ORIGINAL ARTICLE

Comparative evaluation of plasma levels and diagnostic values of macrophage-colony stimulating factor in patients with breast cancer and benign tumors

Sławomir Ławicki[1], Małgorzata Czygier[1], Ewa Będkowska[1], Marek Wojtukiewicz[2], Maciej Szmitkowski[1]

1 Department of Biochemical Diagnostics, Medical Academy, Białystok, Poland
2 Medical Academy Oncology Clinic, Białystok, Poland

KEY WORDS

breast cancer, macrophage-colony stimulating factor, tumor markers

ABSTRACT

INTRODUCTION Macrophage-colony stimulating factor (M-CSF) is one of the glycoproteins called hematopoetic growth factors. The direct production of this cytokine has been reported in tumor cell lines *in vitro* and in solid tumors *in vivo.*

OBJECTIVES In the present study, the levels of M-CSF in patients with breast cancer and in those with a benign breast tumor were evaluated. Moreover, diagnostic values were determined through assesing diagnostic sensitivity and specificity as well as predictive value of positive (PV_{+ve}) and negative (PV_{-ve}) results. The results obtained were compared to the CA 15-3 and a control group.

PATIENTS AND METHODS The study group was made up of 70 patients with breast cancer and 20 patients with benign tumors and the control group of 30 healthy women. M-CSF was assayed using an ELISA method. CA 15-3 was measured by means of an immunoenzymatic method (MEIA) from ABBOT.

RESULTS Statistically higher levels of M-CSF and CA 15-3 were found in breast cancer patients as compared to the benign tumor and control groups. These levels were also significantly higher in patients with more advanced stages of cancer. A positive correlation between M-CSF and CA 15-3 levels was observed. The diagnostic sensitivity of M-CSF (58%), a specificity (93%), PV_{+ve} (94%) and PV_{-ve} (43%) were higher or equal to the values obtained for CA 15-3 (49%, 93%, 93% and 40%, respectively). When both parameters studied were determined jointly, sensitivity increased to 72%.

CONCLUSIONS The above data suggests that M-CSF might be useful in both diagnostics and differential diagnosis of benign tumors and breast cancer (except for the lowest degree of the clinical progression).

Correspondence to:
Sławomir Ławicki, MD, PhD,
Zakład Diagnostyki Biochemicznej,
Akademia Medyczna,
ul. Waszyngtona 15,
15-269 Białystok, Poland,
phone: +48-85-746-87-03,
fax: +48-85-746-85-85;
e-mail: slawicki@amb.edu.pl
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INTRODUCTION Breast cancer is the most common cancer in women. Premature menstruation, delayed menopause, diet, over-exertion, and both endo- and exogenous hormones, such as prolonged oral contraception or hormone replacement therapy, are risk factors for the development of breast cancer.[1,2]

Macrophage-colony stimulating factor (M-CSF) is among the cytokines known as hematopoetic growth factors (HGFs). Firstly, these cytokines regulate the growth and differentiation of hematopoetic cells and accelerate maturation of neutrophils and macrophages.[3,4] The most recent studies have shown that HGFs may stimulate proliferation of non-hematopoetic, e.g. cancer cells.[5,6] Other studies have shown M-CSF, coding mRNA in cancer cell lines.[7,8]

It has been also reported that breast cancer cells secrete cytokines, including SCF[9] and M-CSF[10,11]. Furthermore, the receptors for HGFs in breast cancer cell lines have been located, and the possibility of their proliferation has been demonstrated.[9,11] The expression of mRNA for M-CSF in breast cancer cells has been observed[12], and it has been confirmed that this cytokine may stimulate the progression of a cancer, as found in BT 549, MDA-MB-436 and MDA-MB-231 cancer cell lines[12,13]. Secretion of M-CSF from breast cancer cells is accompanied by an expression of receptors for this cytokine (CSF-1R – approximately in 50% of all tumors, and 90% metastatic tumors), suggesting this cytokine has both an autocrine and endocrine role in the invasiveness of a tumor.[11,13] In addition, elevated levels of M-CSF were shown in plasma of breast cancer patients.[14-17]

Following the analysis of reference reports, the aim of this study was to determine the levels of hematopoetic cytokine (M-CSF) and tumor markers (CA 15-3) in breast cancer patients, as compared to benign tumor patients and a control group. Furthermore, the concentrations of the parameters studied were analyzed depending on the tumor's stage of development. The diagnostic value and correlation between M-CSF and CA 15-3 were also evaluated.

PATIENTS AND METHODS A group of 70 patients with breast cancer, aged 38–77 years (mean 59 years, standard deviation [SD] = 8.4) and one of 20 women with benign breast tumors (30–64 years, SD = 8.4) were enrolled in the study. A study group of women with breast cancer were further divided into subgroups depending on the stage of cancer: group A – 16 patients (stage I – $T_1N_0M_0$), group B – 22 patients (IIA – $T_2N_0M_0$ – 5, IIB – $T_2N_1M_0$ – 14 and $T_3N_0M_0$ – 3), group C – 16 patients (IIIA – $T_2N_2M_0$ – 5, $T_3N_1M_0$ – 4, $T_3N_2M_0$ – 3 and IIIB – $T_4N_2M_0$ – 2, $T_4N_3M_0$ – 2) and group D – 16 patients (distant metastases). The histological type of cancer was established in all patients, i.e. in a majority of patients carcinona intraductale was diagnosed (67 patients), and in the remaining patients – carcinoma lobulare was found (3 patients). The group with benign tumors consisted of the following patients: 6 with adenoma, 2 papilloma intraductale, 9 *fibroadenoma*, 1 masthopathy and 2 adenoma papillare. Blood samples were taken prior to any treatment. Enrollment of the patients into a study or a control group was performed basing on examinations conducted by a gynecologist or oncologist. A control group consisted of 30 women aged 37–74 years (mean 58 years, SD = 8.4). The study was approved by the Bioethics Committee of the Medical Academy in Białystok.

Blood samples were drawn into a solution of sodium salt heparin, and spun in order to obtain platelet poor plasma. The plasma was stored at a temperature of –85°C. M-CSF was determined by an immunoenzymatic method (ELISA) using reagents from R&D. CA 15-3 was detected with the immunoenzymatic method using microparticles (MEIA) from ABBOTT.

A statistical analysis of the results was performed using the STATISTICA program. Since a normal distribution was not confirmed, the evaluation of the statistical significance of differences between the study groups and the control group was performed based on the non-parametric Mann-Whitney's U test. Spearmen's test was used to determine correlations between the parameters studied. Moreover, the parameters for the diagnostic and predictive values of positive (PV_{+ve}) and a negative (PV_{-ve}) results were calculated.

RESULTS TABLE presents concentrations (median + deviation) of M-CSF and of the compared marker, CA 15-3, in breast cancer and benign tumor patients and in the control group. The concentration of M-CSF in the whole group studied (median 440.6 pg/ml) was significantly higher than the benign tumor group (307.22 pg/ml) ($p = 0.00044$) and the control group (298.9 pg/ml) ($p = 0.000039$). No statistically significant difference was found between group A (patients with a low stage of clinical advancement) and the group of benign tumor patients as compared to the control group. However, significant statistical differences were observed between particular groups of patients at a higher stage of clinical advancement: group B (397.5 pg/ml), group C (497.1 pg/ml) and group D (639.4 pg/ml) as compared to the control group, as well as between group C or D and the group with benign tumors. Similar differences in concentrations were observed with CA 15-3.

Furthermore, there were statistically higher concentrations of M-CSF, similar to CA 15-3, in a more advanced stage of breast cancer (III or IV, groups C or D) as compared to stage I or II (groups A or B). In addition, a statistically significant difference was found between groups B and A for the concentrations of M-CSF ($p = 0.015$).

A statistically significant positive correlation between M-CSF and CA 15-3 was also demonstrated ($R = 0.224$; $p = 0.044$).

Evaluation of diagnostic parameters, such as diagnostic sensitivity and specificity, and the predictive value of positive (PV_{+ve}) and negative (PV_{-ve}) results, made it possible to establish a cut-off for the parameters studied (95th percentile of the control group), i.e. 406.84 pg/ml for M-CSF and 25.6 U/ml for CA 15-3.

The diagnostic sensitivity in the total breast cancer group studied (58%), PV_{+ve} (94%) and PV_{-ve} (43%), exceeded CA 15-3 in each case (49%, 93% and 40%, respectively). A combined analysis of diagnostic sensitivity demonstrated an increase in the whole group of breast cancer patients of up to 72%, PV_{+ve} to 95% and PV_{-ve} to 54%. The diagnostic specificity of M-CSF and CA 15-3 was very high and amounted up to 93% for both parameters (FIGURE 1).

TABLE The plasma levels of macrophage-colony stimulating factor (M-CSF) and CA 15-3 in breast cancer and benign breast tumor patients

Test groups	Test markers	
	M-CSF (pg/ml) Median (range)	CA 15-3 (U/ml) Median (range)
Breast cancer		
Group A	326.4 (132.5–466.54)	18.9[a] (7.02–35.61)
Group B	397.1[a,b,c] (210.6–1165.4)	24.4[a,b] (4.5–33.51)
Group C	497.4[a,b,c] (326.2–1074.21)	27.1[a,b] (16.9–168.6)
Group D	640.41[a,b,c,d] (249.0–899.24)	85.41[a,b,c,d] (18.7–250.0)
Total group	440.6[a,b] (136.4–1174.14)	25.22[a,b] (4.6–259.0)
Benign breast tumor	307.22 (158.4–466.92)	16.56 (7.8–29.6)
Control group	298.9 (178.8–438.91)	14.2 (6.6–28.4)

a Statistical significance compared to the control group
b Statistical significance compared to the group with benign breast cancer
c Statistical significance compared to group B or C, or D and group A
d Statistical significance compared to group C or D and group B

The diagnostic sensitivity of M-CSF and CA 15-3, depending on the stage of breast cancer, is shown in FIGURE 2. An apparent increase in sensitivity with the advancement of breast cancer was reported, both in case of M-CSF and CA 15-3. In group A sensitivity to M-CSF was 23%, in B – 48%, in C – 78%, in D – 86% and in each case these values were higher than CA 15-3 (17%, 41%, 63% and 80%, respectively). A combined analysis of M-CSF and CA 15-3 clearly increased the diagnostic sensitivity of the assays, i.e. in group A – to 34%, in B – to 60%, in C – to 88% and in D – up to 91%.

DISCUSSION M-CSF is synthesized by a variety of cells, including endothelial cells, fibroblasts, stromal bone marrow cells, osteoblasts, tymocytes, keratinocytes, astrocytes, mesothelial cells, glandular mucosal cells of uterus and placental cells.[18,19] It has been demonstrated that in an *in vitro* setting, breast cancer cells are capable of producing HGFs[9,11,12], including M-CSF, much like the cells of other malignant tumors, such as prostate cancer[20], ovarian cancer[21] and uterus cancer[23].

In the studies performed, plasma was used as an investigational material, as the blood clotting does not lead to false overestimation of concentrations. A cytokine concentration in serum is higher, as they can be secreted by the cells involved in blood clotting (e.g. thrombocytes). Normal values of M-CSF in serum range from 150 to 500 U/ml or 3–8 ng/ml, and are higher than in plasma.[18,19] High levels of M-CSF, both in serum and plasma, were found in patients with pancreatic cancer[23], uterus cancer[22,24], ovarian cancer[8,25,26] and in soft tissue sarcomas[27]. Elevated levels of HGFs, e.g. SCF and M-CSF, were reported in serum of breast cancer patients.[9,15,24] An increase in M-CSF concentration was demonstrated both in serum and peritoneal or pleural exudate in patients with highly advanced breast cancer.[15,24] Clinical studies have also shown that elevated concentration of M-CSF correlates in the majority of cases with advanced stages of cancer and poor prognoses.[10,24]

In the present study, the concentration of M-CSF in the plasma of breast cancer patients was significantly higher than in benign tumor patients or the control group. Moreover, it is of great importance that no statistically significant differences were demonstrated between patients at the least advanced stage of breast cancer and patients with benign tumors, when compared to the control group. This allows for the accurate differentiation of patients with cancer (except for the patients with the first stage cancer) and patients with benign tumors and healthy individuals, based on M-CSF concentration, which is a highly desirable cancer marker property.[28,29] Similar results were obtained in studies by McDermott et al.[14], in which plasma concentrations of M-CSF in patients with advanced breast cancer were solely compared to a control group of healthy women,

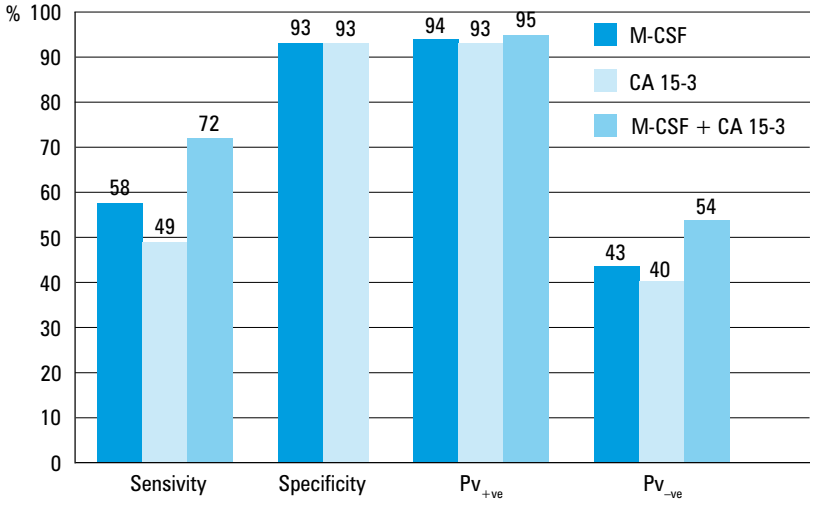

FIGURE 1 Diagnostic parameters of macrophage-colony stimulating factor (M-CSF) and CA 15-3 in breast cancer patients

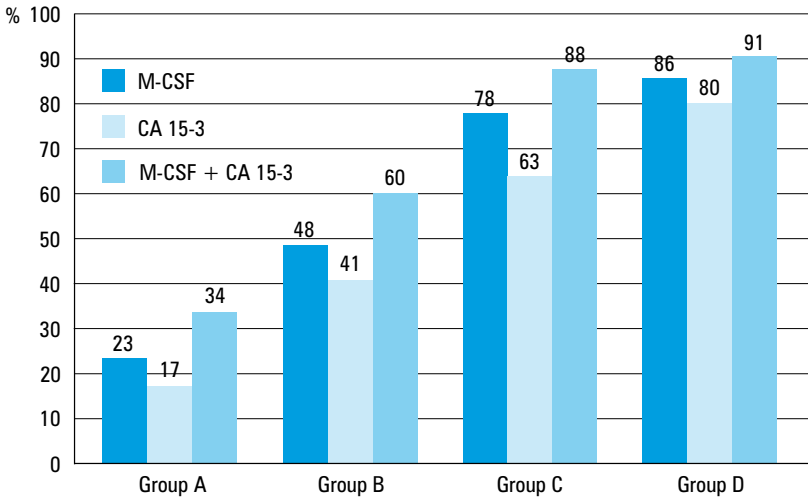

FIGURE 2 Diagnostic sensitivity of macrophage-colony stimulating factor (M-CSF) and CA 15-3 compared to stage of breast cancer

and in other studies with a different selection (number and histology) of the groups investigated[30]. Comparable results were also obtained in patients with other malignancies, e.g. pancreatic cancer[23] or ovarian cancer[26].

Moreover, an increase in M-CSF concentration was found in more advanced stages of breast cancer (stages III and IV) compared to stages I and II (a statistically significant difference). The results are similar to those of other authors.[15,16] Scholl et al.[15] reported significantly higher M-CSF serum concentrations in patients with metastases than in patients with localized tumors, and demonstrated that patients at the early stage of cancer ($T_0/T_1/T_2$) had significantly lower cytokine concentrations than patients with larger tumors (T_3/T_4). Slightly different results were presented by McDermott et al.[14], reporting no statistically significant differences between a group of patients with invasive cancer and one at the pre-invasive stage. The above discrepancies probably derived from the study groups selected.

Spearman's method was applied to analyze the correlation between the cytokine studied and CA 15-3. A statistically significant positive correlation was found between M-CSF and CA 15-3 concentrations, suggesting a similarity in fluctuations of cytokine concentrations in relation to a marker commonly used in breast cancer diagnostics.[31,32] Therefore, M-CSF, much like CA 15-3, may turn out to play a diagnostic role in breast cancer.

An ideal cancer marker should be highly specific, i.e. it should not be detected in healthy individuals; it should be highly sensitive, i.e. it should be detectable in cancer patients; and it should allow for very early detection, even when only single cancer cells are present. It should also have a high predictive value and correlate with the size of a tumor. None of the markers to date meet the criteria of 100% specificity or 100% sensitivity. In the present paper M-CSF, both in the total group studied and in its subgroups related to the stage of breast cancer, displayed a higher sensitivity than CA 15-3, thus confirming our previous studies, performed with a selection of the study and control groups established in a different way.[16,30]

A predictive value of a positive result reflects the probability of cancer diagnosis based on the positive results of the investigation. The predictive value of a negative result introduces the probability of excluding the disease on its basis. In the studies performed higher predictive values were demonstrated in M-CSF than in CA 15-3.

Summing up the results, it should be noted that the M-CSF plasma levels in breast cancer patients (except for group A) were significantly higher than those in benign tumor patients and the control group. However, no differences in the M-CSF plasma levels between the group of benign tumor patients and healthy individuals were reported. This makes differentiation between cancer and cancer-free patients possible. Furthermore, M-CSF diagnostic indicators turned out to be an improvement on CA 15-3 ones, and results of combined detection of both markers seem to be very promising. At present, it seems that future analyses will confirm the usefulness of M-CSF in breast cancer diagnostics; this requires further investigation, however.

Conclusions from the presented study are as follow:

1) The concentration of M-CSF, like CA 15-3, was apparently higher in the plasma of the breast cancer patients (except for patients at the least advanced stage of cancer) than in the benign tumor patients and the control group.
2) M-CSF and CA 15-3 are not useful in differentiating between healthy patients and those with benign tumors.
3) The diagnostic sensitivity of M-CSF in each study group of breast cancer patients was higher than that of CA 15-3 and seemed to increase with the advancement of cancer; a combination of the 2 parameters markedly enhanced diagnostic performance.
4) M-CSF and CA 15-3 demonstrated equally high capacity to exclude and high probability to diagnose breast cancer.
5) M-CSF may be helpful in diagnostics and differentiation of breast cancer patients, especially in patients with advanced stages of cancer; further studies are required, however.

REFERENCES

1 Pieńkowski M. [Breast cancer]. In: Krzakowski M. [Clinical oncology]. T. II. Warszawa, Wydawnictwo Medyczne Borgis, 2001: 87-139.

2 Sasco A. Epidemiology of breast cancer: an environmental disease? APMIS. 2001; 109: 321-332.

3 Munn DH, Cheung NKV. Preclinical and clinical studies of macrophage-colony stimulating factor. Semin Oncol. 1992; 19: 395-407.

4 Dunlop RJ, Campbell CW. Cytokines and advanced cancer. J Pain Symptom Manage. 2000; 20: 214-232.

5 Chao K, Chang C, Yen M, et al. Granulocyte colony-stimulating factor on the growth of human ovarian carcinoma cells in vitro. Gynecol Obstet Invest. 1999; 48: 280-284.

6 Kirma N, Hammes LS, Liu YG, et al. Elevated expression of the oncogene c-fms and its ligand, the macrophage colony-stimulating factor-1,

in cervical cancer and the role of transforming growth factor-beta 1 in inducing c-fms expression. Cancer Res. 2007; 67: 1918-1926.

7 Baiocchi G, Kavanagh JJ, Talpaz M, et al. Expression of the macrophage colony-stimulating factor and its receptor in gynecologic malignancies. Cancer. 1991; 67: 990-996.

8 Asschert JGW, Vellenga E, Hollema H, et al. Expression of macrophage colony-stimulating factor (M-CSF), interleukin-6 (IL-6), interleukin-1β (IL-1β), interlekin-11 (IL-11) and tumour necrosis factor α (TNF-α) in p53-characterised human ovarian carcinomas. Eur J Cancer. 1997; 33: 2246-2251.

9 Hines SJ, Litz JS, Krystal GW. Coexpression of c-kit and stem cell factor in breast cancer results in enhanced sensitivity to members of the family of growth factors. Breast Cancer Res Treatm. 1999; 58: 1-7.

10 Kaciński BM. CSF-1 and its receptor in breast carcinomas and neoplasms of the female reproductive tract. Mol Reprod Dev. 1997; 46: 71-74.

11 Yee DL, Liu LI. The constitutive production of colony stimulating factor 1 by invasive human breast cancer cells. Anticancer Res. 2000; 20: 4379-4384.

12 Mancino AT, Klimberg VS, Yamamoto M, et al. Breast cancer increases osteoclastogenesis by secreting M-CSF and upregulating RANKL in stromal cells. J Surg Res. 2001; 100: 18-24.

13 Lin EY, Gouon-Evans V, Nguyen V, et al. The macrophage growth factor CSF-1 in mammary gland development and tumor progression. J Mammary Gland Biol Neoplasia. 2002; 7: 147-162.

14 McDermott RS, Deneux L, Mosseri V, et al. Circulating macrophage colony stimulating factor as a marker of tumour progression. Eur Cytokine Network. 2002; 13: 121-127.

15 Scholl SM, Lidereau R, Rochefordiere A, et al. Circulating levels of the macrophage-colony stimulating factor CSF-1 in primary and metastatic breast cancer patients. Breast Cancer Res Treat. 1996; 39: 275-283.

16 Ławicki S, Mroczko B, Omyła J, et al. [Macrophage colony-stimulating factor (M-CSF) as a candidate for the tumor marker of breast cancer]. Pol Arch Med Wewn. 2003; 6: 595-600.

17 Ławicki S, Omyła J, Mroczko B, et al. [Plasma level of macrophage-colony stimulating factor (M-CSF) in the course of breast cancer treatment]. Pol Arch Med Wewn. 2004; 4: 1191-1197.

18 Praloran V. Structure, biosynthesis and biological roles of monocyte–macrophage colony stimulating factor (CSF-1 or M-CSF). Nouv Rev Fr Hematol. 1991; 33: 323-333.

19 Shadle PJ, Allen JI, Geier MD, et al. Detection of endogenous macrophage colony-stimulating factor (M-CSF) in human blood. Exp Hematol. 1989; 17: 154-159.

20 Savarese DMF, Valiński H, Quesenberry P, et al. Expression and function of colony-stimulating factors and their receptors in human prostate carcinoma cell lines. Prostate. 1998; 34: 80-91.

21 Parrot JA, Kim G, Skinner MK. Expression and function of kit ligand/stem cell factor in normal human and bovine ovarian surface epithelium and ovarian cancer. Biol Reproduction. 2000; 62: 1600-1609.

22 Kaciński BM, Chambers SK, Stanley ER. The cytokine CSF-1 (M-CSF) expressed by endometrial carcinomas in vivo and in vitro, may also be a circulating tumour marker of neoplastic disease activity in endometrial carcinoma patients. Int J Radiat Oncol Biol Physics. 1990; 19: 619-626.

23 Mroczko B, Szmitkowski M, Wereszczynska-Siemiatkowska U, et al. Stem cell factor and macrophage-colony stimulating factor in patients with pancreatic cancer. Clin Chem Lab Med. 2004; 42: 256-260.

24 Kaciński BM. CSF-1 and its receptor in ovarian, endometrial and breast cancer. Ann Med. 1995; 27: 79-85.

25 Ławicki S, Czygier M, Gacuta-Szumarska E, et al. [The plasma levels and diagnostic utility of granulocyte colony stimulating factor (G-CSF) and macrophage – colony stimulating factor (M-CSF) in ovarian cancer patients]. Pol Merkur Lekarski. 2006; 125: 465-468.

26 Scholl SM, Bascou CH, Mosseri V. Circulating levels of colony-stimulating factor 1 as a prognostic indicator in 82 patients with epithelial ovarian cancer. Br J Cancer. 1994; 69: 342-346.

27 Rutkowski P, Kaminska J, Kowalska M, et al. Cytokine serum levels in soft tissue sarcoma patients: correlations with clinico-pathological features and prognosis. Int J Cancer. 2002; 100: 463-471.

28 Ławicki S, Mroczko B, Szmitkowski M. Cytokiny jako markery nowotworowe raka piersi. Post Hig Med Dośw. 2003; 57: 455-463.

29 Ławicki S, Mroczko B, Szmitkowski M. [Tumor markers of breast cancer]. Post Hig Med Dośw. 2004; 58: 292-300.

30 Ławicki S, Szmitkowski M, Wojtukiewicz M. The pretreatment plasma level and diagnostic utility of M-CSF in benign tumor and breast cancer patients. Clin Chim Act. 2006; 371: 112-116.

31 Kokko R, Holli K, Hakama M. CA 15-3 in the follow-up of localised breast cancer: a prospective study. Eur J Cancer. 2002; 38: 1189-1193.

32 Canizares F, Sola J, Perez I, et al. Preoperative values of CA 15-3 and CEA as prognostic factors in breast cancer: A multivariate analysis. Tumor Biol. 2001; 22: 273-281.