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Detection of Stromelysin in Synovial Fluid and Serum from Patients with Rheumatoid Arthritis and Osteoarthritis

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Summary **Stromelysin levels were measured using a one-step sandwich immunoassay in synovial fluid (SF) obtained from 31 patients with rheumatoid arthritis (RA) (31 samples) and 13 patients with osteoarthritis (OA) (13 samples) and in serum from 81 patients with RA (106 samples), 12 with OA (14 samples), 12 with gouty arthritis (gout) (14 samples), and 8 with osteoporosis (OP) (14 samples) to identify differences in the levels in these diseases as well as correlations with clinical parameters in RA. SF stromelysin levels were significantly higher in RA than in OA, and rose with increasing joint destruction in the former. No significant correlations were found between the SF stromelysin level in RA and various clinical parameters, except for the volume of SF which showed a correlation. Serum levels of stromelysin were highest in RA, gout, OA, and osteoporosis in decreasing order, and in RA were correlated with the Steinbrocker Stage. A significant correlation was also found between the serum stromelysin level and number of swollen joints, and correlations with the Lansbury index, ESR, CRP, WBC and Plt. The stromelysin level in SF was thought to be a useful parameter of local joint involvement and that in serum of the severity of systemic joint inflammation.**

Key words **Stromelysin, Rheumatoid Arthritis, Osteoarthritis, Serum, Synovial Fluid, Lansbury Index.**

INTRODUCTION

Chronic RA and OA are characterized by destruction of joint cartilage, with degradation of extracellular matrix an important factor in both conditions. Neutral matrix metal protease (MMP) plays an important role in the degradation of matrix in RA and OA. Collagenase (MMP-1) digests types I, II and III collagen, while gelatinase (MMP-2) digests gelatins degenerated from collagen and types IV and V collagen (1). Stromelysin (MMP-3) has been clarified to digest proteoglycans, types IV, VII, IX, and XI collagen, and also fibronectin (2). Although type IX collagen is a minor constituent of cartilage collagen, it is believed to play an important role in maintaining the structural integrity of this tissue. Thus, stromelysin, which digests not only proteoglycans but also type IX collagen, is considered to play a significant role in matrix destruction. In the present study, the authors compared stromelysin levels in the SF and serum of patients with RA, OA, and other joint diseases, and determined correlations between these levels and the degree of joint destruction and disease activity in RA.

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MATERIALS AND METHODS

Synovial fluid

Stromelysin was measured in SF obtained from 31 patients with RA (31 samples from 31 knee joints) and 13 patients with OA (13 samples from 13 knee joints). The patients with RA comprised four men and 27 women with a mean age of 61.4 years (range: 50-79 years) and a mean duration of disease of 14.5 years (3-39 years). All of the patients satisfied the conditions of RA based on the diagnostic criteria of the American Rheumatism Association (ARA; 1987). The patients

Table I: *Correlations between stromelysin levels in serum and SF from patients with RA and clinical parameters*

Clinical parameters	Number of patients	Spearman's correlation
Lansbury index	106	0.42($p<0.01$)**
Joint score (Lansbury Index)	106	0.52($p<0.01$)**
CRP	106	0.52($p<0.01$)**
ESR	106	0.38($p<0.01$)**
WBC	106	0.31($p<0.01$)**
Platelet	106	0.32($p<0.01$)**
Number of swollen joints	106	0.43($p<0.01$)**
RA-Test	106	0.10($p>0.1$)
RAHA	106	0.11($p>0.1$)
SF volume*	70	0.38($p<0.01$)**

ESR = erythrocyte sedimentation rate, CRP = C reactive protein, * synovial fluid from knee joints ** $p<0.01$.

with OA consisted of two men and 11 women with a mean age of 70.5 years (33-86 years).

Serum

Serum samples obtained from 81 patients with RA (106 samples including multiple samples from some patients), 12 patients with OA (14 samples), 12 patients with gout (14 samples), and 8 patients with OP (8 samples) were used. The patients with RA comprised 19 men and 62 women with a mean age of 54.1 years (23-80 years) including 12 patients (19 samples) from whom SF samples were obtained at the same time. The patients with OA consisted of two men and ten women with a mean age of 66.8 years (52-78 years). The 12 patients with gout, all of whom were male, had a mean age of 73.0 (48-81 years), while the 8 patients with OP were all female and had a mean age of 67.8 (64-74 years). The samples were immediately frozen at -40°C, and measurements performed as a rule within 3 months.

A one-step sandwich enzyme immunoassay for stromelysin-1 (MMP-3)

MMP-3 concentration in synovial fluid and serum was determined according to the method of Obata et al. (3), as follows. The assay system used two simultaneous immunoreactions using a solid phase monoclonal antibody and a horseradish peroxidase labeled monoclonal antibody (Fab'). The sensitivity of the assay system was 20μg/l and linearity was obtained between 31 and 500μg/l. Active MMP-3 prepared by incubation with APMA or plasmin showed a loss of assay reactivity to 29-47% of the original levels of proMMP-3. When active MMP-3 was complexed with TIMP-1 or TIMP-2, the levels were restored to 54-62% or 33-53%, respectively. ProMMP-3 assay reactivity was not changed in the presence of α2-M. The cross-reactivity of the antibodies utilized in the assay system cross-reacted negligibly with proMMP-1, proMMP-2, proMMP-9, fibronectin, type IV collagen and laminin P1 fragments. The intra-assay and inter-assay C.V.s of normal and pathological serum were 4.5-7.3% and 6.0-8.0% ($n=10$), respectively.

Clinical parameters in RA patients

As indices of disease activity the Lansbury Index (4), CRP (c-reactive protein; mg/dl), ESR (erythrocyte sedimentation rate; mm/hr), RA-test, and RAHA titer (IU/1) were used. As an index of the severity of joint destruction the most recently obtained X-ray films classified according to Steinbrocker (e.g. Roentgenologic Stage) (5) were examined. In addition, serum IgG, IgM, IgA, C3 and C4 (all mg/dl), white blood cells (WBC), platelets (Plt), duration of morning stiffness (MS; min), disease duration, grip strength (GP; mmHg using a manometer), the number of swollen joints, the number of painful joints, and the volume of SF were determined and correlations with stromelysin levels in each disease examined.

Statistical analysis

The stromelysin data were analyzed using a one way analysis (ANOVA). Relationships between the stromelysin level and clinical parameters were examined by Spearman's rank correlation. Correlation coefficients and P values <0.05 were considered statistically significant.

RESULTS

Stromelysin levels in SF from patients with RA and OA

Stromelysin levels in SF were significantly higher in the patients with RA than in those with OA (180.9 ± 18.3μg/ml vs. 29 ± 8μg/ml respectively, $p<0.01$) (Fig. 1). No significant correlations were found between SF stromelysin levels in the RA patients and the Lansbury Index, ESR, CRP, RA-test, RAHA, IgG, IgA, IgM, C3, C4, WBC, Plt, GP, disease duration, or MS, although a correlation was seen with the volume of SF ($r=0.48$, $p<0.01$) (Table I). Stromelysin levels in SF from the patients with RA increased in parallel with increasing Steinbrocker Stage: 62.9 ± 10.6μg/ml in Stage

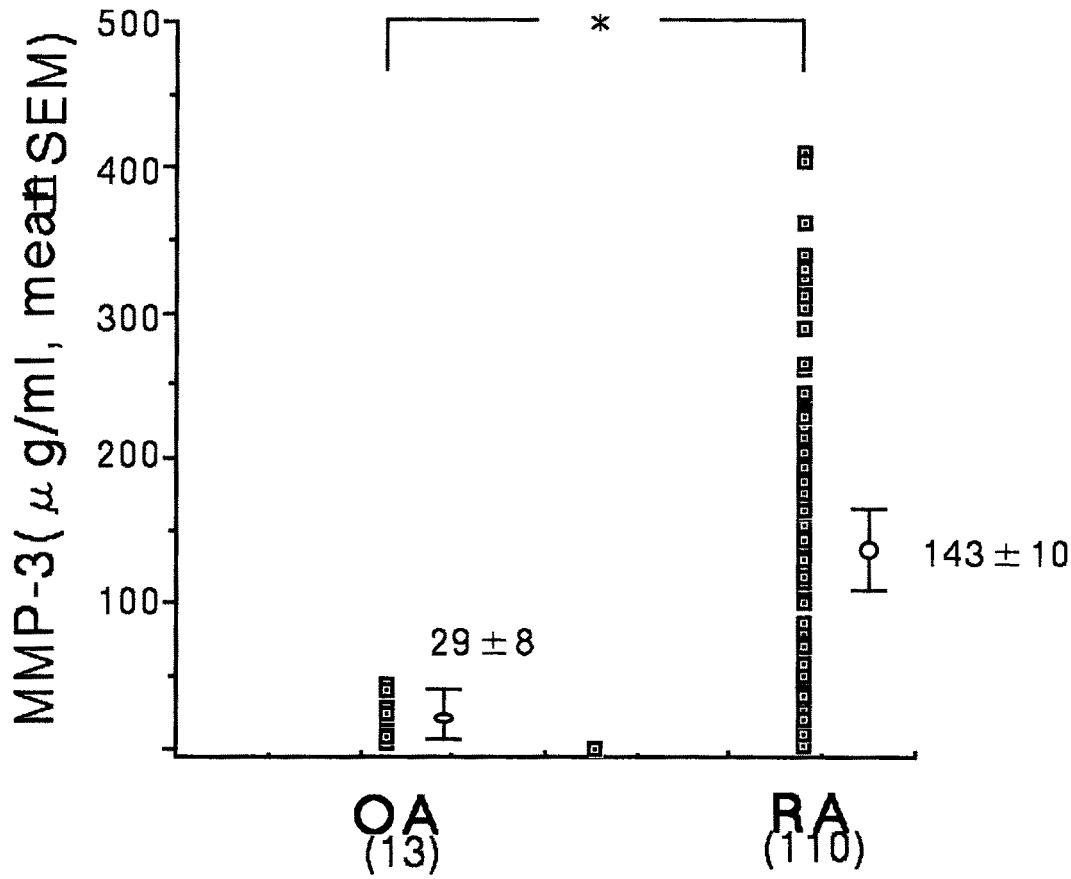

Fig. 1: Stromelysin levels in synovial fluid (SF) from osteoarthritis (OA) and rheumatoid arthritis (RA) patients. Levels of stromelysin were measured using a sandwich EIA. The values are mean±SEM. *p<0.01(ANOVA).

I, 113.1±14.3μg/ml in Stage II, and 250.6±33.0μg/ml in Stage III, and significant differences were found between Stages I and III ($p<0.01$), and Stage II and III ($p<0.05$) (Fig. 2).

Correlations between serum stromelysin levels and clinical parameters in patients with RA

Serum stromelysin levels were significantly correlated with CRP ($r=0.52$, $p<0.01$) and the joint score of the Lansbury Index ($r=0.52$, $p<0.01$), and weakly correlated with the Lansbury Index ($r=0.42$, $p<0.01$), number of swollen joints ($r=0.43$, $p<0.01$), ESR($r=0.38$,$p<0.01$), WBC($r=0.31$,$p<0.01$) and Plt. (0.32,$p<0.01$) (Table I). No significant correlations were found between serum stromelysin levels in the RA patients and disease duration, RA-test, RAHA, IgG, IgA, IgM, C3, C4, GP or MS ($p>0.1$). Stromelysin levels in serum increased in parallel with increasing Steinbrocker stage: 145.9±38.9ng/ml in Stage I, 146.9±40.9ng/ml in Stage II, 358.2±60.7ng/ml in Stage III, and 559.1±129.9ng/ml in Stage IV; the difference between Stages I and IV was statistically significant ($p<0.05$) (Fig. 3).

Stromelysin levels in serum from patients with RA, OA, gout and OP

Stromelysin levels in serum were highest in the patients with RA (348.3±43.94ng/ml) followed by, in decreasing order, those with gout (164.8±26.5ng/ml), OA (68.8±15.9ng/ml), and OP (33.8±12.4ng/ml). Significant differences were found between RA and OA ($p<0.01$), RA and gout ($p<0.01$), and RA and OP ($p<0.01$) (Fig. 4).

Correlation between stromelysin levels in serum and SF from patients with RA

Stromelysin levels in serum and SF obtained at the same time from 12 patients with RA (19 samples) showed a significant positive correlation ($r=0.659$,$p<0.01$).

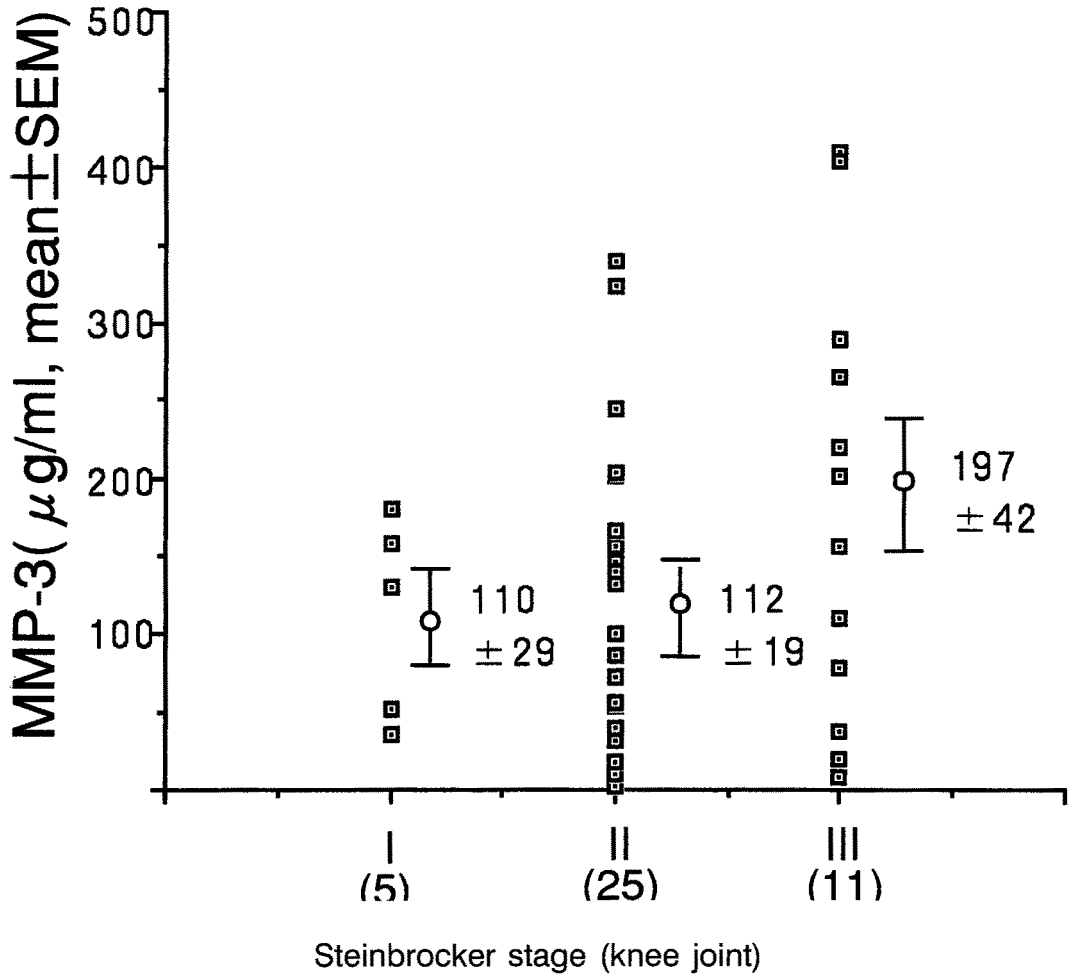

Fig. 2: Stromelysin levels in synovial fluid(SF) from patients with rheumatoid arthritis (RA) classified according to Steinbrocker Stage (knee joint). Stage I(n=5), Stage II-(n=17), Stage III(n=9) Stage IV(n=0). **p<0.01 *p<0.05(ANOVA).

DISCUSSION

Collagen and proteoglycans, which are the major constituents of cartilage matrix, are mainly degraded by a number of matrix-reducing enzymes that are active in the neutral range of pH. First, the intermolecular bridges of collagen fibers are reduced by elastase, then collagen helixes are reduced by collagenase (MMP-1) and form degenerative collagen, and are finally reduced to lower molecules by gelatinase (MMP-2). In addition, proteoglycans are reduced by stromelysin (MMP-3) which acts on the hyaruronate acid binding region of proteoglycans. Stromelysin is secreted by chondrocytes, fibroblasts, and synovial cells as an inactive proenzyme, and then activated by other enzymes and activating proteins (6,7). Recent studies have demonstrated that interleukin-1 (IL-1) and tumour necrosing factor α (TNF-α) are produced by chondrocytes and synovial cells and induce the production of collagenase and stromelysin (8,9), and also interleukin-6(IL-6) dose stimulates the production of TIMP and augments the IL-1 stimulated production of collagenase and stromelysin (10). It is known that these activating systems exist in both RA and OA, but in different degrees. Our results and those of other investigators have shown that stromelysin levels in SF from patients with RA are significantly higher than those from patients with OA (11). Martel-Pelletier, et al. (12) measured stromelysin levels in joint cartilage from patients with OA and reported 3-10-fold higher stromelysin activity as compared to normal cartilage, with increasingly high levels paralleling increasingly severe OA changes, and in their recent study, they found that OA cells (chondrocytes) increased the levels of IL-1 receptor more than those of normal cells (chondrocytes) (13). In the present study as well, stromelysin levels in SF from patients with RA were increased in parallel with the degree of joint destruction, with stromelysin levels in these patients 4-6-fold higher than

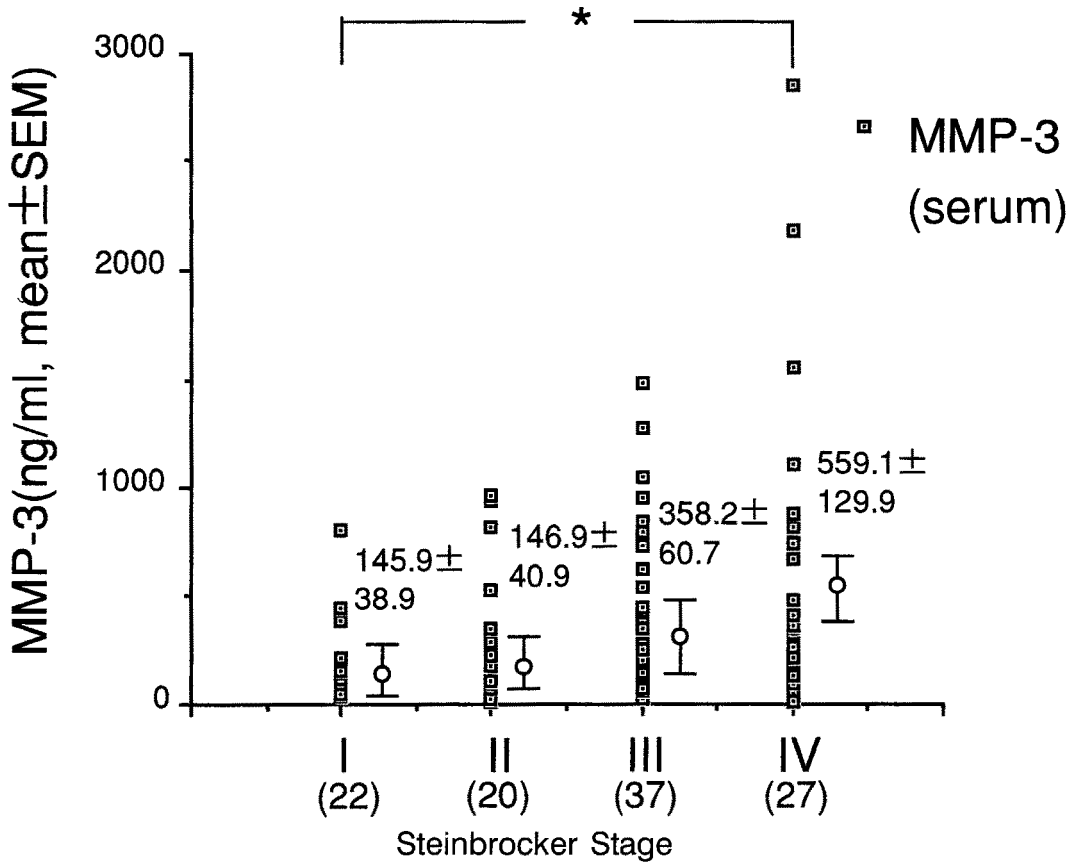

Fig. 3: Stromelysin levels in serum from patients with rheumatoid arthritis (RA) classified according to Steinbrocker Stage. Stage I(n=22), Stage II(n=20), Stage III(n=37), Stage IV(n=27) *p<0.05(ANOVA).

those in patients with OA. However, the stromelysin levels in SF from the patients with RA were not significantly correlated with CRP, ESR, or the Lansbury Index, all of which are markers of disease activity, or in any other parameters. Only the volume of SF from the patients with RA showed a correlation with stromelysin levels in SF, with this result thought to reflect cartilage matrix degradation in the affected joint rather than the degree of systemic inflammation or RA activity.

Serum stromelysin levels were highest in the patients with RA, and tended to increase with increasing Steinbrocker Stage. Stromelysin, most of which is produced within the joint capsule, must pass through the blood-synovial-barrier (BSB) before reaching the systemic circulation. We speculate that enhanced permeability of the BSB induced by the RA disease process leads to increased serum stromelysin levels, which show clear correlations with CRP, Lansbury Index, and number of swollen joints etc.

The above results suggest that the stromelysin level in SF is a useful parameter of local joint involvement and that in serum of the severity of systemic joint inflammation and of the RA disease process.

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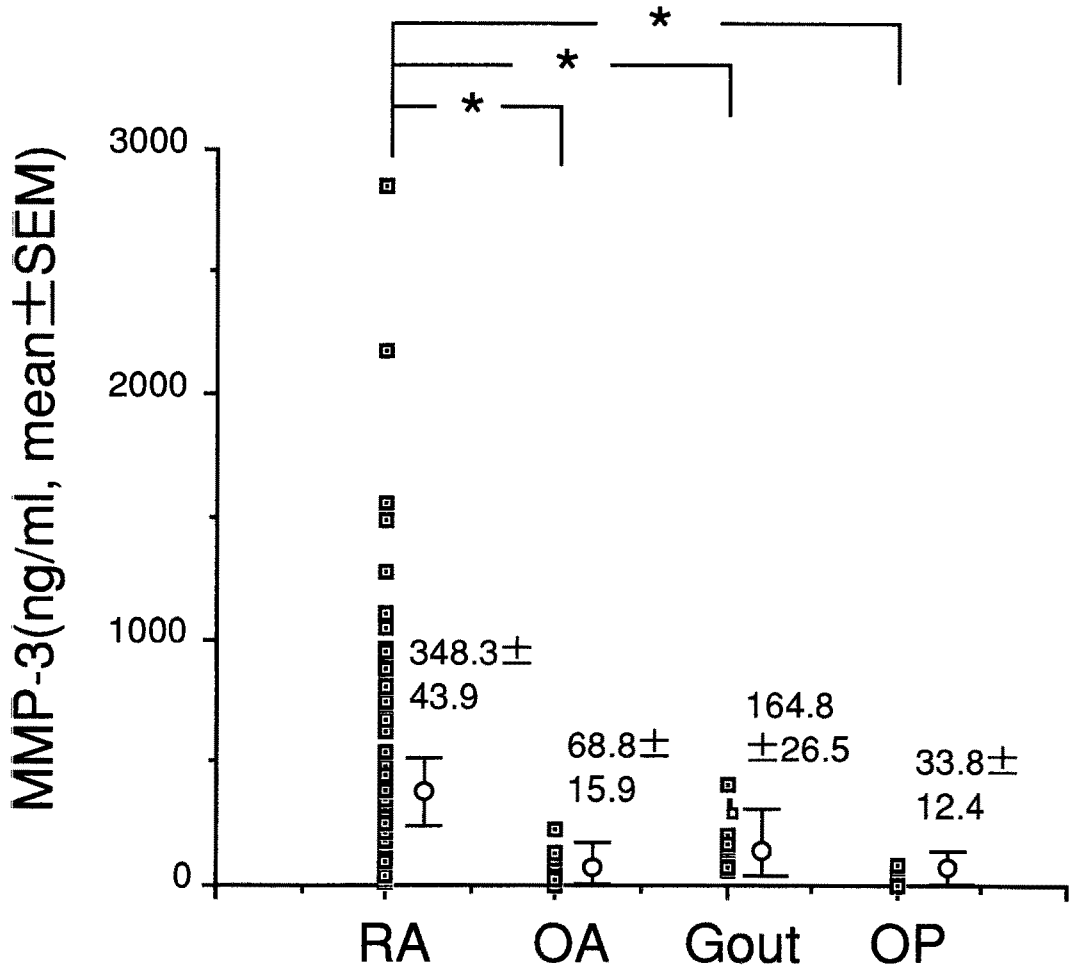

Fig. 4. Stromelysin levels in serum from patients with rheumatoid arthritis (RA), osteoarthritis (OA), gouty arthritis (Gout) and osteoporosis (OP). *p<0.01(ANOVA).

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