

Prognostic and predictive factors

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POSTER

Quantification of CK19 mRNA in peripheral blood (PB) and bone marrow (BM) from primary operable breast cancer (BC) patients pre- and postoperatively to investigate possible shedding of CK 19+ cells during the operation

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Introduction: We previously reported the development of a sensitive and quantitative assay to detect BC cells in PB of BC patients using real-time quantitative RT-PCR (ABI Prism 7700, Taqman[®]) identifying transcripts of the cytokeratin-19 (CK19) gene (Aerts J., et al, Ann Oncol 2001; 12: 39-46). In stage IV - BC patients we found a statistically significant number of transcripts of CK19 compared to healthy volunteers. We further analysed CK 19+ transcripts in bone marrow in a control population and primary operable BC patients. Pre- and postoperatively PB samples of these patients were further analysed to investigate possible shedding of CK 19+ cells during the operation.

Methods: Bone marrow samples of 22 patients with haematological malignancies were taken as control population. In 54 primary operable BC patients (pathological stage I (18pts.), stage II (28pts.), stage III (8pts.)), we analysed 50 BM samples taken preoperatively and 297 PB samples. The PB samples were collected before surgery(-1), immediately after surgery(0), on first(+1), second(+2), fifth(+5) days and one month (+1m) postoperatively.

Results: In the BM of the control population and the BC patients, we detected a median of 28.4 (95% CI [16; 67]) and 568 (95% CI [266; 1573]) CK19 positive cells/5x10⁶ leukocytes respectively (Mann-Whitney p < 0.001). In PB(-1) samples we measured a median of 109 (95% CI [58; 298]) CK positive cells/5x10⁶ leukocytes. Using the upper limit of the 95% CI of the control groups as cut-off, 74% and 52% of BM and PB(-1) samples respectively were considered as showing CK 19 positivity. The relationship between presence of CK 19+ cells in BM and PB(-1) and classical prognostic factors showed no significant correlation with pathological tumour size, nodal involvement, stage, differentiation grade and receptor status. The possible shedding of CK 19+ cells during the operation was investigated and no significant difference between the time points with respect to the average CK 19+ was detected (F=1.21, p= 0.32).

Overview: In primary BC patients, we detected high numbers of CK 19+ cells in BM and PB-1 samples compared to the control population. However, in this study population, no significant correlation between presence of CK 19+ cells in BM and PB(-1) and the classical prognostic factors was found. With this technique, we detected no statistically significant influence of surgical manipulation on the amount of CK 19 positivity.

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Thyroglobulin RT-PCR in fine needle biopsy aspirates from neck lymph nodes for detection of differentiated thyroid cancer metastases

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Cytology examination of fine needle aspirates is a well established tool in detection of cancer recurrence in neck lymph nodes. In patients with differentiated thyroid cancer (DTC) it is combined with serum thyroglobulin (Tg) measurement and radioiodine scintigraphy. However, in some patients all those examinations fail in early detections of neck lymph node recurrence. Demonstration of Tg gene expression in neck lymph nodes may prove the presence of metastatic cells derived from thyroid cancer, when the method of estimation has sufficient specificity and sensitivity.

Aim: Prospective study of thyroglobulin mRNA detection in neck lymph nodes of patients with differentiated thyroid cancer suspected for lymph nodal metastases.

Method: Expression of the thyroglobulin gene in neck lymph nodes was investigated by RT-PCR method in a selected group of 75 DTC patients. Aspirates from patients with other cancers were examined as a control group. Primer spanning exons 3-5 were used with 39 cycles of PCR. RNA isolation control and cDNA amplification were carried out using GADPH starters

Results: Thyroglobulin mRNA was detected by RT-PCR in 23 out of 75 examined nodes. In three patients in whom thyroglobulin mRNA detection was not confirmed by cytology examination, neck recurrence was confirmed a few months later. In two other patients, despite diagnosis of neck recurrence confirmed by cytology and histopathology examination, thyroglobulin expression in fine needle aspirates was not detected. Classical cytology confirmed nodal involvement in 22 (29%) of DTC patients, RT-PCR Tg was positive in 20 of them (92%). Additional 3 positive RT-PCR results were obtained and all of them were confirmed by repeated cytology conducted 4-6 months later, followed by surgery. There were no cases of false positive RT-PCR Tg result in DTC patients, in whom the suspicion of recurrence was excluded by the further observation nor in patients with other types of cancer. Thus, the specificity is 100% up to now. Sensitivity with reference to histopathology examination was 86%.

Conclusions: Thyroglobulin RT-PCR accelerated the diagnosis of lymphonodal involvement in some patients with differentiated thyroid cancer. Although further studies are needed to evaluate its sensitivity, the method is specific enough to be applied in these investigations

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Serum urokinase-type plasminogen activator (uPA) as a prognostic factor in metastatic breast cancer

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Purpose: Urokinase-type plasminogen activator (uPA) is a serine protease involved in tumor cell migration, angiogenesis, and invasion. Expression of uPA has been proposed as a prognostic factor in several types of cancer. In this study serum uPA was evaluated as a prognostic factor in metastatic breast cancer.

Methods: uPA levels were measured in pretreatment sera from 242 metastatic breast cancer patients enrolled in a randomized second-line hormone therapy trial. uPA was measured using a quantitative ELISA assay (Oncogene Science Diagnostics). A cutoff of 1.75 ng/mL (mean + 2SD) was derived from the sera of 29 postmenopausal healthy women. 81/242 (33.5%) patients had elevated serum uPA levels. Response rate, time to progression, and survival with respect to elevated vs. normal serum uPA were analyzed.

Results: The response rate (CR+PR+Stable disease) was not different in patients with elevated levels of serum uPA 69/161 (43%) compared to patients with normal serum uPA levels 26/81 (32.1%) (p=0.11). The time to progression (TTP) was significantly shorter for patients with elevated serum uPA levels when compared to patients with normal levels (p=0.013). Overall survival was significantly shorter for patients with elevated serum uPA levels when compared to patients with normal levels (p=0.03)

Conclusion: These results suggest that metastatic breast cancer patients with elevated serum uPA levels have a shorter time to progression and survival when treated with second-line hormone therapy.

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Development of novel high throughput microsatellite technology for detection of bladder cancer

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Purpose: We are assessing the feasibility of an assay for early detection of bladder cancer employing high-throughput screening techniques based on PCR amplification of urine sediment microsatellite DNA.

Using DNA from healthy individuals and focussing on 20 clinically relevant genomic loci, we sought to identify and quantify potential sources of variation in the calculated 'LOH statistic' (LOHs): a ratio of blood (reference) to urine (test) DNA PCR-product. Recently, two groups have used similar but low-throughput technology for detection of bladder cancer, reporting sensitivities of 95% (n=20) and 84% (n=103)

Methods: The MegaBACE sequencer and custom-designed, fluorescently-labelled primers were used with blood and urine DNA extracted from samples obtained from healthy individuals. The following studies of observed LOHs were carried out: 1) Intra-assay variation arising from

changing PCR conditions ($n \geq 16-32$ per condition) 2) Inter-assay variation within replicate amplifications of a single genome ($n \geq 750$ LOHs for each of the 20 markers) 3) Inter-assay variation arising from amplifications of different genotypes ($n = 940$ LOHs, 20 genotypes). Coefficients of determination (r^2) from regression lines fitted to allele amplification product scatter graphs were used to quantify variation and rank markers for reproducibility. Histograms of LOHs were used to construct various marker-specific probability distributions for confidence intervals.

Results: 1) All PCR conditions affected either the relative extent or quality of allele amplification. 2) Most markers at the chosen loci could be reproducibly amplified ($0.996 > r^2 > 0.885$) with exceptions reflecting natural variation in allele sizes (eg $r^2 = 0.635$), which caused an increase in LOHs variation. 3) LOHs variation correlated with increasing difference in allele sizes. Finally, population homozygosity at some loci rendered the assay less informative.

Conclusion: Many parameters were identified that contribute to variation in LOHs measurement. For microsatellite technology (and LOHs) to be predictive of bladder cancer, optimised amplification conditions need to be strictly adhered to. The number of markers involved in the current protocol provide considerable challenges for sample handling and data interpretation. Results from ongoing trials of clinical samples will determine if the natural variation in observed LOHs is sufficiently low to achieve high sensitivity and specificity in cancer detection.

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p53 overexpression predicts the resistance to chemotherapy in advanced non small cell lung cancer (NSCLC)

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Purpose: Tumors with p53 overexpression have been associated with enhanced resistance to cisplatin-based chemotherapy in limited studies involving NSCLC. Ours is the largest study on p53 and response to chemotherapy in patients (pts) with locally advanced and advanced NSCLC. The relationships and interactions between p53, Rb and bcl-2 immunostaining, clinical parameters and response and overall survival were considered in analysis.

Methods and population: Paraffin-embedded bronchial biopsies and transthoracic agobiopsy specimens of 102 pts who underwent cisplatin based-chemotherapy between 1991 and 1999 were evaluated using an immunostaining method. Pts were 88 males and 14 females, with a mean age of 61.5 ± 9.9 yrs; 47 were stage III and 55 were stage IV.

Results: Median survival was 12.2 months and the 5 year survival was 6.1%. Only 15 specimens (14.7%) were normal for all three markers. Fifty-six tumor samples (54.9%) had normal p53, 41 (40.2%) had normal Rb and 94 (92.2%) had normal bcl2 expression. The univariate analysis indicated that the pts with normal p53 and association of normal p53 and deleted Rb had better response to chemotherapy. The response rate of the p53 positive group was 26% versus 57% of p53 negative group ($p < 0.002$). We also compared the different associations of Rb and p53 protein expression: group I (p53-Rb+), group II (p53-Rb-), group III (p53+Rb+), group IV (p53+Rb-) including 17, 39, 24 and 22 patients, respectively. Group II was found to have the best response rate (62%, $P < 0.003$). Moreover, bcl-2 overexpression, TNM (III vs IV) and normal LDH serum levels were associated with better overall survival ($p < 0.03$, $p < 0.02$ and $p < 0.001$). In multivariate analyses, p53 overexpression for resistance to chemotherapy and TNM for overall survival were identified as independent predictive factors ($p < 0.02$, $p < 0.001$, respectively).

Conclusion: Our study demonstrates a predictive role of p53 for response rate and confirmed TNM as the only predictive factor for survival. It may be important to consider these data for stratification in prospective randomized clinical trials because the difference in the response rate due to p53 expression is too high to be ignored.

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Relationship between a clinical drug resistance and immunohistochemical expression of p-glycoprotein and p53 in small cell lung cancer

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Background: Although small cell lung cancer (SCLC) is in general chemosensitive, nearly 20% of SCLC is refractory to chemotherapy. While resistance to chemotherapy is a major problem in treatment of SCLC, there is no useful predictor of treatment response. One reason of drug resistance is the overexpression of the drug efflux pump, P-glycoprotein and other multidrug resistance protein. On the other hand, inactivation of the p53 tumor suppressor gene is reported to result in chemoresistance due to deletion of p53-mediated apoptosis. Several markers of drug resistance have been described in preclinical models, but the mechanism of drug resistance in lung cancer patients remains unknown.

Purpose: We designed this study to determine the utility of p53, P-glycoprotein and multidrug resistance protein expression in predicting the response to chemotherapy in patients with SCLC, retrospectively.

Material and Methods: We evaluated transbronchial biopsy (TBB) specimens from 62 patients with previously untreated SCLC. Formalin-fixed, paraffin-embedded TBB specimens were immunostained using anti-p53 antibody (DO-1, Oncogene Science, New York, U.S.A.), anti-P-glycoprotein antibody (JSB-1, Nichirei Co., Japan).

Results: The positive rate of P-glycoprotein was 25.8%, and that of p53 was 76.7%. No correlation was observed between P-glycoprotein and p53 immunostaining. The expression of P-glycoprotein and p53 were not correlated with clinical backgrounds including age, sex, disease extension, tumor markers, or chemotherapy regimen. However, the expression of P-glycoprotein was correlated with clinical chemotherapy resistance in SCLC ($P < 0.001$), while that of p53 was not ($P = 0.999$).

Conclusion: These results suggest that immunostaining of P-glycoprotein for TBB specimens may help to predict clinical drug resistance in SCLC. Staining results of other ABC superfamily such as MRP1 and MRP2 are also to be presented.

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Predictive value of p16ink4a and p53 alterations for the prognosis of resected early stage non-small cell lung cancer

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Purpose and Methods: The association of p16 and p53 gene abnormalities with the prognosis of patients with non-small cell lung cancer (NSCLC) has been widely investigated to date, however, this association is still controversial.

Therefore, we investigated the prognostic significance of p16 abnormalities (mutations by sequencing and hypermethylation by methylation-specific PCR), and p53 alterations (by immunohistochemistry and serum p53 antibody assessment, and by direct sequencing through exons 4 to 8) in 72 radically resected stage I-II NSCLCs. Kaplan-Meier estimates of survival were calculated for clinical variables and molecular markers using the Cox model for multivariate analysis.

Results: Sequencing analysis of the p53 gene showed mutations in 32% of stage I-II NSCLC; serum p53 antibodies were detected in 14%, and p53 protein expression in 40%. p16 abnormalities were found in 33% of patients (point mutations in 2 (3%) and promoter hypermethylation in 22 (30%) cases). No correlation was found between p53 and p16 abnormalities and various clinicopathologic factors, including age, sex, histological type of tumour and TNM (I vs. II) stage. The overall survival rate of NSCLC revealed that both the patients with p53 and p16 abnormalities tended to have a poorer prognosis than the patients without p53 ($p < 0.02$) and p16 ($p < 0.01$) abnormalities. In the multivariate analysis, however, when the types of p53 and p16 alterations were analyzed, only p53 gene mutations and p16 hypermethylation were associated with poor prognosis.

Conclusion: These results indicated that p53 point mutation and p16 hypermethylation could be a useful molecular markers for the prognosis of patients with resected early stage NSCLC.