and ≥ 30 was chosen as a positive value (range: 0–300). We used standard statistical procedures (Pearson correlation, linear regression, logistic regression) when distributions proved to be normal, and non-parametric tests otherwise (Spearman correlation, Chi-square test).

Results: In the 139 women, as expected, hormone receptor expression (positive or not) was strongly associated with tumour differentiation. Levels of ER and PR were strongly correlated. We observed no significant association between other variables. Noteworthy, breast weight and tumour diameter were not associated with ER (<30, ≥ 30) or PR expression ($p = 0.452$ and 0.810). In the subgroup of 82 women, breast-weight was strongly correlated with BMI. We performed a logistic regression analysis to examine the relationships between age, BMI, breast weight, tumour diameter and tumour differentiation, and qualitative ER expression (65 ER+ vs 17 ER−). Tumour differentiation only was significantly associated with ER expression. We also performed a linear regression analysis in the 65 ER positive women in order to model ER expression by taking into account the same set of covariates. The model did not fit at all, providing no evidence of any significant relationship between the independent variables considered and ER expression. When we tried to model PR expression by logistic regression analysis, it appeared that tumour differentiation ($p < 0.01$), BMI ($p = 0.051$) but not breast weight and tumour diameter ($p = 0.036$) were positively associated with PR expression. However, after selection of the 50 PR positives women, linear regression analysis failed to identify any variable associated with the magnitude of PR expression.

Conclusion: Using the now generally accepted method to measure steroid hormone expression in invasive breast cancers, immunohistochemistry, in our series of postmenopausal women, we failed to demonstrate any clinically relevant and statistically significant adjusted effect of age, BMI, breast-weight or tumour diameter on ER expression. Tumour differentiation was the only variable associated with ER positivity. Yet, we observed a significant adjusted effect of BMI, tumour diameter and tumour differentiation on PR status, albeit these variables did not appear to influence the level of PR expression in women scoring positive.

277 POSTER

Analysis of urokinase and tissue type plasminogen activators and their inhibitor (PAI-1) in breast cancer tissue: clinical implications

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Purpose: Several studies on predominantly European populations have indicated that uPA, tPA and PAI-1 may be of prognostic importance in breast cancer. We have measured these proteins in tumours from an ethnically mixed population in Kuwait, to assess their clinical usefulness for patient management, in comparison to other markers that are routinely measured as an aid to staging and therapy.

Methods: Cytosol fractions prepared from primary breast cancers surgically removed from 217 women were assayed for uPA, tPA and PAI-1 using a new ELISA method; ER, PR and pS2 were measured by immunoradiometric assay in a subset of these patients. During clinical follow up, serum

levels of CEA and CA153 were periodically determined using EIA. Statistical analyses and inter-relationships of these factors and clinico-pathological parameters were performed using the SPSS package.

Results: Only tPA correlated to nodal status and tumour grade, and PAI-1 to clinical stage. PAI-1 was related to uPA and both were inversely correlated with PR and pS2 (PAI-1 also to ER) which are usually associated with survival advantage. Conversely tPA was directly correlated with ER, PR and pS2. Women with high tumour uPA and PAI-1, but not tPA, had shorter overall, and relapse free, survival. However none of the protein markers were independent predictors in multivariate analysis. Only nodal status and clinical stage retained this feature.

Conclusions: As prognostic indicators, none of the proteins measured added additional information to that achieved from axillary node assessment. However, uPA and PAI-1 were more prognostically informative than ER or PR and, as with these markers, their usefulness may extend to delineation of patients likely to respond to certain types of therapy. The presence of plasminogen activators as a negative influence suggests that anti-proteases may be effective as growth inhibitors, possibly by retarding metastasis.

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278 POSTER

Plasma viscosity and prognosis of breast cancer

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Tumor growth leads to tissue hypoxia and tissue hypoxia, in turn, is a strong stimulus for expression of genes encoding factors that promote tumor growth. A marker of the presence of tissue hypoxia may be the presence of high blood viscosity, which is found in a number of neoplastic diseases. From September 1992 to September 1998, 285 consecutive patients with localized breast cancer admitted for primary surgery were entered into this study and followed over a maximum of 7.3 years. 125 healthy women and 164 women awaiting surgery for benign tumors served as controls. Plasma viscosity (pv) was determined using a capillary tube viscosimeter (KSPV 1 Fresenius, Bad-Homburg). The day prior to primary breast cancer surgery, the mean pv was 1.33 SD:0.13 mPas which was significantly higher when compared to both patients with benign tumors (1.27 SD:0.1mPas;$p<0.0001$) and to healthy women (1.29 SD:0.09 mPas;$p<0.0001$). The total follow-up observation-time was 15,699 patient months (mean: 55.1 months/patient). Within this period, 50 of the 285 patients (17.5%) died. Patients dying of cancer had had significantly higher initial pv (1.40 SD:0.18 mPas;$p<0.0001$) when compared to patients not dying of cancer (1.30 SD:0.10mPas). In multivariate proportional hazard regression analysis, next to tumor size (p=0.03) and nodal status (p=0.004), pv was an independent prognostic marker for overall survival of breast cancer patients (RR=130.2;95%CI:11.6 to 1,460.6; $p<0.0001$). An optimized preoperative cut-off value above 1.40 mPas was significantly associated with poor outcome in the Kaplan Mayer survival-estimates, even in node-negative breast cancer.

Like-wise an increase fibrinogen/fibrin-turnover and breakdown-products characteristically associated with tumor-cell-dissemination contribute to the increased plasma viscosity while the hematocrit, leukocyte-, and platelet count contributed little to the increased blood viscosity in patients with breast cancer.

279 POSTER

Thrombosis - a clue of poor prognosis in primary non-metastatic breast cancer?

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In breast cancer the true incidence of venous thromboembolism (VTE) is not known but after diagnosis of non-metastatic breast cancer VTE will occur in about 6% of patients during adjuvant treatment. An association between the occurrence of thrombosis in a patient with breast cancer and poor prognosis of cancer has been discussed for a long time but not formally tested.

From 1/1991 until 6/1996, 366 consecutive patients with breast cancer admitted for primary surgery, were screened for VTE using non-invasive methods and followed over a maximum of 7.5 years. Eight-teen patients (4.9%) had metastases at the initial evaluation. Of the remaining 348 patients without metastases, 19 (5.5%) developed VTE during adjuvant treatment. Total observation-time was 23,211 patient months (mean of 66.7 months/patient). 126/348 patients (36.2%) developed metastases while 60/348 (17.2%) died. 12/116 patients (10.3%) who developed metastases and 12/60 patients (20.0%) who died of progressive cancer, previously had VTE. Accordingly, patients with VTE had a 2-fold increased risk for subsequent cancer-relapse (Odds Ratio: 2.00; 95%CI: 1.37-2.92; P=0.008) and a more than 4-fold increased risk of dying of cancer compared to patients without VTE (Odds Ratio: 4.32; 95%CI: 2.81-6.67; $P< 0.0001$). Median disease-free (p=0.004) and overall survival (p=0.01) in patients with VTE was significantly shorter as compared to patients without VTE.

VTE occurred more often in the presence of a tumor greater than 5 cm, in nodal positive women having more than 9 positive axillary-lymphnodes, and in women with a history of thrombosis.

These results support the idea that the development of VTE is linked to the progression of cancer. Anticoagulant-treatment and low-molecular-weight-heparin in particular might not only reduce thrombosis incidence during chemotherapy but also improve survival in certain patients.

280 POSTER

The role of depression in the development of breast cancer: a case-control study

A. Montazeri, S. Jarvandi, M. Ansari, M. Jafari, M. Ebrahimi, S. Haghighat.

The belief that developing breast cancer may be associated with psychological distress is not uncommon. This study examined the role of psychological variables in over 3000 women attending a breast clinic for medical examination in Tehran, Iran during a three-year period (1997-1999). All women were interviewed by a trained female nurse before a confirmed diagnosis was made. Data were collected on demographic variables, known risk factors, history of familial psychiatric disorder, history of psychiatric medications, depression (depressed mood and hopelessness), anxiety (mental and somatic sign), overall health and quality of life. In all, 243 patients were diagnosed as having breast cancer. Thus, 243 patients with benign disease were randomly selected from the original cohort as controls. A forward selection uni- and multivariate logistic regression analysis was performed to determine the predictive effect of each factor on the risk of breast cancer. There were significant differences between cases and controls with regard to familial psychiatric disorder (P = 0.01), psychiatric medications (P = 0.01), anxiety (mental sign P = 0.02, somatic sign P = 0.01) and depression (depressed mood $P < 0.00001$, hopelessness P = 0.003), while age, educational and marital status, overall health and quality of life were not. The logistic regression analysis indicated that depressed mood was the most significant predictor of breast cancer (odds ratio = 5.5, $P < 0.00001$). The findings of this study suggest that in addition to the known risk factors, psychological determinants such as depressed mood may play an important role in etiology of breast cancer.

281 POSTER

Analysis of two hundred six cases of bilateral breast carcinoma

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Introduction: Women with unilateral carcinoma of the breast are at increased risk for developing contralateral disease.

The purpose of this study is to evaluate risk factors and outcome for bilateral breast cancer.

Methods: 2189 unilateral and 206 bilateral breast carcinoma (8.6%) were treated surgically in our Department. Median follow up was 80 months. The mean age at presentation of each group was 56 and 53 years respectively.

Results: There was no statistically significant difference regarding the survival between the two groups. In the bilateral group 105 patients (50.9%) developed metachronous carcinoma, which was diagnosed at the same or earlier stage than the first cancer in 76% of cases.

There was no significant difference regarding the stage of the disease, lymph node involvement or the multicentricity of the disease (p= 0.952). Bilateral patients were more likely to have ductal invasive carcinoma than lobular, compared with the unilateral group.

Conclusion: The analysis of our material showed that the presence of synchronous or metachronous carcinoma doesn't affect the overall survival of breast cancer patients.

282 POSTER

Heat shock protein 27 correlates with oestrogen receptor alpha in benign and malignant breast and mammary cell lines

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Heat shock proteins (hsps) are molecular chaperones that are induced in cells in response to various stimuli. Overexpression of hsp27 has been correlated with poor prognosis in many cancers. Dysregulation of hsp27 expression in benign breast lesions may represent an early event in mammary carcinogenesis. We stained 49 biopsy specimens of normal breast, 47 hyperplasia of usual type (HUT), 31 ductal carcinoma in situ (DCIS) and 125 primary breast cancers for hsp27 and ER alpha using mouse monoclonal antibodies with heat pretreatment for antigen unmasking. The percentage of positively stained cells was quantified. The expression of hsp27 was also studied in MCF-7 and T47D ER positive and MDA-MB-231 ER negative breast cancer cell lines by Western blotting and immunohistochemistry using the same antibody. A progressive increase in mean hsp27 expression was seen from normal (6%), through HUT (23.5%) to DCIS and invasive cancer (61.1% and 54.9% respectively). In normal breast, % expression was significantly lower than that in HUT and cancers ($P<0.001$). ER+ cell lines expressed high levels of the protein whereas MDA-MB-231 cells showed low endogenous levels. A strong positive correlation was found between hsp27 and ER in HUT and cancers ($r=0.836$ and 0.714, $P<0.001$). Our data indicate a novel oestrogen dependent dysregulation of hsp27 expression in progression from normal through precancerous breast to malignancy, which may be an early important event in mammary carcinogenesis. These findings provide insight into its role as potential marker of breast cancer risk.

283 POSTER

Breast cancer in Iran: an immunohistochemical approach

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Considering the genetic basis of cancers, immunohistochemistry is a powerful tool to evaluate the gene function. In this regard we analyzed ER, PR, P53, CatD, CerbB2, Bcl2, Ki67 & P21 status of 1500 Iranian primary breast cancer patients.

Mean age of the patients was 47.5. Conventional prognostic factors (PF) included tumor size(mean 3.44), axillary lymph node (ALN) status (N0 36, N1 56 & N2 8%), TNM staging (stages IIB;29.1, IIA; 19.5, IIIB; 17.6, IIIA; 17, I; 14.5, IV; 2.2 & 0; 0.2%), grade (1; 14, 2; 54, & 3; 32%), lymphatic, vascular & perineural invasion (PNI)(61, 25 & 20% res.).

ER, PR, P53, CatD, CerbB2, Bcl2 & P21 were positive in 53.5, 41.6, 30, 79, 62, 27.6 & 31% of cases respectively.

ER expression was significantly ($p<0.001$) associated with older age, lower grade, smaller tumor size, less cellular atypia (CA) & pleomorphism (CP), less nuclear pleomorphism (NP) & less mitotic index (MI) but more PNI. It was also associated with more expression of PR ($p<0.001$) & CerbB2 ($p=0.003$) but less staining of P53 ($p<0.001$) & CatD ($p=0.028$). Similarly, PR expression correlated with lower grade ($p<0.001$), smaller tumor size ($p=0.027$), less CP & CN ($p=0.001$ & $p=0.004$ res.), less NP ($p<0.001$), less MI ($p<0.001$), less involvement of nipple ($p=0.046$) & less P53 staining ($p=0.003$).

Conversely, tumors with positive P53 staining tended to have higher nuclear & histological grades ($p<0.001$), Ki67 score ($p<0.001$) & CerbB2 staining ($p=0.035$).

CerbB2 expression correlated with more involvement of ALN ($p=0.023$) & the correlation increased with more number of nodes involved. CerbB2 staining was noted more among tumors with a background of fibrocystic changes ($p<0.001$).

CatD staining was associated with expression of CerbB2 ($p=0.043$), & Bcl2 expression was associated with nuclear hyperchromasia ($p=0.015$), more vascular invasion ($p<0.001$) & P21 expression ($p<0.001$), which was itself a predictor of ALN involvement ($p=0.032$). Finally, higher Ki67 score correlated with less tubular differentiation ($p=0.002$), more nuclear atypia ($p=0.004$) and more PNI ($p=0.004$).

These data confirm the role of ER & PR as good PFs (with the exception of ER & PNI) & also the role of P53, P21, & Ki67 as indicators of a worse prognosis, but the role of CerbB2, Bcl2 & perhaps CatD seems to be controversial.

284 POSTER

Plasma D-Dimer levels in operable breast cancer and correlation with sentinel lymph node involvement

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Malignancy frequently is accompanied by activated coagulation and systemic fibrinolysis underlining a hypercoagulable state. In patients with operable breast cancer, plasma D-Dimer levels have been shown to correlate with lymphovascular invasion, clinical stage, and axillary lymph node involvement (J.Clin.Oncol. 2000, vol. 18: 600-608).

In order to investigate whether plasma D-Dimer levels can be considered a prognostic marker of lymphovascular invasion we studied a potential correlation between plasma D-Dimer levels and sentinel lymph- node. D-Dimer levels of 240 ng/ml was considered the upper limit of the normal range by testing healthy volunteers.

Preoperative D-Dimer plasma evaluation was performed in 103 women (aged 34-83) affected by breast cancer before having breast resection and sentinel node biopsy.

Plasma D-Dimer levels resulted elevated (> 240 ng/ml) in 42 patients and only 6 had a positive sentinel node. Similarly, in the remaining 61 patients with low D-dimer levels (< 240 ng/ml) sentinel lymph node was positive in 14 patients. Table 1 ($P>0.05$)

Plasma D-Dimer	Sentinel node +	Sentinel node -	Total
>240	6	36	42
<240	14	47	61
			103

In conclusion, although preliminary, these results are not suggestive for a significant correlation between plasma D-dimer levels and extent of sentinel lymph node involvement in patients with operable breast carcinoma.

285 POSTER

Breast cancer: the medial tumor location - an unfavorable disease ! results from 644 patients (1984 - 1995)

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Purpose: The authors demonstrate the unfavorable survival rate in patients with medially located breast tumors (med) compared to patients with cancer in the lateral quadrants (lat).

Patients & Methods: From 1984 to 1995 644 patients with 649 T1-2 tumors were treated. 429 presented with lat and 220 with med (central 55 patients). The prospective treatment method included breast conserving surgery and radiotherapy (45 to 50 Gy) and one interstitial 10 Gy boost. All axillary nodal positive patients underwent systemic therapy (6 x CMF or Tamoxifen). Mean follow up of survivors: 77 months (25 to 158). Survival and local control were calculated by the Kaplan-Meier actuarial method and significance by the log-rank test. From the first 216 patients the cosmetic results were evaluated using a 4 grade scoring system.

Results: Comparing med and lat the actuarial survival parameters are highly significant in favor of the lateral tumors (p-values: overall survival 0.0009, disease specific survival 0.009, disease free rate 0.001). No significance is given in local control ($p = 0.08$). Cosmetic results after surgery were 1.65 in lat and 2.15 in med ($p<0.005$). These values hadn't changed 5 years after radiotherapy with 1.69 and 2.13 respectively ($p<0.025$).

Conclusion: The medial tumor location in the breast is associated with significant lower survival rates and significant unfavorable cosmetic results. The reason may lie in the fact that the pathological stage of the internal mammary chain is unknown, while in lateral tumors all patients with positive axillary nodes underwent systemic therapy and (in part) supraclavicular irradiation. Surgery in the medial part of the breast results in a poorer cosmetic outcome compared to lateral tumors. The medial tumor location has to be considered a negative prognostic parameter. A more agressive systemic therapy may be the therapeutic consequence in these patients.

286 POSTER

Effect of MR-mammography, mammoscintigraphy and whole-body 18F-FDG PET imaging on clinical staging and management of patients with breast cancer

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Correct staging is important in selecting the appropriate treatment for breast cancer (BC) patients. This study compared the accuracy of second line diagnostic procedures: MR-mammography, mammoscintigraphy and whole-body 18F-FDG PET for the identification of primary tumors and metastases and further impact on the management in suspected BC patients. Methods: Gd-enhanced MR-mammography in FLASH-mode, 99mTc-MIBI mammoscintigraphy and 18F-FDG PET were prospectively studied in 132 patients with suspected BC. PET and scintigraphic evidence of primary tumors or metastases was defined as regions of high FDG or MIBI uptake. For MR-studies, tumor lesions were defined as. Management changes were made inter- or intramodality within medical treatment, surgery and radiation. Results: MRI provided a working diagnosis in 86% of patients with equivocal mammography data and clinical examination results and allowed a rational approach to treatment (mastectomy or tumor excision) while demonstrated features compatible with tumor in breast and axillary region. Mammoscintigraphy and PET led to a change in the clinical stage in 46% of patients and, thus to a combination of the management changes. PET did result in management changes (surgical to chemotherapy and radiation) in cases of parasternal lymph nodes detection in 32% of patients. Findings of the MIBI-washout examination that reflect chemotherapy resistance resulted in altered medical management in 42% of patients. Conclusion: This study indicates that combined use of MR-mammography, mammoscintigraphy and FDG-PET can predict the clinical outcome and is helpful to decision making in the treatment strategy of BC patients. These diagnostic modalities are useful for staging as well as for monitoring of therapy.

287 POSTER

Scintigraphic, histologic and immunohistochemical parallels in patients with breast cancer

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Mammoscintigraphy with 99mTc-MIBI has been shown to be useful in the detection and staging of breast cancer. However, tracer uptake varies considerably among tumors and whether MIBI uptake is dependent on tumor features is still unclear. Tumor characteristics defined by histologic and immunohistochemical tissue analysis were compared with preoperative MIBI uptake assessed by mammoscintigraphy to evaluate the prognosis and treatment strategy of patients with BC.

Methods: Our series consisted of 21 BC patients. MIBI uptake in breast tumors was quantified by calculating tumor to normal tissue coefficients (T/NTs). The histologic sections were analyzed for histologic type, histopathologic grading, microscopic tumor growth pattern, percentage of tumor cells, presence of inflammatory cells, tumor cell proliferation (mitotic rate and antibody binding of MIB-1), expression of estrogen and progesterone receptors.

Results: A positive correlation was found between MIBI uptake and the percentage of tumor cells (P = 0.003), microscopic tumor growth pattern (nodular vs. diffuse; P = 0.007), and tumor cell proliferation (MIB-1; P = 0.009). Tumors with diffuse growth patterns had significantly lower T/NTs compared with clearly defined tumors. Lower sensivity to chemotherapy corresponded to higher MIBI washout (P = 0.008). A weak relationship was found between MIBI uptake and histologic tumor type (ductal vs. lobular;P = 0.06). No relationship was found between MIBI uptake and percentage of necrotic, fibrotic, and cystic compounds; presence of inflammatory cells; and steroid receptor status;.

Conclusion: Histologic and immunohistochemical tissue analysis was unable to sufficiently explain the variation of MIBI uptake in breast cancer. So, mammoscintigraphy may not be used to estimate tumor biologic behavior of breast cancer such as differentiation, histopathologic grading, or cell proliferation. At the same time, the MIBI washout degree in malignant transformation is most likely explained by a complex interaction between cellular energy demand tumoral microenvironment and Pgp expression, and MIBI can be used for chemotherapy resistance prognosis. Therefore, the use of hybrid 99mTc-MIBI mammoscintigraphy, histologic and immunohistochemical tissue analysis can predict the clinical outcome and is helpful to decision making in the treatment strategy of patients with BC.

288 POSTER

Clinical significance of E2F1 expression in breast cancer and its relationship with expression of cyclin E and retinoblastoma protein (pRB): integration of tissue microarray technology

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Background: Deregulation of transcription factor E2F1 activity results in the loss of cell cycle check point controls, thereby predisposing cells to malignant transformation and excessive growth. However, biologic role of E2F1 has not been determined in clinical breast cancer.

Methods: The interactions between E2F1 expression and the expression of retinoblastoma protein (pRB) and cyclin E were analyzed using tissue microarray (TMA) technology in 188 breast cancers.

Results: E2F1 was expressed in 79 (42.1%) and cyclin E expression was observed in 99 (52.7%) of 188 breast carcinomas. E2F1 expression was significantly increased in cyclin E-expressing tumors (p=0.023) and its expression was significantly associated with pRB expression (p<0.001). Ki67 labeling index was increased in 74% of E2F1-expressing tumors whereas its frequency was significantly decreased to 57.5% in E2F1-negative tumors (p=0.015). Of 79 patients with E2F1 expressing tumors, 23 patients (29.1%) had recurrent disease and 22 patients (27.8%) died during the median follow-up period of 52 months. Of 109 patients with E2F1-negative tumors, 17 patients (15.6%) had recurrent disease and 14 patients (12.8%) died during the follow-up period. Survival of the patients having E2F1-expressing tumor was significantly decreased compared with the patients having E2F1-negative tumor (p=0.012 by log-rank test).

Conclusions: Expression of cell cycle regulators was interrelated each other and was intimately associated with proliferative index in clinical breast cancers, but it is not clear whether the proteins function in linear pathways as in experimental system. Increased expression of E2F1 seems to be associated with aggressive biologic property of the breast cancer. The results of the current study indicate that integration of TMA technology enables a high-throughput analysis for correlating molecular in situ findings with clinico-pathologic information.

289 POSTER

Predictive factors associated with axillary lymph node metastasis(ALNM) in T1,T2 and T3 breast carcinomas

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Objectives: The purpose of this study is to determine the clinical and pathological factors predictive of ALNM in Turkish patients with T1, T2, and T3 breast carcinomas.

Patients and Methods: We retrospectively analyzed the records of 118 patients who received adjuvant treatment in Marmara University Hospital since 1997. All patients underwent a resection of primary tumor and axillary lymph node dissection. Patient and tumor characteristics including age, tumor size, histological grade, Er/Pr status, laterality of disease, multifocality, lymphatic, vascular and neural invasion are evaluated.

Results: ALNM were detected in 67 (56.8%) patients. The percentages of T1, T2, T3 tumors were 56.9%, 37.3%, 3.9% in node negative (NN) and 27.3%, 56.1%, 16.7% in node positive (NP) patients. The percentages of grade 1, 2 and 3 tumors and the percentages of Er/Pr status in NN and NP patients were similar. Left breast was involved in 61.2% of NP and 41.2% of NN patients. The percentages of lymphatic, vascular and neural invasion were 5.9%,3.9%,5.9% in NN and 34.4%,25%,10.4% in NP patients. In univariate analysis four variables were found to be significantly associated with ALNM; tumor size, lymphatic invasion, vascular invasion, and site of disease. NP patients were more likely to have larger tumors (p=0,004) and left breast involved (p=0,041). Vascular and lymphatic invasion were found to be more frequent in NP patients (p=0,004; p=0,000). In multivariate analysis, ALNM was found to be significantly associated with lymphatic invasion and increasing tumor size.

Conclusion: Lymph node metastasis in Turkish breast cancer patients is significantly associated with the presence of lymphatic invasion as well as the tumor size. Lymphatic invasion alone should also be considered in treatment planning strategy if other prognostic indicators are negative.

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Predictive model of axillary lymph node involvement (ALNI) in stage I-II breast cancer (BC)

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Purpose: To predict ALNI risk and to select optimal sentinel node biopsy (SNB) indications in stage I-II BC by combining tumor size, tumor location, histological subtype and grade.

Methods: We studied 795 stage I-II BC treated between 1980 and 1997 by breast conserving therapy. All cases had axillary dissection (AD) with at least 10 nodes removed. A stepwise logistic regression analysis was performed to build a predictive model of ALNI.

Results: The global ALNI rate was 25.7% (pN_1:10.5%, pN_{2-3}:8.4%, $pN_{>3}$:6.8%), but the estimated probability of ALNI varied from 6% (95% IC: 0–13%) to 45% (95% IC: 37%–52%).

Clinical size	Location	IDC SBR 1 %	IDC SBR 2–3 %	ILC %	other %
T_0	Inner	14	14	15	6
	Central	17	18	17	11
	Outer	19	24	24	16
T_1	Inner	19	20	15	13
	Central	13	20	18	7
	Outer	22	26	23	14
T_2 <4 cm	Inner	26	26	20	19
	Central	28	34	8	13
	Outer	37	45	39	21

Conclusion: The estimated probability of ALNI risk was subdivided in three categories: low (<15%), intermediate (15–25%) and high (>25%). The pN > 2 ALNI risk was in these three categories 3.7%, 12.2% and 20.7%, respectively. Thus, we consider that women with a risk of ALNI < 25% could benefit from SNB with a minimal risk of false negative rate.

291 POSTER

Predictive value of HER-2 receptor, p53, bcl-2 and bax proteins, MIB-1 antibody, estrogene and progesterone receptor expression in breast cancer patients receiving adjuvant tamoxifen

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The aim of the study was to asses the predictive value of several factors on overall and disease-free survival in breast cancer patients receiving adjuvant tamoxifen.

Radical surgical treatment was performed in 243 (150 - stage I and II, and 93 - stage III) breast cancer patients. Tamoxifen as sole subsequent systemic therapy was administered to 78 patients (56 of them had surgery alone, the remaining 22 received neoadjuvant therapy prior to surgery). Tamoxifen was administered for a period of 1.7-56 months (med. 40) and the observation time was 2-66.2 months (med. 44.6). The expression of HER-2 receptor, p53, bcl-2 and bax proteins, MIB-1 antibody, estrogene and progesterone receptor was assessed retrospectively using immunohistochemical methods. Survival curves were estimated using Kaplan-Meier method. The analysis of predictive factors was performed using multivariant proportional regression Cox model.

No statistically significant (p=0.05) factors influencing overall survival were found in a model stratyfied according to neoadjuvant therapy. Multivariant Cox analysis showed that disease-free survival was influenced by: HER-2 (p=0.016), age (p=0.016), p53 overexpression (p=0.018), nodal status (p=0.004).

Disease-free survival in breast cancer patients receiving adjuvant tamoxifen depended on:

HER-2 receptor expression (overexpression accompanied by at least 50% increase of recurrence risk), p53 protein acumulation (overexpression accompanied by at least 40% increase of recurrence risk), age (recurrence risk at least 2% lower in elderly women), - nodal status (metastases to at least 4 nodes accompnied by at least 40% increase of recurrence risk).

292 POSTER

No relationship between oxygenation status (pO2) of malignant T 2/3 breast tumors and serum hemoglobin levels (Hb) before and after primary systemic chemotherapy (PSC)

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Application of PSC for operable breast cancer can be used for testing various biomarkers as predictive factors for tumor remission and as surrogates for patient outcome. In a prospective analysis we are currently investigating the value of pO2 in predicting remission of T 2/3 breast cancers after PSC. After having shown that malignant T 2/3 breast tumors are very hypoxic (median pO2 = 3 mmHg) we examined whether there is a relationship between tumor oxygenation and Hb. Prior to PSC (adriamycin/docetaxel x 4, adriamycin/cyclophosphamide x 4 followed by docetaxel x 4 or adriamycin/paclitaxel x 4 followed by CMF x 4) pO2 was polarographically measured using the Eppendorf histograph. Serum hemoglobin levels were determined before, during and after PSC. Data of 33 patients with T 2/3 breast cancer is available for analysis. The median pO2 level was 2.5 mmHg (0.0 -26.4 mmHg). Tumors with a pO2 < 5 mmHg were defined hypoxic (n = 23), tumors with a pO2 > 5 mmHg were defined non hypoxic (n = 10). Median Hb level prior to PSC was 14.0 g/dl (12.3 -15.0 g/dl). In the patient subgroups with a Hb < 14.0 g/dl and > 14.0 g/dl the median pO2 was the same with 2.6 and 2.2 mmHg respectively. After PSC median Hb was 11.7 g/dl (9.7 - 14.2 g/dl) implicating a significant median decrease of 1.8 g/dl (0.1 -5.1 g/dl). Subgroup analysis of patients with Hb < 11.7 g/dl and > 11.7 g/dl again showed no difference between the median pO2 levels (2.5 vs. 2.2 mmHg). Finally, in the subgroups with a median decrease of Hb during PSC < 1.8 g/dl and > 1.8 g/dl, the median pO2 was equal (2.5 vs. 2.4 mmHg). Median Hb levels pre PSC in hypoxic and non hypoxic tumors showed no difference (14.1 vs. 13.6 mg/dl). Post PSC Hb levels were identical in hypoxic and non hypoxic tumors (11.7 vs. 11.7 g/dl). In conclusion serum Hb pre and post PSC as well as decrease of Hb during PSC and oxygenation status of T 2/3 breast cancer tumors seem to be independent factors.

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Expression of Pgp functional activity can be an additional prognostic factor determining tamoxifen efficacy in breast cancer patients

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Estrogen receptors (ER) are determinants of sensitivity for tamoxifen (Tam) therapy of breast cancer but the treatment is not effective in all the patients (pts) with ER-positive tumor status. We supposed that among the various reasons of that discussed in literature expression of Pgp which extrudes anti-cancer drugs out of the cells and associates with multidrug resistance mechanism can be the important one because Tam is a substrate of Pgp as well. Thus, Pgp can decrease an active intracellular concentration of Tam bound to ER and thereby - antiestrogen therapy efficacy. Aim of the study was to evaluate Pgp expression in ER-positive breast cancer. Pgp functional activity was assayed in 62 pts by a new methodology described by us previously. It allows determination of the transporter activity in intact solid tumor specimens by evaluation substrate of Pgp doxorubicin intracellular accumulation before and after action of verapamil - Pgp specific inhibitor.

Results: Expression of Pgp functional activity was shown in about 70% of the ER-positive tumors. It means that in case of tumors with the same level of ER more than half of pts can have worse prognosis in terms of Tam efficacy as compared to those with no tumor Pgp activity because of decreasing of Tam intracellular concentration under Pgp action. That influence in tumors with low level of ER has to be the most pronounced. Clinical confirmation of the conclusion is under way. Supported by Russian Foundation for Basic Research (01-04-49213).