Correlation of two analytical methods for circulating tumor cells in peripheral blood of patients with primary breast cancer

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Background

While the evidence for circulating tumor cells (CTCs) as a prognostic marker in metastatic breast cancer has been well established, there is still a lack of data in primary disease. In the SUCCESS A trial two different techniques for the detection of CTCs in early breast cancer were prospectively evaluated.

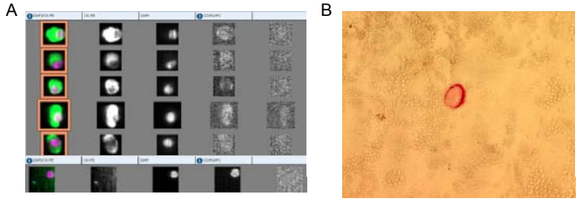

Figure 1:*Sample pictures of CTCs, detected by CSS (A) and MICC (B). A: row 1 – 5: CTCs; row 6: leukocyte.*

Patients

Table1: *Patients characteristics and CTC prevalence with the CellSearch System® (CSS) and the manual immuncytochemistry (MICC).*

	CSS N	MICC N	CSS % pos	MICC % pos
pT1	818	489	19,32	19,22
pT2-4	1159	751	22,69	22,24
pTx	17	9	17,65	33,33
pN0	680	445	19,41	20,22
pN1-3	1314	804	22,22	21,64
Gx	16	8	18,75	12,50
G1	98	59	14,29	23,73
G2	934	613	21,52	21,21
G3	946	569	21,78	20,91
HR -	569	371	21,79	22,10
HR +	1425	878	21,05	20,73
Her-2 x	50	29	18,00	20,69
Her-2 +	489	311	20,25	23,47
Her-2 -	1455	909	21,72	20,35
ductal	1600	1035	20,94	21,06
lobular	237	125	25,74	25,60
mixed	143	82	18,18	15,85
premen	830	529	20,00	21,55
postmen	1164	720	22,16	20,83

Materials & Methods

SUCCESS A compared FEC-Docetaxel vs. FEC-Docetaxel-Gemcitabine and 5 vs. 2 years of treatment with zoledronic acid in primary breast cancer patients and node positive or high-risk node negative disease.

Two different techniques to detect CTCs were prospectively evaluated in two consecutive, but comparable subgroups of the whole study population. In 3515 samples the CellSearch® System (CSS) (Veridex, Warren, USA) was used for CTC detection. Immunomagnetic enrichment with an EPCAM-antibody was followed by labeling with monoclonal antibodies specific for cytokeratin (8, 18, 19) and leukocytes (CD45).

2165 samples were evaluated with a manual immunocytochemistry (MICC) protocol. Cytospins were prepared after mononuclear cell enrichment based on Oncoquick® centrifugation (greiner bio-one, Frickenhausen, Germany). Staining was performed with the monoclonal pancytokeratin antibody A45-B/B3 (Micromet, Munich, Germany) and the APAAP technique. Conventional light field microscopy (Axiophot; Zeiss, Oberkochen, Germany) was used for the detection of stained cells.

For both methods, the cut-off value for positivity was ≥ 1 CTC. All events were evaluated by two independent observers.

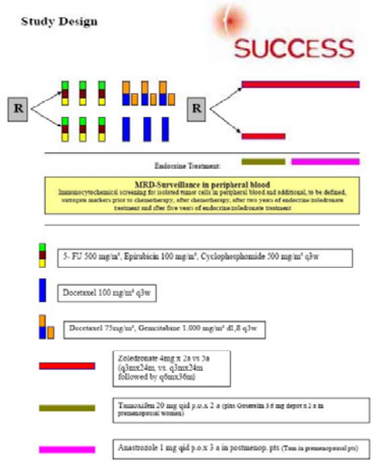

Figure 2: *Study Design of the SUCCESS A trail.*

Results

CTCs were examined in a total number of 3243 patients before and after chemotherapy (CHT). The two subgroups evaluated with one or the other method were well-balanced regarding clinical parameters as tumor size, grading, lymph node-status, hormone receptors and Her2. Furthermore there was no significant correlation between the CTC positivity and one of these clinical parameters using CellSearch® or the MICC, respectively ($p > 0.05$ using the chi square test each time). Before adjuvant CHT 21.3% (424 out of 1994) and 21.1% (264 out of 1249) of the patients were found positive for CTCs using CellSearch® or the MICC respectively, with a mean CTC level of 5.9 (range: 1 to 827) and 3.1 (range: 1 to 256). Immediately after CHT 21.9% (333 out of 1521) and 16.5% (151 out of 916) of the patients were positive for CTCs using CellSearch® or the MICC. The mean CTC level decreased to 3.0 (range: 1 to 124) and 2.1 (range: 1 to 23) in both analytical methods. Using CellSearch® there was a significant correlation between the presence of CTCs before CHT and disease progression ($p = 0.0044$), as well as survival ($p = 0.0001$), whereas the MICC did not predict any of these ($p = 0.3143$ and $p = 0.0801$ respectively; the chi-square test was used each time).

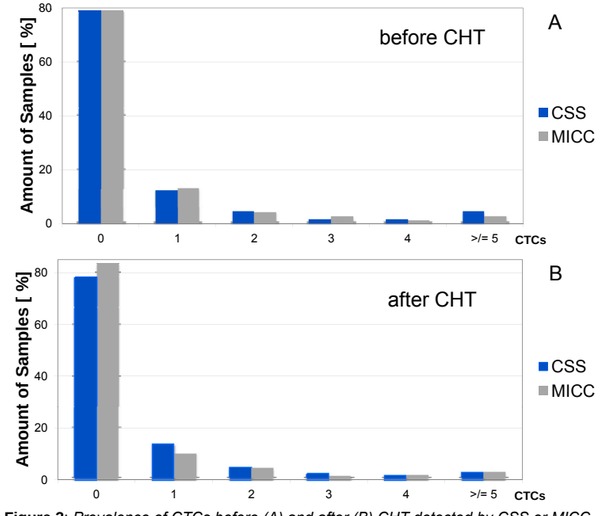

Figure 3: *Prevalence of CTCs before (A) and after (B) CHT detected by CSS or MICC.*

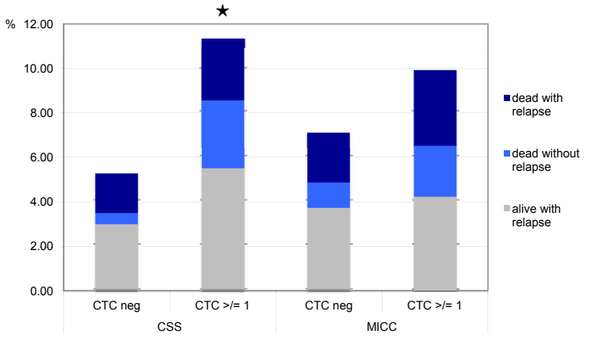

Figure 4: *Percentage of patients who had a progress of their disease or died regarding the CTC-Positivity before CHT with one or the other method (median follow up 35 months). ★ indicates a significant result ($p \leq 0.05$ for disease progression and survival).*

Conclusion

We found comparable prevalence of CTCs before and after adjuvant chemotherapy both with the CellSearch® System or the MICC. However, prognostic relevance could only be shown for CTCs detected with the CellSearch® System. This may be attributed to the high standardization and reproducibility of the automated system, as well as the additional CD45 counterstaining. According to our findings, the FDA approved CellSearch® System should be used as gold standard for CTC detection in future clinical trials.

Institutions

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