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EXPERIMENTAL PAPER

Detection of circulating tumour cells in the breast cancer using CytoTrack system

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Summary

Introduction: Plants are a rich source of healing substances. Cancer is a leading cause of death worldwide while breast cancer is the most common cancer among women. Circulating tumour cells (CTCs) are poten-

tial founder cells for metastasis. Therefore, their assessment may be used for monitoring of treatment as well as detecting cancer metastatis. Hence, it is suggested that the number of CTCs may be a valuable tumour biomarker during therapy.

Objective: The purpose of this study was to detect CTCs in breast cancer and to validate the method of assessment of CTC count using CytoTrack CT11 technology.

Methods: MCF-7 cells were sorted by a FACSARIA flow cytometer from blood samples derived from patients who have not been diagnosed with cancer. Identification and quantitative assessment of MCF-7 cells in blood samples were determined by flow sorting. Then, blood samples containing MCF-7 cells or without MCF-7 were scanned with the use of an automated fluorescence scanning microscope.

Results: In *in vitro* model analysing the glass CytoDisc™ with stained MCF-7 cells, we noted the correlation between the amount of observed tumour cells and expected number of tumour cells. Moreover, coefficient of variation in case of the recovery rate of the assumed number of MCF-7 cells was 30%, 17%, 18% and 15%, respectively.

Conclusion: Our study suggest that CTCs could be predictive factor in patients with metastatic cancer especially in breast cancer.

Key words: ***circulating tumour cells, breast cancer, CytoTrack CT11, liquid biopsy***

Słowa kluczowe: ***wolnokrążące komórki nowotworowe, rak piersi, CytroTrack CT11, płynna biopsja***

INTRODUCTION

Plants are a rich source of healing substances. They have been used for over four thousand years due to the fact that substances of plant origin have a very wide range of therapeutic effects. One of diseases in which they have a healing effect is cancer. Probably the first plants to be found to have anti-cancer effects were meadow clover (*Trifolium pratense*) and autumn cold-floss (*Colchicum autumnale*). Cancers are the second cause of death in the world, which frequently leads to serious clinical consequences. The majority of cancer deaths result from metastasis through circulating tumour cells (CTCs) in the bloodstream of patient [1-3]. A blood sample containing CTCs is often called liquid biopsy. The presence of these cells is proof that a patient has cancer because the circulating tumour cells will not be found in the peripheral blood form healthy individual. Therefore, CTC detection may be a potential prognostic tumour marker in the diagnosis, treatment and monitoring of cancer. It results from the analysis of CTCs because the reduced number of these cells indicates effective treatment. In case of the sensitivity loss of cancer cells to the therapy, the number of CTCs increases as a result of tumour progression [4, 5]. Hence, it is suggested that the analysis of CTCs may improve the effectiveness of cancer treatment. These cells are isolated from peripheral blood drawn from cancer patients. However, used methods are significantly limited to identification and enumeration of CTCs [6]. Hence, the choice of methodology for CTC detection is crucial because it may significantly influence the result depending on sensitivity, specificity and detection limit [1, 7]. Moreover, it is suggested that CTCs are heterogeneous populations of cells and may not reflect a heterogeneous tumour profile. Methods based on CTC characterization are considered to be a promising tool for the implementation of personalized medicine in the cancer treatment [8, 9]. The technology is a new kind of scanner combining flow cytometry and fluorescence microscopy which scans and analyses of rare cells, such as circulating tumour cells immobilized on the round glass disc [10]. The aim of this study was to detect CTCs in breast cancer and to validate the method of assessment of CTC count using CytoTrack CT11 technology.

MATERIALS AND METHODS

Cell culture

The ER-positive human breast cancer cell line,

MCF-7 (ATCC HTB22) was obtained as a gift of Professor M. Zabel, Poznań University of Medical Sciences, Poland. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM-Gibco®) (Sigma-Aldrich) in the incubator at 37°C with 5% CO_2 atmosphere. The medium (DMEM-Gibco®) contained 10% foetal bovine serum (Sigma-Aldrich) and 0.1% penicillin (100 U/ml) (Sigma-Aldrich). The cell growth and viability was assessed by counting viable cells using Countess™ II Automated Cell Counter (Thermo Fisher Scientific). Then, MCF-7 cells were sorted by a FACSARIA flow cytometer (BD Biosciences) from blood samples derived from patients who have not been diagnosed with cancer. Identification and quantitative assessment of cancer cells in blood samples were determined by flow sorting. Blood samples were prepared with 0, 5, 10, 20 and 30 cancer cells derived from human breast cancer cell line (MCF-7) in triplicate and then analysed by CytoTrack CT11 scanner with CytoPicker (CytoTrackApS, Lyngby, Denmark).

Sample processing and scanning

Blood samples containing MCF-7 cells or without MCF-7 (controls) were centrifuged at 2500 g for 15 min. The buffy-coat containing mononuclear cells and tumour cells was transferred to 15 ml tube. Erythrocytes were lysed using FACS lysing solution (BD Biosciences) for 15 min on a rocker at a room temperature and then the samples were centrifuged at 2500 g for 15 min. Subsequently, cells were stained by a mixture of anti-CD45, anti-pan-cytokeratin (FITC) and nuclear-stain 4,6-diaminidino-2-phenylindole (DAPI) (CytoTrackApS, Lyngby, Denmark) for one hour at 2–8°C in darkness. Next, the cells were washed with phosphate-buffered saline (PBS) with 1% BSA and resuspended in 1 ml H_2O. Thereafter, the cell suspension was transferred to a glass CytoDisc™ and air-dried in the dark. On the centre of the CytoDisc™ we added mounting medium (Olink Bioscience, Uppsala, Sweden) and placed a CytoCover™ (CytoTrack). Then, we sealed the CoverDisc™ by applying Fixogum (Marabu Scandinavia, Denmark) along the edge and air-dried in the dark for minimum 2 hours, according to manufacturer's protocol. The glass CytoDisc™ with stained cells was evaluated using scanning fluorescence microscope CytoTrack CT11. Scanning was performed as previously described by Hilling *et al.* [7]. To identify CTCs, we analysed images using the following criteria: cell size >4 μm, DAPI-positive for nucleus, pan-cytokeratin positive, and CD45 negative.

The obtained data were evaluated using the D'Agostino-Pearson K2 test and Gaussian distribution [11]. Values such as mean, SD, percent of recovery of CTCs and percent coefficient of variation (CV%) were also calculated.

Ethical approval: The conducted research is not related to either human or animal use.

RESULS

Analysing of the glass CytoDisc™ with stained MCF-7 cells from cell culture, we noted the correlation between the number of observed tumour cells and expected tumour cells (tab. 1).

Coefficient of variation in case of the recovery rate of the assumed number of MCF-7 cells was 30, 17, 18 and 15%, respectively. The results are summarized in table 1. Additionally, the presence of individual CTCs for each sample was confirmed by imaging using CytoTrack CT11 scanner (fig. 1).

DISCUSSION

Currently, treatment of patients with metastatic breast cancer is based on the characteristics cells of the primary tumour. It is believed that 90% of deaths

Table 1.

Recovery of MCF-7 cells (5, 10, 20, 30 tumour cells) added to whole blood from healthy donors

Number of expected CTC	Number of observed CTC (mean)	Recovery [%]	SD	95% CI	CV[%]
5	3.67	73.4	0.57	3–4	30
10	8	80	1	7–9	17
20	15.67	78.35	1.15	15–17	18
30	25.67	85.57	1.53	24–27	15
Total	–	79.33	–	–	20

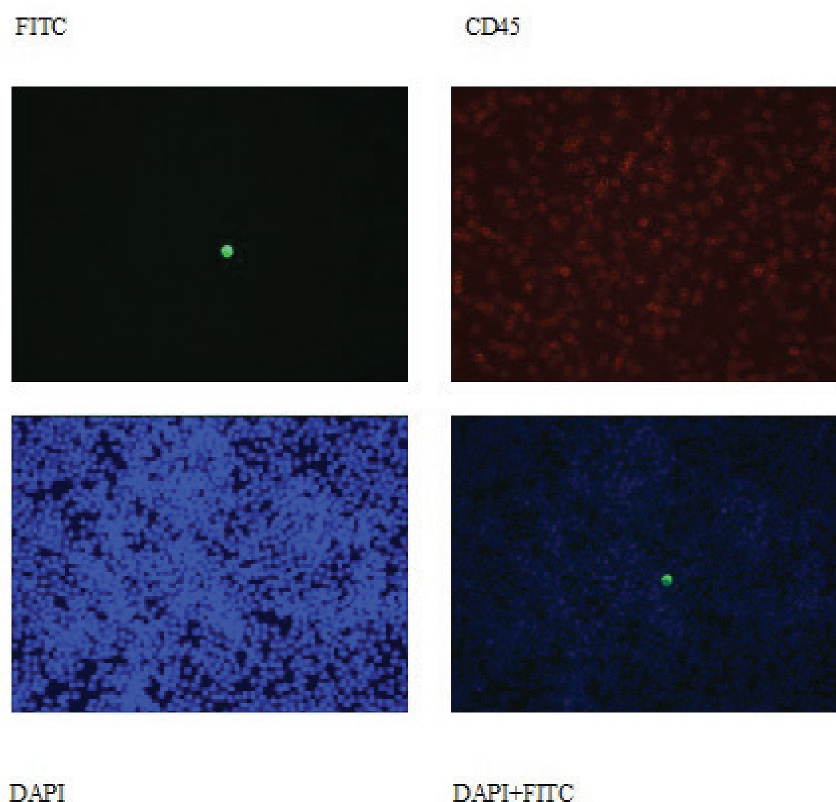

Figure 1
Images galleries of cells detected by CytoTrack CT11 scanner with CytoPicker from patient with breast cancer. The left image shows tumor cell stained with the epithelial marker pan-cytokeratin FITC (green). The right image shows corresponding leukocytes stained with a CD45 antibody (red). The left image below shows corresponding nuclei stained with DAPI (blue). The right image below shows the nuclear marker DAPI with FITC presenting tumor cell (green).

of breast cancer results from the clinical complications related to metastases [12, 13]. Thus, the novel methods for CTC detection are still sought as a tool for characterization of the metastatic cancer because analysis methods of CTCs are often based on their size, selection or enrichment of these cells by immunocapture techniques [14, 15]. Nowadays, CTC analysis is conditioned by cytokeratin staining. However, some tumour cells with decreased cytokeratin content are difficult to detect. Therefore, it is important to use other markers for detection of the cells from epithelial-mesenchymal transition [16]. CytoTrack CT11 as a novel scanning fluorescence microscope allows the study of new CTC markers for detection of these tumour cells in different types of cancer e.g. ovarian cancer, gastric cancer, colon cancer, etc.

In our study, we evaluated a method related to identification and enumeration of CTCs in breast cancer using CytoTrack CT11 technology. This method is based on scanning all nucleated cells (leukocytes, tumour cells) in blood samples without enrichment procedure compared to other methods of detection of CTCs and the ability to recover of these cells from the glass disk for molecular studies using CytoTrack CT11 scanner with CytoPicker. Such a simplified procedure of sample preparation for scanning may provide better CTC recovery. Our study with use of cell culture showed the correlation between the number of observed MCF-7 cells and expected tumour cells. Similar study based on the recovery of MCF-7 cells from drawn blood was conducted by Hilling *et al.* [10]. They showed that the recovery of MCF-7 cells was 68% (95% CI=52-83) whereas the coefficient of variation (CV) in case of the recovery rate for 10, 33 and 100 tumour cells was 59, 32, and 18% (total CV=36%). This study emphasizes the importance of method optimization for detection of CTCs because in patients with low number of tumour cells we can receive false-negative results due to low recovery and high CV%. The authors explain that the variation in number of recovered CTCs may be due to the manner

of collection the buffy coat cells and the lysis of erythrocytes causing a reduction of leukocytes with up to 10% [10, 17, 18].

CONCLUSION

The analysis of CTCs as a liquid biopsy can be used in the treatment and monitoring of cancer. Our study suggest that CTCs could be used as a predictive factor in patients with metastatic cancer, especially in breast cancer. So far, only a few studies have been carried out in obtaining CTCs from patients and analysing them in terms of genetic profile. Therefore, there is a need to conduct further studies on obtaining CTCs from patients with cancer in order to improve the technique of their recovery and to use CTCs analysis in the diagnosis and treatment. In further studies, we plan to recover CTCs for other cancers with method validation and analysis of their genetic profile.

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Conflict of interest: The authors declare no conflict of interest.

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