Hardware-software complex for diagnostics of breast cancer on the basis of flow cytometry

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Abstract. The method of multicolor flow cytometry makes it possible to quantify the disseminated tumor cells (DTC) in patients with solid tumors. Application of the method multicolor flow cytometry showed its efficiency and accuracy in the detection of DTC in patients with breast cancer.

1. Introduction
Breast cancer (BC) occupies the first place in the structure of oncological diseases of the female population of Russia, as in most developed countries. There are significant violations of various parts of the immune response in patients with malignant tumors. Immune profile of intratumoral lymphocytes CD68<sub>low</sub>CD4<sub>high</sub>CD8<sub>high</sub> is characteristic for the group of patients with primary breast cancer with high overall and disease-free survival. In contrast, the immune response of patients with CD68<sub>high</sub>CD4<sub>high</sub>CD8<sub>low</sub> profile corresponds to the group of patients with risk of development of distant metastases and reduced survival.

Thus, it is established prognostically favorable immunological characteristics of intratumoral lymphocytes in breast cancer.

Drug Polyoxidonium (PO) was selected to assess the possible impact on the subpopulation composition intratumoral lymphocytes, bone marrow, and disseminated tumor cells (DTC).

The randomized study on the effect of polyoxidonium on the tolerance to postoperative chemotherapy or chemoradiation therapy in patients with breast cancer was conducted in the N.N. Blokhin Russian Cancer Research Center.

The objective of this paper is to evaluate the effect of polyoxidonium on disseminated tumor cells (DTC) and subpopulation composition of lymphocytes in the bone marrow of patients with operable breast cancer [1-4].

2. Materials and methods
The material for this research work was the results of the studies of 63 patients with breast cancer T1-3N1-3M0 stage. The diagnosis of breast cancer and treatment of patients were conducted in the N.N. Blokhin Russian Cancer Research Center in September-December 2015.

The standard volume of preoperative medical examination of patients with breast cancer included ultrasound and mammography of both breasts in two views, ultrasound examination of the axillary, supraclavicular and subclavial lymph nodes, pelvic organs and liver, and radiography of the chest, a bone scan of the skeleton.
Calculation of cellularity was performed for all bone marrow samples at the cellular analyzer ABX Micros60 (company ABX Diagnostics, USA).

Table 1. MCA combinations.

| tube No | The fluorochrome |
|---------|------------------|
|         | V450 | FITC | PE | Pс5 | PC7 |
| 1       | CD45  | Control | Control |  |  |
| 2       | CD45  | CD3   | CD95 | CD19 | TCR γ/δ |
| 3       | CD45  | HL-DA | CD8  | CD3  |  |
| 4       | -     | CD4   | CD25 | CD3  |  |
| 5       | -     | CD10  | CD5  | CD19 |  |
| 6       | -     | CD5   | CD38 | CD19 |  |
| 7       | CD45  | CD3   | CD56 |  |  |

Figure 1. Lymphocyte gate (CD45⁺) for subpopulation analysis.

Table 2. The MCA specificity.

| No  | Differentiation cluster name | Specificity |
|-----|------------------------------|-------------|
| 1   | CD45                         | All leukocytes |
| 2   | CD3                          | All T-cells |
| 3   | CD4                          | T-helper cells |
| 4   | CD8                          | T-killer cells |
| 5   | CD19                         | All B-cells |
| 6   | CD5                          | B-cells, B1- leukocytes |
| 7   | CD10                         | Common ALL antigen |
| 8   | CD38                         | Activation antigen |
| 9   | CD56                         | N-CAM |
| 10  | CD25                         | IL-2 receptor |
| 11  | CD95                         | FAS/APO1 apoptosis receptor |
| 12  | TCRγ/δ                       | γ/δ T-cells receptor |
| 13  | HLA-DR                       | 2 class histocompatibility molecule |
Determination of DTC was carried out by two methods: 1 – morphologically (myelogram counting by two morphologists; 6 smears of bone marrow stained with hematoxylin and eosin), 2 - flow cytometry using monoclonal antibodies to cytokeratin Cam5.2, labeled FITC (Beckton Dickenson company, USA) in combination with antibodies to panleukocyte antigens CD45, V450 labeled (company Beckton Dickenson, USA). Collection of material was conducted on a flow cytometer Attune (Thermo Fisher Scientific, USA) and flow cytometer Facs Canto II (Beckton Dickenson, USA).

3. Assessment of the subpopulation composition of bone marrow
Assessment of the subpopulation composition of bone marrow was carried out using 4-color flow cytometry with combinations of monoclonal antibodies (MCA) conjugated with fluorochrome (table 1). Analysis of subpopulations was performed within the lymphocyte gate (figure 1). The MCA specificity are presented in table 2.

4. Results
Statistically significant differences in parameters are highlighted in bold.

| Parameter | Frequency | The fluorochrome | Correlation | Significance |
|-----------|-----------|------------------|-------------|--------------|
| CD45+     | 10        | 7.62 ± 2.56      | 8.52 ±4.87  | 0.86         | 0.001        |
| CD45+CD3+ | 9         | 63.35 ± 11.97    | 64.46±10.29 | 0.89         | 0.001        |
| CD3+CD95+ | 3*        | 45.44±12.7       | 63.47±10.29 | 0.97         | 0.13         |
| CD3+TCRγδ | 3*        | 4.82±5.18        | 3.42±4.11   | 0.99         | 0.089        |
| CD3+CD4+  | 11        | 29.53±3.44       | 29.57±5.87  | 0.64         | 0.034        |
| CD3+CD8+  | 11        | 27.42±6.17       | 29.85±9.42  | 0.26         | 0.44         |
| CD4/CD8   | 11        | 1.12±0.28        | 1.07±0.35   | 0.60         | 0.053        |
| CD19/CD5+ | 10        | 8.88±4.21        | 13.78±13.68 | 0.002        | 0.99         |
| CD19/CD10+| 7         | 40.41±16.68      | 26.29±18.00 | 0.38         | 0.41         |
| CD19/CD38+| 9         | 37.95±16.13      | 29.67±20.92 | 0.62         | 0.076        |
| CD3-CD56+ | 3*        | 7.54±3.09        | 7.93±3.92   | 0.98         | 0.13         |
| CD3-CD56+ | 3*        | 8.64±5.25        | 8.37±6.5    | 0.99         | 0.053        |

*Too small a sample.

Statistically significant increase in the total number of lymphocytes (CD45+) and Mature T cells (CD45+CD3+) was revealed. Other statistically significant changes were not detected.

5. Conclusion
We first studied the influence of Polyoxidonium (PO) on disseminated tumor cells (DTC) in the bone marrow – the drug does not cause increase in the number of DTC of breast cancer in the bone marrow. Statistically no significant trend of decrease in DTC in the bone marrow after PO course was noted. Antitumor effect of PO by induction of the pathomorphosis of the tumor tissue of breast cancer was shown in 43.7% cases.

References
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