MMP-2, MMP-9, VEGF and CA 15.3 in Breast Cancer

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Abstract. *Background: Matrix metalloproteinases (MMPs) are a family of extracellular matrix degrading proteinases. Owing to their matrix-degrading abilities and high expression in advanced tumours, MMPs were originally implicated in cancer progression, invasion and metastasis. Patients and Methods: In this study, the correlation was determined between the expression of gelatinases (MMP-2 and MMP-9) in the sera of breast cancer patients from zymographic analysis and serum concentrations of VEGF and CA 15.3, before surgery and after 1 and 6 months; the association of both markers with clinicopathological features including histological type, stage of disease and estrogen (ER) and progesterone (PgR) receptors status were also analysed. In all, 88 breast cancer patients and 20 healthy women were involved in this study. Results: No statistically significant correlation between pro MMP-2, pro MMP-9, VEGF and CA 15.3 serum levels was found (p>0.05). In breast cancer patients, a significant decrease of the pro MMP-2 serum expression 1 month after surgery with respect to serum levels before surgery (p=0.0008) was evident, as well as of CA 15.3 serum levels at baseline and after 1 month (p=0.017). Moreover a strong decrease of pro MMP-9 serum levels was found in 88 breast cancer patients after 1 month (p=0.028) and after 6 months (p=0.009) from surgery. On the other hand, no significant differences in the serum levels of VEGF, CA15.3, pro MMP-2 or pro MMP-9 between 88 breast cancer patients preoperatively and 20 healthy women as controls were found. Our findings did indicate a significant positive association between higher preoperative levels of CA 15.3 and progression of disease (p=0.03), as well as a longer disease-free survival in patients who exhibited a decrease of serum pro MMP-9 expression compared to other biomarkers. No relationship between these four markers and the main clinical and pathological parameters was found. Conclusion: The present study failed to demonstrate any association between serum levels of MMPs, VEGF and CA 15.3 and well-known clinicopathological characteristics of breast carcinoma, while demonstrating the prognostic value of CA 15.3 and pro MMP-9 in the follow-up of breast cancer patients.*

The aggressiveness of cancer comes from the invasion of local tissues and progression to distant sites. A critical event during progression of cancer is the invasive growth of neoplastic cells into the host tissues: this involves a series of complex interactions occuring at the tumor-host interface, including angiogenesis and an extensive remodelling of the extracellular matrix (ECM) (1). Angiogenesis, the process of new capillary formation, occurs in a variety of normal and pathological conditions, such as an embryonic growth, wound healing, tumor growth and metastasis (2). This process comprises several steps including protease secretion and proteolysis of extracellular matrix, proliferation of endothelial cells and migration to form capillary sprouts and lumen closure (3). In the first step of the angiogenic response, some proteases such as matrix metalloproteinases (MMPs) are thought to play an important role (4, 5). The proteolysis of the ECM also represents an important step of tumor invasion and metastasis. Recent studies confirmed that the expression of MMPs in tumor cells is closely correlated to their metastatic activity (6, 7).

Matrix proteoglycans are known to partecipate in the regulation of cell adhesion, migration and proliferation (8). The MMP axis has several areas of overlap with the cytokine

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nertwork; in fact inflammatory cytokines or growth factors can regulate the expression of MMPs. Cytokine and growth factors play an important role in promoting the activation of MMPs from the inactive zymogens to the active enzymes. In a recent analysis, several endothelial growth factors, including vascular endothelial growth factor (VEGF), were demonstrated to promote neovascularization (9). In fact VEGF is a specific mitogen and survival factor for endothelial cells and a key promoter of physiological and pathological angiogenesis (10-12). VEGF presents five isoforms, composed of 121, 145, 165, 189 and 206 amino acids, which can be translated because of alternative mRNA splicing (13). $VEGF_{165}$, the predominant isoform, is a heparin-binding glycoprotein of 45 kDa, induced by hypoxia and oncogenic mutation, which binds to two specific tyrosine-kinase receptors: VEGF1 and VEGF2 (14). These are up-regulated almost exclusively in migrating/proliferating blood endothelial cells. In several studies, patients with gastro-intestinal cancer have been found to have elevated serum or plasma VEGF levels compared to cancer-free controls and VEGF levels have been correlated with stage or prognosis (15). For example, VEGF expression was significantly associated with an increase of the intratumoral microvessel density (MVD) in a variety of human solid cancers (16, 17). Furthermore, intratumoral VEGF concentration was a significant prognostic indicator for primary breast cancer patients (18, 19). It has been demonstrated that VEGF expression levels in primary tumor tissue can reflect the biological aggressiveness of the tumor and provide prognostic information (20, 21). Degradation of the ECM requires the concerted action of a number of extracellular enzymes. Various proteolytic enzymes such as the matrix metalloproteinases, serin proteinases and the cathepsins have been implicated in the proteoliytic process (22).

The MMPs are a family of zinc-containing endopeptidases secreted by various cell types that play a key role in extracellular matrix degradation, cancer cell invasion, metastasis and angiogenesis (23-25). Digestion of the subendothelial basement membrane is the first step toward intravasation and extravasation of cancer cells. Type IV collagen is the main component of the basal membrane and represents the first vital barrier breached by tumor cells when they become invasive; its degradation is favoured by two metalloproteinases, namely the 72 kDa type IV collagen (MMP-2 or gelatinase A) and the 92 kDa type IV collagen (MMP-9 or gelatinase B) (26). Gelatinase A and gelatinase B differ from other MMPs because they have three tandem fibronectin type II repeat sequences that are thought to confer gelatin-binding properties within the amino terminus of the catalytic module that mediates gelatin binding (27). These gelatinases are secreted as zymogens and cleaved to the active form, and their function is tightly regulated by several different mechanisms.

The expression and prognostic significance of MMP-2 and MMP-9 has not been fully clarified, but recent studies indicated that increased gelatinase expression in the primary tumor is associated with aggressive disease and unfavourable outcome (28-29). Kurizaki et al. showed that MMP-2 and MMP-9 expression were frequently co-upregulated with endothelial growth regulators in human breast cancer tissue, which underlines the cooperative function of MMPs in neovascularization; in particular, VEGF expression was significantly related to the expression of actived MMP-2 (30).

As reported by Coussens et al. (31) and Bergers et al. (32), MMP-9 has been shown to be important for angiogenesis in two transgenic models of tumor progression: the K14 HPV16 skin cancer model (31) and the RIP1 TAg2 insulinoma model (32). MMP-9 acts by increasing the biovailability of the pro-angiogenic factor VEGF, although is not known exactly how (32). Surprisingly, MMP-2 is not required for angiogenesis in the RIP1 TAg2 model.

Concerning CA 15.3, it is a mucinous antigen product of the MUC1 gene and defined by two monoclonal antibodies: DF3 raised against a membrane fraction of liver metastases from breast cancer and 115D8 raised against milk fat globule membrane (33, 34). Although the physiological function of MUC1 is unclear, recent data suggest that it plays a role in cell adhesion generating reduced cell-cell and cell-extracellular matrix interactions, immunity and metastasis (35, 36). MUC1 increased expression in primary tumors, acting as antiadhesive molecules and facilitating detachment of malignant cells, both from adjacent normal cells and the ECM in the primary cancer (37). Thus MUC1 might play a role, in the initiation of cancer invasion and metastasis. Evidence of this possibility was the recent finding that breast cancer growth and dissemination was impaired in MUC1-deficient mice (38). Currently, CA 15.3 is the most widely used serum marker for breast cancer (39): it is recommended in the evaluation of response to therapy and in monitoring of the course of breast cancer. There is no evidence for efficacy of screening with this marker in breast cancer: CA 15.3, in fact, is elevated in only 3% of patients with localised cancer while it is elevated in up to 70% of patients with metastatic disease.

To the best of our knowledge, this is the first study to investigate the correlation between the serum expression of two gelatinases and the serum levels of VEGF and CA 15.3 in breast cancer patients before and after surgery, and analyze the association of these markers with clinicopathological characteristics including histological type, stage of disease and positivity to estrogen (ER) and progesterone (PgR) receptors with the aim of clarifying the prognostic significance of these enzymes and their possible usefulness as prognostic tumoral markers.

Patients and Methods

Patients. A total of 88 patients with breast cancer (without clinically apparent metastases) who underwent surgery between 2000 and 2002 at Oncologic Institute "Giovanni Paolo II" IRCCS of Bari were involved in this study. Informed consent for the taking of venous blood was obtained from all of the patients. Women volunteers gave their consensus verbally. Peripheral venous blood samples were collected on the day of surgery in sterile plastic tubes and immediately centrifuged at 3000 rpm for 20 min. Sera was aliquoted and stored at –80°C until further processing.

A computerized database of updated patient clinical data, in addition to receptor status, nodal status, number of positive nodes, primary tumour diameter, age, menopausal status of the patients, grade of tumours, PgR and ER status, was available for statistical analysis. All patients had a histologically confirmed diagnosis of primary breast cancer and received no treatment before surgery. For all patients, the histological diagnosis and the stage of cancer were established by assessment of paraffin sections. Control sera (n=20) were taken from healthy women volunteers among dependents of our Institute.

Detection of VEGF and CA 15.3. Serum VEGF levels were measured using an enzyme-linked immunoassorbent assay (ELISA) (Quantikine Human VEGF Immunoassay; R&D Systems Inc, USA) according to the manufacturer's instructions. Briefly, a microplate was supplied carrying a monoclonal antibody specific for VEGF; standards and samples were pipetted into the wells and any amount of VEGF present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody against VEGF conjugated to horseradish peroxidase was added to the wells. Following a wash, a substrate solution was added to the wells and color developed in proportion to the amount of VEGF bound in the initial step. The color development was stopped and the intensity of the color was read at 450 nm in a microtiter plate spectrophotometer (Multiskan Ascent-LabSystems). CA 15.3 levels were determined using IMMULITE 2000 BR-MA (Medical System S.p.A., Genova, Italy), a two-step sequential chemiluminescent immunometric assay that used an anti-CA15.3 murine monoclonal antibody, with 0.2 U/ml as the lower limit of sensivity. Of the serum donated by healthy women volunteers 99% contained less than 40 U/mL of CA 15.3 (40). Serum samples were stored at –80°C until the assay was performed, but never for longer than two weeks.

Gelatin zymography. Protein concentrations were measured using the bicinchoninic acid method (Pierce Chemical Co, Rockford, IL, USA).

MMP-2 and MMP-9 quantification was performed using gelatin zymography with HT1080-conditioned medium as standard control. This conditioned medium is a well-known standard for detecting MMP-2 and MMP-9 activity (41). Serial dilutions of the HT1080 preparation, as well as MMP-2 and MMP-9 commercial preparations at known concentrations were analyzed using gelatin zymography. In each experiment, a standard curve using HT1080 at known concentrations was included and serial dilutions of the sample were performed with a linear relationship between sample dilution and gelanolytic activity. Gelatin zymography was prepared as described elsewhere (41). Serum samples were normalized for protein concentration, loaded into the gels and then acquired and quantified with an image-analysis software system (Image Master ID Prime, Pharmacia Biotech, UK). Gels were incubated overnight, stained with Coomassie Blue and destained with methanol acetic acid solution until gelatinolytic areas appeared evident as unstained bands in a blue-stained gel. The gels were then acquired and processed as described elsewhere (41).

Hormone receptor expression (IHC). Estrogen and progesterone receptor (ER, PgR) assays were performed with primary monoclonal antibodies to anti-estrogen and anti-progesterone receptors (anti-human estrogen receptor a1D5-DAKO Diagnostics™, Denmark Inc. and anti-human progesterone receptor 1A6-YLEM srl, Rome, Italy respectively). Cases of intraductal infiltrating carcinoma (IDC) with known reactivity for the antibody were used as positive controls. Sections 4-6 mm-thick obtained from paraffin-embedded tissue were placed on slides, pretreated with endogeneous peroxidase, blocked with hydrogen peroxidase then antigen retrieval was performed using a microwave at 650 W for 3 cycles of 8 minutes each and citrate buffer 1X, 0.07 M, pH 6±1. Subsequently, the slides were incubated, firstly with streptavidin-biotinylated ABC (StreptABComplex) overnight at 4°C, and then with ammino-ethyl-carbazolo (AEC) as chromogen. Slides were examined under light microscopy. The results were recorded as the percentage of positively stained target cells, positive samples being defined as those showing more than 10% stained tumor cell nuclei.

Statistical analysis. Analysis was carried out using the median and interquartile range (IQR) to describe quantitative and continuous variables. The reason for this choice was the non approximate quantitative variables examined in compliance with Gaussian distribution (Wilks test: $p<0.05$).

The correlation among quantitative variables was assessed using Spearman's correlation coefficient; the comparison between independent samples was accomplished by means of a non-parametric test *e.g.* Wilcoxon or Kruskal-Wallis tests. Friedman's test was carried out in order to assess the (eventual) difference between the values of the markers examined during the three follow-up stages. A survival rate analysis was also performed, using as an outcome variable the eventual tumour progression, *i.e* metastasis or recurrent tumour. The comparison between survival rate curves was calculated in compliance with the Kaplan-Meier (42) method and Log-Rank test. A Cox regression method (43), with selection based on a score test, was used to assign to each clinical variable a role as a risk factor in tumour progression; the explanatory variables were: decrease in MMP-9 value (decrease=0, no decrease=1) increase in CA 15.3 value beyond a value of 20 (increase=1; other=0), histotype (CDI=1, other=0), stage of disease (stage 3 or higher=1; other=0). Analyses were carried out using the software SASV8.2 (SAS Institute Inc., Campus Drive, Cary, NC, USA).

Results

Our analysis focused on 88 patients with breast carcinoma (average age 54.5 years±11.42 years) and 20 healthy women (average age 48.7 years±13 years).

The main features of the samples examined are reported in Table I. As to the 88 patients with cancer, no statistically significant relationship was found between protease MMP-2

Table I. *Main features of overall series of patients with breast carcinoma.*

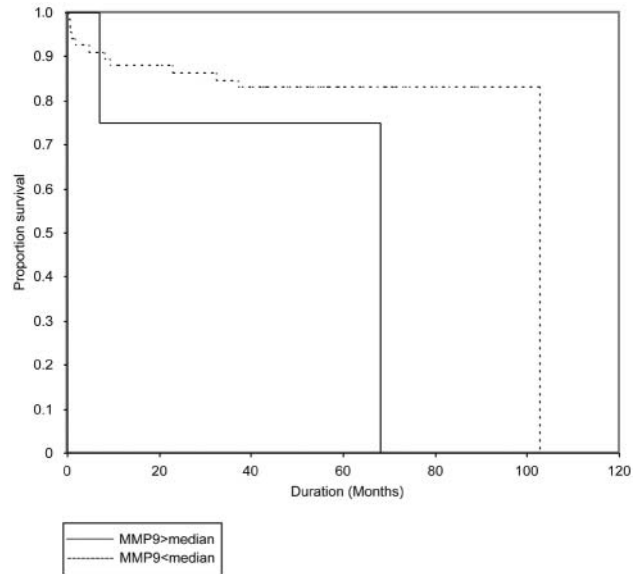| Histotype (n=88)	η	%
Ductal infiltrating carcinoma (DIC)	77	87.5%
Other	11	12.5%
Stage (n=88)		
1	38	43.2%
2	36	40.9%
3	14	15.9%
Grading (n=88)		
1	29	32.9%
2	40	45.5%
3	19	21.6%
ER receptor (n=84)		
Positive	59	70.2%
Negative	25	29.8%
Not available	4	-
PgR receptor (n=88)		
Positive	47	55.9%
Negative	37	44.1%
Not available	4	
Therapy (n=88)		
Chemo	15	17.1%
Radio	1	1.1%
Chemo+radio	25	28.4%
No therapy	47	53.4%
Progression of disease (n=74)		
Relapse or death	14	18.9%
No evidence	60	81.1%

Figure 1. *Overall survival analysis (Kaplan-Meier) of the breast cancer patients according to serum expression of MMP-9 by zymography on the primary tumor.*

and MMP-9 values, MMP-2 and MMP-9 and CA 15.3, MMP-2 and MMP-9 and VEGF.

The only statistically significant correlations found were between basal MMP-2 and MMP-2 one month after surgery (r_s=0.75; p=0.0008) and basal MMP-9 and MMP-9 six months later (r_s=0.44; p=0.009). The relationship between basal CA 15.3 and CA 15.3 after 1 month (r_s=0.57; p=0.01) was noted as significant as well.

The correlation coefficients enable us to note that high values were to be found after only 1 month, in the case of a high protease concentration before surgery.

MMP-2, MMP-9 and CA 15.3 concentrations after 6 months and basal VEGF did not significantly correlate.

The median MMP-2 level was 950.5 ng/ml (range 750-1263 ng/ml) prior to surgery, 946.8 ng/ml (825-1.118 ng/ml) 1 month after surgery and 859.55 ng/ml (694.3-1298.5 ng/ml) 6 months after surgery: the differences in concentrations were not statistically significant (T=0.32, p=0.85; Table II).

The initial MMP-9 level was 150.5 ng/ml (108.5-215.9 ng/ml), which decreased to 107.7 ng/ml (59-153 ng/ml) 1 month later and finally reached 60.4 ng/ml (42.7-129.8 ng/ml) six months later. This decrease was statistically significant (T=16; p=0.0003).

Monitoring of CA 15.3 levels gave 27.8 U/ml (20.2-39.4 U/ml), 23.7U/ml (18.3-35.8U/ml), 23.2U/ml (14-33.6 U/ml) prior to surgery, 1 month or 6 months later respectively, but did not show any statistically significant trend (T=0.66; p=0.716) during the 3 monitoring stages. Concentrations of MMP-2, MMP-9, VEGF and CA 15.3 were compared between different histotypes (ductal infiltrating carcinoma *vs.* other; p>0.05), cancer stage (stage 1 *vs.* other; p>0.05), presence of estrogen or progesterone receptors (p>0.05), but there were no differences between groups. Higher concentrations of CA 15.3 were found in patients with tumor progression (median: 36.2; IQR: 24.8-51.3) with respect to patients without (median: 25.6; IQR: 18.3-35); this difference was statistically significant (W=679.5; p=0.03).

VEGF levels, detected in the early stages of our study and analysed using the Kruskal-Wallis test, did not reveal any statistically significant difference as to any eventual estrogen or progesterone status, histotype (CDS *vs.* others) or illness stage. It is important to note that levels in patients with tumour progression were not significantly different from those in patients without any tumour progression.

No statistically significant differences were found when comparing VEGF levels between 20 healthy women and 88 breast cancer patients (W=1024, p=0.6).

The median value of MMP-9 concentration, when comparing basal values and follow-up, decreased by 30% for each time period, therefore, the role of this decrease in predicting tumour progression should be assessed. For this

reason, it was categorized as "change in MMP-9", with a value of "1" when a decrease in MMP-9 concentration (cut-off threshold ≥100 at first month and ≥60 after the sixth month) was observed, and a value of "0", when no decrease in MMP-9 concentration took place in any follow-up moment. The median survival rate was 102 months for members of group 0, 0.68 months for patients belonging to group 1, although this difference is not to be considered statistically significant (χ^2=2.438, p=0.119; Figure 1). To assess the risk of illness progression linked to some clinical features such as the lack of MMP-9 decrease during follow-up, the increase in CA 15.3 value beyond the threshold 40U/ml, the histotype and stage of disease, a one-variable and multivariate Cox regression scheme was adopted, whereas its selection depended on a Score test. This model was not statistically significant (χ^2=3.47; p=0.0627) and demonstrates that a lack of MMP-9 decrease, an increase in CA 15.3 value, stage ≥3 of tumour and histotype are not statistically significant risk factors.

Discussion

Among more than 15 species of MMPs, MMP-2 and MMP-9 have been most characterized and have been reported to play a crucial role in the degradation of macromolecule structures of connective tissue such as collagens, proteoglycans, laminin and fibronectin (44).

Tumour growth involves alteration in the stromal ECM and malignant tumors often induce a fibroproliferative response in the adjacent stroma (45). The formation of tumour stroma is often viewed as a non-specific host attempt to wall off the tumour and it is thought to have a negative influence on tumour progression. During the process of metastasis formation, malignant cells detach from the primary tumour, invade the stromal tissue, enter the circulation, arrest at the peripheral vascular bed, extravasate, invade the target organ interstitium and parenchyma, and form a metastatic colony. Tumour cells must escape the host immune surveillance and therefore only a fraction of circulating tumor cells successfully initiate metastatic colonies (46). Angiogenesis is a crucial component in the growth invasive progression and metastatic spread of solid tumours. Tumour-induced angiogenesis is essential for growth of the primary tumour and metastases, and new blood vessels are also frequent sites for tumor cell entry into the circulation. It is conceivable that several factors are responsible for degradation and angiogenesis. Several potential regulators of angiogenesis have been identified.

The proangiogenic cytokine, VEGF, not only stimulates the proliferation and migration of endothelial cells, but also activates inactive pro-MMPs to active MMPs and influences the activity of tissue inhibitor of MMPs (TIMPs); the MMPs degrade the vascular basal membrane and ECM proteins

Table II. *Median and interquartile range (first quartile Q1 and third quartile Q3 are reported) of markers in different groups of patients.*

| | | Histotype | | Stage | | | ER Receptor | | PgR Receptor | | Progression of disease | |
		IDC n=77	Other n=11	1 n=38	2 n=36	3 n=14	Positive n=59	Negative n=25	Positive n=47	Negative n=37	Evidence n=14	No evidence n=60
VEGF	Median	328	528.3	272.9	442	560.7	328	420.1	323.4	420.2	477	330.6
	Q1-Q3	186.6-699.7	335.6-923.5	151.3-482.5	223.8-737.7	297.1-787.5	183.6-699.7	243.9-769.7	179.6-705.8	243.9-681.4	305.5-791.1	187.8-701.5
MMP-9 (baseline)	Median	148.8	181.6	175.3	144.7	108	153	143.8	153	143.8	113.7	152.6
	Q1-Q3	106.5-209.4	116.1-246	119.4-198.7	110.6-248.7	69.1-130.2	114.7-230.1	91.8-211.6	112-240.1	106.5-211.6	78.1-203.8	111.3-263.1
MMP-9 (1 month)	Median	105.7	144.5	144	107.7	45	107.7	111.2	109.7	105.7	69.5	103
	Q1-Q3	58-152	93.5-232.2	85-181	60-127	20-81	59-152	79.5-174.5	59-169	59-148	38-110.5	59.5-144.5
MMP-9 (6 months)	Median	59.1	137.9	56.6	62	94.5	68.4	59.1	73.5	57.2	55.3	69.6
	Q1-Q3	41.3-119.3	49.7-170.5	40.3-117.9	42.3-127.6	55.7-135.8	43.9-123.7	41.7-133.9	48.1-132.2	40-129.8	36.6-99.9	46.6-134.4
MMP-2 (baseline)	Median	952	942.3	918	957	1124	941	957	869	996	970.5	953.5
	Q1-Q3	753-1242	589-1284	723-1140	750-1314	759-1383	747-1364	753-1224	736-1284	893-1344	738-1383	737-1215
MMP-2 (1 month)	Median	957.6	903.9	966.5	990.1	817.5	957.6	893.3	946.8	939.3	976.1	905.5
	Q1-Q3	825-1125.5	839.3-988.4	853.7-1148.9	883.5-1118.4	738-825	825-1118.4	831.9-1115.8	825-1172.2	831.9-1112.3	450-1118.4	835.6-1013.6
MMP-2 (6 months)	Median	953	848.6	1041.3	891.4	806.2	898.5	955.9	894.1	955.9	998.1	851.1
	Q1-Q3	722.5-1315.5	601.5-920.1	771.5-1320.9	694.3-1315.5	586.9-1108.8	694.3-1298.5	762-1315.5	694.3-1206	735.3-1405.3	806.4-1343.4	686.6-1156.2
CA 15.3	Median	28.4	20.7	26.2	27.4	37.6	28.2	26.2	26.7	32	36.2*	25.6*
	Q1-Q3	20.8-39.7	15.7-33.7	20.3-37.8	17.2-40.1	29.5-44.9	19.8-41	20.3-35.7	16.9-39.2	20.7-41	24.8-51.3	18.3-35
CA 15.3	Median	23.4	25.7	21	23.1	43.7	24.4	23.7	23.1	30	23.1	22.5
(1 month)	Q1-Q3	18.1-35.8	18.9-48.1	18.3-26.2	17.8-40.8	43.3-45.6	17.9-35.5	18.9-36.1	18.1-32.3	18.6-46.8	22.8-43.3	17.9-35.9
CA 15.3	Median(a)	25.8	19.7	18.5	25.8	33.1	25.3	19.8	22.8	25.8	25.3	22.8
(6 months)	Q1-Q3	14-33.6	10.9-23	12.1-30.2	15-33.6	22.8-34.7	17.3-35.8	11.9-31.2	14-35.9	13-33.1	10.9-45.7	13.8-33.6

*Statistically significant.

and, therefore, enable the migration of endothelial cells and formation of new blood vessels (47, 48).

In many studies the expression of MMPs and VEGF in resected tumour tissue was determined.

Many authors, admitting that serum levels reflect their expression in tumour tissue, measured serum levels of MMPs and VEGF to evaluate their role in neoplastic growth. Lamari *et al.* have reported the effect of luteinizing hormone-releasing hormone (LHRH or GnRH) on regulation of expression of MMPs and their tissue inhibitor (TIMP-2) in the MCF-7 breast cancer cell line (49). The determination of angiogenic cytokine levels in the blood may help to assess which of them can be applied as diagnostic and prognostic markers and to evaluate the efficacy of MMP inhibitors (50-53).

In the present study, we investigated the correlation between serum expression of gelatinases and serum levels of VEGF and CA 15.3 before surgery and after 1 and 6 months.

Our data show no correlation between MMP-2 serum levels before and after surgery suggesting that this gelatinase may not play a significant role in the development of tumours. MMP-9 serum levels showed significant changes in patients with breast cancer, in fact, MMP-9 drastically decreased in all patients after surgery both at 1 and 6 months, suggesting that MMP-9 may be of prognostic significance. The statistically significant decrease of MMP-9 levels was noted in breast cancer patients with respect to healthy women suggesting that this protease could be a marker of the tumour presence. We did not find any relationship between VEGF levels in the sera and the stage of disease, or with survival, but the limited number of patients might account for these results. Nor do our results show a significant correlation between VEGF levels before surgery and the outcome of disease.

Several authors reported that CA 15.3 is one of the most specific tumour markers in the differentiation of malignant from benign disease, particularly for breast cancer; we found that circulating levels of CA 15.3 were higher in breast cancer patients with progression or metastatic disease.

It is possible that MMPs are not directly produced by cancer or stromal cells, but there may be other sites responsible for increased levels of MMP-9 that correlate with the existence of tumour tissues.

The lack of a correlation between MMP, VEGF, CA15.3 and clinicopathological characteristics suggests that these proteases and cytokines are dependent on neoplasm activity and not on the stage of disease or histological type of tumour.

In conclusion, this study suggests that high CA 15.3 and MMP-9 levels in the sera of breast cancer patients, as demonstrated in our previous study (54), correlated with a poor prognosis and survival, while a large numbers of patients will be necessary to establish whether high levels of VEGF and MMP-2 are predictive of a more aggressive tumour.

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