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Two Methods for the Detection of Breast Cancer Cells in Blood Samples

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Background: Disseminated tumor cells (DTCs) in bone marrow and circulating tumor cells (CTCs) in blood are found in patients with epithelial carcinomas (breast cancer) and are often correlated with poor prognosis of the disease.

In current models circulating tumor cells (CTCs) dissolved from the primary tumor are thought to be responsible for the occurrence of metastases.

However, the detection of CTCs is still a technical challenge. In this study, two methods for tumor cell detection of patients' samples are presented (Real-Time-PCR and immunohisto-chemical staining). Both are known methods with a high sensitivity and a spread marker panel.

Materials and Methods: For the implementation of both methods, different breast cancer cell lines have been used (Cama-1, MCF-7; ZR-75−1). For Real-Time-PCR, blood samples of a healthy donors were spiked with different cell counts (0, 10, 100, 1000, 10,000 and 100,000) per ml blood sample. Total RNA was isolated from the samples, reversely transcribed into cDNA and used for TaqMan Real-Time-PCR reaction with probes against CK8, 18 and 19, while 18S was used as reference. Relative Quantification Curves were drawn by Microsoft™ Excel®.

For immunohistochemical staining, cytospins were prepared from spiked blood samples, fixed with acetone, air dried and stained with antibodies against Her2- and Thomsen-Friedenreich-Antigen (CD176). In a second staining step fluorescently labelled secondary antibodies were applied. Nuclei were counterstained with DAPI, TF-Antigen was stained by Cy2 and Her2-Antigen by Cy3. The staining was controlled and documented by an epifluorescence microscope.

Results: The curve of Relative Quantification for MCF-7 and ZR-75−1 cells shows an increasing slope starting from 1000 cells. For the Cama-1 cell line this trend is already seen from 10–100 cells. In ZR-75−1 all three genes analysed reveal this trend, whereas in Cama-1 and MCF-7 cells a strong increase in Relative Quantification is especially seen for CK8 and 18.

In the immunohistochemical staining, the cells were considered as tumour cells if they showed staining with the antibody-combinations used. Stained cells were counted and recovery rates were determined. For ZR-75−1, 17 of 30 cells which were spiked in the blood samples were recovered. For MCF-7, 18 cells were found in average, and for Cama-1 23 cells were located per slide. The recovery rates calculated from these numbers are 56.6% and 60.0% for ZR-75−1 and MCF-7, for Cama-1 the recovery rate reaches 76.6%.

Conclusion: It seems that Cama-1 cells are a better model than MCF-7 and ZR-75−1 for Real-Time PCR quantification of mamma carcinoma tumor cells in blood samples. MCF-7 and ZR-75−1 cells tend to react more likely immunologically with blood cells of the donor (agglutination between blood cells and cancer cells). The Cama-1 cell line shows also advantages in the detection of tumor cells using immunohistochemical staining. Therefore it will be necessary to test both methods on patient samples to proof their benefit.

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MicroRNAs as Emerging Biomarkers for Micrometastasis Detection in Breast Tumors

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Background: Quantitative Real Time PCR (qPCR) is a highly sensitive method commonly used for the detection of circulating tumor cells (CTC) in peripheral blood of patients with malignant breast tumors. The rationale for using microRNAs (miRNA) as potential therapeutic targets is explained by the fact that miRNAs overexpression in cancer cells has a pathogenic effect. miRNAs may constitute a promising new class of cancer biomarkers for CTC detection. Our primary purpose is to use this approach to identify microRNAs with diagnostic and prognostic value in peripheral blood of patients suffering from breast cancer (BC).

Material and Methods: We used different online bioinformatics tools allowing us to select a panel of microRNAs with high expression in breast tumors while low or no expression in control peripheral blood (PB) and bone marrow. The usefulness of potential up-regulated miRNAs from previous bioinformatic analysis was validated in human breast cell lines: BT549, MCF7, MDA-MB-231, PM1 and T47D; hematopoietic cell lines: JURKAT, KG1 and K562; human breast total RNA (Ambion®), and healthy blood samples (n = 19). *mirVana miRNA* and *RiboPure Blood* isolation kits (Ambion®) were used. Using qRT-PCR miRNAs tumor-related were amplified. Genex 5.0.1 (MultiD Analyses) was used as qPCR data analysis software.

Results: Among a panel of 12 microRNAs analyzed highlight 5 upregulated in breast tumors: miR-32, miR-200a, miR-200b, and the cluster miR-200c/141 fulfilled the premises of bioinformatic searches. For instance, relative expression of mir-32 and mir-141 was 1.09×10^3 and 1.08×10^3 respectively, higher in T47d cells than in control PB (n = 19).

Conclusions: These results suggest that this bioinformatic approach is an useful high-throughout method to identified BC associated miRNAs. Actually our group is actively involved in the study of selected miRNAs for their potential as markers for CTC detection in peripheral blood of BC patients and age-matched healthy control subjects. Supported by Grants PI06/1541 and PI07/0477 from Fondo de Investigaciones Sanitarias (FIS), Instituto de Salud Carlos III. Cancer research in our laboratory is supported by the 'Fundación do CHU A Coruña'.

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The Expression of RANTES (CCL5) is Inversely Correlated with Lymph Node Involvement in Breast Cancer

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Background: Lymph node involvement is the most important prognostic factor in primary operable breast cancer and is mainly related to tumor size. However, it is unclear whether the host affects the process of lymph node metastasis. The chemokine CCL5 plays a role in chemoattraction and activation of immune cells (Weinberg RA; Nature 2007). Its exact functions in tumor biology are somewhat controversial and still not completely understood. We investigated the correlation between the expression of CCL5 in plasma and the presence of lymph node metastases in primary operable breast cancer.

Material and Methods: We measured preoperative plasma levels of CCL5 in 213 postmenopausal breast cancer patients using the BioPlex© technology and compared the expression of CCL5 by the patients lymph node status (negative versus positive). The association between levels of circulating CCL5 and lymph node status was analyzed using a two way ANOVA, allowing an interaction between tumor size and lymph node involvement.

Results: We included 213 patients; 56% were lymph node negative and 44% lymph node positive. Mean tumor size was 28 mm (range 1−90 mm). Across all patients, mean CCL5 levels were 1239 pg/ml (range 158–6814 pg/ml). When allowing the effect of RANTES to differ between different tumor sizes, there was statistical evidence of an association between RANTES and lymph node status (p = 0.048). More specifically, for small tumors the inverse correlation was highly significant (p = 0.014 at tumor size 20 mm) whereas for large tumors, the difference was not found to be significant (p = 0.790 at tumor size 50 mm).

Conclusions: CCL5 concentration in plasma in postmenopausal women with a small primary operable breast cancer are inversely correlated with lymph node involvement. This finding suggests an effect of the host's immune response on the process of lymph node involvement. Our results are currently being validated on a larger cohort of plasma samples of breast cancer patients.

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Quantitative Assessment of Her-2 Expression of Circulating Tumor Cells in Patients with Metastatic and Non Metastatic Breast Cancer

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Background: Circulating tumor cells (CTC) can provide the basis for a real-time liquid biopsy and may guide the use of targeted therapies. We report on unbiased quantification of Her-2 protein expression of CTC.

Materials and Methods: Her-2 assessment of CTC was performed using the CellSearch® system in 103 metastatic (M1) and 88 non metastatic (M0) breast-cancer patients. Digital images of Cytokeratin-PE, DAPI, CD45-APC, and Her-2-FITC from these samples were stored. Expression of Her-2 on CTC was determined by manual review and an automated