Flow cytometric analysis of CTCs in breast cancer patients before and after chemotherapy

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Introduction: Breast cancer if one of the most common types of malignancy regarding females. The most prevalent methods for detecting breast cancer include mammographs, MRI, ultrasound and serum tumor markers. In the recent years a new method for detecting malignancies has emerged. It involves detecting circulating tumor cells (CTCs) in the peripheral blood using flow cytometry. It has been shown in many studies that the detection of CTCs has great value in the early diagnosis, prognosis and treatment plan of cancer patients. The present study makes use of a multiparameter flow cytometric panel to achieve highly specific and sensitive of CTCs in breast cancer patients before and after chemotherapy.

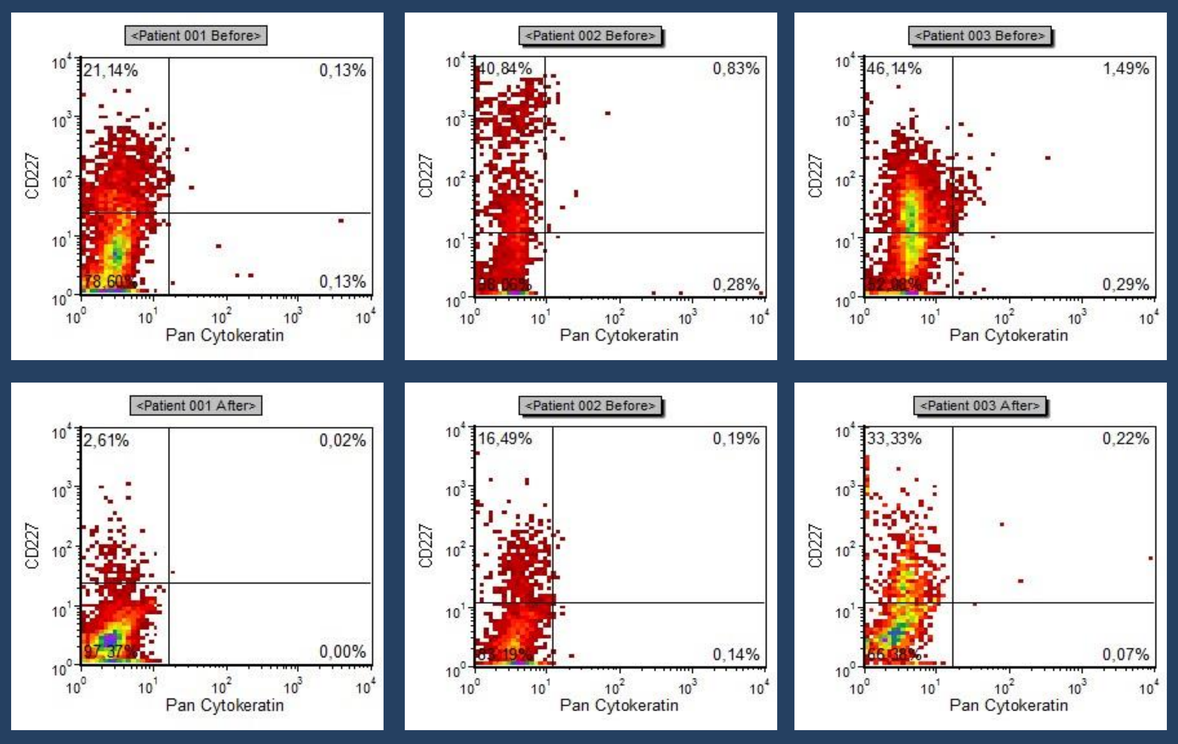

Figure 1: CTC detection in 3 patients. Top row shows CTC percentages before chemotherapy and bottom row after chemotherapy. Both tumor related markers (CD227-PanCytokeratin) show significant reduction.

Results: CTCs were detected in all patients that were studied. Before chemotherapy the CTCs that were detected ranged from 5 to 11.5 with a median of 6.44 cells per 7.5 ml of peripheral blood and a standard error of ±0.35. After chemotherapy their numbers ranged from 2 to 9.4 cells per 7.5 ml of blood with a median of 4.67 and a standard error of ±0.3. Cut off point was 5 cells per 7.5 ml. All patients studied had a decreased CTC count after their chemotherapy with a p=0.003 . Also, 46.7% of them were below the cutoff point of 5 cells and were considered negative.

Discussion: According to the data from this study, we were able to detect a decrease in CTC count using the multiparameter flow cytometric panel before and after chemotherapy. The four antibodies that were used increased the sensitivity and specificity of the method, by better immunophenotyping the cancer cells. This could be a useful diagnostic tool, which along with histopathology and imaging techniques, can be used to monitor cancer treatment in patients.

Methods:

Study subjects: A total of 30 female patients were selected for this study, aged 37 to 82 with a median of 59 years. All patients were histopathologically diagnosed with breast cancer and had performed a CTC detection test prior to having chemotherapy. Cancer types were identified as invasive ductal, invasive lobular and other types. Their chemotherapeutic program included Fluorouracil, epirubicin, Cytoxan and tamoxifen. The study was approved by the Ethics Committee of RGCC. Signed consent forms were obtained from each patient.

Flow cytometry: The panel that was used to stain patients' samples included CD45-PE/Cy7, CD31-RPE, pancytokeratin-PE/Cy5 and MUC-1(CD227)-FITC. Data acquisition was performed on a Beckman Coulter FC500. 50000 events were acquired for each patient. Gating strategy was performed as to identify the CTCs as $CD45^-/CD31^-/PanCK^+/MUC1^+$. Data analysis was performed using FCSExpress v3 from Denovo Software.

Statistical analysis: Data were analysed using SPSS software. T test was used to compare the two groups. A significance level of $p<0.05$ was set as a statistically significant difference.

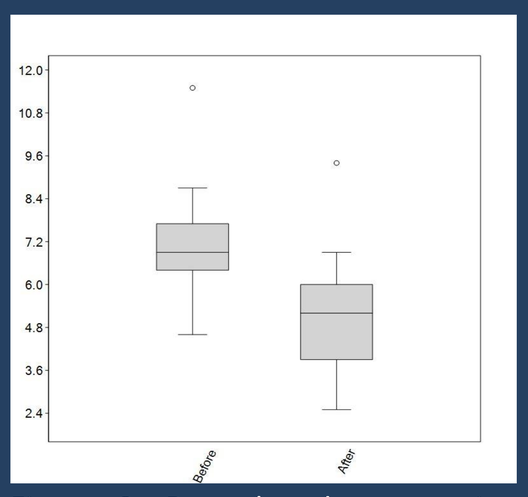

Figure 2: Box plot demonstrating mean values of CTCs before and after chemotherapy in all 30 patients

Stage	No. of Patients	Median Before	Median After
I	7	5,3 ±0,8	3,3 ± 0,44
II	5	5 ± 0,34	3,62 ± 0,67
III	6	6,63 ± 0,36	5,3 ± 0,27
IV	7	6,84 ± 0,39	5,2 ± 0,49
N/A	5	8,68 ±0,75	6,08 ± 0,9

Table 1: Categorization of patients according to stage of cancer. Table also shows median values of CTC numbers for each stage

Selected references

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