Streszczenie w języku angielskim

OBJECTIVE: The aim of the study was to assess levels of heparanase, selected angiogenesis markers (VEGF-A, sVEGFR1, sVEGFR2), number of circulating endothelial progenitor cells with the immunophenotype CD45-/CD34+/CD31+/CD133+, hormones released by the adipose tissue (adiponectin, leptin), inflammatory marker- YKL-40 protein, and the activity of tissue factor (TF) in a group of patients with primary, unilateral breast cancer with no distant metastases. Additionally, correlations between these parameters and selected anthropometric and clinical-pathological features, including: TNM classification, assessment of histological grade, breast cancer molecular subtype, tumour diameter and expression of the proliferation marker Ki67 were determined. The influence of surgical procedures and standard adjuvant therapies on change in heparanase levels was also assessed. A final objective of the study was an attempt to determine a prognostic value of the analysed parameters for predicting neoplastic disease relapse.

MATERIAL AND METHODS: In publication no. 1, 86 women aged 40 to 71 (median age 55) with newly diagnosed unilateral breast cancer stage IA-IIB (with no distant metastases) were enrolled in the study. In the study group, the most common molecular type of cancer was luminal type A (58% patients). As part of the study, levels of heparanase, vascular endothelial growth factor (VEGF-A), soluble forms of receptor 1 and receptor 2 for VEGF (sVEGFR1 and sVEGFR2), and the number of circulating endothelial progenitor cells with the immunophenotype CD45-/CD34+/CD31+/CD133+ were determined. **In publication no. 2**, 81 women with diagnosed, unilateral, invasive breast cancer grade IA-IIB, median age 54 (Q1/Q3 – 49/59 years) were included in the study. With regard to histological type, the most common breast cancer was invasive duct cancer (IDC) (69 patients). In the study group, the median tumour diameter was 1.67 cm (Q1/Q3 – 1.2/2.1 cm). 62 patients showed no nodal involvement. As part of the study, the levels of adiponectin, leptin and YKL-40 protein, and the activity of tissue factor (TF) were determined. **In publication 3**, 80 patients with confirmed, operable, primary and unilateral breast cancer with no distant metastases were enrolled in the study: 53 postmenopausal patients (66%), and 27 premenopausal patients (34%). Median follow-up was 55 months (Q1/Q3 – 49/59 months). The most commonly observed molecular type of breast cancer was luminal type A (59% of the study group). Moreover, the following incidence of selected clinical-pathological signs was observed in the

study group: tumour size < 2 cm in 53 patients (66%), and no nodal involvement in 61 patients (76%). The majority of the study group, 65 patients (81%) received breast-conserving treatment. And as part of adjuvant therapy, chemotherapy was used in 38 subjects (47.5%), and hormonal treatment in 68 patients (85%). In the whole study group, the heparanase level was determined twice: a day before the surgical procedure, and about 8 months post surgery (Q1/Q3 – 6.2/10.7).

RESULTS: As a result of statistical analyses, **in the first publication,** depending on the status of oestrogen (ER) and progesterone receptors (PR), a significantly higher heparanase level was observed among patients with a negative status of a ER and PR receptors, as compared to patients with a positive status of these receptor. A subgroup of patients with T2 tumours showed significantly higher heparanase level than the subgroup with T1 tumours. Depending on low, medium and high level of heparanase, an increase in the level of circulating EPCs and level of sVEGFR2 was observed, together with an increase in the heparanase level. Additionally, an increase in the heparanase level was associated with a decrease in the anti-angiogenic potential expressed by the sVEGFR1/VEGF-A ratio, and increased levels of the sVEGFR2/sVEGFR1 ratio were observed. The analysis of ROC curves showed that the heparanase and sVEGFR1 levels may be useful in the assessment of the neoplastic disease relapse. And the analysis of the ROC curve created for a model accounting for levels of heparanase, circulating EPCs, VEGF-A, sVEGFR1 and sVEGFR2 shows a strong diagnostic potential of this model in predicting neoplastic disease relapse. Cox regression analysis showed that the most significant predictors of neoplastic disease relapse in the analysed models are VEGF-A and sVEGFR2 levels. The Kaplan-Meier survival curves revealed a much higher rate of disease relapse in patients with high heparanase level of > 200 pg/ml vs subgroups with low and medium heparanase levels. **The second publication** showed lower adiponectin level in patients with expression of cell proliferation marker Ki67 ≥ to 20% as compared to the group with expression less than 20%. Additionally, higher adiponectin levels were observed in the case of luminal subtype A versus triple-negative subtype. The activity of tissue factor also differed depending on the molecular subtype, and was the highest in the subgroup with HER2(+) luminal B breast cancer. The Kaplan-Meier survival curves demonstrated a higher rate of disease relapse in patients with high YKL-40 level of > 2.50 ng/ml and in patients with high TF activity > 24 pM vs subgroups with low and medium levels of these parameters. A shorter relapse-free survival was noticed in the group of patients with

BMI < 25 kg/m^2 compared to the group of patients with BMI of 25-30 kg/m^2. Furthermore, in patients with high leptin levels > 12.58 ng/ml a significantly higher incidence of disease relapse was revealed. A similar relationship was also found among patients with low adiponectin levels < 27.05 ng/ml. **In the third publication,** almost four times reduction in the heparanase level was observed in the whole study group, resulting from the use of adjuvant treatment. It was shown that heparanase level before and after treatment differed, depending on the type of the hormonal therapy applied. The results of survival analysis obtained in study 3 confirmed the results achieved in study 1. A higher rate of disease relapse was observed in the group of patients with high pre-treatment heparanase level (> 181 pg/ml). In a univariate analysis, patients with overweight and patients with T2 tumours had a lower chance of low pre-treatment heparanase level, while patients with a preoperative positive ER and PR status had a higher chance of low baseline heparanase level.

CONCLUSIONS: In summary, the studies confirm a relationship between high heparanase level with increased vasculogenesis and angiogenesis. A high level of heparanase can lead to high mobilization of circulating EPCs including by increasing the release of VEGF. Additionally, a reduction of the heparanase level regardless of the therapy model applied was demonstrated. It was also observed that high pre-treatment heparanase level, high pre-treatment YKL-40 level and high pre-treatment TF activity are independent negative prognostic factors, and are associated with shorter survival rate. Also high pre-treatment YKL-40 level and serum leptin level combined with normal BMI, as well as normal BMI combined with low adiponectin level was associated with poor prognosis. Moreover, it was observed that patients with breast cancer who are overweight showed a better prognosis, regardless of YKL-40, leptin and adiponectin levels.