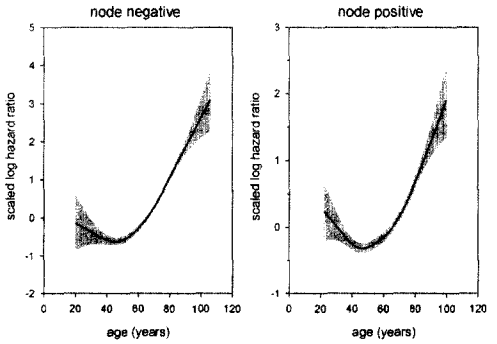
was assessed. The age covariate was then iteratively fitted with parametric functions until the linearity condition of the PH model was satisfied. Finally, the parametric function of age found in N0 was verified in a PH model applied to node-positive (N+) cases.

Results: The analysis by martingale residuals was performed on 58,139 N0 cases. Age was significantly non-linear in PH models. The graph of functional form showed a U-shape of the effect of age on mortality (Fig1). An appropriate transform was obtained with the function: Age + |Age-50|$^{1.5}$. Modeling based on 25,665 N+ cases found a similar U-shape functional form. The transform applied to a PH model based on N+ cases improved the model, but the linearity condition of PH was satisfied only by using Age+|Age-50|$^{1.8}$.



Discussion: The U-shape functional form indicate an abnormal age pattern in which younger patients experienced the same mortality as much older patients, e.g. patients aged 20 had the same relative mortality risk as patients aged 60-65. The modeling suggests that the age pattern has two components: a linear log hazard ratio which represents the normal aging process, and a non-linear component which might represent the disease related age process. The non-linear component is more intense the farther away from the peri-menopause period as expressed by the absolute difference |Age-50|, and more intense in N+ than in N0 as expressed by the larger 1.8 exponent. We hypothesize that efficacy of cancer treatment might be detected by a change in the non-linear component. The modeling approach might represent in that case a powerful measurement of treatment effects.

406 POSTER

Genomic DNA amplification of decoy receptor 3 (DcR3) correlates with lymphatic invasion and lymphnode metastasis in breast cancer.

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Background: Decoy receptor 3 (DcR3) shows inhibitory effect to Fas-mediated apoptosis (Nature 1998; 396 (6712): 699-703). We have reported positive relationship between DcR3 mRNA expression and the gene amplification in breast cancer tissues (The 23rd Annual San Antonio Breast Cancer Symposium; abstract#380), suggesting that breast cancer, in some part, express DcR3 under the gene amplification to evade the apoptotic mechanism. In the present study, we examined the relationship between DcR3 genomic amplification and clinicopathologic factors to clarify its effect(s) in human breast cancer.

Materials & Methods: One hundred patients who underwent operations for primary breast cancer at Niigata University Hospital between 1996 and 2000 were selected for the present study. Genomic DNA of 100 breast cancer tissues and 14 normal breast tissues was extracted respectively from paraffin embedded sections of surgical specimens by microdissection under light microscope. Real-time quantitative PCR was performed to measure genomic amplification of DcR3 by standardizing with b-globin gene. The results were expressed as DcR3/b-globin ratio (D/b), and compared with clinicopathologic factors, disease free survival (DFS) and overall survival (OS) of patients. D/b of both cancer tissues and normal tissues were also compared, and genomic amplification in cancer tissue was defined as D/b > 1.55; greater than mean + 2SD of normal breast samples. Statistical analysis was performed by Mann-Whitney U-test and Breslow- Graham-Wilcoxon test, and the statistical significance was defined as $P < 0.05$.

Results: D/b was significantly higher in cancer tissues compared to normal tissues ($p<0.0001$). In cancer tissue, D/b was significantly higher in the lymphatic invasion positive group compared to negative group ($p=0.0056$), and was also significantly higher in the lymphnode metastasis positive group compared to negative group ($p=0.0396$). There was no significant association between D/b and other clinicopathologic factors, such as age, tumor size, venous invasion or hormone receptor status. The DFS was significantly lower in the genomic amplification positive group compared to negative group ($p=0.0397$), however, the OS showed no statistical difference with or without genomic amplification.

Conclusion: These results suggest that DcR3 gene amplification in breast cancer might be involved in both lymphatic invasion and lymphnode metastasis of cancer cells, and might decrease DFS.

407 POSTER

Abnormalities of erbB oncogenes in locally advanced breast cancer

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Background: The *erbB* family of protooncogenes (*erbB-1, erbB-2, erbB-3, erbB-4*) and receptors encoded by them play an important role in normal cell growth and in neoplastic transformation. Literature data indicate that some abnormalities of *erbB* oncogene family (amplification, deletion) have special importance in breast cancer development, correlate with tumor aggressiveness and with worse clinical outcome. Therefore, these abnormalities may be potentially useful for determining prognosis and for optimizing breast cancer treatment.

Aim of the study: This study was designed to determine gene dosages of *erbB* oncogene family in breast cancer. The relationship of these abnormalities with (CA)n dinucleotides polymorphism and with loss of heterozygosity (LOH) in *erbB1* was examined. Molecular parameters were analyzed in relation to clinical and pathological features of the tumors and to chemotherapy response.

Matherials and methods: Study subjects included 32 chemotherapy naive patients (pts) with primary inoperable locally advanced breast cancer (any T,N_2, any N,T_4). All pts were managed with induction chemotherapy. Tumor (incisional or core needle biopsy) and blood samples were taken prior to treatment and frozen immediately for further analysis. Chemotherapy regimens included ET (docetaxel 100 mg/m^2, epirubicin 90 mg/m^2; 6 pts), FEC (5-Fu 500 mg/m^2, epirubicin 100 mg/m^2, cyclophosphamide 500 mg/m^2; 8 pts) and FAC (5-Fu 500 mg/m^2, doxorubicin 50 mg/m^2, cyclophosphamide 500 mg/m^2; 18 pts). Tumor measurement was performed after each cycle and at the completion of induction chemotherapy. Double differential PCR (ddPCR) was used for detection of *erbB* oncogene family abnormalities (gene amplification/deletion). Microsatellite polymorphism of *erbB-1* was examined by PCR with fluorescently labeled primers, followed by capillary electrophoresis and quantitative analysis of PCR product with GeneScan system, using automated sequencer ABI PRISM 310.

Results: Amplifications of *erbB-1, erbB-2, erbB-3, erbB-4* (defined as AGCN value >1.6) were detected in 5.9%, 26.5%, 2.9% and 2.9% of examined cases, respectively. Deletions, defined as AGCN value <0.4 occurred only in *erbB-1* and was found in 26.5% of all cases. There was a polymorphic simple sequence repeat region of 12-20 CA repeats detected in the first intron of *erbB-1*. Homozygotes comprised 31% of the examined group. The majority of the homozygous pts revealed 14/14 CA repeat combination. LOH (most frequently affecting shorter allele) was determined in breast cancer heterozygotes and occurred in 50% of cases. Correlation between these findings and clinical outcomes in extended group of 50 pts will be presented at the meeting.

408 POSTER

New sequence variants, recurrent BRCA1/BRCA2 mutations and new aberrations in BRCA1 promoter region in breast and ovarian cancer cases from Upper Silesia in Poland.

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Purpose: Germline mutations within BRCA1 and BRCA2 are responsible for a significant fraction of hereditary breast and ovarian cancer cases. BRCA

mutation spectrum might be more dispersed than previously observed. Aberrations within BRCA1/2 promoters, which can result in BRCA1/2 protein decrease, could be also associated with an increased risk of the disease.

Patients and Methods: One hundred and fifty unrelated probands with strong family history of breast and ovarian cancer, bilateral or early-onset breast cancer we screened by direct sequencing of all exons and exon/intron boundaries.The sequence of BRCA1 promoter region were also analysed in 87 breast/ovarian cancer cases without mutation in BRCA1/2 genes.

Results: We found 2 families with deletions in beta-promoter of BRCA1 (No.Ac U37574, 2223delAAAAA) and 5 polymorphisms in BRCA1 promoter region (No.Ac U37574, 2642A>G, 2743T>C, 1895G>C, 1983G>C, 1873G>C). Any important aberrations in this region have not been reported previously. We also found 5 different disease predisposing mutations within BRCA1 gene (185delAG, 300T/G, 4153delA, 5382insC, 5528del1+IV22-6). The results confirms the presence of two strong BRCA1 founder mutations in Polish population-5382insC and 300T>G. The BRCA1 (5528del1+IV22-6) mutation were not reported previously and might be specific to the southern Polish population while the others were recurrent. We also detected two new sequence variants in BRCA1 introns (IVS12-4 and IVS21-31). Two disease predisposing mutations were detected in BRCA2 (6174delT, 9631delC). In addition, eight new unclassified sequence variants were found within BRCA2 exons (3431T>C, 3446A>G, 3655G>C, 4846G>T, 4988C>T, 6188A>G, 8335A>T, 8341A>T) and 6 new variants in BRCA2 introns (IVS4+67, IVS4+147, IVS12+157, IVS12+183, IVS18+13, IVS24-36).

Conclusion: Identified novel aberrations in the BRCA1 promoter suggest that mutation and polymorphisms in this region might be responsible for significant fraction of breast and ovarian cancer cases. Our results lend further support to the need for more detailed functional and epidemiological studies aimed at understanding the role of BRCA1 and BRCA2 promoters in the etiology of breast and cancer.

409 POSTER

Clinical prognosis of BRCA1-associated breast cancer: a cohort study from the upper silesia region in Poland.

R. Suwinski[1], K. Behrendt[1], E. Grzybowska[2], H. Zientek[2], B. Utracka-Hutka[3], J. Rogozinska-Szczepka[1], M. Kazimierczak-Maciejewska[1]. [1] *Radiation Oncology,* [2] *Tumor Biology,* [3] *Chemotherapy, Center of Oncology, Gliwice, Poland*

Background: To compare the pathologic characteristics, survival, and the incidence of second cancer in three groups of patients with breast cancer: A) BRCA1 mutation carriers, B) BRCA1 non-carriers, C) patients who never had genetic counseling.

Material/Methods: Women affected with breast cancer were encouraged to attend genetic counseling if they had a family history of breast or ovarian cancer, and/or presented with bilateral breast cancer. Screening for BRCA1 mutations was performed using ASA-PCR. Forty-six carriers of BRCA1 mutation were identified: 5382insC, and 9631delC constituted 82%. From the database of patients who had genetic counseling, but had no identified BRCA1 mutation we matched a control group of 46 patients using a year of the diagnosis as a sole stratification criterion. Likewise, 46 patients who never had genetic counseling were matched to create a second control group.

Results: Patients in groups A and B were younger at the diagnosis than those in group C. Tumour grade was higher in group A than in group B and C. Group C presented at more advanced clinical stage than group A and B. Eighty percent of patients in group A lacked estrogen receptor expression vs. 40% and 30% in group B and C. A high incidence of second breast cancer in group A and B (43% vs. 43%) compared to group C (2%) was attributable to the counseling criteria. The actuarial 10-years metastases-free survival was 82%, 65% and 54% in groups A, B, C respectively. The corresponding actuarial 10-years local recurrence-free survival was 95%, 80% and 75%. Eight patients from group A developed ovarian cancer compared to only 1 and 0 from groups B and C. The overall 10-year actuarial survival was significantly lower in group C (35%), but did not differ significantly in groups A and B (80 vs. 81%).

Conclusions: These data show a low incidence of distant metastases and local recurrences among BRCA1 mutation carriers, in spite of unfavorable pathologic features and high incidence of second breast cancers in this group. However, the overall survival of BRCA1 mutation carriers did not differ significantly from non-carriers due to frequent occurrence of ovarian cancers which eventually relapsed. Patients who never had genetic counseling presented in most advanced stages, and carried the most unfavorable prognosis. This shows that the counseling may carry the bias of artificially increasing survival times by excluding patients with most advanced disease at the diagnosis.

410 POSTER

Correlation between BLC-2 protein expression and the clinical, pathological and biological characteristics of 483 breast cancer patients

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Introduction: Apoptosis, or programmed cell death, plays a critical role in the development of cancer. The BCL-2 oncogene is currently believed to be important in suppressing apoptosis and, among the recently proposed putative prognostic markers of breast cancer, considerable attention has been given to the products of the BCL-2 proto-oncogene.

Patients and methods: We evaluated the immunohistochemical expression of BCL-2 in 483 stage I and II breast cancer patients and assessed its relationship with a number of clinicopathological outcome predictors.

Results: BCL-2 immunoreactivity was observed in 413 cases (85%), being significantly higher in node-negative ($p<0.001$), ER- and PgR-positive ($p<0.001$), slowly proliferating and well-differentiated tumours ($p<0.001$). BCL-2 immunostaining did not correlate with pT, c-erbB2, vascular invasion or age, nor with the other proteins involved in regulating cell death and tumour proliferation, such as p53 and p21. The same analyses were repeated in 69 cases of *in situ* ductal carcinoma, and showed a close correlation between BCL-2 and ER/PgR positivity.

Conclusion: The immunohistochemical expression of BCL-2 protein in breast cancer patients is associated with a prognostically favourable phenotype and seems to be related to hormonal regulation. The prognostic data will be avaliable at the meeting.

411 POSTER

Highly sensitive detection of the MGB1 transcript(mammaglobin) in the peripheral blood of breast cancer patients

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Background: In recent years, several reverse-transcriptase polymerase chain reaction (RT-PCR) assays using epithelial [(e.g. citokeratin 19 (*CK19*), citokeratin 20 (*CK20*)] and supposedly mammary-specific markers [e.g. maspin (*SERPINB5*)] have been developed for the detection of disseminated breast cancer cells, but the sensitivity of these molecular markers is controversial and their specificity limited by the fact that these genes are expressed at low levels in blood cells, a phenomenon known as illegitimate transcription. Recently, the mammaglobin gene (*MGB1*) was described to be a potentially specific marker for the detection of circulating breast tumor cells since his expression was reported to be restricted to the mammary epithelium

Material and Methods: A new One-Step Nested reverse-transcriptase polymerase chain reaction (RT-PCR) assay for the detection of the mammaglobin (*MGB1*) gene transcript in the peripheral blood of breast cancer patients was applied to the study of 54 breast cancer patients. The control group included 38 peripheral blood samples from healthy donors and 18 samples from patients with hematopoietic malignancies.

Results: The *MGB1* transcript could be detected in the peripheral blood of 24 of 54 (41%) breast cancer patients prior to any therapy. Our method, using specific primers for cDNA synthesis, proved to be more sensitive (10^{-6} to 10^{-11}, usually 10^{-7}) than previously reported methodologies. This increased sensitivity was achieved without compromising specificity, as the *MGB1* transcript was not detected in 38 blood samples of healthy donors and in only one of 18 blood samples of patients presenting with hematological malignancies. A positive correlation was seen between *MGB1* positivity and breast cancer stage: 0/3 (0%) in stage 0, 3/13 (23%) in stage I, 6/17 (35%) in stage II, 5/10 (50%) in stage III, and 8/11 (73%) in stage IV ($p=0.003$).

Conclusion: We have found that the proportion of breast cancer patients with *MGB1* peripheral blood positivity increases with disease stage: 0% in stage 0, 23% in stage I, 35% in stage II, 50% in stage III, and 73% in stage IV. Although t he linear trend for *MGB1* positivity according to clinical stage was statistically significant ($p = 0.003$), the prognostic value of this marker, especially in clinically localized disease, must be evaluated after long-term clinical follow-up of these patients.