Summary

Introduction: Breast cancer is one of the most common cancer in women, whose incidence rate is significantly increasing worldwide. Mortality has been reduced over the past few years, thanks to constantly improving diagnostic methods, full-scale mammography screening as well as comprehensive treatment. The success rate of treatment is known to depend on the earliness of diagnosis of breast cancer. Therefore, the scientists endeavour to find and optimize laboratory diagnosis of tumors using serum or tissue tumor markers. However, thus far, there is no biomarker suitable for screening or diagnosis of the early stage of breast cancer.

Aim of the study: Our main goal was to study classic tumor markers and many other molecules associated with the process of carcinogenesis, namely markers of angiogenesis and lymphangiogenesis, growth factors, multifunctional proteins and proteases to assess their benefit of evaluation of tumor aggressiveness, extent of surgery, choice of the subsequent therapy and recurrence detection. A partial objective was to compare preoperative levels of these biomarkers (CEA, CA 15-3, CYFRA 21-1, TPA, TK, MonoTotal, VEGF, EGF, IGF-1, IGF-BP3, Osteopontin, Osteoprotegerin, Matrix Metalloproteinases MMP-2 and MMP-9) between a group of patients with malignant and benign breast disease. In patients with malignant disease we evaluated the levels of biomarkers depending on the clinical stage as well as the condition of the lymph nodes and their benefit of determination of the extent of surgery. The next aim was to propose an optimal algorithm of biomarker screening for recurrence detection and the final objective was to evaluate overall survival (OS) and progression free survival (PFS) in breast cancer patients.

Methods: It was a prospective, non-randomized study involving 206 women with breast cancer treated primarily with surgery and 43 women with benign breast tumors (control group) from June 2012 to June 2015 followed by monitoring these patients in the course of four years and five months.

Besides, breast cancer patients (N=206) were divided in two ways, according to clinical stages into Group A (Stage I), Group B (Stage IIA) and Group C (Stage IIB and III) from the prognostic point of view and in accordance with lymph node status and type of axillary performance into four groups. The first group included sentinel node-negative patients, the second group involved women with a positive sentinel node without axillary dissection. In the third group, there were patients with a positive sentinel node and completed axillary dissection, and the fourth group consisted of patients with primary axillary dissection. One blood sample was taken in all patients in the morning before surgery. Afterwards, levels of chosen biomarkers were determined from these samples (see above). Results were statistically processed and statistically significant differences were evaluated between particular groups. Moreover, prognostically useful biomarkers were determined in relation to progression free survival (PFS) and overall survival (OS).

Results: A statistically significant differences between preoperative levels in the malignant and benign groups were detected in biomarkers CEA, CA 15-3 and CYFRA 21-1 (**CEA 1.3 vs. 0.8ng/mL, p < 0,0001, CA 15-3 12.0 vs. 9.0 kIU/L, p = 0,0037, CYFRA 21-1 1.3 vs. 1.0 µg/L, p = 0.0289**). According to the clinical stage, the assessment of biomarker levels in the malignant group showed the only statistically significant difference in **CEA (1.1 vs. 1.6 ng/mL, p = 0.0299**) between Group A and Group B and in **TPA (28 vs. 46.5 IU/L, p = 0.0081)**, **CYFRA 21-1(1.4 vs. 1.72 µg/L , p = 0.0004) and MonoTotal (51.25 vs. 63.35 IU/L, p = 0.0232)** between Group B and Group C. Based on the clinical stage, there was no statistically significant difference between groups related to the tumor marker CA 15-3. Although the evaluation of biomarker levels in four groups depending on lymph node status detected statistically significant higher levels of **IGF-1(195 ng/ml, p = 0.0337 vs. 157 ng/ml)** and **VEGF (166 ng/ml, p = 0.0438 vs. 69.4 ng/ml)** in sentinel node positive patients versus negative sentinel node patients, there was no difference between levels of IGF-1 and VEGF in patients with negative sentinel lymph node and positive sentinel lymph node related to less aggressive tumors where axillary dissection was not finished. A statistically significant difference of MMP-2 levels was found in a smaller number of patients with positive sentinel lymph node (they were lower). Further, the results showed that the combination of CA 15-3 and CYFRA tumor markers is the best option to detect recurrence or progression, while CEA determination is worthless.

Besides, it was proved that statistically significantly shorter overall survival (OS) and progression free survival (PFS) could be found in Group C (Stage IIB and Stage III) having a poorer prognosis, in comparison with Group A (Stage I) and Group B (Stage IIA). The same results were also seen in Group 4 (patients with primary axillary dissection, but without statistical significance. Furthermore, the study showed that patients who had completed axillary dissection in case of metastatic sentinel lymph node from preoperative biopsy (Group 3) had only 1% better time to progression (PFS) and 8% better overall survival (OS) than Group 2 without axillary dissection. This result is not statistically significant.

Our results also showed statistically significant differences in overall survival (OS) in the malignant group, depending on levels of TPA, TK and CYFRA 21-1. The differences in survival depending on levels of other tumor markers and growth factors (CA 15-3, CEA, MonoTotal, OPN, OPG, IGF-BP3, IGF-1, EGF and VEGF) were not found to be statistically significant.

It was also proved that pre-operative levels of all tumor markers above the cut-off (CA15-3, CEA, TPA, TK and MonoTotal), above the median (CYFRA 21-1, Osteopontin, Osteoprotegerin, MMP - 2 and MMP - 9) and below the median (IGF-BP3, IGF-1, EGF and VEGF) where the Hazard ratio is ≥1 means shorter PFS and OS.

Conclusion: Our study showed that screening of particular biomarkers or their combination might be justified in clinical practice, especially in early diagnosis of recurrence (disease recurrence is predicted several months earlier than it is by using imaging methods) or breast cancer progression as well as in case of advanced stages to intensify dispensary care or to change oncological therapy. Nevertheless, these statements have to be verified in a larger clinical trial. The combination of multiple biomarkers increases a sensitivity of recurrence detection. The combination of CA 15-3 and CYFRA 21-1 appeared to be auspicious in our study. Deciding to perform axillary surgery, it is worth considering using the determination of IGF-1 and VEGF biomarkers in patients with suspicious axillary lymph nodes for their lymphangiogenesis ability followed by tumor dissemination by lymphatic pathways. In conformity with literary data, the

statistical assessment of our group also showed that staging was the most important prognostic factor in terms of cancer progression and overall survival, which we managed to confirm.

Key words: breast cancer - prognostic factors – biomarkers -– progression free survival (PFS) - overall survival (OS)