

Detection and characterization of circulating tumour cells in blood and disseminated tumour cells in bone marrow of breast cancer patients

Background

The main reason for breast cancer associated death are remote metastases, that are due to cells, that dissolve from the primary tumour, and are found in blood (circulating tumour cells, CTCs) and bone marrow (disseminated tumour cells, DTCs). The detection of these cells in breast cancer patients is mostly linked to a worse prognosis.

We present an immunohistochemical staining method for the detection of DTCs from bone marrow by using the cancer-associated Thomsen-Friedenreich antigen (TF) in combination with Her-2, and stem cell markers (Muc-1, ALDH1A1), and a highly sensitive RT-PCR based approach for CTC-detection from peripheral blood of adjuvant breast cancer patients using the established cytokeratin markers CK8, 18 and 19.

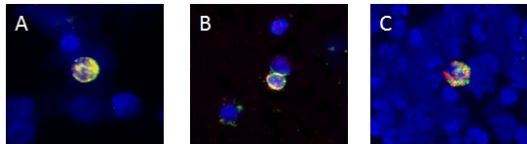


Figure 1: Fluorescent double staining of DTCs from bone marrow samples (merged pictures)

A) TF and HER-2; B) TF and Mucin-1; C) TF and ALDH1A1

Blue: DAPI nuclear counterstain; Green: TF-Antigen; Red: HER-2, Mucin-1 or ALDH1A1 respectively

	TF single staining	X single staining	TF + X double staining
X = Her-2 (%)	17,00	36,86	46,14
X = Muc-1 (%)	47,40	34,45	18,15
X = ALDH1A1 (%)	24,68	59,65	15,68

Table 1: Frequencies of single and double staining of TF and the respective cancer cell marker antigens.

	TF-Her-2	TF-Muc1	TF-ALDH1A1
Σ patient samples	41	40	40
# of patient samples with positive stainings	32	35	32
% positive stainings	78,05	87,50	80,00
Significances	p < 0,001	p < 0,001	p < 0,001

Table 2: Overall staining frequencies of bone marrow samples

Patients and Methods

A total of 20ml blood and 2-3ml of bone marrow were withdrawn from 20 respectively 40 primary breast cancer patients during surgery. For this investigation we used only samples of patients with at least one CTC already detected with the FDA approved VERIDEX CellSearch System®.

For both methods an enrichment of mononuclear cell fraction, containing DTCs and CTCs, was carried out by density gradient centrifugation.

For immunohistochemical staining cells were spun down on coverslips, fixed and stained with antibodies against Thomsen-Friedenreich-Antigen and Her-2, MUC1 or ALDH1A1.

For Real-Time PCR RNA was isolated from the cell pellet obtained by density gradient centrifugation, reversely transcribed to cDNA and RT-PCR was run with Taq-Man Primers against Cytokeratin (CK) 8, 18 and 19. The housekeeping gene 18S was used as internal reference, blood samples from healthy donors were withdrawn, treated equally and used as negative control group.

Results

For the staining of Her-2 and TF, 78,05% of all samples showed at least one stained cell. The biggest subgroup within these samples was the double stained one with 46,14%.

In the other two experimental settings respectively the biggest cohorts were only single stained with TF (47,4% for TF-MUC1) or ALDH1A1 (59,65% for TF-ALDH1A1).

Within the PCR trials, the investigation between the negative control group and adjuvant breast cancer blood samples showed significant correlations for the expressions of CK8 (p<0,047) and CK18 (p<0,041). CK19 showed borderline significance (P<0,057) of correlation.

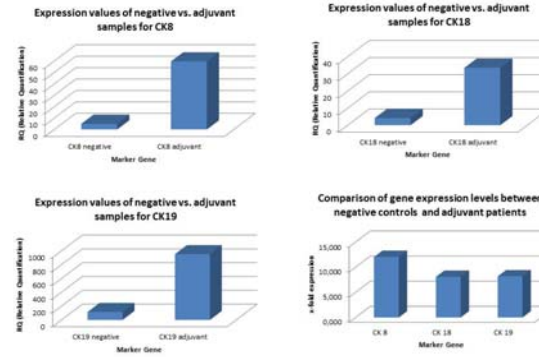


Figure 2: Comparison of gene expression values in negative control and adjuvant breast cancer samples measured by RT-PCR

Marker gene	Group	Valid Cases	Significance (p-Value)
CK8	Control	N = 19	0,047
	Adjuvant	N = 19	
CK18	Control	N = 20	0,041
	Adjuvant	N = 20	
CK19	Control	N = 20	0,057
	Adjuvant	N = 20	

Table 3: Statistically significant differences in gene expression values

Conclusion

The immunostaining-combination of diverse cell surface antigens for detection and characterization of DTCs, especially the TF antigen, in combination with Her-2, Muc-1 and ALDH1A1 is an excellent way to detect DTC's in bone marrow due to its significant correlations (p<0,001).

The verification of CTC's by using RT-PCR is possible by using epithelial marker genes like the cytokeratins, especially CK8 and 18.

Both methods can be a step towards modern personalized medicine and lead to new ways of early tumour detection. Additionally an improved tumour characterization might ease the decision on specific medical treatment (eg. against Her-2).

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