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The evaluation of the inflammatory status and systemic antioxidant-oxidant balance of women with breast cancer during adjuvant chemotherapy

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ABSTRACT

Background: Chemotherapy may cause systemic inflammation. Therefore, reliable markers monitoring inflammation during cancer treatment are intensively investigated. In our study, we analyzed the concentration of high-sensitivity C-reactive protein (hs-CRP) and selected oxidative stress markers, such as malondialdehyde (MDA), glutathione peroxidase activity (GPx), and total antioxidant capacity (TAC), in breast cancer women before and during adjuvant chemotherapy.

Materials and methods: The study included 90 women with breast cancer stratified according to clinicopathological and anthropometric features. Blood samples were taken before and after two cycles of adjuvant chemotherapy.

Results: During adjuvant chemotherapy, a significant increase in hs-CRP concentration was noticed in the entire group of patients with breast cancer. After division into appropriate groups, a twofold increase in hs-CRP concentration was particularly observed in patients not expressing steroid hormone receptors and those without metastases in regional lymph nodes. A significant rise in hs-CRP was observed in patients with smaller tumor sizes (2 cm ≤) and with a lower stage of disease [I–IIA according to the tumor–node–metastasis (TNM) classification]. Adjuvant chemotherapy resulted in a significant decrease in GPx activity, especially in patients diagnosed with larger (> 2 cm) and more advanced tumors (IIB–IIIC according to the TNM classification), without metastasis in regional lymph nodes, and without HER-2 expression. A significant decrease in glutathione peroxidase (GPx) activity during adjuvant chemotherapy was also observed in patients with abnormal body mass index (BMI) and body fat content. TAC and MDA values remained unchanged in the entire group of patients and individual subgroups during adjuvant chemotherapy.

Conclusion: Our study showed that adjuvant chemotherapy causes systemic inflammation, manifested by increased hs-CRP and altered markers of oxidative stress in the blood of breast cancer patients. The severity of inflammatory processes during adjuvant chemotherapy may depend on specific characteristics of breast cancer and body composition.

Key words: breast cancer; adjuvant chemotherapy; inflammation; high sensitivity C-reactive protein; oxidative stress; total antioxidant capacity; glutathione peroxidase; malondialdehyde

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Introduction

Breast cancer is the most common malignant tumor worldwide and one of the leading causes of cancer-related death in women [1]. A growing number of studies indicate the critical role of chronic inflammation in the development and progression of various cancers, including breast cancer [2, 3]. Inflammation can be caused by bacterial and viral infections and lifestyle factors, especially smoking habits, stress, and obesity. Chronic inflammation is a response to pro-inflammatory cytokines secreted by a tumor [4]. The massive damage to cancer cells and healthy tissues accompanying anti-cancer treatment causes leakage of intracellular molecules known as damage-associated molecular patterns (DAMPs) that strongly activate the immune response [5]. An activated immune system can support eliminating cancer cells; conversely, various cytokines, reactive oxygen species (ROS), and growth factors secreted by immune cells create a tumor microenvironment favorable to progression and the formation of metastases [5]. Some studies indicate that systemic inflammation induced by cancer treatment is an unfavorable prognosticator that negatively affects clinical outcomes [6–8].

High-sensitivity C-reactive protein (hs-CRP) is the most popular parameter for monitoring inflammation [9]. Due to the simplicity and low assessment cost, hs-CRP is routinely used in clinical practice. It is also possible to estimate the intensity of ROS production accompanying inflammation using a wide range of oxidative stress markers, including antioxidant enzymes, low- and high-molecular-weight antioxidants, and oxidatively damaged biomolecules. Glutathione peroxidase (GPx) is one of the most important agents of the antioxidant defense system in the human body [10]. GPx catalyzes the reduction of hydroperoxides, including hydrogen peroxides, and protects the cell from oxidative damage. Antioxidant proteins and small-weight molecules contribute to the total antioxidant capacity (TAC), which reflects the systemic ability to counteract ROS [11]. An uncontrolled increase in ROS, often caused by the depletion of the body's antioxidant reserves, intensifies oxidative processes, leading to oxidative damage to essential biomolecules. Oxidative processes catalyzed by ROS within lipids lead to their peroxidation, damaging cell membranes. Malondialdehyde (MDA), one of the final products of lipid peroxidation in the cells, is commonly known as a marker of oxidative stress in cancer patients [12, 13].

So far, markers of inflammation and oxidative stress have been assessed primarily as risk factors for the development of breast cancer [14, 15]. In other studies, pro-inflammatory factors have been evaluated in pre-treatment breast cancer patients to determine the impact of tumor stage, grade, and subtype on their levels [16, 17]. There have been few longitudinal studies following patients from pre- to mid- or post-treatment, with inconsistent results depending on the panel of inflammatory markers used, treatment type, and patient population characteristics [18, 19]. The limited number of studies and the lack of clear conclusions prove that the issue of the pro-inflammatory properties of cancer therapies requires further thorough analysis. Patients with breast cancer who underwent tumor resection are usually qualified for adjuvant chemotherapy, which is an integral element of systemic treatment. Despite the substantial evidence for the effectiveness of adjuvant chemotherapy in treating breast cancer, little is known about its effect on the immune system and antioxidant-oxidant balance of breast cancer patients. Bower et al. showed statistically significant increases from pre- to posttreatment in five of the six inflammatory markers assessed: tumor necrosis factor alpha (TNF-α), soluble tumour necrosis factor receptor type II (sTNF-RII), interleukin 6 (IL-6), interleukin 8 (IL-8), and interferone gamma (IFN-γ) in women diagnosed with early-stage breast cancer, those who received adjuvant chemotherapy. In contrast to the other markers, no increases in CRP were observed after chemotherapy [20]. However, the authors draw attention to the influence of age, race, number of patients, and individual characteristics on the results obtained. It is known that breast cancer is characterized by high phenotypic heterogeneity and a multitude of molecular types. The influence of the tumor's clinicopathological features on the inflammatory response's strength during treatment is also unknown. A growing number of reports also indicate that obesity and excessive fat content may be a factor promoting inflammation not only at diagnosis but also during anti-cancer therapy. The crosstalk be-

tween adipocytes, macrophages, and proinflammatory cytokines may promote immunometabolic dysregulation and immunosuppressive phenotypes in breast tumors, which may be correlated to treatment resistance [21].

Due to many doubts related to the effect of adjuvant chemotherapy on systemic inflammation and a small number of reports on this subject our study aimed to analyze the effect of six-week adjuvant chemotherapy on hs-CRP concentration and selected markers of oxidative stress (GPx, TAC, MDA) in women with breast cancer. We also examined the impact of clinicopathological and anthropometric characteristics of patients on systemic inflammatory status during chemotherapy.

Materials and methods

Patients

The study included 90 women (aged 31–76 years) from the Greater Poland Cancer Center in Poznan with primary breast cancer who underwent tumor resection and were qualified for adjuvant chemotherapy in the AC regimen (doxorubicin + cyclophosphamide) from December 2015 to November 2017. Exclusion criteria from the study included comorbidities such as diabetes, cardiovascular diseases, autoimmune diseases, kidney and liver diseases. Patients with distant metastases, infections within six weeks preceding the study, and those using stimulants, such as regular smoking or drinking alcohol, were also excluded from the analysis. Patients did not take vitamin or herbal supplementation before and during adjuvant chemotherapy. The preoperative diagnosis based on a core biopsy was confirmed by examination of tissue material collected during tumor resection. The levels of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER-2) were all determined by immunohistochemistry performed pre-and postoperatively. The size of the tumor was estimated by mammography and confirmed during tumor resection. The status of axillary lymph nodes was found to be positive by histopathologic examination. The clinical tumor–node–metastasis (cTNM) cancer staging was performed preoperatively with reference to the 7th edition of the American Joint Committee on Cancer (AJCC) and confirmed by a pathological histopathology report (pTNM). According to clinicopathological features, patients were divided into appropriate subgroups. Patient classification included biological tumor type (luminal A, luminal B, triple negative breast cancer and HER2-enriched), tumor size (≤ 2 cm or > 2 cm), presence of metastases in regional lymph nodes (present or absent), expression of steroid and HER2 receptors, (positive or negative), overall TNM classification (I–IIA or IIB–IIIC). The type of previous surgery was also taken into account, dividing patients into those who had undergone radical mastectomy (n = 49), those who had undergone breast-conserving surgery (n = 40) and without data (n = 1). The study was conducted in accordance with the principles of the Declaration of Helsinki. The protocol used in this study was approved by the Local Bioethics Committee of the Medical University of Poznan (approval no. 245/15 and 1016/16). All patients gave written consent to participate in the study.

Biochemical analysis

Biochemical tests were performed twice: before and during the administration of adjuvant chemotherapy (before administering the third cycle of chemotherapy). Blood samples (10 mL) were drawn in the early morning from the arms of breast cancer patients following overnight fasting. Two neutral vacuum tubes of blood were collected from each patient into ethylenediaminetetraacetic acid (EDTA) anticoagulant. After 30 minutes, EDTA test tubes were centrifuged at 3.000 rpm for 15 minutes, and the obtained plasma was stored at -80°C until all assays were performed. Plasma hs-CRP concentrations were determined using the immunoenzymatic method (DRG Instruments GmbH, Germany), according to the instructions provided by the manufacturers. The MDA concentration was determined using the thiobarbituric acid reactive substance (TBARS) reaction. MDA was quantified calorimetrically following its controlled reaction with thiobarbituric acid (Cayman Chemical Company, USA). GPx activity was assayed using the Cayman's Glutathione Peroxidase Assay Kit (Cayman Chemical Company, USA). The oxidized glutathione (GSSG) formation reaction catalyzed by GPx is coupled with the glutathione disulfide reductase (GR) reaction, which regenerates GSH at the expense of nicotinamide adenine dinucleotide phosphate (NADPH) oxidation. NADPH oxidation decreases absorbance at 340 nm, measured spec-

trophotometrically (Cayman Chemical Company, USA). Plasma TAC was determined using a colorimetric kit based on the inhibition of the oxidation of ABTS® (2,2-Azino-di-[3-ethylbenzthiazoline sulphonate]), which is subsequently quantified as mmol Trolox equivalent (Cayman Chemical Company, USA).

Anthropometric analysis

Anthropometric analysis was performed twice: before and during the administration of adjuvant chemotherapy (before the administration of the third cycle of chemotherapy) using a certified, 8-electrode Tanita BC-418 MA body composition analyzer. Patients were divided into subgroups according to BMI (≤ 25 kg/m^2 or > 25 kg/m^2) and body fat percentage (≤ 33% or > 33%).

Statistical analysis

Statistical analysis was performed using the GraphPad Prism 6.0 (GraphPad Software, San Diego, CA) and PQStat Software (2023). The normality of quantitative variables was assessed using the Kolmogorov-Smirnov or Shapiro-Wilk test. All tested parameters were not normally distributed, therefore they were presented as median and interquartile range. Comparisons between the relevant categories of patients were made using the unpaired Mann-Whitney U test. Comparison of the analyzed parameters before and after chemotherapy was performed using the Wilcoxon test. $p \leq 0.05$ was considered statistically significant.

Results

Patients baseline characteristics

The mean age of patients included in the study was 56 ± 10 years. Based on immunohistochemical studies, hormone-dependent and HER-2-positive tumors accounted for 78% and 30% of cases, respectively. In 48% of women, the tumor size did not exceed 2 cm. 53% of patients had metastases in regional lymph nodes. The stage of the disease (based on the TNM classification) was at level I–IIA in 58% of patients, IIB–IIIC — in 38% of patients, and 4% was without data. The study was dominated by patients after radical mastectomy — (54%), 44% underwent breast-conserving surgery, and for one patient we have no information about the surgical treatment performed. Taking into account the molecular subtypes of breast cancer, 70% of the study group were patients with luminal B, 11% with HER2-enriched, 9% with luminal A and 9% with triple negative breast cancer. Most women qualified for the study were overweight; the average BMI was 27.4 ± 5.7 kg/m^2 and increased during treatment to 27.7 ± 5.7 kg/m^2 ($p < 0.0001$). The average body fat content in the study group was 34.22 ± 8.1% and remained stable throughout the six weeks of adjuvant chemotherapy.

The effect of six-week chemotherapy on hs-CRP concentration, and selected markers of oxidative stress in the entire group of breast cancer patients

Changes in the concentration of hs-CRP, and selected oxidative stress markers in the entire study group are presented in Figure 1. hs-CRP concentration increased significantly from 3.06 (0.04–56.95) mg/L to 4.52 (0.10–44.25) mg/L during six weeks of adjuvant chemotherapy ($p = 0.0425$). GPx activity decreased significantly from 101.5 (10/09–398.10) nmol/min/mL to 91.89 (12.23–454.20) nmol/min/mL during six weeks of treatment ($p = 0.0063$). Values of the other oxidative stress markers: MDA (MDA before — 4.28 (2.00–8.33) μM, MDA after — 4.06 (1.76–9.56) μM; $p = 0.9603$) and TAC (TAC before — 2.82 (0.62–15.68) mM, TAC after — 2.90 (0.02–10.12) mM; $p = 0.6894$) remained unchanged during six weeks of adjuvant chemotherapy.

The effect of 6-week chemotherapy on hs-CRP concentration, and selected markers of oxidative stress depending on the clinicopathological and anthropometric profile of breast cancer women

Changes in the concentrations of hs-CRP, MDA, GPx activity, and TAC value during the 6-week chemotherapy, depending on the selected clinicopathological and anthropometric features of breast cancer patients, were presented in Table 1. A significant increase in hs-CRP was observed in patients with smaller tumor sizes (≤ 2 cm) and with a lower stage of disease (I–IIA according to the TNM classification) compared to those with larger tumors (> 2 cm) and a higher stage of cancer (IIB-IIIC according to the TNM classification). A statistically significant increase in hs-CRP also occurred in patients

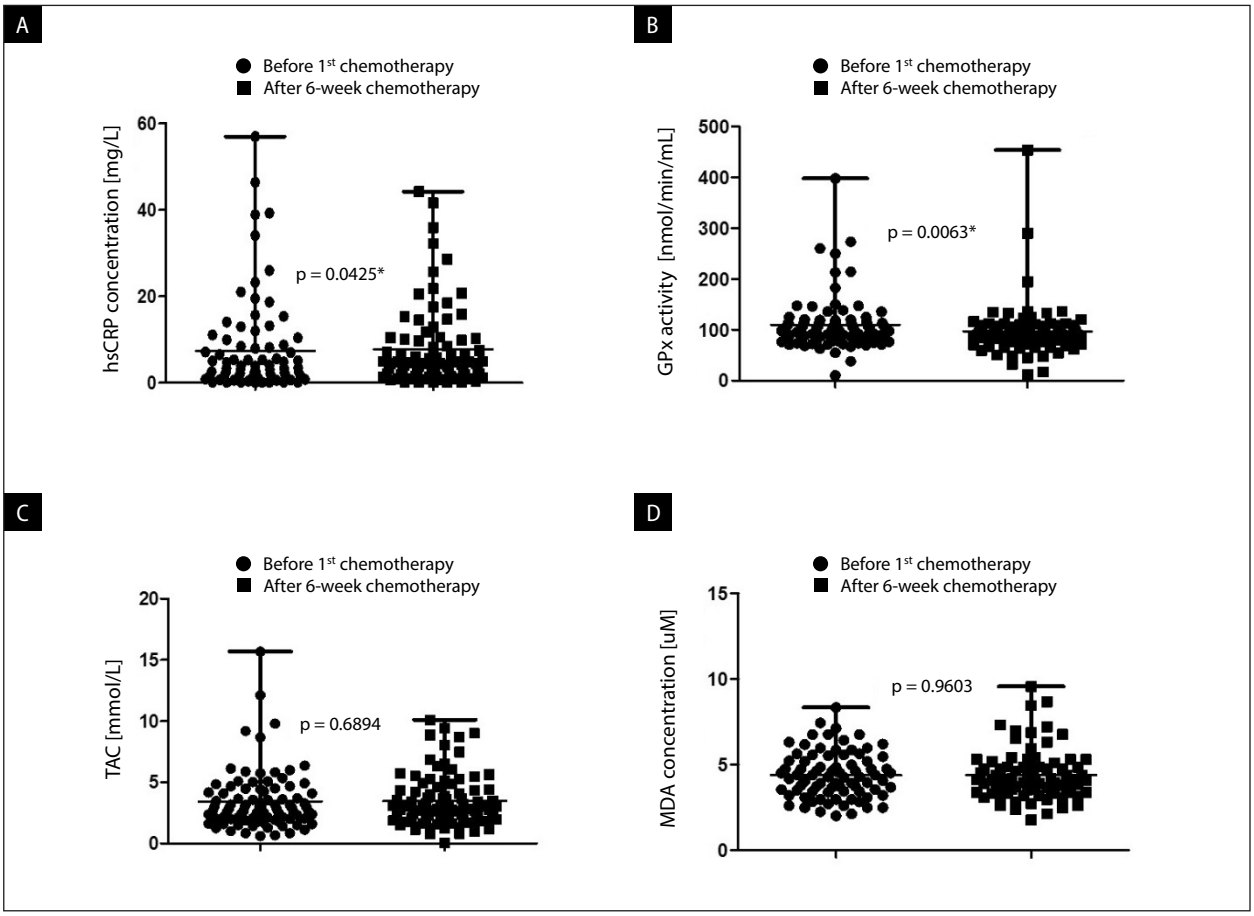

Figure 1. Changes in the concentration of high-sensitivity C-reactive protein (hs-CRP) and selected oxidative stress markers in the entire study group during adjuvant chemotherapy. Results presented as median and interquartile range and analyzed using Wilcoxon test. $p \leq 0.05$ was considered statistically significant. GPx — glutathione peroxidase activity; TAC — total antioxidant capacity; MDA — malondialdehyde

without lymph node metastasis compared to those with positive lymph nodes. Hormone-independent and HER2-enriched breast cancer were also associated with an increase in hs-CRP. There were no differences in hs-CRP concentration depending on BMI and body fat content, HER2 activity and type of surgery. Among oxidative stress markers, only changes in GPx activity were observed during six weeks of adjuvant chemotherapy. A significant decrease in GPx activity occurred in patients with larger (> 2 cm) and more advanced tumors (IIB–IIIC according to the TNM classification). Patients without tumoral HER-2 expression or with hormone-dependent carcinoma also presented reduced GPx activity during six weeks of adjuvant chemotherapy. A statistically significant decrease in GPx activity was observed in women without metastasis in the regional lymph nodes, similarly to the group of patients after radical mastectomy and with luminal B biological subtype of breast cancer. It was noticed that during six weeks of treatment, the reduction in GPx activity was also related to abnormal body composition, i.e., increased BMI and body fat content. After taking into account clinicopathological and anthropometric features, the values of other oxidative stress markers — MDA and TAC – remained unchanged during chemotherapy.

Discussion

Adjuvant chemotherapy benefits the patient by destroying cancer cells that have not been completely eliminated during surgery but, at the same time, negatively affects other healthy organs. Recent studies have provided evidence suggesting that the inflammatory response plays a pivotal role in breast cancer chemotherapy-induced comor-

Table 1. Changes in high-sensitivity C-reactive protein (hs-CRP) concentrations and glutathione peroxidase activity (GPx) activity in a group of women with breast cancer during adjuvant chemotherapy depending on clinicopathological and anthropometric criteria

Value		hsCRP [mg/L]		GPx [nmol/min/mL]	
		p	Value	p	Value
Tumor size					
< 2 cm	Before CT	2.38 (0.10–34.12)	0.0026	105.40 (38.20–214.20)	0.3813
	After CT	4.11 (0.10–41.62)		94.54 (31.99–454.20)	
> 2 cm	Before CT	4.91 (0.04–56.95)	0.5880	98.82 (10.09–398.10)	0.0061
	After CT	4.62 (0.10–44.25)		87.92 (12.23–194.90)	
HER2/neu expression					
Positive	Before CT	1.94 (0.04–18.69)	0.1117	101.40 (10.09–213.20)	0.7823
	After CT	3.72 (0.10–35.87)		92.91 (17.01–454.20)	
Negative	Before CT	3.31 (0.10–56.95)	0.1817	102.60 (38.20–398.10)	0.0005
	After CT	4.52 (0.10–44.25)		91.59 (12.23–136.60)	
Hormonal sensitivity					
Positive	Before CT	3.16 (0.04–56.95)	0.4659	102.10 (38.20–273.70)	0.0168
	After CT	3.16 (0.10–44.25)		90.52 (12.23–290.00)	
Negative	Before CT	2.60 (0.10–13.19)	0.0009	100.10 (10.09–398.10)	0.2250
	After CT	5.38 (0.19–20.73)		97.90 (17.01–454.20)	
TNM classification					
I–IIA	Before CT	2.60 (0.10–39.28)	0.0019	100.90 (38.20–398.10)	0.0669
	After CT	4.47 (0.10–41.62)		92.20 (31.99–454.20)	
IIB–IIIC	Before CT	6.06 (0.04–56.95)	0.8217	103.40 (10.09–273.70)	0.0386
	After CT	4.52 (0.10– 44.25)		89.14 (12.23–194.9)	
Lymph node metastases					
Present	Before CT	4.11 (0.04–56.95)	0.5609	106.80 (66.83–273.70)	0.1439
	After CT	4.52 (0.10–44.25)		95.05 (12.23-290.00)	
Absent	Before CT	2.43 (0.10–34.12)	0.001	97.85 (10.09–398.10)	0.0158
	After CT	4.43 (0.10–41.62)		89.19 (17.01–454.20)	
Type of surgery					
Breast conserving surgery	Before CT	3.40 (0.10–46.39)	0.2482	95.66 (38.20–398.10)	0.1205
	After CT	4.52 (0.10–44.25)		93.12 (31.99–136.0)	
Radical mastectomy	Before CT	2.48 (0.04–56.95)	0.1151	107.20 (10.09–273.7)	0.0252
	After CT	4.43 (0.10–35.87)		90.67 (12.23–454.20)	
Molecular subtype					
Luminal A	Before CT	7.00 (0.63–39.28)	0.3125	105.30 (74.68–398.10)	0.8885
	After CT	2.87 (0.97–8.27)		98.11 (87.72–136.60)	
Luminal B	Before CT	3.11 (0.04–56.95)	0.1050	102.60 (38.20–273.7)	0.0093
	After CT	4.52 (0.10–44.25)		90.36 (12.23–290.0)	
HER2-enriched	Before CT	1.21 (0.10–13.19)	0.0273	98.11 (10.09–138.6)	0.9568
	After CT	5.89 (0.19–20.73)		95.56 (17.01–454.2)	
Triple negative	Before CT	3.28 (2.14–7.01)	0.1484	103.2 (76.61–115.8)	0.1069
	After CT	4.52 (1.85–11.73)		90.92 (76.71–115.8)	

→

Table 1. Changes in high-sensitivity C-reactive protein (hs-CRP) concentrations and glutathione peroxidase activity (GPx) activity in a group of women with breast cancer during adjuvant chemotherapy depending on clinicopathological and anthropometric criteria

Value		hsCRP [mg/L]		GPx [nmol/min/mL]	
		p	Value	p	Value
Body mass index					
< 25 kg/m^2	Before CT	1.72 (0.10–56.95)	0.2757	110.60 (38.20–398.10)	0.1462
	After CT	2.09 (0.10–44.25)		94.49 (31.99–454.20)	
> 25 kg/m^2	Before CT	5.25 (0.04–46.39)	0.1781	95.25 (10.09–273.70)	0.0147
	After CT	5.87 (0.10–41.62)		89.65 (12.23–290.00)	
Fat content					
< 33%	Before CT	1.99 (0.04–56.95)	0.1118	104.80 (10.09–398.10)	0.1315
	After CT	2.70 (0.10–44.25)		17.01–194.90)	
> 33%	Before CT	4.91 (0.10-46.39)	0.1587	98.31 (62.55–273.7)	0.0327
	After CT	5.40 (0.19–41.62)		90.16 (12.23–454.20)	

All results shown as median and interquartile range. Wilcoxon test was used for comparison. TNM — tumor–node–metastasis; CT — chemotherapy

bidities such as peripheral neuropathy, gastrointestinal distress symptoms, and liver injury [22]. In addition, elevation in the serum inflammatory biomarkers during treatment in the adjuvant setting has been found to be significantly and independently associated with an increased risk of relapsing in some types of breast cancer [23, 24]. For this reason, the need to monitor the inflammatory response during chemotherapy and determine its optimal indicators is increasingly being postulated. In our study, we assessed the effect of adjuvant chemotherapy on the level of hs-CRP, the most popular marker of systemic inflammation. Since inflammation is inextricably linked to the overproduction of ROS, the fluctuations of selected oxidative stress markers were also examined. Undoubtedly, the clinicopathological features of the tumor, including its molecular subtype, clinical advancement, and degree of malignancy, as well as factors related to the patient's lifestyle, may significantly impact the severity of inflammatory processes both at the time of diagnosis and individual stages of treatment. Although tumor resection reduces the impact of tumor-derived agents on the host immune system, recovery of immune homeostasis takes time. Moreover, surgery does not remove all cancer cells; some circulate in the bloodstream or are scattered in distant anatomical areas. Hence, they can still disrupt the functioning of the immune system and promote systemic inflammation. Therefore, in addition to assessing the impact of adjuvant chemotherapy on inflammatory parameters, we also monitored how their changes were influenced by baseline tumor characteristics, patients' BMI, and fat tissue content.

We have shown that adjuvant chemotherapy is associated with increased systemic inflammation measured by hs-CPR levels. This result is in agreement with the findings of Hasan et al. who demonstrated that hs-CRP is higher among post-chemotherapy breast cancer patients as compared to its pre-chemotherapy value for both investigated AC (adriamycin, cyclophosphamide) and AC-T (adriamycin, cyclophosphamide, and taxane) chemotherapy regimens [25]. An almost two-fold increase in hs-CRP levels during adjuvant chemotherapy was demonstrated in patients with hormone-independent breast cancer in our study. In contrast, in those with hormone-dependent tumors, hs-CRP concentration was hardly stable. The estrogen receptor-positive is the most common histological subtype, representing 70% of new cases yearly [26]. Estrogen binds to the estrogen receptor (ER) in these tumors, and genomic ER signaling induces the expression of genes involved in cell proliferation and survival. ER also influences inflammatory pathways mediated by nuclear factor kappa B (NF-κB), which regulates various pro-inflammatory mediators [27]. NF-κB activation in breast cancer cells via loss of estrogen ER expression makes these cells able to secrete various cytokines and growth factors responsible for developing local and systemic inflammation [28]. This inverse relationship between the ER and the NF-κB may

explain the higher levels of hs-CRP observed in patients with hormone-negative breast cancer during adjuvant chemotherapy in our study. The severe impairment of the immune system caused by NF-kB activation in this type of tumor may hinder the rapid restoration of immune homeostasis even after tumor resection. The reactive immune system may respond more strongly to subsequent immunostimulants, such as adjuvant chemotherapy, which may increase hs-CRP in hormone-negative breast cancer.

Surprisingly, during adjuvant chemotherapy, hs-CRP concentration increased significantly in patients with smaller and less advanced tumors. Larger and more advanced tumors, which, according to previous reports, are accompanied by severe inflammation [29], may reduce systemic sensitivity to pro-inflammatory stimulants. For this reason, the levels of inflammatory markers, including hs-CRP, in patients with larger and more advanced tumors may fluctuate less during adjuvant chemotherapy. As a result, after six weeks of adjuvant chemotherapy, hs-CRP obtained a similar value regardless of its initial pre-chemotherapy value observed in patients differing in tumor size and stage.

Previous studies used CRP as an inflammatory marker in women with breast cancer, showing no significant changes in its concentration. Our results are opposite, which may be due to some critical factors. First of all, the hs-CRP can react stronger and faster to developing inflammation compared to the CRP protein, so changes in the concentration of the former can be detected earlier. Some authors examined the effect of radiotherapy on CRP levels in women with breast cancer, obtaining results inconsistent with ours [19]. This contradiction may indicate a different impact of the treatment regimen on the inflammatory status, already postulated by others [20]. Bower et al., who investigated the impact of different treatment regimens in breast cancer patients, including adjuvant chemotherapy, showed no changes in CRP [20]. However, as our research indicates, inflammation and its dynamics may be influenced by the initial characteristics of patients, which the authors did not consider in their study.

It is known that chemotherapy disturbs the intracellular redox balance in favor of pro-oxidant processes. Chemotherapeutic agents primarily induce intracellular mitochondrial ROS production, which promotes ROS-mediated cell injury in cancer [29]. This mechanism may enhance the anti-cancer effects of therapy. However, our research focused on the systemic redox balance, which is affected by various biological systems, including the immune system. Some studies have revealed that human peripheral polymorphonuclear leukocytes from patients receiving chemotherapy for hematological and solid malignancies produce more hydrogen peroxide and superoxide anion in vitro than healthy control subjects [30]. Inflammatory cells activated by chemotherapy can disturb the systemic redox balance, including the tumor microenvironment's oxidative-antioxidative balance, thus promoting chemoresistance [31]. This effect may harm the effectiveness of treatment, which is confirmed by clinical studies in which blood markers of oxidative stress are strongly correlated with a poorer prognosis in some cancers [32].

In our study, we observed stable MDA concentrations during adjuvant chemotherapy in the entire group of patients and individual subgroups differing in the initial clinicopathological and anthropometric features. Similar results were obtained by Hewala et al. who revealed a non-significant increase in serum MDA levels after six cycles of adjuvant chemotherapy compared with its baseline levels in breast cancer patients [33]. The lack of effect of chemotherapy on MDA concentration has also been demonstrated by Mohan et al. in lung cancer patients [34]. However, other findings contradict these reports and indicate that chemotherapy causes an increase in MDA in patients with various cancers, including breast cancer [35, 36]. These discepacies are difficult to explain and may be related to the chemotherapy regimen used, which varies depending on the study and may have a different impact on the MDA level. Some authors who, similarly to us, observed unchanged MDA concentrations during adjuvant chemotherapy postulate that this result may be influenced by the previous surgery, which is associated with such a high degree of oxidative damage that it reaches a plateau phase and does not progress during further treatment [37].

In this study, we demonstrated unchanged TAC during adjuvant therapy. A similar effect of chemotherapy on the TAC value was obtained by Mohan et al. in patients with lung cancer [34].

Although we found no changes in MDA concentrations, the increased oxidative stress during chemotherapy cannot be denied. We assume that the consumption of antioxidants in the neutralization of ROS mobilizes compensatory mechanisms that restore the proper antioxidant barrier so that the TAC value may remain constant. This stable antioxidant barrier reflected by constant TAC value may prevent the progression of oxidative damage, which may also explain the constant MDA level during adjuvant chemotherapy We also cannot exclude the influence of diet and antioxidant intake on the TAC value during adjuvant treatment. The impact of consumption and supplementation of various antioxidants and nutrients on their circulating levels has not yet been clearly established in cancer. Zabłocka-Sowińska et al. demonstrated that TAC in lung cancer is associated with levels of endogenous antioxidants and disease stage rather than lifestyle factors [38]. This conclusion was supported by Terrence et al. who did not prove any correlation between intake of dietary antioxidants and TAC value in patients with prostate cancer [39]. Statistically insignificant correlations between TAC and dietary habits observed in cancer patients are consistent with several other studies performed with normal subjects [40]. These findings may indicate that diet-provided antioxidants may have a limited effect on the persistently stable TAC levels observed during chemotherapy in our study but cannot be completely excluded. However, there is no doubt that diet significantly impacts inflammation, which is confirmed by numerous research. It appears that a more appropriate approach is to focus on the immunomodulating properties of dietary patterns rather than individual nutrients. Therefore, the number of studies that have explored various dietary patterns in relation to inflammatory biomarkers has constantly grown [41].

Regular physical activity is another factor influencing the immune system. Wärnberg et al. described various studies that had observed decreased systemic inflammation after an intervention of physical activity in humans [42]. Further reviews by You et al. [43] and Nicklas et al. [44] suggest that exercise training reduces chronic inflammation independently of weight loss. One of the mechanisms proposed by You et al. [43] is a reduction in adipose tissue hypoxia that occurs due to increased angiogenesis and an increase in blood flow as a result of exercise training. This evidence suggests that accurate dietary intake and physical activity assessment are essential for high-quality research on the immune system. However, consistent and precise estimation of both factors remains one of the most critical challenges. Several subjective and objective measures of dietary intake and physical activity assessment exist, each with its own limitations and biases [45]. Due to the lack of uniform and validated assessment methods, we decided not to consider these factors in our work to avoid problems with comparison with the results of other authors and false conclusions.

Six weeks of adjuvant chemotherapy reduced GPx activity in women with breast cancer, which is consistent with previous reports by Kadam et al. [46]. Junior et al. demonstrated that GPx activity remains decreased in all cycles when compared with healthy women and after the second and fourth chemotherapy cycles compared to baseline [47]. In our study, this decrease was particularly significant in patients with Luminal B breast cancer, larger and more advanced tumors, with the presence of hormonal receptors, and without regional lymph node metastases. In addition, excess weight and inappropriate body fat content were associated with lower GPx activity during adjuvant treatment. The oxidative state in obesity is well documented in the literature, and evidence suggests that it is closely linked to pro-inflammatory cytokines secreted by adipose tissue, which can trigger oxidative stress in a vicious cycle [48]. Therefore, in obese patients, chemotherapy may cause a greater decrease in GPx activity due to the initially weakened antioxidant barrier caused by long-term oxidative stress.

GPx, like other antioxidant enzymes, is the first line of defense against ROS generated in the body, explaining its faster noticeable changes compared to other oxidative stress indicators [49]. In our study, a more pronounced decrease in GPx activity during chemotherapy was observed in patients who had previously undergone mastectomy. Meanwhile, in those who had previously undergone breast-conserving surgery, its activity was stable during chemotherapy. It is likely that mastectomy, as a more invasive procedure, may lead to increased production of ROS, which results in the depletion of antioxidant resources, primarily antioxidant enzymes such as GPx. Further intensive involvement of GPx in the neutralization of ROS during adjuvant che-

motherapy may completely deplete its reserves, which may be reflected by a decrease in its activity in the serum of patients. The situation in which ROS are first neutralized by GPx has a protective effect on other high- and low-molecular-weight antioxidants, which are therefore depleted more slowly. Moreover, the decreasing activity of GPx is a signal promoting the expression of genes encoding other antioxidants, which increases their concentration in the body [50]. These mechanisms may explain the constant TAC value despite the reduced GPx activity observed in our study. The rapid action of GPx, which eliminates excess ROS and maintains the proper level of other antioxidants, may be another, apart from the previously presented, explanation for the stable MDA concentration during adjuvant treatment.

In summary, some features of the tumor and the abnormal body composition may promote systemic inflammation not only at the time of diagnosis, as previous studies have shown, but also during anti-cancer treatment, as we observed in the present work. Some significant trends emerge from our research. In patients with less advanced breast cancer, i.e., smaller tumor size and stage, adjuvant chemotherapy caused stronger fluctuations in hs-CRP, ultimately equalizing its value after six weeks with that observed in those with larger and more advanced tumors. Furthermore, hormone-independent breast cancer appears to be associated with increased inflammation during adjuvant treatment. The adjuvant treatment causes a more visible disturbance of the oxidative-antioxidant balance measured by GPx activity in patients with larger and more advanced tumors. Our research has shown that the intensification of oxidative processes during chemotherapy is also influenced by overweight and abnormal fat content.

Our study has several limitations. We could not determine the level of inflammatory markers after completion of adjuvant chemotherapy. We also did not know the baseline values of the analyzed parameters at the diagnosis. Only considering these missing checkpoints would provide complete insight into the kinetics of inflammatory processes at individual stages of anti-cancer treatment. However, monitoring inflammation from diagnosis through all stages of treatment is time-consuming and requires significant financial resources. Moreover, interpretation difficulties may arise due to the fact that treatment regimens are modified and selected individually and sometimes shortened due to the patient's deteriorating health condition. Therefore, obtaining a homogeneous group of patients undergoing the same therapeutic scheme becomes complicated. Thus, in our study, like some other authors [33, 34, 46], we focused only on one of the stages of treatment, i.e., adjuvant chemotherapy, which allowed us to eliminate these issues and obtain a relatively consistent group of patients subjected to the same chemotherapy regimen. Another limitation of our study is assessing the impact of only the first two cycles of adjuvant chemotherapy on inflammatory status. However, it should be considered that this is a pilot study that aimed to identify potential candidates for markers of chemotherapy-induced inflammation. The usefulness of these molecules, particularly hs-CRP and GPx, which showed such potential in our work, should be confirmed in larger studies monitoring their fluctuations during and after adjuvant chemotherapy completion. The patient population we classified was also relatively small and heterogeneous, which could influence our results. Although our study aimed to assess the impact of different breast cancer characteristics and anthropometric factors on inflammation, the number of patients presenting each feature was limited. Despite these limitations, our study provides the background for further research on inflammation during cancer treatment to select its best markers. Knowledge about them could help monitor and manage inflammation, which would reduce the development of inflammation-related complications during the therapeutic process in cancer patients.

Conclusions

Our study showed that adjuvant chemotherapy causes systemic inflammation, manifested by increased hs-CRP and altered markers of oxidative stress in the blood of breast cancer patients. The severity of inflammation during adjuvant chemotherapy may depend on specific characteristics of the tumor and the patient's body composition.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Local Bioethical Committee of

Poznan University of Medical Sciences (approval no. 245/15 (March 5, 2015) and 1016/16 (October 5, 2016).

Conflicts of interests

The authors have no relevant financial or non-financial interests to disclose.

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References

1. Łukasiewicz S, Czeczelewski M, Forma A, et al. Breast Cancer-Epidemiology, Risk Factors, Classification, Prognostic Markers, and Current Treatment Strategies-An Updated Review. Cancers (Basel). 2021; 13(17), doi: 10.3390/cancers13174287, indexed in Pubmed: 34503097.
2. Zhao H, Wu L, Yan G, et al. Inflammation and tumor progression: signaling pathways and targeted intervention. Signal Transduct Target Ther. 2021; 6(1): 263, doi: 10.1038/s41392-021-00658-5, indexed in Pubmed: 34248142.
3. Danforth DN. The Role of Chronic Inflammation in the Development of Breast Cancer. Cancers (Basel). 2021; 13(15), doi: 10.3390/cancers13153918, indexed in Pubmed: 34359821.
4. Morris RM, Mortimer TO, O'Neill KL. Cytokines: Can Cancer Get the Message? Cancers (Basel). 2022; 14(9), doi: 10.3390/cancers14092178, indexed in Pubmed: 35565306.
5. Hernandez C, Huebener P, Schwabe RF. Damage-associated molecular patterns in cancer: a double-edged sword. Oncogene. 2016; 35(46): 5931–5941, doi: 10.1038/onc.2016.104, indexed in Pubmed: 27086930.
6. Jiang C, Xiu Y, Yu X, et al. Prognostic value of a modified systemic inflammation score in breast cancer patients who underwent neoadjuvant chemotherapy. BMC Cancer. 2022; 22(1): 1249, doi: 10.1186/s12885-022-10291-2, indexed in Pubmed: 36460981.
7. Schauer T, Henriksson A, Strandberg E, et al. Pre-treatment levels of inflammatory markers and chemotherapy completion rates in patients with early-stage breast cancer. Int J Clin Oncol. 2023; 28(1): 89–98, doi: 10.1007/s10147-022-02255-0, indexed in Pubmed: 36269530.
8. Kaźmierska J, Bajon T, Winiecki T, et al. Significance of neutrophil to lymphocyte ratio as a predictor of outcome in head and neck cancer treated with definitive chemoradiation. Rep Pract Oncol Radiother. 2023; 28(3): 389–398, doi: 10.5603/RPOR.a2023.0042, indexed in Pubmed: 37795402.
9. Asegaonkar SB, Asegaonkar BN, Takalkar UV, et al. C-Reactive Protein and Breast Cancer: New Insights from Old Molecule. Int J Breast Cancer. 2015; 2015: 145647, doi: 10.1155/2015/145647, indexed in Pubmed: 26693355.
10. Lubos E, Loscalzo J, Handy DE. Glutathione peroxidase-1 in health and disease: from molecular mechanisms to therapeutic opportunities. Antioxid Redox Signal. 2011; 15(7): 1957–1997, doi: 10.1089/ars.2010.3586, indexed in Pubmed: 21087145.
11. Silvestrini A, Meucci E, Ricerca BM, et al. Total Antioxidant Capacity: Biochemical Aspects and Clinical Significance. Int J Mol Sci. 2023; 24(13), doi: 10.3390/ijms241310978, indexed in Pubmed: 37446156.
12. Barrera G. Oxidative stress and lipid peroxidation products in cancer progression and therapy. ISRN Oncol. 2012; 2012: 137289, doi: 10.5402/2012/137289, indexed in Pubmed: 23119185.
13. Erejuwa OO, Sulaiman SA, Ab Wahab MS. Evidence in support of potential applications of lipid peroxidation products in cancer treatment. Oxid Med Cell Longev. 2013; 2013: 931251, doi: 10.1155/2013/931251, indexed in Pubmed: 24369491.
14. Agnoli C, Grioni S, Pala V, et al. Biomarkers of inflammation and breast cancer risk: a case-control study nested in the EPIC-Varese cohort. Sci Rep. 2017; 7(1): 12708, doi: 10.1038/s41598-017-12703-x, indexed in Pubmed: 28983080.
15. Tobias DK, Akinkuolie AO, Chandler PD, et al. Markers of Inflammation and Incident Breast Cancer Risk in the Women's Health Study. Am J Epidemiol. 2018; 187(4): 705–716, doi: 10.1093/aje/kwx250, indexed in Pubmed: 28641369.
16. Jadoon SK, Soomro R, Ahsan MN, et al. Association of neutrophil-to-lymphocyte ratio with clinical, pathological, radiological, laboratory features and disease outcomes of invasive breast cancer patients: A retrospective observational cohort study. Medicine (Baltimore). 2023; 102(20): e33811, doi: 10.1097/MD.0000000000033811, indexed in Pubmed: 37335707.
17. Kaur RP, Banipal RP, Vashistha R, et al. Association of elevated levels of C-reactive protein with breast cancer, breast cancer subtypes, and poor outcome. Curr Probl Cancer. 2019; 43(2): 123–129, doi: 10.1016/j.currproblcancer.2018.05.003, indexed in Pubmed: 29921457.
18. Shi W, Misra S, Li M, et al. Inflammatory Biomarkers, Hematopoietic Stem Cells, and Symptoms in Breast Cancer Patients Undergoing Adjuvant Radiation Therapy. JNCI Cancer Spectr. 2020; 4(5): pkaa037, doi: 10.1093/jncics/pkaa037, indexed in Pubmed: 33134822.
19. Hu JJ, Urbanic JJ, Case LD, et al. Association Between Inflammatory Biomarker C-Reactive Protein and Radiotherapy-Induced Early Adverse Skin Reactions in a Multiracial/Ethnic Breast Cancer Population. J Clin Oncol. 2018; 36(24): 2473–2482, doi: 10.1200/JCO.2017.77.1790, indexed in Pubmed: 29989859.
20. Bower JE, Ganz PA, Irwin MR, et al. Acute and Chronic Effects of Adjuvant Therapy on Inflammatory Markers in Breast Cancer Patients. JNCI Cancer Spectr. 2022; 6(4), doi: 10.1093/jncics/pkac052, indexed in Pubmed: 35900175.
21. Devericks EN, Carson MS, McCullough LE, et al. The obesity-breast cancer link: a multidisciplinary perspective. Cancer Metastasis Rev. 2022; 41(3): 607–625, doi: 10.1007/s10555-022-10043-5, indexed in Pubmed: 35752704.
22. Brown T, Sykes D, Allen AR. Implications of Breast Cancer Chemotherapy-Induced Inflammation on the Gut, Liver, and Central Nervous System. Biomedicines. 2021; 9(2), doi: 10.3390/biomedicines9020189, indexed in Pubmed: 33668580.
23. McAndrew NP, Bottalico L, Mesaros C, et al. Effects of systemic inflammation on relapse in early breast cancer.

NPJ Breast Cancer. 2021; 7(1): 7, doi: 10.1038/s41523-020-00212-6, indexed in Pubmed: 33483516.
24. Sparano JA, O'Neill A, Graham N, et al. Inflammatory cytokines and distant recurrence in HER2-negative early breast cancer. NPJ Breast Cancer. 2022; 8(1): 16, doi: 10.1038/s41523-021-00376-9, indexed in Pubmed: 35136076.
25. Hasan D, Ismail Y, Al Tibi A, et al. Serum Biomarkers for Chemotherapy Cardiotoxicity Risk Detection of Breast Cancer Patients. Asian Pac J Cancer Prev. 2021; 22(10): 3355–3363, doi: 10.31557/APJCP.2021.22.10.3355, indexed in Pubmed: 34711013.
26. Gennari A, André F, Barrios CH, et al. ESMO Guidelines Committee. ESMO Clinical Practice Guideline for the diagnosis, staging and treatment of patients with metastatic breast cancer. Ann Oncol. 2021; 32(12): 1475–1495, doi: 10.1016/j.annonc.2021.09.019, indexed in Pubmed: 34678411.
27. Van Laere SJ, Van der Auwera I, Van den Eynden GG, et al. NF-kappaB activation in inflammatory breast cancer is associated with oestrogen receptor downregulation, secondary to EGFR and/or ErbB2 overexpression and MAPK hyperactivation. Br J Cancer. 2007; 97(5): 659–669, doi: 10.1038/sj.bjc.6603906, indexed in Pubmed: 17700572.
28. Xia Y, Shen S, Verma IM. NF-κB, an active player in human cancers. Cancer Immunol Res. 2014; 2(9): 823–830, doi: 10.1158/2326-6066.CIR-14-0112, indexed in Pubmed: 25187272.
29. Yang H, Villani RM, Wang H, et al. The role of cellular reactive oxygen species in cancer chemotherapy. J Exp Clin Cancer Res. 2018; 37(1): 266, doi: 10.1186/s13046-018-0909-x, indexed in Pubmed: 30382874.
30. Sangeetha P, Das UN, Koratkar R, et al. Increase in free radical generation and lipid peroxidation following chemotherapy in patients with cancer. Free Radic Biol Med. 1990; 8(1): 15–19, doi: 10.1016/0891-5849(90)90139-a, indexed in Pubmed: 2157633.
31. Seebacher NA, Krchniakova M, Stacy AE, et al. Tumour Microenvironment Stress Promotes the Development of Drug Resistance. Antioxidants (Basel). 2021; 10(11), doi: 10.3390/antiox10111801, indexed in Pubmed: 34829672.
32. Boakye D, Jansen L, Schöttker B, et al. Blood markers of oxidative stress are strongly associated with poorer prognosis in colorectal cancer patients. Int J Cancer. 2020; 147(9): 2373–2386, doi: 10.1002/ijc.33018, indexed in Pubmed: 32319674.
33. Elsoud MA, Hewala T. The clinical significance of serum oxidative stress biomarkers in breast cancer females. Med Res J. 2019; 4(1): 1–7, doi: 10.5603/mrj.a2018.0039.
34. Mohan A, Poulose R, Gupta T, et al. Impact of chemotherapy on symptom profile, oxidant-antioxidant balance and nutritional status in non-small cell Lung Cancer. Lung India. 2017; 34(4): 336–340, doi: 10.4103/0970-2113.209230, indexed in Pubmed: 28671164.
35. Sri Phani TP, Mannangatti M, Nekkala R, et al. Oxidative stress in breast cancer after chemotherapy. Bioinformation. 2022; 18(12): 1141–1145, doi: 10.6026/973206300181141, indexed in Pubmed: 37701507.
36. Amin KA, Mohamed BM, El-Wakil MA, et al. Impact of breast cancer and combination chemotherapy on oxidative stress, hepatic and cardiac markers. J Breast Cancer. 2012; 15(3): 306–312, doi: 10.4048/jbc.2012.15.3.306, indexed in Pubmed: 23091543.
37. Vera-Ramirez L, Sanchez-Rovira P, Ramirez-Tortosa MC, et al. Oxidative stress status in metastatic breast cancer patients receiving palliative chemotherapy and its impact on survival rates. Free Radic Res. 2012; 46(1): 2–10, doi: 10.3109/10715762.2011.635658, indexed in Pubmed: 22035543.
38. Zabłocka-Słowińska K, Porębska I, Gołecki M, et al. Total antioxidant status in lung cancer is associated with levels of endogenous antioxidants and disease stage rather than lifestyle factors - preliminary study. Contemp Oncol (Pozn). 2016; 20(4): 302–307, doi: 10.5114/wo.2016.61850, indexed in Pubmed: 27688727.
39. Vance TM, Azabdaftari G, Pop EA, et al. Intake of dietary antioxidants is inversely associated with biomarkers of oxidative stress among men with prostate cancer. Br J Nutr. 2016; 115(1): 68–74, doi: 10.1017/S0007114515004249, indexed in Pubmed: 26521663.
40. Record IR, Dreosti IE, McInerney JK. Changes in plasma antioxidant status following consumption of diets high or low in fruit and vegetables or following dietary supplementation with an antioxidant mixture. Br J Nutr. 2001; 85(4): 459–464, doi: 10.1079/bjn2000292, indexed in Pubmed: 11348560.
41. Barbaresko J, Koch M, Schulze MB, et al. Dietary pattern analysis and biomarkers of low-grade inflammation: a systematic literature review. Nutr Rev. 2013; 71(8): 511–527, doi: 10.1111/nure.12035, indexed in Pubmed: 23865797.
42. Wärnberg J, Cunningham K, Romeo J, et al. Physical activity, exercise and low-grade systemic inflammation. Proc Nutr Soc. 2010; 69(3): 400–406, doi: 10.1017/S0029665110001928, indexed in Pubmed: 20598198.
43. You T, Arsenis NC, Disanzo BL, et al. Effects of exercise training on chronic inflammation in obesity : current evidence and potential mechanisms. Sports Med. 2013; 43(4): 243–256, doi: 10.1007/s40279-013-0023-3, indexed in Pubmed: 23494259.
44. Nicklas BJ, You T, Pahor M. Behavioural treatments for chronic systemic inflammation: effects of dietary weight loss and exercise training. CMAJ. 2005; 172(9): 1199–1209, doi: 10.1503/cmaj.1040769, indexed in Pubmed: 15851714.
45. McClung HL, Ptomey LT, Shook RP, et al. Dietary Intake and Physical Activity Assessment: Current Tools, Techniques, and Technologies for Use in Adult Populations. Am J Prev Med. 2018; 55(4): e93–e9e104, doi: 10.1016/j.amepre.2018.06.011, indexed in Pubmed: 30241622.
46. Charushila K. Evaluation of Serum Antioxidants during Adjuvant Chemotherapy of Breast Cancer- A Prospective Observational Study. Biochem Analyt Biochem. 2015; 04(02), doi: 10.4172/2161-1009.1000171.
47. Gomes Júnior AL, Paz MF, da Silva LI, et al. Serum Oxidative Stress Markers and Genotoxic Profile Induced by Chemotherapy in Patients with Breast Cancer: A Pilot Study. Oxid Med Cell Longev. 2015; 2015: 212964, doi: 10.1155/2015/212964, indexed in Pubmed: 26576218.
48. Jovanović M, Kovačević S, Brkljačić J, et al. Oxidative Stress Linking Obesity and Cancer: Is Obesity a 'Radical Trigger' to Cancer? Int J Mol Sci. 2023; 24(9), doi: 10.3390/ijms24098452, indexed in Pubmed: 37176160.
49. Ighodaro OM, Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role

in the entire antioxidant defence grid. Alexandria J Med. 2019; 54(4): 287–293, doi: 10.1016/j.ajme.2017.09.001.
50. Melnikov F, Botta D, White CC, et al. Kinetics of Glutathione Depletion and Antioxidant Gene Expression as Indicators of Chemical Modes of Action Assessed in Vitro in Mouse Hepatocytes with Enhanced Glutathione Synthesis. Chem Res Toxicol. 2019; 32(3): 421–436, doi: 10.1021/acs.chemrestox.8b00259, indexed in Pubmed: 30547568.