Isolation and characterization of CTCs from breast cancer patients with and without metastasis

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Introduction: Breast cancer if one of the most common types of malignancy regarding females. 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. The present study makes use of a multiparameter flow cytometric panel and magnetic bead separation methods to characterize CTCs and isolate them from breast cancer patients with and without metastasis.

Methods:

Study subjects: A total of 20 female patients were selected for this study, aged 37 to 82 with a median of 59 years.

Flow cytometry: The panel included CD45-PE/Cy7, CD31-RPE, pancytokeratin-PE/Cy5, c-met-PE and MUC-1(CD227)-FITC. CTCs were identified as $CD45^-/CD31^-/PanCK^+/MUC1^+$ and metastatic cells as $CD45^-/c\text{-}met^+$.

CTC Isolation and cultivation: PBMCs from patients were isolated using ficoll centrifugation methods and incubated with EpCAM magnetic beads to isolate CTCs. Cells were cultured in serum free RPMI medium. Microscope images were taken each day to determine CTC growth.

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

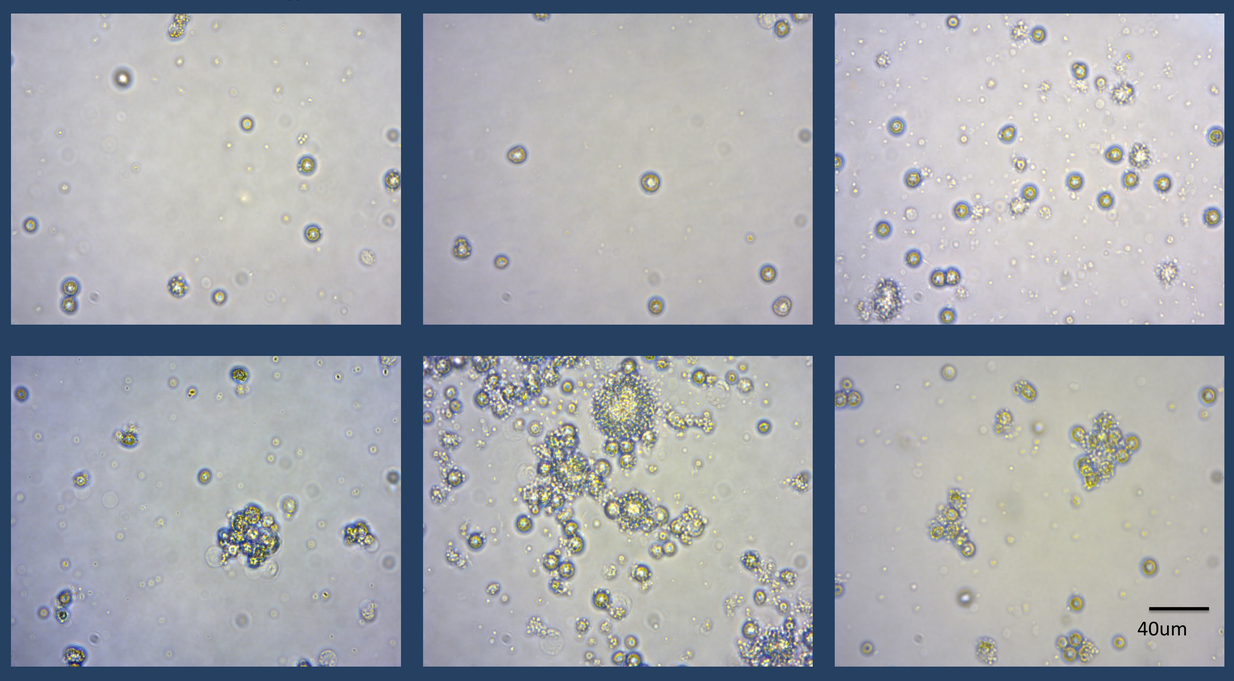

Figure 1: Pictures of isolated $EpCAM^+$ cells (CTCs). Top row show CTCs isolated from non metastatic breast CA patients with stages I-II. Bottom row shows CTCs from metastatic breast CA patients, which are beginning to grow in clumps. All pictures were taken at 48h after isolation.

Discussion: According to the data from this study, CTCs can be used as a tool to monitor disease progression and possible metastatic potential of the cells. Isolating and cultivating the cells can help us better understand CTC physiology. Successful culture of the cells can also be used for further applications such as drug testing, which can lead to more personalized medicine for cancer patients. Flow cytometric analysis using a multiparameter panel opens up new ways in cancer diagnostics and therapeutics.

Results: CTCs were isolated from all patients that were studied. CTCs cultures from metastatic breast cancer patients showed greater cell growth. Also CTCs tended to grow in semi-adherent spheres. CTC number for metastatic patients ranged from 5.7 to 9.3 with a mean of 6.8 and a standard error of ±0.34 while CTC number for non-metastatic patients ranged from 1 to 5.2 with a mean of 3.5 and a standard error of ±0.39. The two groups had a statistically significant difference ($p=0.001<0.05$).Flow cytometric analysis showed greater expression of tumor specific markers in these patients compared to non-metastatic ones.

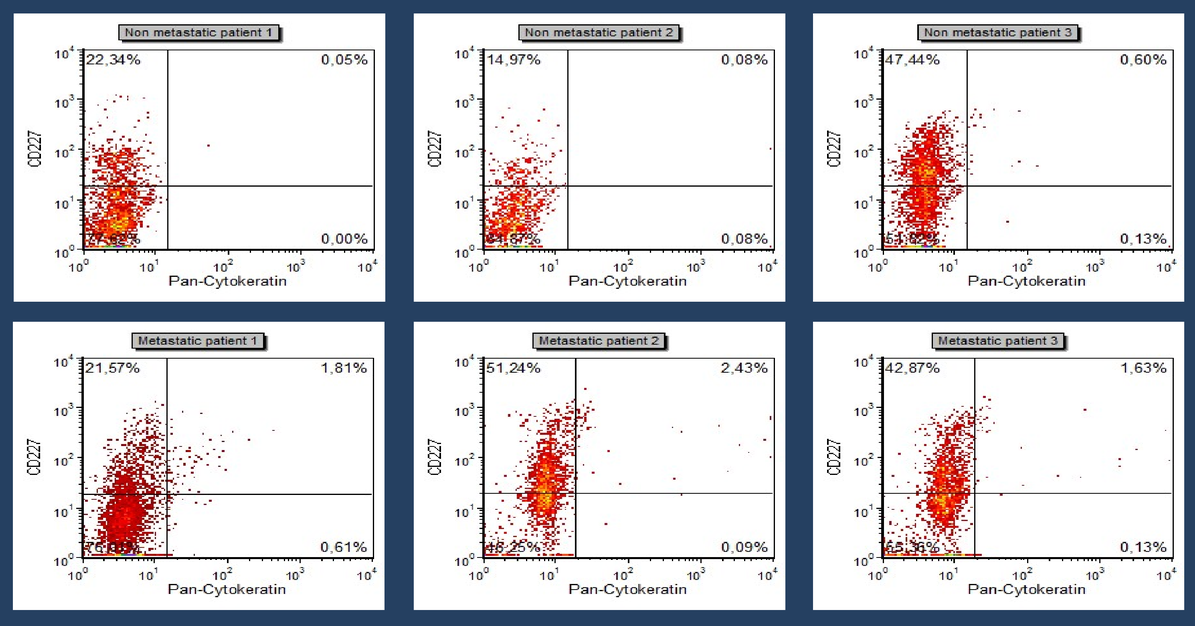

Figure 2: CTC detection in breast cancer patients. Top row show $CD45^-/CD31^-/PanCK^+/MUC1^+$ cells in non metastatic patients, while bottom row shows CTCs in metastatic patients

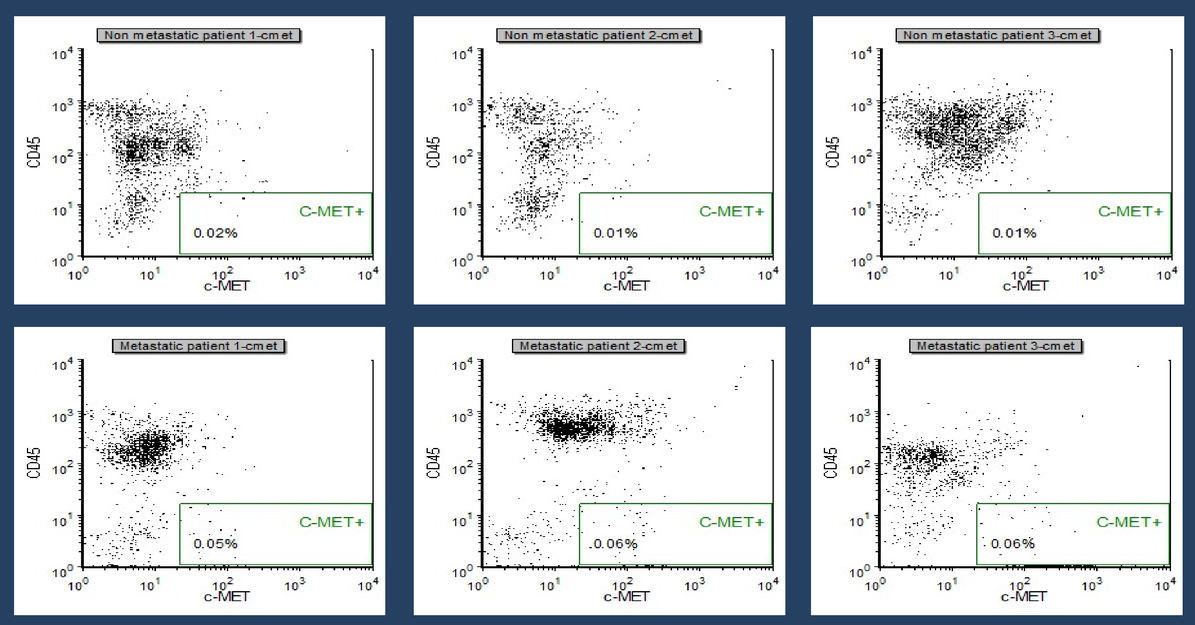

Figure 3: CTC detection in breast cancer patients. Top row show $CD45^-/cMET^+$ cells in non metastatic patients, while bottom row shows cMET positive cells in metastatic patients. P value was $0.007<0.05$ which shows that there is statistically significant difference between the two groups

Selected references

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