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Systemic immune response and peripheral blood cell count in patients with a history of breast cancer

Abstract: The aim of the present study was to evaluate the association between changes in peripheral blood cell count and neopterin concentrations in patients with a history of breast cancer. Peripheral blood cell count, serum ferritin, serum neopterin and urinary neopterin concentrations were determined in 61 patients with a history of breast cancer and 74 control subjects. Hemoglobin, relative lymphocyte count and absolute lymphocyte count were lower, and relative neutrophil count, ferritin and serum neopterin concentrations were significantly higher in breast cancer patients than in controls. Compared to controls, the difference in hemoglobin concentration was statistically significant only in patients with active disease. Significant negative correlations were observed between urinary neopterin and hemoglobin as well as between serum neopterin and relative lymphocyte counts in breast cancer patients. Increased ferritin concentrations were associated with a history of hypertension, but higher absolute lymphocyte counts were associated with hypertension only in subjects without history of cancer. In conclusion, in patients with a history of breast cancer, anemia is associated with disease activity and systemic immune activation. Ferritin concentrations are increased in subjects with hypertension.

Keywords: breast cancer; hemoglobin; neopterin.

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Introduction

Breast cancer is the most common malignant disorder in women. Currently, the majority of patients affected with this tumor are cured, and survivorship issues, including long-term sequels of therapy and quality of life, are of increasing importance. A more effective therapy is considered to be one of the principal factors responsible for improved outcome of patients with breast cancer. However, administration of anticancer agents is associated with many side effects. The administration of cytotoxic agents plays an important role in the management of patients with breast cancer in the adjuvant, neoadjuvant or metastatic setting. Hematologic toxicity is the principal side effect of the administration of chemotherapy. Moreover, changes in hematologic parameters often accompany cancer even in the absence of therapeutic intervention. It has been demonstrated earlier that the anemia of cancer is associated with systemic inflammatory and immune responses [1, 2], and that inflammatory activity may also be involved in the pathogenesis of anemia associated with the administration of cytotoxic agents [3]. Changes

in laboratory parameters that are associated with tumor growth or anticancer therapy also involve leukocytes, in particular lymphocytes [4]. These alterations have a profound effect on the general condition of the patient and on the quality of life [5, 6].

The aim of the present study was to evaluate the association between changes in peripheral blood cell count and urinary or serum neopterin concentrations in patients with a history of breast cancer compared with a cohort of patients followed for benign breast disorders.

Patients and methods

Sixty-one female patients with a history of histologically verified invasive breast cancer were included in the present study. Twelve patients had active recurrent or metastatic disease, while 49 patients were breast cancer survivors without signs of disease activity. The control group consisted of 74 females followed for benign breast disorders, including benign cysts or fibroadenoma, or followed because of a family history of breast cancer. The association between laboratory parameters of risk of atherosclerosis and intima-media thickness in the present cohort has been analyzed in a separate study. The investigations were approved by the institutional ethics committee, and patients signed an informed consent.

Systolic and diastolic blood pressure was measured using a digital upper arm blood pressure monitor (Hartmann, Heidenheim, Germany) in sitting position after 5 to 10 min of rest. The body mass index (BMI) was calculated by the following formula: weight (kg)/[height (m)]^2. Blood samples were drawn from a peripheral vein after an overnight fast. Peripheral blood cell count was determined using the Coulter LH 750 (Beckman Coulter, Fullerton, CA, USA) and the Sysmex XE-2100 (Sysmex, Kobe, Japan) hematology analyzers according to the instructions of the manufacturers. Hemoglobin was measured by photometric method using sodium lauryl sulfate. Leukocyte counts, including differential counts, were measured with the flow cytometry method using a semiconductor laser. Erythrocytes were detected by an impedance method using hydrodynamic focusing.

The samples for biochemical analyses were transferred immediately to the laboratory and centrifuged (1600×*g* for 8 min at 16°C); the serum was separated and analyzed immediately or frozen at –20°C until analysis. Quantitative determination of ferritin was performed by chemiluminescent microparticle immunoassay using the Abbott Architect i2000SR analyzer (Abbott Laboratories, Chicago, IL, USA). Serum neopterin was measured by the Neopterin ELISA Kit (IBL International, Hamburg, Germany) using an automated microplate processor for enzyme immunoassays (EVOLIS, Bio-Rad Laboratories, Hercules, CA, USA).

Early morning urine samples were collected and stored at –20°C until analysis. Urinary neopterin was determined using a modification of the method described earlier [7]. Briefly, after the centrifugation (5 min, 1300×*g*) and dilution of 100 µL of urine specimens with 1.0 mL of the mobile phase containing disodium-EDTA (2 g/L), the samples were filtered using a microtiter (AcroPrep 96 Filter Plate 0.2 µm/350 µL; Pall Life Science, Ann Arbor, MI, USA) and a vacuum manifold (Pall Life Science) and then injected onto a column. Neopterin was determined using a high-performance liquid chromatography system (Prominence LC20, Shimadzu, Kyoto, Japan) consisting of a rackchanger/C-special autosampler for microtitration plates, a DGU-20A5 degasser, two liquid chromatograph pumps (LC-20 AB), an auto sampler (SIL-20 AC), a column oven thermostat (CTO-20AC), a fluorescence detector (RF-10 AXL), a diode array detector (SPD-M20A) and a communications bus module (CBM-20A). Phosphate buffer (15 mmol/L, pH 6.4), with a flow rate of 0.8 mL/min, was used as the mobile phase. Separation was performed using a hybrid analytical column (Gemini Twin 5 µm, C18, 150×3 mm; Phenomenex, Torrance, CA, USA) at 25°C; injection volume was 1 µL. Neopterin was identified by its native fluorescence (353 nm excitation and 438 nm emission wavelengths). Creatinine was monitored simultaneously in the same urine specimen with a diode array detector at 235 nm. Time of analysis for urine neopterin and creatinine was 6 min, and the analytes were quantified by external standard calibration. The results were expressed as neopterin/creatinine ratio (µmol/mol creatinine).

Differences between patients and control group or subgroups of subjects were analyzed by the Mann-Whitney U-test. Correlations were analyzed using Spearman's rank correlation coefficient. Statistical significance was set at the p=0.05 level. The analyses were performed using the NCSS software (Number Cruncher Statistical Systems, Kaysville, UT, USA).

Results

Hemoglobin concentration, relative lymphocyte counts and absolute lymphocyte counts were lower, and relative neutrophil counts, ferritin and serum neopterin concentrations were significantly higher in patients with a history of breast cancer than in controls (Table 1). The difference in hemoglobin concentration was statistically significant only in patients with active disease, and hemoglobin was also significantly lower in patients with active disease than in patients with no disease activity. Compared to controls, absolute lymphocyte counts were significantly lower and serum ferritin concentrations were significantly higher in both patients with active disease and patients with no signs of disease activity. The difference in relative neutrophil counts, relative lymphocyte counts and serum neopterin concentrations reached statistical significance only in patients with no signs of disease activity. No significant differences were observed in the other investigated parameters (Table 1). A total of 29 patients had a history of chemotherapy. Absolute lymphocyte counts were significantly lower in patients with a history of chemotherapy than in patients with no history of chemotherapy (1.42±0.54 vs. 1.66±0.44×10^9/L; p=0.026).

Different patterns of correlations between the hematologic parameters and neopterin concentrations were observed between patients and the control group. In controls, hemoglobin exhibited a positive correlation with serum neopterin concentrations, while a significant negative correlation was observed between urinary neopterin and hemoglobin concentrations in breast cancer patients

Table 1 Hematologic parameters and neopterin concentrations in the control group and patients with breast cancer.

Parameter	Controls		Patients		Active disease		No signs of disease activity		p-Value[a]	p-Value[b]	p-Value[c]	p-Value[d]
	n	Mean±SD (range)	n	Mean±SD (range)	n	Mean±SD (range)	n	Mean±SD (range)				
Hemoglobin, g/L	74	138±8 (122–153)	61	133±13 (102–164)	12	120±10 (102–134)	49	136±11 (111–164)	*0.010*	*0.000002*	0.287	*0.0001*
Leukocytes, 10^9/L	74	6.4±1.5 (3.4–12.1)	61	6.2±2.2 (2.8–13.8)	12	6.3±3.5 (2.8–13.8)	49	6.1±1.7 (2.9–12.7)	0.121	0.138	0.232	0.345
Neutrophils, %	74	58.6±8.3 (42.9–92.1)	61	61.7±10.4 (37.0–88.5)	12	63.4±12.6 (37.0–88.5)	49	61.2±10.0 (37.8–74.9)	*0.016*	0.085	*0.035*	0.670
Absolute neutrophil count, 10^9/L	74	3.75±1.44 (1.49–11.10)	61	3.91±1.84 (1.00–10.20)	12	4.24±2.89 (1.00–10.20)	49	3.83±1.51 (1.04–9.00)	0.863	0.663	0.702	0.670
Lymphocytes, %	74	30.3±7.5 (3.1–45.3)	61	26.7±8.8 (6.8–50.3)	12	25.2±10.6 (6.8–43.5)	49	27.1±8.4 (10.9–50.3)	*0.005*	0.071	*0.012*	0.592
Absolute lymphocyte count, 10^9/L	74	1.89±0.59 (0.20–3.30)	61	1.54±0.50 (0.70–2.90)	12	1.35±0.50 (0.70–2.60)	49	1.59±0.50 (0.70–2.90)	*0.00008*	*0.0008*	*0.001*	0.080
Monocytes, %	74	7.5±1.8 (0.4–10.9)	61	8.7±3.7 (3.8–28.4)	12	8.6±3.7 (4.4–16.3)	49	8.7±3.8 (3.8–28.4)	0.187	0.690	0.168	0.624
Absolute monocyte count, 10^9/L	74	0.50±0.16 (0.27–1.43)	61	0.54±0.32 (0.20–2.15)	12	0.54±0.42 (0.20–1.70)	49	0.54±0.29 (0.20–2.15)	0.764	0.132	0.783	0.163
Platelets, 10^9/L	74	260±54 (153–436)	61	248±58 (127–401)	12	260±61 (172–362)	49	245±58 (127–401)	0.208	0.906	0.155	0.531
Ferritin, µg/L	74	74±64 (6–390)	61	124±116 (5–743)	12	195±189 (51–743)	49	106±84 (5–393)	*0.0005*	*0.0007*	*0.008*	*0.025*
Serum neopterin, nmol/L	68	7.27±2.53 (1.90–12.81)	61	8.62±3.32 (2.69–27.39)	12	9.88±6.13 (2.69–27.39)	49	8.30±2.13 (4.62–15.81)	*0.017*	0.116	*0.031*	0.479
Urinary neopterin, µmol/mol creatinine	74	169±67 (77–440)	61	162±59 (43–460)	12	195±74 (96–360)	49	155±52 (43–279)	0.988	0.166	0.554	0.112

[a]Controls vs. patients with a history of breast cancer (patients). [b]Patients with active disease vs. controls. [c]Patients with no signs of disease activity vs. controls. [d]Patients with active disease vs. patients with no signs of disease activity

p-Values with statistically significant difference are italicized.

(Table 2; Figure 1). A significant negative correlation was observed between relative lymphocyte counts and serum neopterin concentrations in patients with a history of breast cancer.

Significant differences were observed between subjects with or without history of hypertension (Table 3). In control subjects, history of hypertension was associated with higher leukocyte counts, absolute lymphocyte counts and absolute monocyte counts. Significant differences between patients and controls in hemoglobin concentrations, leukocyte counts, relative neutrophil counts, relative and absolute lymphocyte counts, and absolute monocyte counts were observed only in subjects with hypertension. Ferritin concentrations were significantly higher in subjects with hypertension both in the control group and among breast cancer patients, and among subjects with hypertension ferritin was significantly higher in patients with a history of breast cancer. In addition, a significant correlation was observed between ferritin concentrations and systolic blood pressure in controls, and between ferritin concentrations and diastolic blood pressure in patients (Table 4).

Correlations between ferritin concentrations and other investigated parameters differed between patients with a history of breast cancer and the control group (Table 4). While a significant positive correlation was observed between ferritin concentrations and age, BMI, hemoglobin and leukocyte counts in the control group, these correlations were absent in patients with a history of cancer.

Discussion

As expected, hemoglobin concentrations and lymphocyte counts were markedly decreased in patients with a history of cancer. While decreased hemoglobin concentrations were evident only in patients with active disease, significantly lower lymphocyte counts were observed both in patients with active cancer and in breast cancer survivors without evidence of tumor activity.

These changes were associated with systemic immune activation, as reflected by the increased neopterin concentrations. Neopterin is a heterocyclic compound produced by activated macrophages, and neopterin concentrations reflect systemic immune and inflammatory responses across a spectrum of disorders, including, for example, cancer, autoimmune diseases, transplant rejection, acute myocardial infarction or trauma [7–10]. In patients with different primary cancers, increased serum or urinary neopterin concentrations have been previously shown to herald poor prognosis [11, 12]. Although neopterin concentrations are increased only in a minority of patients with breast cancer [13, 14], similarly to tumors of other primary location, increased neopterin concentrations predict poor prognosis [13, 15]. Increased neopterin levels have been also shown to be associated with higher risk of death in a general population, e.g., in elderly subjects [10]. In an earlier study of breast cancer survivors, increased serum neopterin concentrations and increased circulating T-cell counts were associated with persistent fatigue [6]. In the present study, both serum and urinary neopterin concentrations were studied. Serum neopterin concentrations reflect, among other factors, renal function, and neopterin concentrations are increased in patients with renal disease [9]. While urinary neopterin concentration expressed as neopterin/creatinine ratio corrects for renal function, this correction is not performed routinely for serum neopterin. Consequently, urinary and serum neopterin concentrations correlate only relatively weakly in

Table 2 Correlation between neopterin concentrations and hematologic parameters.

Parameter	Serum neopterin (nmol/L)		Urinary neopterin (μmol/mol creatinine)	
	Controls	Patients	Controls	Patients
Hemoglobin, g/L	*0.290 (0.016)*	−0.028 (0.830)	0.148 (0.209)	*−0.360 (0.004)*
Leukocytes, 10^9/L	0.130 (0.290)	0.152 (0.243)	0.169 (0.151)	0.001 (0.992)
Neutrophils, %	0.132 (0.283)	0.191 (0.140)	0.101 (0.391)	0.075 (0.565)
Absolute neutrophil count, 10^9/L	0.195 (0.110)	0.173 (0.183)	0.193 (0.100)	0.000 (1.000)
Lymphocytes, %	−0.172 (0.161)	*−0.257 (0.045)*	−0.067 (0.571)	−0.151 (0.246)
Absolute lymphocyte count, 10^9/L	−0.024 (0.847)	−0.045 (0.730)	0.111 (0.346)	−0.127 (0.330)
Monocytes, %	0.024 (0.849)	−0.026 (0.844)	−0.088 (0.458)	−0.086 (0.510)
Absolute monocyte count, 10^9/L	0.147 (0.233)	0.224 (0.082)	0.086 (0.464)	−0.087 (0.507)
Platelets, 10^9/L	−0.142 (0.248)	0.193 (0.135)	−0.127 (0.279)	0.151 (0.246)
Ferritin, μg/L	0.107 (0.385)	0.140 (0.283)	0.069 (0.557)	0.111 (0.393)

Shown are the values of Spearman's rank correlation coefficient (r_s) with the p-value in parentheses. Statistically significant correlations are italicized.

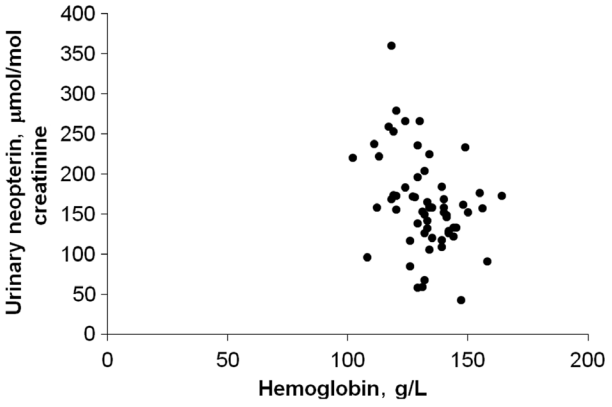

Figure 1 Correlation between hemoglobin and urinary neopterin concentrations in patients with a history of breast cancer (r_s=−0.360, p=0.004).

cohorts of patients with some renal dysfunction, and different correlations with other parameters may be observed for serum and urinary neopterin as was the case in the present study.

Anemia and lymphocytopenia are common in cancer patients even in the absence of interventions that result in myelosuppression, e.g., administration of cytotoxic agents. Moreover, hematologic toxicity is the most common side effect of systemic chemotherapy, followed by gastrointestinal toxicity [16, 17]. A negative association between hemoglobin and urinary neopterin concentrations in cancer patients has been well documented previously [3, 18]. In the present study, this association was not evident in the control group and, interestingly, serum neopterin concentrations even exhibited a positive correlation with hemoglobin levels in the control group. A negative correlation between lymphocyte counts and neopterin reached statistical significance only for the correlation between relative lymphocyte counts and serum neopterin in patients with breast cancer. A negative association between systemic immune activation, reflected in neopterin concentration, and the counts, phenotype or function of peripheral blood lymphocytes has been amply documented previously [4, 19, 20]. Significant decrease in absolute lymphocyte counts was also associated with a history of chemotherapy.

Marked differences in lymphocyte counts were observed in subjects with or without evidence of hypertension. In the control group, hypertension was associated with markedly increased lymphocyte counts, while in patients with cancer no significant differences were noted, with a trend of higher counts in patients without evidence of hypertension. A marked difference in absolute lymphocyte counts between patients and controls was observed only in subjects with a history of hypertension.

Table 3 Hematologic parameters in subjects with or without hypertension.

Parameter	Controls			Patients	p-Value[a]	p-Value[b]	p-Value[c]	p-Value[d]
	No hypertension (n=49)	Hypertension (n=25)		No hypertension (n=24)	Hypertension (n=37)			
Hemoglobin, g/L	137±9 (122–152)	138±8 (122–153)		136±14 (102–164)	131±12 (108–158)	0.913	0.165	0.507	*0.011*
Leukocytes, 10^9/L	6.1±1.5 (3.4–12.1)	7.0±1.4 (3.6–10.5)		6.7±2.3 (3.7–12.7)	5.8±2.1 (2.8–13.8)	*0.013*	0.161	0.428	*0.002*
Neutrophils, %	59.3±8.8 (42.9–92.1)	57.3±7.0 (45.4–74.6)		60.3±11.5 (37.8–88.5)	62.6±9.7 (37.0–74.9)	0.244	0.223	0.711	*0.007*
Absolute neutrophil count, 10^9/L	3.63±1.50 (1.49–11.10)	3.99±1.30 (1.80–7.43)		4.12±2.02 (1.04–9.10)	3.77±1.73 (1.00–10.20)	0.080	0.652	0.318	0.336
Lymphocytes, %	29.7±7.9 (3.1–45.3)	31.5±6.7 (17.0–45.0)		27.3±10.3 (6.8–50.3)	26.3±7.8 (12.7–43.5)	0.280	0.757	0.211	*0.007*
Absolute lymphocyte count, 10^9/L	1.74±0.56 (0.20–3.01)	2.19±0.52 (0.90–3.30)		1.70±0.65 (0.70–2.90)	1.44±0.35 (0.70–2.20)	*0.001*	0.165	0.589	*<0.000001*
Monocytes, %	7.4±2.0 (0.4–10.9)	7.8±1.2 (5.9–10.2)		9.6±5.2 (4.4–28.4)	8.1±2.3 (3.8–15.8)	0.360	0.388	0.120	0.977
Absolute monocyte count, 10^9/L	0.47±0.18 (0.27–1.43)	0.54±0.12 (0.30–0.78)		0.59±0.31 (0.30–1.70)	0.50±0.32 (0.20–2.15)	*0.005*	0.085	0.096	*0.017*
Platelets, 10^9/L	260±57 (153–436)	261±48 (164–346)		248±57 (127–342)	248±60 (145–401)	0.775	0.637	0.751	0.173
Ferritin, µg/L	63±50 (6–234)	97±81 (13–390)		95±89 (5–334)	142±128 (35–743)	*0.049*	*0.010*	0.105	*0.045*

[a]Controls without history of hypertension vs. controls with a history of hypertension. [b]Patients without history of hypertension vs. patients with a history of hypertension. [c]Controls without history of hypertension vs. patients without history of hypertension. [d]Controls with a history of hypertension vs. patients with a history of hypertension. p-Values with statistically significant difference are italicized.

Table 4 Correlations of ferritin with investigated parameters.

Parameter	Controls	Patients
Age, years	*0.411 (0.0003)*	0.089 (0.487)
BMI, kg/m^2	*0.337 (0.003)*	0.183 (0.159)
Systolic blood pressure, mm Hg	*0.269 (0.020)*	0.058 (0.657)
Diastolic blood pressure, mm Hg	−0.020 (0.864)	*0.286 (0.026)*
Hemoglobin, g/L	*0.368 (0.001)*	−0.119 (0.359)
Leukocytes, 10^9/L	*0.252 (0.030)*	−0.009 (0.947)
Neutrophils, %	0.104 (0.380)	0.137 (0.293)
Absolute neutrophil count, 10^9/L	0.222 (0.058)	0.044 (0.739)
Lymphocytes, %	−0.080 (0.495)	−0.126 (0.333)
Absolute lymphocyte count, 10^9/L	0.073 (0.535)	−0.148 (0.255)
Monocytes, %	−0.076 (0.518)	0.004 (0.973)
Absolute monocyte count, 10^9/L	0.108 (0.359)	0.066 (0.611)
Platelets, 10^9/L	−0.179 (0.128)	−0.084 (0.519)

Shown are the values of Spearman's rank correlation coefficient (r_s) with the p-value in parentheses. Statistically significant correlations are italicized.

The immune and inflammatory responses are thought to play an important role in the pathogenesis of hypertension [21, 22], which may be dependent on the presence of T lymphocytes [23]. In fact, higher absolute lymphocyte counts have been described in animal models of spontaneous hypertension [24]. Hypertension represents an important issue in breast cancer patients, specifically in relation to the introduction of agents targeting the vascular endothelial growth factor (VEGF) pathway into the therapy of this tumor [25]. Moreover, VEGF has potent suppressive activity on the immune system [26], and the administration of anti-VEGF agents may result in the augmentation of immune response. Thus, these observations are of interest with regard to development of drugs targeting the VEGF pathway in the therapy of breast cancer.

As discussed above, the negative correlation observed between urinary neopterin and hemoglobin concentrations in patients with a history of breast cancer is in agreement with earlier reports [3, 18]. Denz et al. [18] also reported a negative correlation between neopterin and iron concentration and a positive correlation between neopterin and ferritin levels [18]. In normal subjects, serum ferritin is an indicator of body iron stores and correlates with hemoglobin levels as reflected by the significant correlation between serum ferritin and hemoglobin concentrations in the control group in the present study. Ferritin expression is increased by pro-inflammatory cytokines [27, 28], and in conditions associated with systemic inflammatory response, including cancer, the presence of anemia is associated with high circulating ferritin concentrations. The term anemia of chronic disease has been coined to denote anemia associated with systemic immune and inflammatory responses [1, 2]. It is well documented that ferritin concentrations are increased in patients with cancer, including breast cancer [29, 30]. In patients with cancer, circulating ferritin is considered to be produced, similarly to neopterin, predominantly by macrophages [29, 31]. Ferritin is thought to promote cancer progression, and high serum ferritin concentrations are associated with poor prognosis [29]. In contrast to the report by Denz et al. [18], no correlation between urinary or serum neopterin and ferritin concentrations was observed in the present study. The absence of this correlation may be due to the fact that most patients had no active disease and other stimuli were responsible for increased ferritin concentrations in the patients with a history of breast cancer. Besides the well-known correlation of serum ferritin with age or BMI [29], a strong association with the presence of hypertension was observed. Ferritin may also be regarded as a biomarker of cardiovascular risk. Ferritin concentrations have been shown to be associated with hypertension [32] and to represent an independent risk factor for stroke [33].

In conclusion, in patients with breast cancer, anemia is associated with disease activity and systemic immune activation, while lymphocyte counts also inversely correlate with immune activation, but are decreased independently of disease activity. Increased ferritin concentrations were linked with a history of hypertension, but higher absolute lymphocyte counts were associated with hypertension only in subjects without history of cancer.

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