of serum versus tumour levels of IGFBP-3. It is possible that future characterisation of breast tumours may include fibronectin and IGFBP-3 production, so that clinical response to agents targeting the EGF pathway may be predicted, resulting in a more targeted use of such therapies.

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167 POSTER

HER2 polymorphism and the risk of breast and ovarian cancer

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Introduction: Breast cancer is a major public health problem around the world, and its carcinogenesis is not yet well understood. The Human Epidermal growth factor Receptor-2 (HER2) seems to play an important role in the development of this neoplasia, and genetic alterations in this gene, such as point mutations and polymorphisms have been detected in breast cancer patients, as well as ovarian cancer patients. The aim of our study was to analyze the frequency of a single nucleotide polymorphism in the HER2 gene in a southern European population.

Materials and Methods: The study included 152 patients with breast cancer, 139 ovarian cancer patients and a control group of 146 healthy donors. DNA extracted from peripheral blood was submitted to PCR-RFLP, in order to identify the possible HER2 genotypes; Ile/Ile, Ile/Val and Val/Val. The restriction fragments were analyzed in a 3% agarose gel, stained with ethidium bromide.

Results: A twofold increase in risk of breast cancer was found among women who are carriers of a Val allele genotype – Ile/Val and Val/Val genotypes (OR = 2.00; 95% CI: 1.23–3.25; p=0.005). As for the ovarian patients, we also found an increased risk in ovarian cancer, with an OR of 1.59 (95% CI: 0.96–2.63).

Discussion: Our results indicate an association between the presence of the Val allele in the HER2 polymorphism and the risk of breast and ovarian cancer. Further studies are needed to evaluate the role of this polymorphism in the behavior of breast and ovarian cancer.

168 POSTER

DNA diagnosis of hereditary breast and ovarian cancer in Latvia

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Background: Breast cancer is the leading cancer site in Latvian women and ranks as the first highest cause of cancer-related death. Since the discovery of BRCA1 and BRCA2 genes, the mutation analysis of these genes is widely used for the identification of women with a high risk of breast and ovarian cancer and developing management strategies. The BRCA1 and BRCA2 mutation spectrum and frequencies vary significantly in different populations and geographic regions. Therefore the criteria for BRCA1/2 genetic testing should be optimised for each population.

The objective of this study was to develop effective BRCA1 gene mutation detection strategy in Latvia based on characterisation of mutation profile in breast and ovarian cancer patients.

Material and methods: Mutation analysis of entire BRCA1 gene was performed in DNA from 75 breast cancer patients and 30 ovarian cancer patients from Latvian Oncology Center selected by early onset of disease or family history of breast/ovarian cancer. Most of patients tested have insignificant cancer history in family. The analysis was performed by SSCP/HDA in polyacrylamide gels and automatic direct sequencing (ABI PRISM 310) of variants detected. The screening for recurrent mutations was performed as well in early onset breast/ovarian cancer patients unselected for family history.

Results: 5 different deleterious mutations have been detected by the analysis of entire BRCA1 gene. Three of mutations detected were recurrent. Missense-mutations, registered in BIC database as unclassified variants and common polymorphisms, have been found in this study as well. High proportion of mutation carriers were found in this study regardless insignificant cancer histories in families of patients tested. Altogether 20 mutation carriers were detected by the analysis entire BRCA1 gene and 28 by the screening for recurrent mutations.

Conclusions: Breast cancer diagnosed before the age 48 is suggestive for DNA testing to be offered to patients in Latvia, regardless cancer history in the family. The identification of three founder mutations in Latvian population allows rapid and cost-effective detection of mutation carriers. Further study of founder mutations could be useful for understanding the role of these mutations in the incidence of breast and ovarian cancer in order to provide individual risk assessment and to design better therapeutic strategies.

169 POSTER

Are lobular carcinomas more often steroid receptor positive?

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Background: Invasive lobular carcinomas (LC) and invasive ductal carcinomas (DC) differ with respect to their expression of a variety of molecular tumour markers including oestrogen (ER) and progesterone receptor (PR) expression. LC are generally referred to be more likely ER and PR positive compared to DC. We analysed whether tumour grade affects differences in ER/PR expression by histologic tumour types.

Patients and Methods: Charts from 1472 consecutive female patients diagnosed with primary operable invasive breast cancer (Jan. 2000–May 2003) were reviewed, excluding those who received neoadjuvant therapy. The highest tumour grade was retained for each case and each tumour was classified according to its histological type as LC or non-lobular carcinoma (non-LC). Immunohistochemical stains for ER (antibody 6F11/2) and PR (antibody 312) were categorised using the H-score as follows: <50/300 negative; ∗ 50/300 positive.

Results: 204 (13.8%) of invasive tumours were classified as LC. LCs were more frequently ER/PR positive than non-LCs ($p<0.001$ – table 1). The great majority of LCs (85.3%) were grade 2 where only 40% of non-LC were classified as grade 2. When we only classified grade 2 tumours by receptor state, there was no difference in incidence of ER/PR positivety between LC and non-LC (table 2); differences however, were significant for grade 3 lesions.

Table 1

Type	ER-positive	PR-positive
LC (n=204)	92.7%	79.5%
non-LC (n=1268)	80.0%	62.4%

Table 2

Type	ER-positive	PR-positive
Gr 2 LC (n=174)	94.3%	79.9%
Gr 2 non-LC (n=508)	94.7%	76.0%
Gr 3 LC (n=25)	76.0%	72.0%
Gr 3 non-LC (n=535)	59.1%	42.9%

Conclusion: Regarding the frequency of positive steroid receptors in invasive breast cancer, grade 2 LCs do not differ from grade 2 non-LCs. The difference for ER/PR positivety between both histologic tumour types lies in grade 3 lesions; grade 3 LCs are more often ER/PR-positive than grade 3 non-LCs.

170 POSTER

The progesterone receptor has a prognostic value in oestrogen receptor negative breast cancers

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Background: Considering oestrogen (ER) and progesterone receptor (PR) expression in breast cancers, the ER-negative (−ve) PR-positive (+ve) phenotype is the least common variant. Some believe that non-expression of ER in the presence of PR is a laboratory error whereas others consider this as a separate category. The aim of this study is to analyse whether prognostic factors are differently expressed in ER−ve breast cancers with or without PR expression.

Patients and methods: Charts from 1358 women who underwent primary breast surgery and complete axillary clearance for invasive breast cancer between Jan 2000 and June 2003 (excluding those who had neoadjuvant therapy and those with sentinel lymph node only) were examined. We compared age, mean tumor size, histologic type, incidence of grade 3 lesions, axillary lymph node status, HER-2/neu expression [immunohistochemical (IHC) measurement] and menopausal status between ER−ve PR+ve and ER−ve PR−ve tumours. Steroid receptors were measured by IHC using the H-score and defined negative with a score of less than 50/300.

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.

172 POSTER

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).