Prognostic and predictive factors

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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 C K19+ 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