of mitogenesis, invasion, neoangiogenesis and metastatization with combined chemoradioimmunotherapy after circumvention of chemo- and radioresistant mechanisms such as hypermethylation of oncosuppressor genes, overexpression of oncogenes and inhibition of endothelin induced signal transduction pathways

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p27 Deregulation as a prognostic marker in inflammatory breast cancer

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Introduction: Deregulation of p27 manifested by low protein cellular concentration has been shown to be an independent poor prognostic factor in breast cancer. The objective of this study was to evaluate whether the deregulation of p27 is a prognostic factor in patients with inflammatory breast carcinoma (IBC).
Patients and methods: Fifty-eight IBC patients were treated between January 1994 and July 2002 in clinical trials. Thirty-eight patients with baseline biopsy specimens and adequate follow-up data were included in this study. Patients were treated with preoperative chemotherapy with FAC (5-fluorouracil 500 mg/m^2, doxorubicin 50 mg/m^2 and cyclophosphamide 500 mg/m^2) (N = 13, 34%), or FAC followed by a taxane (paclitaxel 175–250 mg/m^2, or docetaxel 100 mg/m^2 every 21 days) (N = 25, 66%). All patients obtained objective clinical responses and underwent mastectomies. Eight patients received adjuvant paclitaxel; all patients received radiotherapy and hormonal treatment when indicated.
Expression level of p27 was evaluated by standard indirect immunoperoxidase procedure (nuclear staining). Tumors with p27 staining in less than 50% of the neoplastic cells were called p27-deregulated; tumors with p27 staining in equal or more than 50% of the neoplastic cells were called p27-normal or not-deregulated.
Results: Median age at diagnosis was 49 years, (21–73 years). Thirty-two patients (84%) had p27-deregulated tumors and 6 patients (17%) had p27-normal tumors. Six patients (17%) achieved a pathologic complete response (pCR). At a median follow-up of 43 months, 25 recurrences (66%) and 27 deaths (71%) had occurred. Patients with p27-deregulated tumors had fewer pCR (deregulated: 3/32–9%; not-deregulated 3/6–50%; $P = 0.03$) and had lower 4-year RFS (23% vs. 83%, $P = 0.03$) and OS rates, (36% vs. 83%, $P = 0.01$). Due to the small number of patients, the multivariate analysis failed to show any independent predictors of RFS or OS.
Conclusions: This retrospective analysis demonstrates that p27 deregulation manifested by low protein cellular concentration may represent an adverse prognostic marker in IBC and may provide a valuable tool for selecting treatment for this aggressive disease.

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High frequency of population-specific mutations of BRCA genes among breast cancer patients in the Czech Republic

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Introduction: Breast cancer is the most frequent malignancy in women in the Czech republic. Search for high-risk patients is in the focus of oncologists and gynecologists. A part of breast carcinomas (5–10%) arises due to hereditary disposition. The main cancer susceptibility genes responding for more than 80% of these cases are the *BRCA1* and *BRCA2* genes. Several hundred different mutations were detected in these genes to date. In certain populations, there is limited pool of mutations – one or several few mutation comprise majority of detected alterations. F.e. in Ashkenazi Jews, 1% of women are carrier one of the three mutations (185delAG, C61G or 5382insC), in Island population almost all the hereditary breast cancer cases are due to one *BRCA2* mutation (999del5). In population of the Czech Republic, three inactivating *BRCA1* mutations (c.5385dupC – 40%, c.3819_3823delGTAAA – 10%, c.300T>G – 10%) comprise 60% of all the alteration detected in both *BRCA* genes (Pohlreich et al.: Med Princ Pract 2003; Foretova et al.: Hum Mutat 2004).
Methods: We analyzed two population-specific mutations – c.5385dupC and c.3819_3823delGTAAA – in both a group of unselected breast cancer patients and group of unaffected age-matched women. The analysis of c.5385dupC was carried out by PCR-mediated site-specific mutagenesis: a mismatch nucleotide was by modified primer introduced during PCR into amplified fragment near the site of mutation. This mismatch together with non-mutated sequence made up a recognition site for restriction endonuclease Dde I. The wild-type (non-mutated allele was restricted, while the mutated allele remained uncleaved. In the detection of c.3819_3823delGTAAA mutation, we took advantage of spontaneous generation of heteroduplexes during PCR with low annealing temperature and the PCR products were simply analyzed by agarose gel electrophoresis. Suspected mutations were confirmed by direct sequencing of appropriate PCR products.
Results and conclusions: The c.5385dupC and the c.3819_3823delGTAAA mutations were analyzed in 592 patients and 474 controls. Seven mutations were detected in a group of patients (1.18%) and no mutation was detected in controls. None of positively tested patients fits the selection criteria for complete BRCA1/2 genetic analysis. Based on these and previously reported data, we can approximate the frequency of *BRCA1/2* mutations in women affected with breast cancer to be one in fifty cases (2%). At this time, the analysis proceeds and large number of patients and other population-specific mutations (c.300T>G in *BRCA1* and c.5991dupT in *BRCA2*) are screened. Although the complete mutational analysis of both *BRCA1* and *BRCA2* genes is very difficult and time-consuming approach and should be available only for risk patients indicated according consensus criteria, targeted analysis of population-specific mutation is extremely fast, simple and cheap and can be used for population screening (in accord with the frequency of mutation occurrence) of breast (and perhaps ovarian) cancer patients.

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Evaluation of blood based detection methods for NMP66, a breast cancer associated nuclear matrix protein complex

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Background: We investigated the performance of two methods of detecting NMP66, a nuclear matrix protein complex, in discriminating between serum from women with and without breast cancer in a cohort of 298 prospectively collected samples.
Materials and Methods: The NMP66 nuclear matrix protein complex has been demonstrated to be present in the sera of breast cancer patients and absent from the sera of women with no evidence of breast disease (Exp. Rev. Mol. Diag. 2:23, 2002). A signature protein of the complex was first isolated on a nickel affinity surface and identified by time of flight mass spectrometry using a SELDI-TOF instrument (Ciphergen Corp., Inc.). Subsequently, a prototype two-site colorimetric sandwich immunoassay for the identified protein was developed and a qualitative reverse transcriptase-polymerase chain reaction (RT-PCR) assay was constructed using probes specific for unique nucleic acid sequences found within the NMP66 complex (Development of a Blood Based Immunoassay to Detect the NMP66 Breast Cancer Associated Nuclear Matrix Protein, 4th European Conference: Perspectives in Breast Cancer, Seville, Spain 2004). The current investigation evaluated the performance of these methods in discriminating between samples from patients with and without breast cancer. Pre-biopsy blood samples were prospectively collected from 208 women who had suspicious mammograms and/or palpable breast lesions. Subsequent pathological examination determined that 55 had cancer (15 DCIS, 6 LCIS, 3 invasive lobular, 26 T1, 3 T2, 1 T3, 1 T4) and 153 had benign breast conditions. Fifteen samples collected from women with metastatic breast cancer and 75 from women who had no evidence of disease on two sequential mammograms in two years were added to the cohort. All samples (N = 298) were blinded by a third party and tested using both methods for detection of NMP66.
Results: The qualitative RT-PCR correctly identified over 70% of the cancer samples, and the colorimetric immunoassay demonstrated good differentiation of noncancer samples, ruling out 80% of those from patients with benign breast disease or no evidence of disease. There was a 67% greater likelihood of cancer being present when the colorimetric test was positive compared to cancer being present when the test was negative among patients who had clinical or biopsy confirmation of breast disease (RR 1.67). This effect was even greater for postmenopausal women, among whom there was a 75% likelihood of cancer being present upon pathological confirmation when the immunoassay result was positive (RR 1.75).
Conclusions: These preliminary results suggest that measurement of the NMP66 protein and associated complexes or products could provide utility in diagnosis of patients with a cost-effective blood-based method. Additional investigations and data are being compiled.