883 ORAL

Relationship of p53, uPA, pS2, EGF-R, and c-ERBB2, with response to systemic treatment in recurrent breast cancer patients

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Purpose: Based on our and other people's work, several cell biological parameters appeared recently to be associated with response to systemic therapy, but have not been compared by multivariate analysis.

Methods: Of 368 patients, 287 patients received first-line treatment with tamoxifen, and 81 received CMF or FAC as first-line treatment for recurrent disease. The membrane levels of EGF-R and c-erbB2, and cytosolic levels of p53, uPA and pS2, of primary breast tumors were determined by quantitative immunoassays (EGF-R, c-erbB2: Oncogene Science; p53: Sangtec Medical; uPA: American Diagnostica; pS2: Cis bio international).

Results: In logistic regression analysis for response to first-line tamoxifen therapy, of the modern parameters studied, high levels of c-erbB2 (16% of pts) or EGFR (78% of pts) showed the worst response to treatment, with respective odds ratio's of 0.22 ($p < 0.001$) and 0.44 ($p < 0.01$). In multivariate analysis for response to tamoxifen and for the length of progression-free survival, corrected for all relevant clinical and biological factors, c-erbB2 was the only significant biological parameter, together with ER. The odds ratio for response was 0.29 ($p = 0.001$), the relative progression and death rates were 2.28 ($p < 0.0001$) and 1.84 ($p = 0.001$) resp. None of parameters studied showed a significant association in analyses for response and survival on first-line chemotherapy.

Conclusion: The membrane c-erbB2 level is the most potent (and also) independent predictor of response on tamoxifen therapy in recurrent breast cancer in addition to ER. *(supported by the Dutch Cancer Society, Grant DDHK 96-1234).*

884 POSTER

Immunohistochemically detected lymph node micrometastases (N1A-IHC) in breast cancer (BC)

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Purpose: Whereas lymph node involvement is one of the most powerful prognostic factors in BC, the prognostic value of N1a-IHC in conventionally node negative BC patients (pts.) is controversial.

Methods: 3,616 axillary lymph nodes of 202 pts. with pT1-2N0M0 BC were examined for micrometastases using a monoclonal pancytokeratine antibody. In addition to histological findings, ER, PR, S-Phase, ploidy, EGF-R, p53, Ki-67, HER-2/neu, Cathepsin D and pS2 were determined. The mean follow-up time was 52 ± 19 months.

Results: Micrometastases were detected in 76 of 3,616 (2.1%) lymph nodes resulting in a conversion of conventional N0 to N1a-IHC in 10.4% (21/202) pts. Compared with N0-IHC, these patients had a prognostic disadvantage for distant metastases-free survival ($p = 0.003$) and overall survival ($p = 0.006$). By multivariate analysis tumor size, severe loss of differentiation and vessel invasion were confirmed as independent prognostic factors in N0 pts..

Conclusion: N1a-IHC in axillary lymph nodes are of prognostic importance. However, they do not represent independent prognostic factors in N0 pts. and are not helpful in treatment decisions.

885 POSTER

Changes in expression of the adhesion molecule CD66a in different stages of human breast carcinogenesis

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Purpose: CD66a is the human homologue of the well-defined cell adhesion molecule (Cell-CAM) of the rat. It was our aim to characterise for the first time the distribution of CD66a in human breast epithelia at different stages of malignant transformation.

Methods: The expression of CD66a was studied in 31 benign lesions, 15 in situ carcinomas and 100 invasive carcinomas of the mammary gland. Formalin-fixed, paraffin-embedded specimens and a monoclonal antibody (MAb4D1/C2) specific for CD66a were used for immunohistochemistry.

Results: CD66a was expressed at the apical sites of normal epithelial cells and in myoepithelia, but changed gradually from low to high grade in situ carcinomas into a uniform membranous staining. Apical staining was observed in only 11 out of 77 CD66a positive invasive carcinomas, while 23 cases showed no expression at all. A correlation was found between the apical CD66a staining and high differentiated carcinomas ($p = 0.03$) or tumour types like tubular and papillary carcinomas ($p = 0.0002$).

Conclusion: The uniform membranous expression of CD66a might be due to a loss or reduction of the interaction of the adhesion molecule with its binding molecules. Our findings indicate that not the loss of CD66a expression alone, but the change in expression patterns characterise an important event in breast cancer carcinogenesis.

886 POSTER

Cytological grading of breast carcinoma

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Purpose: Fine needle aspiration cytology (FNAC) is widely used in the diagnosis of breast carcinoma. The aim of this study was to devise a system for grading breast carcinoma based on cytological features alone. Such a system would be helpful in the selection of patients for appropriate therapy.

Methods: Diagnostic FNAC smears taken from 100 patients with breast carcinoma were studied. There were usually at least to two slides from each patient. The cytological preparation were studied without knowledge of the subsequent grade and type of the tumors. The features assessed were the nuclear diameter (compared with adjacent white blood cells), nuclear pleomorphism, the presence of easily visible nucleoli, mitoses, the degree of cell clustering and necrosis. Each of the features studied was scored separately and compared to the histological grade of the tumors following excision.

Results: Significant associations between worsening cytological features and increasing histological grade were found with nuclear diameter, nuclear pleomorphism, mitoses and presence of nucleoli. Discriminant analysis confirmed these findings, showing that a combination of the scores for these four parameters gave the best correlation with histological grade. The sensitivity of this method of classification was 83.6% and the specificity 85.8%.

Conclusion: This simple system of grading breast carcinoma is possible from FNAC smear specimens which correlates with histological grade. With the current trend toward less aggressive diagnostic methods definitive treatment may be carried out without prior biopsy and histological examination.

887 POSTER

Amplification of ERBB-2 (HER-2/NEU) oncogene as a potential prognostic marker for breast but not ovarian cancer in Russian group of patients

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Purpose: Amplification of ERBB-2 (HER-2/NEU) oncogene is widely accepted to be a frequent event (25–30%) and an indicator of unfavourable outcome for breast and ovarian cancer patients. Nonetheless some reports failed to confirm such phenomena due to unknown reasons. The purpose was to evaluate possible benefits from this test for North-Western Russia.

Methods: ERBB-2 copy number was determined by Southern-blot.

Results: In our study ERBB-2 oncogene was often amplified in breast carcinomas (BC) (36 of 142 (25%)), but not in ovarian malignancies (1 of 36 (3%)). There was a lack of association between ERBB-2 copy number and tumour size, lymph node involvement stage of disease, age of onset, estrogen and progesterone receptor level and family history in BC cohort. ERBB-2 extradosage was shown to have a prognostic importance in the group of 32 BC patients with sufficient follow-up (more than 40 months): 6 of 7 amplification-positive, but only 2 of 25 amplification-negative women relapsed ($P < 0.00005$).

Conclusions: Thus the data of the same research team are in agreement with the high occurrence of ERBB-2 activation in breast tumours, but contradict to similar reports on ovarian carcinomas. Therefore the peculiarities of the groups tested rather than technical variations in the gene copy num-

ber estimation contribute to the controversies around ERBB-2 amplification incidence and prognostic role.

888 POSTER

Factors predisposing to chronic pain after breast cancer treatment

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Purpose: The study was designed to assess the factors that predispose to chronic post-treatment pain in the breast area and in the ipsilateral arm in patients treated surgically for breast cancer.

Methods: A total of 93 patients with non-metastasised breast cancer who were treated during 1993–1994 answered the questionnaire about pain in the operated breast and in the ipsilateral arm one year after surgery. They were also asked to rate the severity of the acute postoperative pain of their breast surgery on a 5-point verbal rating scale. The Bayesian multivariate model was used. The factors which were included in the analysis were: age, type of operation, size of the tumour, number of lymph nodes removed, involvement of lymph nodes, number of lymph nodes involved, complications of surgery, inensity of the acute postoperative pain remembered by the patient, number of doses of analgesics, number of months from surgery, adjuvant radiotherapy, chemotherapy and endocrine treatment, state and trait anxiety and depression.

Results: The intensity of the acute postoperative pain remembered by the patient, trait anxiety and depression were the most important factors predisposing to chronic pain in the ipsilateral arm. State anxiety, trait anxiety and depression and breast radiotherapy were the factors included in the model of chronic pain in the breast area.

Conclusion: Patients who are anxious and depressed and who experience more acute pain after breast surgery (surgical damage?) and who have radiotherapy are at risk of developing chronic pain.

889 POSTER

Alteration in NK cell activity during the progression of breast cancer and its modulation by cytokines

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Purpose: In malignant processes alterations in immunological parameters are commonly present. As their impairment is associated with disease progression, the aim of this work was to investigate the activity of natural killer (NK) cells and the possibility of their stimulation with different cytokines in breast cancer patients.

Methods: Native NK cell activity and the one after in vitro treatment of PBL (4.0×10^6/ml of RPMI 1640) with rh IL-2, IL-7, IL-12 and TNF a (100 and 200 U/ml) was determined by the 51-chromium release method for breast cancer patients in clinical stages I–IV.

Results: The activity of NK cells is decreased in all breast cancer patients and it deteriorates with the advance of the malignancy. Treatment with IL-2 significantly enhanced ($p < 0.01$) NK cell activity of all patients. Contrary to this, IL-7, IL-12 and TNF a did not have this effect, however, when combined with IL-2 they gave similar activation as IL-2 alone.

Conclusion: The activity of NK cells, which are important antitumor effector cells, is depressed in breast cancer patients but it can be significantly augmented by in vitro treatment with IL-2. These results should encourage the application of IL-2 in these patients as adjuvant therapy, especially in advanced stages of breast cancer, where other therapy does not give sufficient results.

890 POSTER

Analysis of the ability of cytotoxic granules and B7-1 molecule to inhibit nodal metastases of cancer cells in patients with breast and lung cancers

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Purpose: we analyzed for the ability of granzyme B and perforine contained in cytotoxic granules and B7-1, a cell-surface molecule to inhibit nodal metastases of cancer cells in cancer patients.

Methods: Tumor specimens obtained from 23 breast cancer patients and 13 lung cancer patients were studied for granzyme B and perforine productions and B7-1 expressions, using an immunohistochemical technique. Percentages of positive cells for their expressions and the nodal involvement in each case were compared.

Results: 61%, 22% and 61% in breast cancer patients and 100%, 69% and 38% in lung cancer patients were positive for granzyme B, perforine and B7-1 expressions, respectively. Interestingly, granzyme B and perforine released by NK cells or cytotoxic T lymphocytes were shown to be located in the cytoplasm of cancer cells at different stages. B7-1 molecule was shown to be expressed on cancer cells rather than on tumor infiltrating mononuclear cells. In breast cancer patients, percentages of cancer cells containing either granzyme B or perforine inversely correlated with the nodal metastases. In lung cancer patients, only perforine production was related to the suppression of nodal metastases.

Conclusions: Granzyme and perforine contained in cytotoxic granules were suspected to enter cancer cells following released by effector cells in tumors. This event occurred in the early stages of cancer progression. These cytotoxic factors were thought to play crucial roles in the suppression of cancer cells metastasizing regional nodes in breast and lung cancer patients.

891 POSTER

Changes in radiation exposed women's mammary glands

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The research was carried out in accordance with the complex program "Poligon" in 1990–92. The goal of the research was to study 281 women that had lived on the area affected by the nuclear explosion in 1949 and to see the condition of their mammary glands and hormones status. That time they had been 5 to 36 years old. Minimum dose was 400, maximum 2430 mZB. At the examing time (after 41–43 years) they were ages from 48 to 77. Also 187 women from the unaffected area got included into control group. Mammography, ultrasound diagnostic of the mammary glands, immunity tests were given to all of the 468 women.

The analysis of the results showed that radiation exposed women have changes such as adenosis, diffuse or local fibrosis occurrence 3 time higher than the changes in the compared group. 129 women (46%) had corpus luteum insufficiency, hyperestrogenemia laboratory proved. In 34 cases (12%) was found hyperprolactinemia. 19% had hypothyreosis on the diffusus thyreoid hypertrophy background. Immunologic research in the exposed group revealed the symptoms of the secondary immunodeficiency.

Summerizing it up, we can say that radiation-exposed women present a high risk group of developing mammary gland cancer. They need chemio- and diet prevention, observation for a timely malignancy diagnostic.

892 POSTER

HLA genotype can be predictive for the breast cancer susceptibility and outcome

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Purpose: Does HLA-DR genotype contribute into breast cancer risk and aggressiveness?

Methods: DRB-HLA alleles distribution was analyzed by Southern-blot analysis in 44 breast cancer (BC) patients and 120 healthy donors (HD).

Results: The frequency of DRB homozygotes was significantly higher in BC group than in control (31.8% vs. 7.5%). In addition DRB-11 allele was overrepresented in the tumour cohort (25.0% vs. 11.7%; $p = 0.007$). Moreover, DRB allele distribution correlated with clinical parameters of the disease. DRB homozygosity and the presence of DRB-4 allele were associated with unfavourable prognosticators. In particular, DRB homozygous genotypes occurred more often in women with large tumour size (>5 cm), than in those with moderate tumour size (<5 cm) (75% vs. 18.2%; $p = 0.00001$). The tendency to correlation between DRB homozygosity and lymph node positiveness was also observed (38.1% vs. 20.0%; $p = 0.07$). The presence of DRB-4 allele was more typical for large (25.0% vs. 7.6%; $p = 0.07$) and advanced (23.1% vs. 6.7%; $p = 0.05$) carcinomas. On the contrary, DRB-3-1 and DRB-7-2 alleles were associated with rather small tumours.

Conclusions: The data might imply possible benefits of HLA-DRB genotyping for the determination of breast cancer risk and outcome.