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RESEARCH ARTICLE

Low serum MMP-1 in breast cancer: a negative prognostic factor?

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Abstract

In this study we investigated the prognostic significance of serum matrix metalloproteinase (MMP)-1 levels in early-stage breast cancer patients and correlated these levels with various clinicopathologic parameters. MMP-1 levels were determined by enzyme-linked immunosorbent assay. MMP-1 serum levels in patients ($n = 60$) were significantly lower than in healthy subjects ($n = 20$, $p < 0.0001$). We found significant negative correlation between serum levels of MMP-1 and several negative prognostic factors of breast cancer. Kaplan–Meier analysis showed significantly shorter 5-year survival in patients with lower values of MMP-1 compared to those with high levels of MMP-1 ($p = 0.0147$). Our results suggest a negative prognostic role of low serum MMP-1.

Keywords: Proteases, prognosis, clinicopathological correlation

Introduction

Invasive breast cancer is one of the most common forms of cancer in Europe and worldwide. In Croatia, breast cancer accounts for 27% of all cancers in women and is the second major cause of cancer deaths (Cancer registry 2011). Currently, prognosis and choice of treatment is based on histological tumor type, grade, tumor size, lymph node involvement, steroid hormone receptor expression and HER-2 status. However, assessment of these clinical and pathological features does not enable us to fully capture the heterogeneous clinical course of breast cancer. Therefore, there is a continuous search for new biomarkers of invasion and metastases.

The extracellular matrix (ECM) is regarded as a barrier to tumor progression. Cleavage of its components by proteases is assumed to remove the physical obstruction and allow cell migration and tumor invasion. The tumor microenvironment is expansively modified and remodelled by proteases (Mbeunkui & Johann 2009). The matrix metalloproteinases (MMPs) in humans are a family of zinc dependent endopeptidases, with 23 family members, that have classically been associated with remodelling of the ECM and can degrade virtually all ECM components (Vu & Werb 2000, Egeblad & Werb 2002). MMPs belong to the family of zinc endopeptidases collectively referred to as metzincins. The metzincin super-family is distinguished by a highly conserved motif containing three histidines that bind to zinc at the catalytic site and a conserved methionine that sits beneath the active site. The metzincins are subdivided into four multigene families: seralysins, astacins, ADAMs/adamalysins, and MMPs. There are five main subgroups of MMPs, according to their substrate specificity and domain structure: collagenases, gelatinases, stromelysins, matrilysins, and membrane-type MMPs. Because of their involvement in processing of the ECM, MMPs were implicated in cancer invasion and metastasis. Consistent with this hypothesis, multiple data from model systems suggested that specific MMPs were causally involved in metastasis (Fingleton 2006). Tissue MMPs may enter into the blood stream and increase their circulating levels (Zucker et al. 1999). Thus, MMP levels in the blood may serve as biological markers for disease onset or progression, and allow monitoring of the disease. Several studies

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have evaluated the diagnostic and prognostic value of circulating MMP-2 and MMP-9 in breast cancer because of their important role in tumor invasion and metastasis (Vihinen & Kähäri 2002, Turpeenniemi-Hujanen 2005).

The involvement of MMPs in cancer dissemination led to the development of MMP inhibitors (MMPIs) for the treatment of malignancy (Watson & Tierney 1998, Fingleton 2007). Results from clinical trials were disappointing due to poor efficacy and toxic side-effects.

In this study, we evaluated circulating levels of MMP-1 as diagnostic and prognostic markers in breast cancer. We also correlated MMP-1 levels with standard prognostic indicators.

Material and methods

Patients

The study included 60 patients with primary invasive breast carcinoma operated between March and September 2003 at the University Hospital Center Zagreb, Croatia. All specimens were obtained through routine surgery. No patient received irradiation treatment or chemotherapy before surgery. Blood samples and clinical information were obtained under Institutional Review Board approval. Our patients received therapy in accordance with national guidelines (based on St. Gallen's recommendations). We analyzed the following data of each patient: age patients, tumor size, axillary lymph node metastases, histological grade of the tumors, concentrations of estrogen receptor (ER) and progesterone receptor (PR), cathepsin D concentration in cytosol, HER-2 protein expression in tumor, serum levels of HER-2/neu ECD, and anti-p53 antibodies. Patient's data are listed in Table 1.

Table 1. Characteristics of patients and tumors.

	$N = 60$	
Patients with breast carcinoma	Number	(%)
Age (years)		
<50	37	62
>50	23	38
Size		
<2 cm	39	65
>2 cm	21	35
Axillary lymph node		
Negative	39	65
Positive	21	35
Histological grade		
Well differentiated (I)	18	30
Moderately differentiated (II)	20	33
Poorly differentiated (III)	22	37
Estrogen receptor (ER)		
<10 fmol/mg proteins	29	48
>10 fmol/mg proteins	31	52
Progesteron receptor (PR)		
<20 fmol/mg proteins	27	45
>20 fmol/mg proteins	33	55
Cathepsin D		
High value (>45 fmol/mg proteins)	25	42
Low value (<45 fmol/mg proteins)	35	58
HER-2		
Negative	46	77
Positive	14	23
HER-2/neu ECD		
High (>15 μg/mL)	28	47
Low (<15 μg/mL)	32	53
Anti-p53Abs		
<15 U/mL	40	66
>15 U/mL	20	34

Serum

Blood samples were taken from 60 patients with breast cancer and 20 women without malignancy and allowed to clot. Blood samples were retrieved before operation. After centrifugation serum was removed and stored at –20°C until required.

Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay (ELISA) for serum MMP-1 was performed using commercially available ELISA kit (ELISA Cat #QIA30 kit, Amersham Pharmacia Biotech, Buckinghamshire, UK). The analysis was performed according to the manufacturer's instructions. Calculation of results was achieved by the construction of standard curve. The cut-off value was defined with receiver operator characteristic (ROC) analysis as 4.52 ng/mL.

Steroid receptors

ER and PR were determined by using the ligand-binding method according to Horwitz and McGuire (Horwitz & McGuire 1977). A positive receptor status for ER and PR was defined as the presence of 10 fmol/mg of cytosol proteins and 20 fmol/mg of cytosol proteins, respectively.

Cathepsin D

Cathepsin D was determined by immunoradiometric assay. Cathepsin D values higher than 45 pmol/mg of cytosol proteins were considered as high.

HER-2/neu ECD

HER-2/neu ECD values in the serum were determined using ELISA method. The cut-off value was defined as 15 μg/mL.

Anti-p53 antibodies

Anti-p53 antibodies (Anti-p53Abs) in the serum were assessed using ELISA method. The cut-off value was defined as 15 U/mL.

Statistical analysis

Statistical analysis was performed using MedCalc software. Spearman rho correlation coefficient was used to determine the significance of the different clinical and pathohistological factors. Distribution of serum MMP-1 for the group of breast cancer patients and the control

group is shown using box-and-whisker plot. $p < 0.05$ was considered statistically significant. Survival curves were calculated by Kaplan–Meier method and the statistical significance was determined by the log-rank test. The MMP-1 cut-off value was determined by the ROC analysis.

Results

ROC analysis showed a good diagnostic efficiency of MMP-1 in differentiation between breast cancer patients and the control group. Area under the curve (AUC) was 0.952 (95% confidence interval 0.880–0.987) with a sensitivity of 82% and specificity of 100% at a cut-off value of 4.52 ng/mL (Figure 1). Of 60 patients with invasive breast cancer, 47 (79%) had MMP-1 value lower than 4.52 ng/mL (range from 2.12 to 4.50) and 13 (21%) patients had a value higher than 4.52/mL (range from 4.52 to 6.61). Out of 20 healthy subjects, none had a lower value of 4.52 ng/mL in serum (average value was 5.71 ng/mL, range from 4.53 to 6.2). MMP-1 levels in serum of patients with breast cancer were significantly lower than in healthy subjects (Figure 2, $p < 0.0001$).

We found a statistically significant negative correlation between levels of MMP-1 in serum and tumor size, involvement of axillary lymph nodes, the value of cathepsin D in tumors and the concentration of antibodies to p53 in serum (Figures 3–6). There was no correlation between levels of MMP-1 in serum and patient age, histological grade of tumor, HER-2 protein expression in tumor, concentration of estrogen and progesterone receptors or the presence of the extracellular domain of HER-2 in serum.

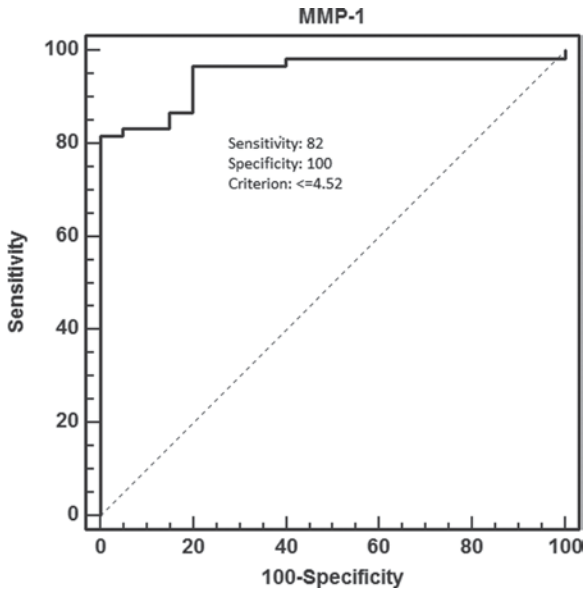

Figure 1. Reciver operator characteristic (ROC) curve for MMP-1.

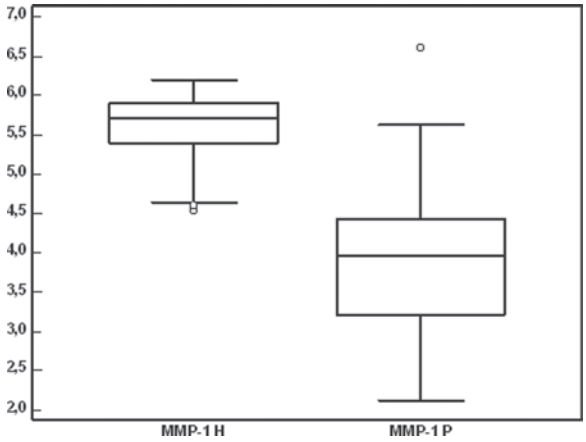

Figure 2. Distribution of serum MMP-1 for the control group and the group of breast cancer patients (box-and-whisker plot, $p < 0.0001$). The concentrations of MMP-1 are shown on the y-axis (ng/mL) (H, healthy control; P, patients).

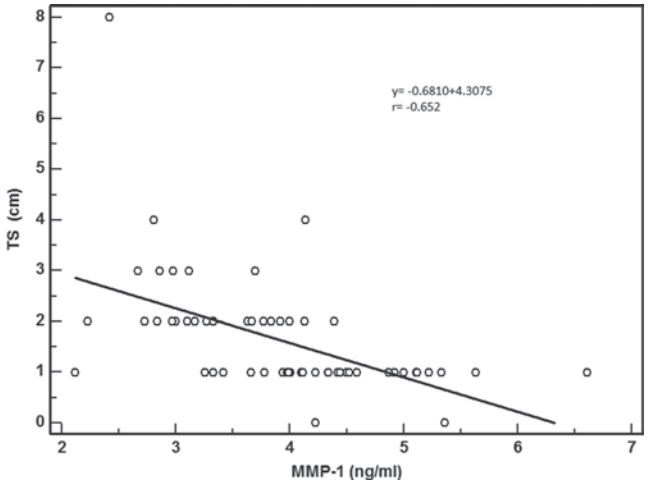

Figure 3. Correlation between serum MMP-1 and the tumor size (TS, tumor size; $p < 0.0001$, 95% CI 0.777–0.477).

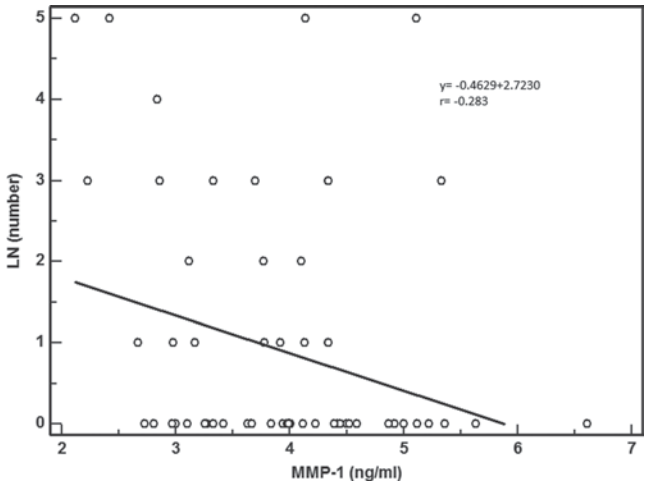

Figure 4. Correlation between serum MMP-1 and the number of involved axillary nodes (LN, number of lymph nodes; $p = 0.0295$, 95% CI 0.501–0.0317).

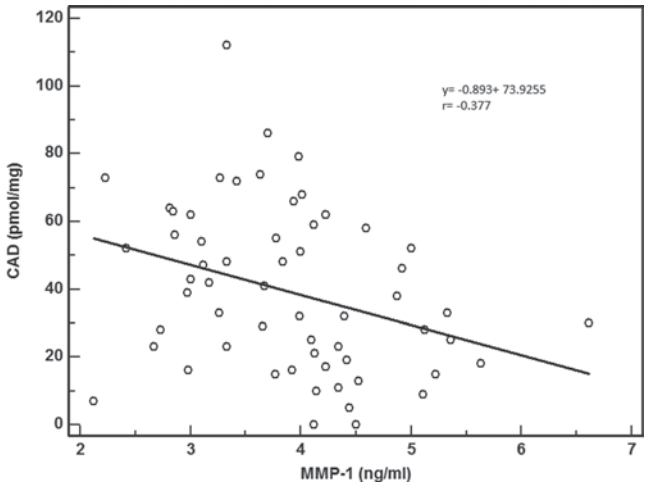

Figure 5. Correlation between serum MMP-1 and the cytosol concentration of cathepsin D. (CAD, Cathepsin D; $p = 0.0038$, 95% CI 0.576–0.136).

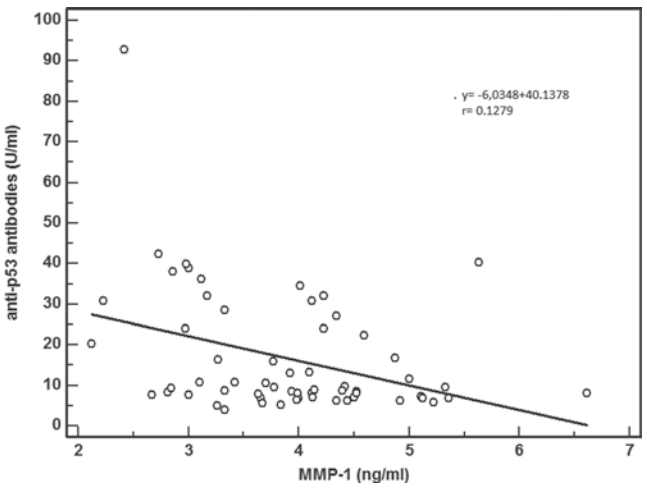

Figure 6. Correlation between serum MMP-1 and serum anti-p53 antibodies. ($p = 0.0089$, 95% CI 0.574–0.0948).

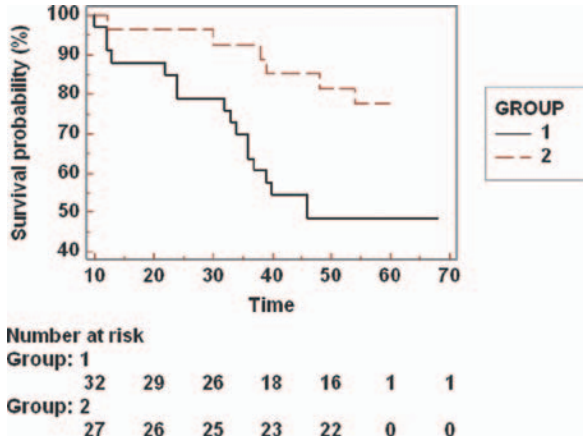

Figure 7. Kaplan–Meier survival curves according to the serum concentrations of MMP-1 in breast cancer patients ($p = 0.0147$). (Group1 = MMP-1 < 4.52 ng/mL, Group2 = MMP-1 > 4.52 ng/mL).

Kaplan–Meier analysis showed a worse prognosis (5-year survival) in patients with lower values of MMP-1 in serum compared to those with high levels of MMP-1 in serum (Figure 7, $p = 0.0147$). Median duration of follow-up was 60 months.

Discussion

Several studies have evaluated prognostic value of serum and plasma MMP levels in breast cancer (Vihinen & Kähäri 2002, Turpeenniemi-Hujanen 2005). In our study we have found differences in total MMP-1 levels in serum from patients with different clinical and pathological characteristics.

We found significantly lower serum MMP-1 levels in breast cancer patients than in healthy controls ($p < 0.0001$). ROC analysis revealed an AUC of 0.952 with a sensitivity of 80% and specificity of 100% at a cut-off value of 4.52 ng/mL serum MMP-1. These high sensitivity and specificity rates suggest that serum MMP-1 may be a good candidate biomarker for breast cancer in clinical use. In the study of Decock et al. the cut-off value was very similar to ours: 4.24 ng/mL, with a sensitivity of 80% and specificity of 24%. The authors concluded that low specificity hampers the clinical use of circulating MMP-1 due to possible significant overtreatment of healthy individuals (Decock et al. 2008). Despite of these differences in specificity, it cannot be excluded that serum MMP-1 measurement in combination with assessment of other biomarkers may improve breast cancer diagnosis.

In our study tumor size correlates inversely with serum MMP-1 levels ($p < 0.0001$, $r = -0.652$). Patients with greater tumors have significantly lower serum MMP-1 levels than those with smaller one. It is concordant with some earlier studies which have also shown a trend of lower plasma MMP-1 levels with increasing tumor size (Decock et al. 2008). Similarly, we have found a greater serum MMP-1 levels in patients with lower cathepsin D in tumor tissue ($p = 0.0038$, $r = -0.377$). We have found a trend of lower MMP-1 levels with increasing number of involved lymph nodes ($p = 0.0295$, $r = -0.283$) and also with increasing concentration of serum anti-p53 antibodies ($p = 0.0089$, $r = -0.348$). In some disease models p53 has been shown to play a role in ECM homeostasis and inflammatory response (Ghosh et al. 2004, Marsolais et al. 2007). The importance of this observation for tumorigenesis remains to be elucidated.

Analyzing prognostic significance of serum MMP-1 levels on overall survival (OS) of patients with primary breast cancer, we have found that subgroup of patients with serum MMP-1 levels 4.52 ng/mL or higher has better prognosis than those with lower serum levels. This seems to be in contradiction with previous results demonstrating a clear correlation between MMPs overexpression and poor prognosis. McGowan and Duffy (2008) have shown in their study that increased

expressions of MMP-1, -9, -12, -14, and -15 were significantly associated with poor OS. The recent study of Boström et al. (2011) showed that MMP-1 expression in breast cancer cells is significantly associated with poor clinical outcome. However, our results are compatible with those of Remacle et al. These authors observed that patients with higher levels of a 50 kDa gelatinase had a significantly better survival than patients with low levels (Remacle et al. 1998).

Besides components of ECM, other substrates of MMPs have been identified. They include: growth factors, growth factor-binding proteins, cell surface receptors, cell adhesion molecules, chemokines and cytokines (Fowlkes et al. 1994, Mañes et al. 1999, Rifkin et al. 1999, Noë et al. 2001, Sternlicht & Werb 2001, McQuibban et al. 2002). The result of these activities may be additional to pro-tumor effect of MMPs. However, some protective, anti-tumor activities of some MMPs have been described. In experimental models of breast cancer, increased expression of a collagenase MMP-8 resulted in decreased metastatic potential (Agarwal D et al. 2003, Montel et al. 2004). Some MMPs play role in generation of anti-angiogenic molecules including angiostatin, endostatin and tumstatin (Folkman 2004, Martin & Matrisian 2007). McQuibban et al. (2002) reported that some MMPs (including MMP-1) cleaved monocyte chemoattractant proteins generating CC chemokine receptor antagonists with anti-inflammatory properties. Inflammation has been recognized as an important factor that may play protumorigenic role (Hanahan & Weinberg 2011).

In conclusion, our results suggest relationship between low levels of serum MMP-1 and adverse prognosis in breast cancer patients. We also found an inverse correlation between serum levels of MMP-1 and some negative established or potential prognostic factors such as: tumor size, lymph node involvement, cathepsin D and serum anti-p53 antibodies. Thus, we speculate that low serum MMP-1 levels may present a negative prognostic factor in breast cancer patients. Our observation needs validation in a study with a larger number of patients. Studying of various aspects of MMPs functions is important for better defining whether certain MMP presents a target or an anti-target for cancer therapy.

Declaration of interest

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