Analysis of Treg cell population in patients with breast cancer with respect to progesterone receptor status

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Introduction

Breast cancer is the most frequently diagnosed type of cancer in women worldwide. The prognosis for breast cancer is determined by age, lymph node involvement, tumour grade, nuclear expression of estrogen receptor (ER), progesterone receptor (PR), and membrane expression of human epidermal growth factor receptor 2 (HER2) [1, 2]. The development and progression of the cancer is related to tumour evasion of the immune system through a process called cancer immune-editing consisting of three phases: elimination, equilibrium, and evasion. During the elimination phase, also called immunosurveillance, innate and adaptive immune responses cooperate to destroy the growing tumour cells before they become clinically apparent. In the equilibrium phase rare tumour cell variants that have escaped elimination outgrow. The cancer cells that have acquired resistance to the elimination enter the escape phase. During this phase cancer cells continue to grow and expand in an uncontrolled manner, enabled by several mechanisms including development of a suppressive cancer microenvironment. One of those mechanisms is recruitment of T regulatory cells exerting a regulatory effect on the immune system. At this phase the tumour becomes clinically apparent. Among the tumour-infiltrating lymphocytes (TILs) in breast cancer patients are regulatory T cells (Treg), which are identified by the nuclear factor forhead box P3 (FOXP3) [3]. FOXP3+ TILs are correlated with a high risk of negative clinicopathological factors, such as ER negativity and high tumour grade [4, 5]. Treg cell infiltration of breast cancer has been considered as an independent negative prognostic factor [6, 7]. However, in a study conducted by West et al. with a cohort of 175 women with estrogen-receptor-negative breast cancers, FOXP3+ TILs were demonstrated as an independent positive prognostic factor in ER-negative breast cancer [8]. Tylor et al. demonstrated that the recruitment of Tregs to the cancer microenvironment inhibits an effective antitumour immune response, and in patients with claudin-low breast cancer, these tumours were found to be highly enriched with Tregs.
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[9]. On the other hand, FOXP3+ TILs were also identified as an independent factor for improved survival and progression-free survival in triple-negative breast cancer [10]. In the present study, we aimed to evaluate the percentages of Treg cell populations in the peripheral blood of patients with breast cancer with respect to progesterone-receptor status.

Material and methods

The study included 27 patients who were treated surgically for breast cancer in 2017 in the Clinical Department of Breast Cancer and Reconstructive Surgery of the Lukaszczyk Oncological Centre, Bydgoszcz, Poland.

Patients were treated in line with the accepted management standard; in all cases, this involved combination treatment. All 27 patients underwent surgical treatment with radiotherapy of the breast. In every case, breast-conserving treatment (BCT) with sentinel lymph node biopsy (SLNB) was applied [11, 12]. Each patient underwent radical surgery. According to current recommendations, this involved removal of the tumour within the limits of healthy tissues (no ink on the tumour) [11, 12], and this was confirmed by the histopathological examination of the state of surgical margins. The SLNB procedure identified the patients without the presence of metastatic lesions in the axillary (cN0 group). The pre-operative assessment of the clinical condition required for this purpose included a physical examination of the patients supplemented by an ultrasound examination of the axilla. The isotope method was used to identify the sentinel lymph node. The surgical procedure was preceded by lymphoscintigraphy using 99mTc radionuclide with 75–100 MBq activity on the albumin carrier (Nanocol). The isotopic marker was administered intradermally at the margin of the nipple envelope (in the breast quadrant where the primary change was located) approximately 2–3 hours before the surgery. For intraoperative identification of places of increased accumulation of radiotracers in the axillary cavity, and to measure the radiation value of the lymph nodes, a handheld gamma ray detector was used. The lymph node with the highest level of radiation was considered the sitter node sought during the surgical procedure. According to the “10% rule” established by Martin et al. [13], lymph nodes displaying elevated radiotracer collection greater than 10% of the radiation value obtained for the sentinel node (nodes of the heart) were also removed.

The patient’s consent was obtained in each case. Additionally, approval for the research program was granted by the Ethical Committee of the Nicolaus Copernicus University Ludwig Rydygier Collegium Medicum in Bydgoszcz (KBET/364/B/2015).

All the patients in our study had an invasive ductal breast cancer. From these patients two groups were selected: 19 patients with invasive breast cancer luminal type A: ER (+) PR (+) HER (-), Ki 67 until 15% and eight patients with non-luminal (HER-positive) invasive breast cancer ER (-) PR (-) HER (+), Ki 67 in each case, according to Saint Gallen Consensus 2017. No statistically significant differences in tumour stage, lymph node status, tumour grade, and HER status were observed between the two groups of patients. The characteristics of the patient groups are presented in Table 1. From each of the patient peripheral blood samples were collected one day before the surgical procedure.

Flow cytometry

The samples for the cytometric evaluation of the Treg cell population in the whole blood of breast cancer patients (luminal A type) were prepared using Becton Dickinson reagents, according to the manufacturer’s instructions. At first, the following antibodies for the detection of surface antigens were added to 100 μl peripheral blood collected on EDTA: 5 μl CD3 APC-Cy7, 20 μl CD4 FITC, 20 μl CD25

Table 1. Characteristics of the patient group

| Characteristic               | Number of patients (%) |
|------------------------------|------------------------|
| Age 57 years (range: 31–64)  |                        |
| Tumour stage according to TNM 2010 |                      |
| T1b                          | 9 (33)                 |
| T1c                          | 17 (62)                |
| T2                           | 1 (3)                  |
| Lymph node status according to TNM 2010 |                    |
| Without lymph node involvement N0 | 22 (81)               |
| With lymph node involvement N1 | 5 (19)                 |
| Tumour differentiation grade |                        |
| Grade 1                      | 1 (3)                  |
| Grade 2                      | 25 (92)                |
| Grade 3                      | 1 (3)                  |
| Histopathological assessment |                        |
| Carcinoma ductale            | 27 (100)               |
| After operation margin       |                        |
| > 1 cm                       | 27 (100)               |
| < 1 cm                       | 0 (0)                  |
| Hormonal status              |                        |
| ER positive                  | 19 (70)                |
| ER negative                  | 8 (30)                 |
| PR positive                  | 19 (70)                |
| PR negative                  | 8 (30)                 |
| HER status                   |                        |
| HER positive                 | 8 (30)                 |
| HER negative                 | 19 (70)                |
| Ki-67 expression             |                        |
| < 10%                        | 0 (0)                  |
| 10–15%                       | 19 (70)                |
| > 20%                        | 8 (30)                 |
| Type of treatment            |                        |
| BCT operation                | 27 (100)               |
| Radiotherapy                 | 27 (100)               |
| Brachytherapy                | 26 (96)                |
| Chemotherapy                 | 1 (4)                  |
PR (+) and PR (–) breast cancer tumours within CD3+/CD4+ T cells in patients with progesterone receptor

Fig. 1. The percentage of CD25+/FOXP3+/CD127 (–/low) T cells in patients with progesterone receptor (PR) (+) and PR (–) breast cancer tumours.

The authors declare no conflict of interest.
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