for uPA is 0.709 (95% C.I. 0.616–0.791), and for PAI-1 is 0.731 (95% C.I. 0.639–0.81) which shows good discriminatory efficacy. Limit values obtained by ROC analysis were 0.92 for uPA, and for PAI-1 1.44 ng/mg protein.

Curves of survival were obtained by Kaplan-Meier analysis. Survival curves of 72 patients with uPA<=0.92 and 41 patients with uPA>0.92 ng/mg proteins showed statistically significant difference (P<0.001). Statistical probability of difference in curves of 62 patients with PAI-1<=1.44 and 51 patients with PAI-1>1.44 ng/mg protein (P<0.001) is the same. Corelation coeficient of uPA and PAI-1 is 0.714 (P<0.0001).

Our research confirmed prognostic significance of both parameters.

**431** POSTER

**Associated changes of lipid peroxidation and transglutaminase activity in the evolution of breast tissue to cancer**

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**Background:** Lipid peroxidation and tissue transglutaminase (tTG) have been indipendently implicated in tissue damage associated with several disease including breast cancer. We have investigated lipid peroxidation products, such as malondialdehyde (MDA) and 4-hydroxy-nonenal (HNE)-protein adducts, and tTG activity in tissues from breast surgical specimens to study the evolution from the normal to the cancerous tissue.

**Methods:** We have collected breast tissues from surgical specimens affected by primary invasive ductal breast carcinomas (C), fibroadenoma (F) and atypical ductal hyperplasia (H). The samples were obtained during surgical resection, directly frozen in liquid nitrogen and stored at −80°C until use. The tissue was suspended in hypotonic buffer Tris-HCl 10mM, EDTA 0.1mM and $MgCl_2$ 5mM, PMSF 90μM, 2-β mercaptoethanol 0.1mM (pH 7.5). The suspensions were homogenized by a Ultradurax T25 basic, then they were centrifuged at 13,000 rpm for 10 min. Biorad protein assay, spettrofluorimetric analyses of HNE-proteins adducts and tTG activity were conducted on the supernatants. The quantitation of the fluorescence intensity at 360 nm excitation/430 nm emission was taken as an indirect measure of the HNE-protein adduct (AFU/mg protein) The pellets were added of 500 μl urea 6M, SDS 8.1% buffer. After mixing the samples were centrifuged at 15,000 rpm for 15 min and 100 μl of supernatants were used for the TBA test in buffer TCA 15%, TBA 0.3%, HCl 0.12 N.

**Results:** The MDA levels and HNE-protein adducts of normal tissues obtained from specimens affected by breast cancer were compared with benign breast disease. In the breast cancer the values were respectively 60±5 nmol/g and 2.2 AFU/mg protein while in the normal tissue belonging to specimens with atypical ductal hyperplasia the values were 30±2.5 nmol/g and 79±4.5 AFU/mg protein. In the control group represented by fibroadenoma affected one the values were 12.4±1.6 nmol/g and 11.5 AFU/mg protein. The tTG activity increased only in normal tissues obtained from specimens affected by breast cancer.

**Conclusion:** Oxidative stress can damage many biological molecules; indeed, proteins and DNA are more significant targets than are lipids, and lipid peroxidation often occurs late in the injury process. In fact HNE-protein adducts increased in precancerous tissue, while higher MDA values are shown in cancer tissue damage. We speculated that these biochemical parameters together with the tTG activity may be a diagnostic index for cancer research.

**432** POSTER

**Prediction of axillary lymph node status in breast cancer patients by the presence of cancer emboli in the primary tumor's vessels**

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**Objective:** To identify the presence of vascular emboli of breast cancer histologic specimens and validate its significance for the axillary lymph node (LN) involvement.

**Methods:** Two hundred and twenty-four patients with invasive breast cancer, who underwent modified radical mastectomy or breast-conserving surgery and standard axillary dissection (level I and II) at the Metaxa Memorial Anticancer Hospital the last 3 years, were assessed for the presence of cancer emboli in at least one vascular structure, irrelevantly of the size of the vessel. Then we applied chi-square test and logistic regression analysis (univariate and multivariate analysis) for the evaluation of the statistical association between the vascular invasion and the number of the metastatic infiltrated LN. In the present study the 224 patients, which participated, had a tumor size until 3 cm. We excluded patients with larger tumors owing to their likelihood of positive LN (74%).

**Results:** Vascular 'invasion (VI) was seen in 29.9% of 127 and 54.6% of 97 patients with ⩽2 cm and >2 cm tumors respectively. 81.5% of the patients with VI and tumors ⩽2 cm had axillary lymph node metastases, compared with 35.9% of patients without VI. Also for tumors >2 cm 77.3% of patients with VI had at least one positive LN, compared with 52.2% of patients without VI.

**Conclusions:** By multivariate analysis axillary lymph node metastases are significantly related to VI (p-value: <0.001 for tumors ⩽2 cm and p-value <0.05 for tumors >2 cm). The absence of VI can be considered as a favourable prognostic factor for the axillary status.

**433** POSTER

**PAI-1 and PAI-2 as predictive factors in breast cancer**

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**Introduction:** The prognostic value of PAI-1 and PAI-2 in breast cancer has already been confirmed. However, the factors that predict the response to therapy are even more important. The latest observations made in the patients with operable breast cancer have shown that PAI-1 may have predictive value in breast cancer.

**Aim:** To evaluate the correlation between PAI-1 and PAI-2 measured in primary tumor and the efficacy of systemic therapy with cytostatics and hormonal therapy in advanced breast cancer.

**Patients and methods:** The patients treated for advanced disease with chemotherapy (ChT) (CMF, vepeside, taxane or platinum-based) or hormonal therapy (HT) (tamoxifen or aromathase inhibitors) were included in the study. PAI-1 and PAI-2 values were determined in the primary tumor using ELISAs (American Diagnostica Inc.; CT). High and low levels of PAI-1 as well as PAI-2 were dichotomized using median value for PAI-1 and optimal cut-off level for PAI-2. Those who achieved complete or partial response by RECIST were considered responders, whereas in patients treated by HT, stable disease for more than six months (mo) was also regarded as response. Differences in response were calculated using chi-square test, time to progression (TTP) was presented by Kaplan-Maier curves and differences in TTP calculated by log rank test.

**Results:** In 45 patients treated by HT, a higher response rate, though statistically not significant, was observed in the patients with high PAI-1 levels compared to the patients with low PAI-1 levels (60% vs. 50%). In these, prognostically unfavourable group of patients, the median TTP was even longer compared to the group of patients with low PAI-1 levels (6.7 vs. 4.1 mo; p=NS). A slightly better response to treatment was observed in the patients with low PAI-2 levels (56% vs. 44.4; p=NS). However, no difference in TTP was observed. The correlation between the PAI-1 and PAI-2 levels and the efficacy of ChT was estimated in 144 patients. The patients with low PAI-1 levels responded better to ChT compared to the patients with high levels of PAI-1 (52.8% vs. 46.2%; p=NS). However, no difference in TTP was observed. A better response to chemotherapy was observed in the patients with low PAI-2 levels (49.1% vs. 38.9%; p=NS). In these, prognostically unfavourable group of patients, also a longer median TTP was observed (6.3 vs. 5.7 mo), this difference was just over the limit of statistical significance (p=0.095).

**Conclusion:** The results of our study although based on a small number of breast cancer patients pointed out that high level of PAI-1 may be predictive for a better response to HT whereas low level of PAI-2 may be predictive for a better response to ChT.

**434** POSTER

**Reading the prognosis of the individual according to the exact NPI value**

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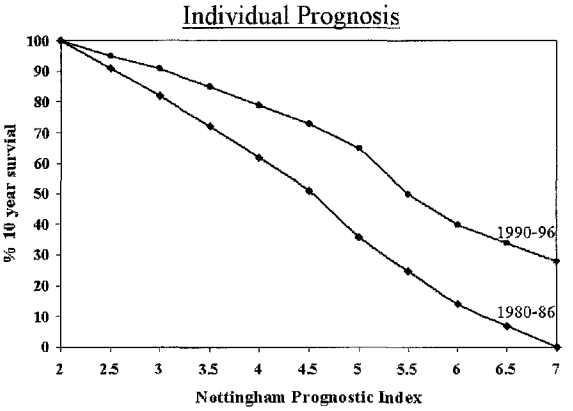
The NPI has until now been used to group women to initially 3 and later 5 prognostic groups, with any two adjacent groups separated by 10–20% in their 10 year survival predictions. This is usually satisfactory for making therapeutic decisions. The prognosis is inversely related to the NPI level. The survival figures for 8 NPI values at which are clustered enough patients, has been plotted. From the resultant graph the 10 year survival prediction for any individual may be read to one decimal point NPI value. Figure 1 shows lines based on 1980–86 (pre-adjuvant therapy) and 1990–96 (adjuvant local and systemic treatments used selectively): Two important uses lie (1) in the design of a computer programme which applies the relative risk reductions from the EBCTCG overview to the individual prognosis without adjuvant therapy from the 1980–86 line, to estimate the expected absolute gain from an adjuvant therapy for the individual and (2) to calculate life expectancy in legal cases.




**435** POSTER

**Tumor angiogenesis as a prognostic indicator in node-negative breast carcinoma**

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The biological aggressiveness of breast carcinomas may be related to prognosis. We investigated the significance of tumor angiogenesis in a retrospective study which included 137 primary node-negative breast cancer patients (median age: 54 years, range: 31–78). The duration of follow-up ranged from 87 to 200 months for cases who alived and 2–152 months for those deceased. Angiogenesis was assessed by counting vessel density with hematoxylin-eosin staining, based on the method published previously (Acta Sterol 1998; 17: 1–8). Histological slides were evaluated to identify "hotspots" of angiogenesis at 100× magnification. Microvessel profiles count were performed at ×400 magnification, using a grid eyepiece graticule; within each "hotspots" (area of field=0.490mm$^2$) were counted. In the same manner we counted microvessels in fields in mean area. The highest single field and the highest average for a "hotspots" value were recorded for each case and the same for fields in mean area. Patients were stratified into high and low microvessel groups (respectively: >6 and 0–5 profiles per field) and their survival compared. As a results we can state: 1) microvessel counts did not corrrelate with primary tumor features, such as histological type, grade, and size; 2) no relationship was found between vascularity in "hotspots" and relapse-free survival; 3) significant correlation was found between vascularity in "hotspots" for older and post-menopausal patients and overall survival ($p<0.05$); 4) no relationship was found between vascularity mean area and overall survival. Our results probably reflects the heterogeneity which exists between different tumours in their ability to induce angiogenesis. Additionally, the study gives some evidence that angiogenesis is possibly related to patient age and menopausal status.

**436** POSTER

**Sister chromatid exchange and micronuclei frequency in early-stage breast cancer patients: preliminary results of a prospective observational study**

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**Background and study aim:** Spontaneous chromosomal instability has been correlated with a risk of developing cancer. We evaluated sister chromatid exchange (SCE) and micronuclei (MN) frequency in peripheral blood lymphocytes of early-stage breast cancer patients to see if it can be considered a disease biomarker.

**Materials and Methods:** In 20 evaluable pts, aged 38 to 81 years (median 57.5), SCE and MN were measured both before and four weeks after conservative surgery. While, in those 10 pts who had previously received chemotherapy (CT), testing was done immediately before radiotherapy (RT). Further controls were done 8 weeks after RT on all pts and at 6 months in 9 cases. All pts will be studied regularly during follow-up. There were 15 ductal infiltrant carcinomas (13 G2, 2 G3), 1 medullary carcinoma, 1 adenocarcinoma and 3 intraductal carcinomas with microinfiltration. Median tumor size was 12 mm (range 7–27 mm). In 16 and 11 cases estrogen and progestinic receptors were positive, respectively. Five pts had axillary positive nodes and in three of these cases they were four or more. Ten pts underwent adjuvant chemotherapy (CMF or FEC). Hormonotherapy was prescribed to 14 cases. RT was delivered to the breast ± sovraclavicular nodes; single dose was 1.8–2 Gy, total dose 50.4–50 Gy; a 10 Gy boost was delivered to the tumor bed. Student's *t* test compared SCE and MN basal values to both those from a healthy control group of 7 women and those values obtained from treatment/follow-up times.

**Results:**

SCE and MN mean values

| | Basal | Post surgery | Before RT* | Post RT | 6 months after RT |
|---|---|---|---|---|---|
| SCE | 8.2±0.9 | 7.8±1.2 | 10.8±2.9 | 8.6±1.3 | 8.7±1.1 |
| MN | 23.2±10.7 | 24.1±10.1 | 23.1±14.2 | 64±17 | 40.6±27.4 |

*Only in pts previously receiving chemotherapy.

SCE value reduction after surgery, though not statistically significant, (p 0.07) seems to be a result of tumor removal while the SCE increase after chemotherapy (p 0.04) is most likely to be a result of cytotoxic damage. MN increase after RT ($p<0.01$) is most likely due to genotoxic damage. A statistically significant difference (p 0.04) was observed between SCE basal and control group values.

**Conclusions:** The frequency of SCE as a cancer biomarker was confirmed by the difference obtained comparing basal testing to control group values. Results here suggest that SCE and MN must be an index of damage due to CT and RT, respectively. In the future, this study will seek to determine if SCE and MN frequency measurements during follow-up are disease progression predictors.

**437** POSTER

**Opportunities of an individual approach to postoperative treatment in breast cancer patients**

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**Background:** In breast cancer treatment the surgical method is the basic. However frequently it is supplemented with use of various ways of antineoplastic therapy, for example, chemotherapy. With this purpose we investigated activity of Thymidine kinase (TK) – the recognized marker of proliferation. Thymidine phosphorylase (TP) is used as the indicator of sensitivity to same chemopreparations. Activity of Adenosinedeaminase (ADA) connected with differentiation and apoptosis of a cell on which effect some preparations.

**Materials and methods:** Activity of TK, TP and ADA is invstigated in blood serum, bioptate of tissues and in lymphocytes of breast cancer