Analysis of Circulating Tumor Cells in Patients with Triple Negative Breast Cancer during Preoperative Chemotherapy

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The presence of circulating tumor cells in the blood of patients with triple negative breast cancer (early and locally advanced cancer) before and after preoperative chemotherapy was assessed using expression markers. Before therapy, circulating tumor cells were detected in 5 of 13 (38%) patients with early cancer and in 7 of 17 (41.2%) patients with locally advanced cancer. After therapy, the circulating immune cells were detected in one patient with locally advanced cancer, who had no circulating cells before therapy. The tumor was resistant to chemotherapy and the disease progressed. The detected circulating tumor cells were HER-2-positive, while the primary tumor was HER-2-negative. It was concluded that the circulating immune cells can be a potential marker of the efficiency of therapy and predictors of the disease course, while their phenotype can differ from the phenotype of the primary tumor.

Key Words: *circulating tumor cells; breast cancer*

Recent advances in oncology, in particular, in the therapy of breast cancer (BC) are related to the development of individual approach to the treatment with consideration to detectable prognostic and predictive factors, first of all, expression of receptors for steroid hormones and HER-2 in the tumor. However, the disease sometimes progresses even in cases of early cancer despite chemotherapy supplemented with targeted preparation (hormone therapy, anti-HER-2 therapy). Tumors with triple-negative phenotype, *i.e.* not expressing receptors of steroid hormones and *HER-2* and therefore carrying no targets for targeted therapy, are characterized by the worst prognosis. Hence, additional prognostic markers are required for refining indications for more or less aggressive treatment. Predictive markers showing tumor sensitivity to the therapy are also important.

The possibility of using circulating tumor cells (CTC) as prognostic and predictive markers attracts now much attention. According to some studies, CTC can be detected even at the early stages of the disease, which is associated with high risk of relapse (incidence of CTC detection varies from 9.4 to 48.6%) [4,7,8]. Recent surge in using neoadjuvant therapy in BC allows *in vivo* evaluation of tumor sensitivity to treatment. It is known that survival rate in patients with triple negative BC in case of attaining complete pathomorphological regression approaches that observed in more favorable forms of the disease. Apart from evaluation of the degree of pathomorphological regression, measurement of CTC content in the blood before and after preoperative chemotherapy can be an additional marker of treatment efficiency.

CTC represent a heterogeneous population of tumor cells released into the bloodstream that according

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to recent data can be representatives of tumor stem cells and can undergo phenotypic changes, the so-called epithelial-mesenchymal transition that enables their migration to the sites of metastasis formation and makes them insensitive to usual cytostatic agents.

The blood content of CTC is a variable parameter, while CTC lifetime in the blood is 1-2 days. The number of detected CTC in the blood depends on the chosen markers (primarily, surface antigens) and varies from two to several thousand cells per 7-10 ml blood. The reported ranges of normal values vary due to the absence of unique CTC markers and common nomenclature. According to some methods, CTC detection in the blood of healthy donors is acceptable, because some combinations of CTC antigens are also expressed in normal cells.

The main CTC markers in BC are surface antigens, including epithelial cell adhesion molecules EpCAM and MUC-1 that are encoded by *GA733-2* and *Muc-1* genes, respectively [1,2,9]. Another marker of CTC in BC is expression of *HER-2* gene, which is of special interest, because different status of *HER-2* in the primary tumor and CTC was reported. Expression of *HER-2* can be determined in CTC, while the primary tumor can be HER-2-negative according to immunohistochemical and FISH analysis.

The aim of the present study was to detect the presence and phenotype of CTC in the blood of patients with triple negative BC receiving preoperative chemotherapy by using markers *GA733-2*, *Muc-1*, and *HER-2*.

MATERIALS AND METHODS

Blood samples from 30 women with triple negative BC (HER-2$^-$, ER$^-$, PR$^-$), patients of the Department of Clinical Pharmacology and Chemotherapy, N. N. Blokhin Cancer Research Center, Russian Academy of Medical Sciences. Inclusion criteria: 18-75-year-old women with first diagnosed BC, triple negative tumor phenotype, possibility of core-biopsy and extended immunohistochemical analysis, without serious concomitant pathologies. Exclusion criteria: previous therapy for BC, history of tumor diseases over the last 5 years (except basal cell carcinoma and *in situ* cervical cancer), pregnancy, and lactation. The study included patients at the age of 28-72 years (median 44 years). The patients were divided into two groups depending on the stage of the tumor process: patients with early BC (T1-2N0-1M0) and patients with locally advanced BC (T2-4N2-3M0). Patients with early BC received neoadjuvant chemotherapy according to the following scheme: cisplatin (30 mg/m^2), doxorubicin (25 mg/m^2), and paclitaxel (100 mg/m^2) intravenously through a dripper once a week for 8 weeks with G-CSF support on days 3-5. Patients with locally advanced BC received induction therapy by the following scheme: carboplatin (AUG2), paclitaxel (60 mg/m^2) intravenously through a dripper weekly during weeks 1-9, doxorubicin (25 mg/m^2) intravenously through a dripper weekly, cyclophosphamide (50 mg) and capecitabine (1500 mg) *per os* daily during weeks 10-18.

The degree of pathomorphological regression was evaluated by the method of Chevallier: class 1 corresponded to the absence of macroscopic and microscopic signs of the tumor (Ch1); class 2 corresponded to the presence of carcinoma *in situ* only in the mammary gland without invasive tumor and tumor cells in lymph nodes (Ch2); class 3 corresponded to the presence invasive carcinoma with stromal changes such as fibrosis and sclerosis (Ch3); and class 4 indicated the absence or minimum changes in the tumor structure [3]. The combination of morphological signs of class 1 and class 2 therapeutic pathomorphosis was considered as complete pathomorphological regression.

The blood was tested for the presence of CTC at the moment of diagnosis and after completion of the course of neoadjuvant therapy (in 21 patients). CTC were detected using Breast Select and Breast Detect kits (Adnagen) for cell isolation and identification of isolated cells by the expression of marker genes, implying magnetic sorting on antibodies to EpCAM and MUC-1 antigens for enrichment of blood cell population with CTC followed by verification of CTC by the expression of *GA733-2*, *Muc-1*, or *HER-2* genes.

RESULTS

The groups of early BC and locally advanced BC included 13 and 17 patients, respectively. At the moment of diagnosis, CTC were detected in 5 of 13 (38%) patients with early BC and in 7 of 17 (41.2%) patients with locally advanced BC. The incidence of CTC detection did not differ in these groups.

In the group of early BC, complete pathomorphological regression was observed in 9 of 12 patients and in 1 patients neoadjuvant therapy was continued. Of 9 patients with complete pathomorphological regression, CTC before therapy were detected in 4 and not detected in 5 patients.

In the group of locally advanced BC, complete pathomorphological regression was observed in 6 of 8 patients and in 9 patients neoadjuvant chemotherapy was continued, and 1 patient was excluded because of disease progression. Of 6 patients with complete pathomorphological regression, CTC before therapy were detected in 2 and not detected in 4 patients.

After neoadjuvant therapy, in none of the patients CTC were detected except one woman with locally

advanced BC. In the only case when CTC were found after treatment (but were not detected at the moment of diagnosis), progression of the disease during chemotherapy was observed. The scheme of therapy was changed because of disease progression, but the tumor was resistant to chemotherapy. CTC were *HER-2*-positive despite the fact that the primary tumor was negative by ER, PR, and HER-2 by the results of immunohistochemical analysis.

The level of CTC in the course of neoadjuvant therapy was analyzed in only few studies. In GeparQuattro trial, the levels of CTC were analyzed at diagnosis and after neoadjuvant chemotherapy in 213 patients with large operable tumors and locally advanced tumors. The incidence of CTC detection before the therapy was 21.6% and after the therapy it decreased to 10.6%. CTC were not detected in 15% patients initially positive for CTC, whereas 8.3% patients without CTC at the moment of diagnosis turned out CTC-positive. However, no significant correlations between the presence of CTC and tumor response to therapy were revealed [5]. Similar results were obtained in the previous study [6] including 118 patients with stage II-III BC. In 23 and 17% patients, CTC were assayed before the start of neoadjuvant chemotherapy and after treatment, respectively. Changes in CTC status did not correlate with tumor response to chemotherapy. However, at the observation median of 18 months, the presence of CTC (p=0.017), negative receptor status, and large tumor size were independent negative prognostic factors for survival without distant metastases [6]. The absence of correlation between changes in the CTC status and response to neoadjuvant chemotherapy can be explained by different biological characteristics of the primary tumor and CTC and consequently different sensitivity to therapy. For instance, different status of HER-2 in the primary tumor and CTC were reported: CTC can express *HER-2*, whereas the primary tumor can be HER-2-negative. Anti-HER-2 therapy can be effective in these patients.

Thus, CTC can be a potential marker of neoadjuvant therapy efficiency and disease course prognosis. CTC phenotype can differ from the primary tumor phenotype. In patients with triple negative BC, CTC expressing *HER-2* can be found in the blood. Further studies are required for elucidation of the role of CTC occurrence in the blood of patients with triple negative BC and the phenotype of these cells during preoperative chemotherapy.

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