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Hematopoietic cytokines as tumor markers in breast malignancies. A multivariate analysis with ROC curve in breast cancer patients

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ABSTRACT

Purpose: Plasma levels of selected hematopoietic cytokines: interleukin 3 (IL-3), stem cell factor (SCF), granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF) and macrophage colony-stimulating factor (M-CSF), and the tumor marker carcinoma antigen 15-3 (CA 15-3) in breast cancer (BC) patients were investigated and compared to control groups: benign breast tumor patients and healthy subjects.
Material/Methods: Cytokine levels were determined by ELISA, CA 15-3 – using the CMIA method.
Results: A significant differences in the concentration of cytokines (with the exception of IL-3) and CA15-3 between the groups of BC patients, benign breast tumor patients and the healthy controls have been demonstrated. M-CSF has demonstrated higher or equal to CA 15-3 values of diagnostic sensitivity, specificity and the predictive values of positive and negative test results. The M-CSF area under the ROC curve (AUC) was the largest from all the cytokines tested and marginally lower than the AUC of CA 15-3.
Conclusion: These findings suggest the usefulness of M-CSF in diagnosing breast cancer, especially when discriminating between cancer and non-carcinoma lesions.

Key words: breast cancer; CA 15-3; HGFs; tumor markers

INTRODUCTION

Breast cancer (BC) is one of the most prevalent forms of cancer in women worldwide [1]. It is also one of the most aggressive malignant tumors associated with poor prognosis, rapid clinical progression, and a high rate of metastasis with the highest mortality [2]. Therefore, finding new substances, known as tumor markers, which are capable of detecting malignant cell transformations early is of vital importance to researchers [3,4].

Stem cell factor (SCF), interleukin 3 (IL-3), granulocyte-macrophage-colony stimulating factor (GM-CSF), granulocyte-colony stimulating factor (G-CSF), and macrophage-colony stimulating factor (M-CSF) are members of a group of cytokines called hematopoietic growth factors (HGFs). These cytokines regulate the growth and differentiation of hematopoietic progenitor cells and activate functions of mature neutrophils or macrophages [5]. The effect of HGFs is not only limited to bone marrow cells. It has been shown that HGFs can also stimulate the proliferation

of nonhematopoietic cells *in vitro*, for instance, cancer cells [6,7,8].

Breast cancer cells are capable of producing HGFs, for example SCF [9] and M-CSF [10,11,12], constitutively. Furthermore, receptors for HGFs have been detected in breast cancer cell lines and the stimulation of these receptors induces proliferation of tumor cells, which suggests a crucial role that cytokines may play in tumor invasion [13]. Moreover, some studies have shown M-CSF mRNA expression in breast cancer cells [14] and some recent studies have demonstrated higher levels of M-CSF or G-CSF in breast cancer patients [3,15].

The aim of this study was to determine plasma levels of hematopoietic cytokines (IL-3, SCF, GM-CSF, G-CSF, M-CSF) and CA 15-3 (carcinoma antigen 15-3–the comparative tumor marker) in breast cancer patients in relation to control groups–benign breast tumor patients and healthy subjects. Additionally, the diagnostic criteria: sensitivity (SE), specificity (SP), the predictive value of a positive (PV-PR) and negative (PV-NR) test result were defined in the study. Furthermore, the study defined: the receiver-operating characteristics (ROC) curve for all the parameters tested and the Spearman rank correlation coefficient between the cytokines and CA 15-3. This study is a continuation of our earlier investigations of HGFs, but the groups tested were enlarged twofold. The data obtained in the present study may be used in evaluating the usefulness of the HGFs tested in the diagnostics and differentiation of breast cancer patients.

MATERIAL AND METHODS

Patients

The study included 110 breast cancer female patients diagnosed by the Oncology Group. The patients were treated in the Department of Oncology, Medical University, Białystok, Poland. None of the patients had received chemo- or radiotherapy before blood sample collection.

Pretreatment staging procedures included physical and blood examinations, mammography, mammary ultrasound scanning, breast core biopsies and chest X-ray. In addition, radioisotopic bone scans, an examination of bone marrow aspirates, and brain and chest computed tomography (CT) scans were performed when necessary. Breast cancer histopathology was established in all cases by tissue biopsy of the mammary tumor or, post-surgery, from tumor cancer tissues.

Tab. 1 shows the groups tested. Tumor classification and staging were established in accordance with the International Union Against Cancer Tumor-Node-Metastasis (UICC-TNM) classification. Moreover, each breast cancer was classified histopathologically according to its morphology: 110 patients (100%) with ductal breast cancer (characteristics – *Tab. 1*). The control groups included 40 patients with a benign breast tumor and 40 healthy, untreated women. The patients underwent a mammary gland examination conducted by a gynecologist prior to blood collection. In addition, mammary ultrasound scans were performed when necessary. Benign breast tumor histopathology was established in all cases either by tissue biopsy of the mammary tumor or post-surgery.

The study was approved by the local Ethics Committee of the Medical University of Białystok. All patients gave their informed consent for the participation in the study.

Biochemical analyses

Venous blood samples were collected from each patient. Blood was collected into a heparin sodium tube, centrifuged 1000 rpm for 15 minutes to obtain plasma samples, and stored at -85^0 C until assayed. Hematopoietic cytokines were measured with the enzyme-linked immunosorbent assay (ELISA) (SCF, GM-CSF, G-CSF, M-CSF–Quantikine Human HGFs Immunoassay, R&D systems; IL-3 – Human ELISA kit, Invitrogen Corporation), according to the manufacturer's protocols. Duplicate samples were assessed for each patient. The intra-assay coefficient of variation (CV%) of IL-3 is reported to be 5.3% at a mean concentration of 87.2 pg/ml, standard deviation–SD = 4.6, SCF – to be 2.0% at a mean concentration of 655 pg/ml, SD=12.9, of GM-CSF – to be 9.5% at a mean concentration of 3.68 pg/ml, SD=0.35, of G-CSF – to be 6.8% at a mean concentration of 60 pg/ml, SD=4.1, of M-CSF – to be 3.4% at a mean concentration of 227 pg/ml, SD=7.7.

The inter-assay coefficient of variation (CV%) of IL-3 to be 6.9% at a mean concentration of 85.3 pg/ml, SD = 5.9, SCF – to be 5.1% at a mean concentration of 703 pg/ml, SD=36.0, of GM-CSF – to be 23.5% at a mean concentration of 2.8 pg/ml, SD=0.66, of G-CSF – to be 8.3% at a mean concentration of 62.6 pg/ml, SD=5.2, of M-CSF – to be 3.1% at a mean concentration of 232 pg/ml, SD=7.3. The assay showed no significant cross-reactivity or interference with a variety of other human cytokines and other growth factors.

Plasma concentrations of CA 15-3 were measured by chemiluminescent microparticle immunoassay (CMIA) (Abbott, Chicago, IL, USA). The intra-assay CV for CA 15-3 is reported to be 2.2% at a mean concentration of 27.0 U/ml, SD=0.6. The inter-assay CV for CA 15-3 is reported to be 2.6% at a mean concentration of 27.0 U/ml, SD=0.7.

Statistical analysis

The statistical analysis was performed using the STATISTICA 8.0 PL program. A preliminary statistical analysis (Chi-square test) revealed that the distribution of cytokine and tumor marker levels did not follow normal distribution. Consequently, the Mann-Whitney U-test was used for the statistical analyses between cancer and control groups. Additionally, statistical analyses between the groups with different BC stages were performed using the Kruskal-Wallis test, and a multivariate analysis of various data with the use

Table 1. **Characteristics of breast cancer patients and control groups.**

Group tested	Stage of breast cancer by TNM		Number of patients	Age Median Range
Breast cancer patients	I - $T_1N_0M_0$		25	46 38-78
	IIA – $T_2N_0M_0$ - 10 persons IIB – $T_2N_1M_0$ – 18; $T_3N_0M_0$ – 7		35	
	IIIA – $T_2N_2M_0$ – 8; $T_3N_1M_0$ – 6; $T_3N_2M_0$ - 4 IIIB – $T_4N_2M_0$ – 5; $T_4N_3M_0$ - 2		25	
	IV - patients with metastases (M_1)		25	
Control groups	Benign breast tumor	adenoma - 15 papilloma intraductale - 4 fibroadenoma - 15 mastopatia - 4 adenoma papillare - 2	40	42 30-65
	Healthy subjects		40	44 32-72

of the post-hoc Dwass-Steele-Crichlow-Flinger test. Data were presented as median and range. The Spearman rank correlation was used in the correlation analyses. Statistically significant differences were defined as comparisons resulting in $p<0.05$ [1].

Diagnostic sensitivity (SE), specificity (SP), the predictive value of a positive (PV-PR) and negative (PV-NR) test result were calculated using the *cut off* value of the 95th percentile in the control group (calculated from healthy blood donors): IL-3 – 1.31 pg/ml; SCF – 1137.94 pg/ml; GM-CSF – 2.73 pg/ml; G-CSF – 29.19 pg/ml; M-CSF – 450.85 pg/ml; CA 15-3 – 23.63 U/ml. The construction of the ROC curves was performed using GraphRoc program for Windows and the area under the ROC curve (AUC) was calculated.

RESULTS

Tab. 2 shows the median and range of plasma levels of the HGFs investigated and CA 15-3 in the groups tested. The medians of G-CSF (23.81 pg/ml) and M-CSF (497.80 pg/ml) levels, similarly to the level of the commonly accepted tumor marker (CA 15-3 – 28.70 U/ml), in the total group of breast cancer patients were significantly higher when compared to the healthy subjects (18.12 pg/ml; 294.50 pg/ml; 15.05 U/ml) ($p=0.44$; $p<0.001$; $p<0.001$; respectively). Moreover, significantly higher levels of GM-CSF, G-CSF were observed in the benign breast tumor group (1.02 pg/ml; 22.80 pg/ml) in comparison with the healthy subject. Similar results were obtained for CA 15-3 ($p=0.003$; $p=0.007$; $p=0.044$). Additionally, the median levels of M-CSF and CA 15-3 in the total group of cancer patients were statistically higher than in the group of benign breast tumor patients ($p=0.014$; $p=0.044$).

Similarly, we observed statistically significantly higher concentrations of M-CSF and CA 15-3 in all the groups analyzed relative to the stage of breast cancer advancement in comparison to the healthy women and benign tumor group (with the exception of stage I) (p values for all differentiations were below 0.010). G-CSF levels in stages III and IV of BC were statistically significantly higher than in the healthy subjects group, but in stage I they were statistically significantly lower ($p=0.020$) and in stage IV significantly higher ($p=0.023$) than in the benign breast tumor group. GM-CSF levels in stage IV and in the total group of breast cancer were statistically significantly lower than in the benign tumor group ($p=0.038$ and $p=0.003$). SCF plasma concentration was statistically lower in stage I of cancer advancement than in healthy subjects ($p=0.021$).

Furthermore, we noticed statistically higher plasma levels of M-CSF in all tumor stages, which we demonstrated in the comparison of stage III or IV with stage I or II of tumor advancement (in all cases $p<0.001$). We observed similar data for CA 15-3 ($p<0.001$; $p=0.014$). Additionally, we noticed significantly higher plasma levels of SCF in stage IV of cancer than in stage I ($p=0.010$).

The Spearman rank correlation was used in the dependence analyses between the cytokines investigated and CA 15-3. We observed a significant positive correlation between G-CSF ($R = 0.30$; $p = 0.001$) or M-CSF ($R=0.46$; $p=0.004$; respectively), or SCF ($R=0.21$; $p=0.028$) with CA 15-3 concentrations in the total group of BC patients. Additionally, we noticed statistically positive correlation between SCF and M-CSF ($R=0.27$; $p=0.004$) or SCF and G-CSF ($R=0.21$; $p=0.026$), or G-CSF and M-CSF ($R=0.34$; $p=0.004$). We also observed significant positive correlations in the control group (healthy subjects) between IL-3 and M-CSF ($R=0.39$; $p=0.012$) or GM-CSF ($R=0.55$; $p=0.006$), or SCF ($R=0.46$; $p=0.003$). Furthermore, a significant positive correlation in the group of patients with a benign tumor between IL-3 and GM-CSF ($R=0.40$; $p=0.046$) was also observed. Significant negative correlations were observed between SCF and GM-CSF in the

Table 2. **Plasma levels of HGFs and CA 15-3 in patients with breast cancer and in control groups.**

Groups tested		IL-3 (pg/ml)	SCF (pg/ml)	GM-CSF (pg/ml)	G-CSF (pg/ml)	M-CSF (pg/ml)	CA 15-3 (U/ml)
Breast cancer Median Range	I stage	0.26 0.26-29.69	[1] 659.50 362.40-1404.00	0.51 0.26-3.05	[2] 18.75 9.4-31.96	[1] 350.80 134.50-536.20	20.10 7.10-34.20
	II stage	0.26 0.26-35.37	753.80 450.50-2000.00	0.42 0.26-3.23	19.80 7.66-85.00	[1/2/4] 436.30 213.70-1175.80	[1/2/4] 25.90 4.60-48.10
	III stage	0.26 0.24-80.58	877.40 433.5-1393.00	0.60 0.26-3.40	[1] 29.60 10.83-52.50	[1/2/5] 710.00 308.90-1073.20	[1/2/5] 42.90 17.50-310.00
	IV stage	0.26 0.22-1.26	[5] 879.00 481.60-1258.30	[2] 0.40 0.27-1.82	[1/2] 29.80 12.10-60.90	[1/2/5] 717.00 444.2-1477.00	[1/2/5] 84.77 18.50-259.00
	Total group	0.26 0.22-80.58	779.50 362.4-2000.00	[2] 0.50 0.26-3.40	[1] 23.81 7.66-85.00	[1/2] 497.80 134.5-1477.00	[1/2] 28.70 4.60-310.00
Control groups Median Range	Benign breast tumor	0.28 0.22-2.90	837.40 387.90-1111.40	[3] 1.02 0.29-2.76	[3] 22.80 15.52-46.68	350.80 158.40-671.90	[3] 20.90 7.80-30.40
	Healthy subjects	0.28 0.24-2.19	879.10 398.60-1241.10	0.38 0.26-7.65	18.12 4.62-46.43	294.50 119.60-719.80	15.05 6.60-27.20

[1] statistically significant when BC patients compared with healthy subjects;
[2] statistically significant when BC patients compared with benign breast tumor group;
[3] statistically significant when benign breast tumor group compared with healthy subjects;
[4] statistically significant when BC patients stage II compared with BC patients stage I;
[5] statistically significant when BC patients stage III or IV compared with BC patients stage I or II;

breast cancer (R=-0.21; p=0.029) and in the benign lesions group – GM-CSF and CA 15-3 (R=-0.43; p=0.032).

Tab. 3 shows the HGFs diagnostic criteria: SE, SP, PV-PR, PV-NR in the patients with breast cancer. We indicated that the SE of HGFs in the total group with breast cancer was the highest for M-CSF (60%)–higher than for CA 15-3 (53%) and distinctly higher than for the remainder of the cytokines tested (IL-3–9%, SCF–8%, GM-CSF–5%, G-CSF–36%). The combined use of hematopoietic cytokines with antigen CA 15-3 resulted in an increase in sensitivity values. A maximum value for the total BC group was obtained for the combination of M-CSF and CA15-3 (85%) (*Tab. 4*). Among all the cytokines tested, M-CSF showed the highest SE at every stage of cancer advancement, and it was repeatedly higher than for other factors. Furthermore, only the sensitivity of M-CSF (and, to a lesser extent, G-CSF) increased markedly with cancer advancement stage. The combined use of hematopoietic cytokines and antigen CA 15-3 resulted in an increase in sensitivity for every stage of breast cancer. A maximum value was obtained for the combination of M-CSF and CA 15-3 (III–92%, IV–100%).

The diagnostic specificity for SCF, M-CSF and G-CSF (all equal to 95% and identical as for CA 15-3) were higher than the SP for IL-3 and GM-CSF (equal to 93%).

The predictive value of a positive test result in the total group of breast cancer patients was the highest for M-CSF and CA 15-3 (97%), and was higher than for the other cytokines tested. The M-CSF PV-PR values were the highest in all cancer stages and they increased in line with the stage of BC only for macrophage colony stimulating factor and G-CSF, similarly to CA 15-3. The combined use of the cytokines tested with the breast tumor marker resulted in a decrease in the PV-PR range (*Tab. 4*).

The predictive value of a negative test result in the total group of breast cancer patients was repeatedly higher for M-CSF (43%) than for other cytokines and slightly higher than for CA 15-3 (42%) (*Tab. 3*). The combined use of hematopoietic cytokines and antigen 15-3 resulted in an increase in the PV-NR in all cases, e.g. with M-CSF – to 57%, with G-CSF to 50% (BC total group). In every group of breast cancer patients PV-NR was the highest for M-CSF and increased with cancer stage for M-CSF and G-CSF only. We observed similar data for the commonly accepted tumor marker–CA 15-3 (*Tab. 3*). The combined use of hematopoietic cytokines and antigen 15-3 resulted in an increase of the PV-NR in every stage of cancer. Maximum ranges (as high as

Table 3. **Diagnostic criteria of HGFs tested and CA 15-3 in breast cancer patients.**

Parameters tested	Diagnostic criteria (%)	Breast cancer				
		I stage	II stage	III stage	IV stage	Total group
IL-3 Cut off=1.31 pg/ml	Sensitivity	12	11	8	4	9
	Specificity	93	93	93	93	93
	PV-PR	50	57	40	25	83
	PV-NR	63	54	63	61	27
SCF Cut off=1137.94 pg/ml	Sensitivity	8	9	8	8	8
	Specificity	95	95	95	95	95
	PV-PR	50	60	50	50	82
	PV-NR	62	54	62	62	27
GM-CSF Cut off=2.73 pg/ml	Sensitivity	4	9	4	4	5
	Specificity	93	93	93	93	93
	PV-PR	33	50	25	25	67
	PV-NR	61	63	61	61	26
G-CSF Cut off=29.19 pg/ml	Sensitivity	12	29	52	56	36
	Specificity	95	95	95	95	95
	PV-PR	60	83	87	88	95
	PV-NR	63	60	76	78	35
M-CSF Cut off=450.85 pg/ml	Sensitivity	24	46	84	92	60
	Specificity	95	95	95	95	95
	PV-PR	75	89	91	92	97
	PV-NR	67	67	90	95	43
CA 15-3 Cut off=23.63 U/ml	Sensitivity	20	43	68	84	53
	Specificity	95	95	95	95	95
	PV-PR	71	88	89	91	97
	PV-NR	66	66	83	90	42

95%-100%) were obtained for the combination of M-CSF and CA 15-3 in stage III or IV of BC (*Tab. 4*).

The relationship between the diagnostic sensitivity and specificity was illustrated by the ROC curve (*Tab. 5*). The area under the ROC curve (AUC) indicates the clinical usefulness of a tumor marker and its diagnostic power. From the viewpoint of practice only markers with AUC higher than 0.7 or even 0.8 may have some diagnostic utility. It shows that the M-CSF area (0.8376) under the ROC curve in the total group of breast cancer patients is the largest from HGFs but still slightly lower than the AUC of CA 15-3 (0.8765). Moreover, the areas under the ROC curve for GM-CSF, G-CSF, M-CSF, similarly to CA 15-3 were statistically significantly larger in comparison to AUC=0.5 (borderline of the diagnostic usefulness of the test) ($p=0.038$; $p=0.001$; $p<0.001$; $p<0.001$; respectively). Additionally, our study confirmed that M-CSF had the highest receiver operating characteristic curves among all the HGFs investigated in all the groups analyzed relative to the stage of BC, and its value was marginally lower than that of CA 15-3 (stages I, II and III), but was the largest for stage IV. Furthermore, the areas under the ROC curve in stage I for SCF, GM-CSF, M-CSF, similarly to CA 15-3 were statistically significantly larger ($p=0.016$; $p=0.009$; $p<0.041$; $p<0.001$; respectively). AUC values of tested parameters in I stage of cancer were below 0.7 value, therefore in this stage CA 15-3 and all hematopoietic cytokines have small diagnostic usefulness, but represent additional information and they are helpful in the diagnosis of early breast cancer. The AUCs in stages II, III and IV for M-CSF and CA 15-3 and in III and IV for G-CSF were statistically significantly larger ($p<0.001$ in all cases) (*Tab. 5*).

DISCUSSION

HGFs are synthesized by a variety of cell types including endothelial cells, fibroblasts, bone marrow stromal cells, osteoblasts, thymic epithelial cells, keratinocytes, astrocytes, myoblasts, mesothelial cells, endometrial gland cells, and the placenta-trophoblast decidual stroma [5,16,17]. *In vitro* breast cancer cells are also capable of producing hematopoietic cytokines (HGFs) constitutively [18], similarly to cells of several malignant tumors [19] including prostate cancer [8]

Table 4. **Diagnostic criteria of HGFs tested in combined analysis with CA 15-3 in breast cancer patients.**

Parameters tested	Diagnostic criteria (%)	Ovarian cancer				
		I stage	II stage	III stage	IV stage	Total group
IL-3+CA15-3	Sensitivity	24	54	72	84	58
	Specificity	88	88	88	88	88
	PV-PR	55	79	78	81	93
	PV-NR	65	69	83	90	43
SCF+CA15-3	Sensitivity	28	49	72	84	57
	Specificity	90	90	90	90	90
	PV-PR	64	81	82	84	94
	PV-NR	67	67	84	90	43
GM-CSF+CA15-3	Sensitivity	24	49	68	84	55
	Specificity	88	88	88	88	88
	PV-PR	55	77	77	81	92
	PV-NR	65	66	92	90	42
G-CSF+CA15-3	Sensitivity	28	60	88	96	67
	Specificity	90	90	90	90	90
	PV-PR	64	84	92	86	95
	PV-NR	67	72	92	97	50
M-CSF+CA15-3	Sensitivity	44	69	92	100	85
	Specificity	90	90	90	90	90
	PV-PR	73	86	85	86	95
	PV-NR	72	77	95	100	57

Table 5. **Diagnostic criteria of ROC curve for all parameters tested.**

Parameters tested	ROC criteria	Breast cancer				
		I stage	II stage	III stage	IV stage	Total group
IL-3	AUC	0.5010	0.5239	0.5890	0.6160	0.5540
	SE	0. 0804	0.0753	0.0779	0.0753	0.0657
	p (AUC=0.5)	0.990	0.750	0.253	0.123	0.411
SCF	AUC	0.6690	0.5864	0.5090	0.5640	0.5493
	SE	0.0701	0.0675	0.0736	0.0722	0.0569
	p (AUC=0.5)	**0.016**	0.200	0.902	0.375	0.386
GM-CSF	AUC	0.6730	0.6107	0.6290	0.5650	0.6186
	SE	0.0670	0.0661	0.0691	0.0722	0.0572
	p (AUC=0.5)	**0.009**	0.093	0.061	0.367	**0.038**
G-CSF	AUC	0.5300	0.5850	0.7780	0.7995	0.6515
	SE	0.0787	0.0674	0.0626	0.0613	0.0471
	p (AUC=0.5)	0.702	0.207	**p<0.001**	**p<0.001**	**0.001**
M-CSF	AUC	0.6770	0.7993	0.9370	0.9825	0.8376
	SE	0.0722	0.0516	0.0311	0.0140	0.0333
	p (AUC=0.5)	**0.041**	**p<0.001**	**p<0.001**	**p<0.001**	**p<0.001**
CA 15-3	AUC	0.7490	0.8425	0.9615	0.9665	0.8765
	SE	0.0640	0.0500	0.0209	0.0204	0.0280
	p (AUC=0.5)	**p<0.001**	**p<0.001**	**p<0.001**	**p<0.001**	**p<0.001**

and ovarian cancer [20,21]. High serum or plasma levels of M-CSF and other hematopoietic cytokines were observed, for example, in patients with endometrial cancer [22,23,24], ovarian cancer [25], colorectal cancer [26,27]. Enhanced levels of HGFs, for example SCF and M-CSF, have been demonstrated in the serum of breast cancer patients [12,28,29]. Elevated levels of M-CSF were also noted in the serum, ascites and pleural effusion of patients with an advanced stage of breast cancer [10,15]. Clinical studies have also shown that M-CSF expression was found in ~75% of cases investigated, and the extent of expression in these tumors correlates with a high tumor grade and poor prognosis [30].

In the present study plasma levels of G-CSF and M-CSF in the total group of breast cancer patients were statistically significantly higher compared with the healthy subjects. Similar significant data were observed in our study for CA 15-3 and also in the study of McDermott et al. [12]. These authors compared only the plasma level of M-CSF in pre-invasive and advanced breast cancer patients in relation to the control group. Similar results were observed in our earlier studies, but the groups tested were far smaller [31,32]. We observed higher plasma levels of G-CSF in stages III and IV of BC in comparison to stages I and II and to the healthy subjects. It should be emphasized that some authors explained increased levels of G-CSF in cancer patients by leukocytosis [33], which is very common in acutely ill patients, although we excluded all patients with this condition in order to eliminate its influence on our results (in BC, benign lesions patients and healthy controls). Other authors received identical results regarding other cancer types, for example colorectal or ovarian cancer [25,27].

Additionally, we noticed higher plasma levels only of M-CSF among all the HGFs tested in BC group in comparison to the group with benign lesions (statistical significance), similarly to CA 15-3. These data are almost identical with the results of the study by Scholl et al. [30] in which the authors found that M-CSF serum levels were significantly higher in a metastatic population as compared to patients with primary tumors. Additionally, they suggested that patients with early stage tumors (T0/T1/T2) had significantly lower M-CSF levels than patients with tumors of a larger size (T3/T4). The results of our present study were obtained in groups twice the size as those investigated formerly (breast cancer and control groups) and are in agreement with our previous studies [15].

Furthermore, we observed significantly higher levels of GM-CSF and G-CSF in the benign lesions group in comparison with the healthy subjects, similarly to CA 15-3, but we did not observe similar data for M-CSF plasma levels. Interestingly, only M-CSF was capable of discriminating between all the groups tested: breast cancer from non carcinoma groups (benign lesions or healthy subjects). Our previous investigations concerning M-CSF in endometrial cancer patients confirm this cytokine's ability to discriminate malignant disease from myoma uteri [24], but are in opposition to the results obtained in our previous study on a smaller breast cancer group [15].

Nevertheless, we demonstrated that the concentrations of SCF in all the BC patients investigated, and also in every stage of disease advancement, were lower in comparison to the healthy women group. Additionally, SCF plasma levels were lower in the total group of cancer patients than in the patients with a benign breast tumor. Comparable results were reported by other authors who, for example, confirmed lower concentrations of other cytokines in cancer patients than in healthy controls or patients with benign lesions [26,32]. Other researchers reported different results [3,7]. It is difficult to find published research results offering an explanation concerning reduced levels, especially of SCF, but it offers an interesting hypothesis that SCF could be a "negative" cancer marker.

The Spearman rank correlation was used in the dependence analyses between the cytokines investigated and CA 15-3. There were significant positive correlations between G-CSF, M-CSF, SCF and CA 15-3 concentrations. Plasma levels of G-CSF and M-CSF followed the levels of the established tumor marker (CA 15-3), which is a commonly accepted breast cancer marker [32]. Therefore, these two cytokines, similarly to CA 15-3, can play a potential role in the diagnosis of this type of cancer.

In the present study, M-CSF sensitivity (60%) was the highest from all the HGFs tested and it was higher than CA 15-3 (53%). These data bear considerable similarity to the results published in our previous papers on breast [15] and endometrial cancer [23]. Similarly, SP values were as high as in our earlier publications regarding endometrial cancer [24].

The PV-PR reflects the probability of diagnosing cancer on the basis of a positive result of the test. The PV-NR indicates the probability of excluding the disease based on a negative test result. In this investigation, M-CSF proved to have the highest predictive values from all the HGFs tested, which was marginally higher than or equal to CA 15-3.

The most important criterion for tumor marker diagnostic efficacy is the sensitivity/specificity diagram: the ROC curve. The area under the ROC curve (AUC) indicates the clinical usefulness of a tumor marker. The larger the area under the ROC curve the greater the efficacy of a tumor marker. In the present study, the ROC area of M-CSF was the largest from all the cytokines tested, but marginally lower than the ROC area of CA 15-3. Additionally, we observed statistically significantly larger AUCs for M-CSF, G-CSF, and CA 15-3 compared to AUC=0.5. Our results show that the diagnostic power of hematopoietic cytokines, especially M-CSF, in the group of breast cancer patients was higher than the diagnostic power of M-CSF in the course of endometrial cancer [24], lung cancer [35] and pancreatic cancer [36].

CONCLUSIONS

Our present results indicate the usefulness of M-CSF in the diagnostics of breast cancer patients (especially in combination with CA 15-3) and in discriminating between patients with breast cancer and non-carcinoma lesions.

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