Results: There are 230 (16.9%) women with an ER−ve PR−ve breast cancer and 20 (1.5%) women with an ER−ve PR+ve tumour; the other 1108 patients (81.6%) have ER+ve tumours. Compared with women with a ER−ve PR−ve tumour, patients with ER−ve PR+ve breast cancer are younger (mean age 48.45 years vs 55.03 years; P=0.037) and more likely premenopausal (78.9% vs 34.3%). Such tumours are larger (39.05 mm vs 27.66 mm) and more likely of lobular type (20% vs 7.8%; P=0.002). Tumours in the ER−ve PR+ve category are less likely grade 3 (60% vs 86.1%, P=0.002), more frequently lymph node positive although not statistically significant (50% vs 38%; P=0.291) and more frequently overexpress HER-2/neu especially in the non-lobular type breast cancers (62.5% vs 35%; P=0.028).

Conclusion: ER−ve PR+ve breast cancers are less likely grade 3, appear more often in younger and premenopausal women and express more frequently HER-2/neu compared with the ER−ve PR−ve phenotype. Our findings suggest ER−ve PR+ve breast cancers have typical characteristics separating them from other breast cancers.

171 POSTER

Expression analysis of VEGF-C in breast cancer – correlates with expression of LYVE-1 gene and some prognostic factors

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Background: Metastatic spread of cancer cells is a major risk factor linked to the clinical prognosis. Despite of numerous clinicopathological studies on VEGF-C and LYVE-1 expression in various malignancies, little studies have investigated the correlation between them and its relationship with other prognostic factors in human breast cancer. The aim of this study was to assess the expression levels of VEGF-C and LYVE-1 gene and protein in human breast cancer, and to compare the correlation between these factors, to analyes the the expression of VEGF-C protein and VEGF-C, LYVE-1 mRNA to compare related data with clinico-pathological findings, to consider as valuable prognostic factor of VEGF-C in breast cancer patients.

Material and Methods: RT-PCR was carried on VEGF-C, LYVE-1 mRNA drawn from three adjacent normal breast tissue, MCF-7 breast cancer cell line and 39 breast cancer tissues and imunohistochemical staining to detect the expression of VEGF-C protein on 39 cancer tissues and 5 benign tissues using well preserved paraffin embedded blocks. Clinico-pathological findings were reviewed for menopausal status, axillary nodal status, lymphatic invasion by tumor cells, hormonal status, p53, c-erbB2, retrospectively. **Results:** RT-PCR analysis revealed the expression of VEGF-C mRNA in 23 of 39 (60.0%) and LYVE-1 mRNA in 19 of 39 (48.7%). The expression of VEGF-C mRNA was positive in all cases except one in LYVE-1 mRNA positive group, this revealed good correlation between two genes. The expression of VEGF-C and LYVE-1 mRNA did not significantly correlate with expression of VEGF-C protein. Immunohistochemical analysis revealed but VEGF-C protein is expressed only in breast cancer cells, specific VEGF-C staining was evident in 10 of 39 (25.6%). There was no significant correlation between VEGF-C, LYVE-1 mRNA expression and other prognostic variables. However, VEGF-C protein expression was negative in the group of premenopausal status, positive estrogen receptor, negative c-erbB2, statistical significantly.

Conclusions: VEGF-C seems to have a significant role in the mechanisms of lymphatic spread of breast cancer cells, the expression of its protein may help to evaluate the patient's prognosis.

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Oxidative stress products and soluble adhesion molecules in patients with breast cancer

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Backgrounds: Identification of clinically useful prognostic markers and markers of activity could contribute to the improvement of therapy of patients with breast cancer, mainly to the identification of subgroups of patients in higher risk of formation of metastases and early detection of relapses of the disease. In patients with breast cancer oxidative stress may modify membrane lipids which may then become the target of some autoantibodies. Some receptors (including EGF receptor and apo1/Fas) and adhesion molecules (standard and/or variant CD44 and P-selectin) may detach from the surface of tumor cells and increased levels of their soluble forms may be also identified in sera.

Methods: In our study serum levels of soluble EGF receptor, soluble standard and variant CD44 (CD44s and CD44v6, respectively), soluble P-selectin, soluble Apo-1/Fas, advanced oxidation protein products (AOPP), advanced glycation end-products (AGEs), pregnancy associated plasma protein (PAPP-A) and IgG and IgM anticardiolipin antibodies (ACA) were studied in 76 patients (pts) with newly diagnosed mostly non-metastatic breast cancer (3 pts in stage 0, 37 pts in stage I, 18 pts in stage IIA, 12 patients in stage IIB, 4 patients in stage III and 2 patients in stage IV) and compared with 8 age-matched healthy women.

Results. Patients with breast cancer had significantly higher serum levels of soluble standard form of CD44 (CD44s, 581.5±281.1 vs. 406.4±48.9 ng/ml, p<0.05), but not soluble variant form most common on breast cancer cells (CD44v6, 171.4±48.4 vs. 160.1±48.3 ng/ml, p=n.s.). Serum levels of soluble P-selectin (248.1±137.0 vs. 125.5±32.0 ng/ml, p<0.05) and serum levels of soluble Apo-1/Fas (852.9±1593 vs. 541.5±124.5 pg/ml, p<0.05) were also significantly increased in patients with breast cancer. Concerning the markers of oxidative stress patients with breast cancer had higher AOPP (93.6±46.8 vs. 68.5±23.1 μmol/l, p<0.05), but there was no difference in AGEs, PAPP-A and IgM and IgG ACA. We were not able to find any significant difference in serum levels of soluble EGF receptor (3.2±3.1 vs. 3.6±2.0 ng/ml, p=n.s.). None of measured parameters was able to discriminate the patients with different stages of breast cancer.

Conclusions: Patients with breast cancer (including those in early stages of the disease) may have increased serum levels of some soluble adhesion molecules (sCD44s, sP-selectin), markers of apoptosis (sApo-1/Fas) and oxidative stress (AOPP). Further follow-up should demonstrate the response of these markers to hormonal therapy/chemotherapy and putative prognostic significance of increased levels of these markers in order to improve the current possibilities to monitor the activity of the disease and to predict its outcome.

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173 POSTER

A new look at the prognostic value of the presence of estrogen, progesterone and epidermal growth factor receptors in breast cancer tissue of women patients

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The aim of the study was to evaluate the influence of the presence or absence in tumours of estrogen (ER), progesterone (PR) and epithelial growth factor receptors (EGFR) on the survival of women with breast cancer. The receptors were determined by biochemical radiocompetitive methods. In order to analyse disease-free survival (DFS) and overall survival (OS) we applied Cox's proportional hazard model, in which we analysed both the presence of receptors and clinical and morphological parameters of survival.

The tumour size, metastatic lymph nodes and the presence of cancer infiltrations outside lymph nodes were negative prognostic factors. The mean relative risk (RR) were between 1.50 and 3.91.

The table presents the influence of 8 different variants of receptor status of breast cancer tissue on the survival of the patients.

No of patients n=184	Variables				DFS		OS	
				Significance	RR Exp(B)	Significance	RR Exp(B)
	ER	PR	EGFR	0.0074		0.0394	
28	+	+	+	0.0855	0.45	0.0248	0.22
9	−	+	−	0.4783	0.63	0.1895	0.26
64	+	+	−		1.00		1.00
11	−	−	−	0.9297	1.07	0.6914	1.16
11	−	+	+	0.5020	1.46	0.0516	3.16
33	+	−	−	0.0350	2.14	0.0454	2.56
21	−	−	+	0.0337	2.23	0.0712	2.32
7	+	−	+	0.0332	3.31	0.0383	3.95

Our results suggest, that both disease free survival and overall survival is directly related to the concomitant presence or absence of ER, PR and EGFR in breast cancer. It was found that patients with receptor status ER+PR+EGFR+ ER− PR+EGFR−; ER+PR+EGFR−; and ER− PR− EGFR− had better parameters of DFS and OS (RR for DFS or OS were between 0.22–1.16). The patients with receptor status: ER− PR+EGFR+; ER+PR− EGFR−, ER− PR− EGFR+ and ER+PR− EGFR+ presented a more aggressive disease course (RR for DFS and OS were between 1.46 – 3.95).

The presence of EGFR in breast cancer tissue is not always a negative prognostic factor for survival. It's coexistence with ER and PR is related to the best survival parameters (the group ER+PR+EGFR+, RR for DFS – 0.45 and for OS – 0.22).

The survival of patients with only PR receptors or no receptors (ER–PR–EGFR–) within breast cancer tissue do not differ significantly from the parameters found in the reference variable ER+ PR+EGFR–, RR for DFS and OS are, respectively, less than 1 (0.63 and 0.26) or only slightly greater than 1 (1.07 and 1.16).

174 POSTER

Significance of MAGE-A gene expression in primary breast cancer

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Background: The melanoma antigen (MAGE)-A genes are expressed in various malignant tissues including breast cancer, but not in normal tissues other than the placenta and testis. MAGE-A family consists of several subtypes from MAGE-1 to MAGE-12. We intended to investigate the significance and the correlation with clinicopathological factors of MAGE-A gene expression in primary breast cancer.

Methods: We collected 23 breast cancer tissues and 12 breast benign lesion tissues and kept at −70° until total RNA isolation. MAGE gene expression was assessed by nested reverse transcription-polymerase chain reaction assay using Cancer Hunter Core kit (iC&G Co., Daegu, Korea) which contained multiple MAGEs recognizing primers that can bind to the sequences of cDNA of MAGE-1, -2, -3, -4, -5 and -6 together. We determined estrogen receptor (ER), progesterone receptor (PR), P53 and c-erb B2 status by immunohistochemistry.

Results: The MAGE gene expression was positive in 15 (65.2%) of 23 cancer tissues, which correlated with the tumor size (P=0.0086) and inversely correlated with the ER status (P=0.0007). No association was observed for MAGE-A gene expression and lymph node metastasis, grade, TNM stage, PR, P53 and c-erb B2 status. The expression of MAGE genes is not recognized in benign tissues.

Conclusions: Our data suggest that the MAGE-A gene expression in primary breast cancer relates to potential involvement in tumor progression and may be associated with unfavorable prognosis. The detection of multiple MAGE gene expression together seems to be more useful for the diagnosis of MAGE-expressing cancers.

175 POSTER

Transforming growth factor-beta1 and steroid receptor status in breast cancer

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Background: In despite of controversial results of transforming growth factor-beta1 (TGF-beta1) role and importance in breast cancer, it is generally accepted that TGF-beta1 is molecular biomarker of breast cancer progression. It is suggested that estrogen receptor (ER) and progesterone receptor (PR) phenotypes as markers of breast cancer estrogen dependence, are inherently different in relation to biological and clinical features. The aim of this study was to examine the relationship between the expression of TGF-beta1 and ER/PR status.

Materials and methods: This study included 52 breast cancer patients (I, II, III and IV stage) and 36 healthy women donors, as controls. Informed consent was obtained from each woman, according to the National Health Regulations. Determination of TGF-beta1 levels in platelet-poor plasma samples, was performed with commercial Quantikine ELISA kit (R&D,USA). Steroid receptor content was determined by classical biochemical ligand binding assay.

Results: Statistically significant higher expression of TGF-beta1 was found in breast cancer patients in relation to healthy donors. In that analyzed breast cancer group of patients, expression of TGF-beta1, there was a statistically significant difference between PR-positive and PR-negative subgroups, as well as between ERPR-negative and ERPR-positive subgroups. Higher levels of TGF-beta1 were found within unfavorable steroid receptor phenotypes. It is important to point out that in separated group of patients with metastasis (stage IV) statistically significant higher levels of TGF-beta1 were found even in the ER-negative subgroup as well, in comparison with ER-positive subgroup.

Conclusion: These findings indicate that TGF-beta1 could be considered as a marker of progression of disease and possible marker of more aggressive hormonally independent breast cancer (ER–, PR–, ER–PR–).

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Loss of fhit expression is associated with higher malignant phenotypes in breast cancer

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Background: Breast cancer originates trough a series of genetic events, with a multistep pattern which includes alterations of tumor suppressor gene. Fhit gene has been identificated as a gene with a role of suppression in human cells.

Method: 225 breast tumor have been analyzed about the expression of fhit protein with an himmunohistochemical assay.

Results: In the present study was noted a negative-weak expression of fhit protein in 46% of patients, according to the criteria of fhit immunohistochemical assay score, and a moderate–strong reaction in 54%. As regards fhit vs grading we had 9 (39%) cases with negative–weak reaction and 14 (61%) with a moderate or strong reaction among G1; instead among G2 fhit positive staining moderate or strong was detected in 42 cases (45%) and reduced (negative–weak) in 21 (72%). Among G3 fhit expression was moderate–strong in 8 (28%) cases and weak negative in 21 cases (72%). Fhit protein is widely reduced in poor differentiated carcinomas. As regards fhit protein expression and nodal status we we detected a weak negative expression in 34 cases (63%) and a moderate strong expression in 20 cases (37%). On the contrary a weak negative reaction was detected in 48 cases (44%) and a moderate strong in 62 (56%) among N–. Therefore if nodal status is positive fhit protein is more often alterated. Finally we had examined fhit vs hormonal receptors. 73 cases (42%) of ER-positive had a negative–weak reaction and 101 (58%) a moderate–strong reaction. Instead 21 cases (73%) of Er-negative was negative–weak and 11 (27%) was moderate–strong. In a similar way among patients with a pg-positive status fhit expression was weak–negative in 64 cases (43%) and moderate–strong in 85% (57%). Among patients with pg-negative status a strong moderate reaction was detected in 27 cases (40%) and weak–negative in 40 (60%) cases. That is fhit protein is more expressed in patients with a positive status of hormone receptors.

Conclusions: In our study Fhit expression was inversely correlated in a statistically significant manner with histological grade (c2=6.2 for 2 grad of liberty, $p<0.05$) negative nodal status (c2=4.6; $p<0.05$) negative ER receptors (c2=12.3; $p<0.01$) negative PG receptors (c2=4.5; $p<0.05$). Loss of fhit expression could be associated with higher malignant phenotypes and appear to be a prognostic factor in breast cancer.

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Mammaglobin A, a novel marker of minimal residual disease in early stages breast cancer

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Background: Mammaglobin A, in contrast to other factors, is a breast specific member of uteroglobin gene family. Expression is restricted to normal and neoplastic breast epithelium. We examined Mammaglobin A expression using RT-PCR in the bone marrow of patients with breast cancer.

Methods: There were examined bone marrow aspirates of 37 patients (median age 51) with stage I (40%), II (58%) and III (2%) breast cancer who underwent either immediate complete curative surgery or neoadjuvant therapy with subsequent radical procedure. mRNA was isolated using QIAamp RNA blood mini kit (Qiagen®). Subsequently two-step nested RT-PCR for the expression of Mammaglobin A was performed.

Results: Mammaglobin A was detected in samples from 5 (14%) out of 37 patients. With a median follow-up of 24 month (range: 4–32) we noted only 2 recurrences. However, one was observed in the patient with positive bone marrow. These data are obviously too immature to observe statistical differences.

Conclusion: We have shown that RT-PCR assay for Mammaglobin A may be used for detection of occult breast cancer cells in the bone marrow. Clinical and prognostic value of this method should be further investigated in the prospective fashion. At the meeting we will demonstrate updated results of 65 patients.

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