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Serum Matrix Metalloproteinase-3 as Predictor of Joint Destruction in Rheumatoid Arthritis, Treated with Non-biological Disease Modifying Anti-Rheumatic Drugs

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Background: Rheumatoid factor (RF), anti-citrullinated peptide antibody (ACPA), C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) have been studied extensively as prognostic markers of rheumatoid arthritis (RA). However, despite the fact that matrix metalloproteinase-3 (MMP-3) is linked to RA activity, few studies have evaluated MMP-3 as prognostic marker.

Objective: To evaluate the performance of MMP-3 as predictor of joint destruction in RA treated with non-biological disease modifying anti-rheumatic drugs.

Methods: In a retrospective study of 58 early to moderate stage RA patients who consulted the Department of Clinical Pathology and Immunology, Kobe University Hospital between May 2002 and April 2009, we evaluated the performance of MMP-3 and other biomarkers as predictors of joint destruction, by comparing them between radiographically progressive and non-progressive group.

Results: Serum levels of RF at entry and ACPA, but not MMP-3 at entry, were significantly higher for the progressive group. Ratios of patients with MMP-3 levels higher than healthy control were not significantly different for the two groups. However, cutoff values determined through receiver operating characteristic analysis showed that the ratio of patients with elevated RF was significantly higher in the progressive group (p=0.001), while MMP-3 (p=0.092), ACPA (p=0.052), CRP (p=0.056), and ESR (p=0.069) tended to be more elevated in the progressive group. Multiple logistic regression analysis using the cutoff value identified MMP-3 positive and RF positive, but not ACPA, CRP or ESR, as significant factors for radiographic progression (OR 16.79 [95% CI: 1.34-414.19]).

Conclusion: MMP-3 can be a useful marker for prediction of joint destruction.

Rheumatoid arthritis (RA) is a systemic disorder that primarily affects the joints and is chronic and destructive in nature (1). It is well established that accumulation of joint destruction in RA leads to functional impairment, deterioration in quality of life, and work disability (2,3,4,5). The delay and minimization of joint destruction caused by RA is therefore a prudent strategy for the treatment of RA patients.

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Several studies have reported on the efficacy of tumor necrosis factor (TNF)-blocking agents, a biological disease modifying anti-rheumatic drugs (DMARDs), for retarding the progression of joint destruction quantified by radiographic scores (6,7,8,9). Although TNF-blocking agents are highly effective in preventing damage to the joint structure, they are very expensive and are associated with several serious side effects (10,11,12,13). Since some patients are good responders to non-biological DMARDs in terms of radiographic progression (14,15,16), it may be preferable to use biological DMARDs for selected patients who are at high risk of joint destruction, assuming these patients are being treated with non-biological DMARDs alone. Markers to identify RA patients at high risk of aggressive joint destruction would therefore be very useful in clinical practice.

The multi-step process in the development to RA proposed by Klareskog, suggests that markers related to RA appear at different stages in the development of RA, implies that each marker represents a different role in RA. These markers may be non-specific markers such as C-reactive protein (CRP) or erythrocyte sedimentation rate (ESR), or specific markers such as anti-citrullinated peptide (ACPA), and rheumatoid factor (RF), or matrix metalloproteinase-3 (MMP-3, stromelysin 1)) (17).

MMP-3 is a proteolytic enzyme which is thought to play a pivotal role in joint destruction in RA. In this disorder, MMP-3 is locally produced in the inflamed joint, and released into the blood stream. Several studies have suggested that serum MMP-3 levels correlate with MMP-3 levels produced by the synovium, and thus reflect the level of activity of rheumatoid synovitis (18,19). The serum MMP-3 level is therefore a systemic marker which reflects local inflammation of joints. In addition, serum MMP-3 can be a specific inflammatory marker of the synovium, unlike CRP, which is a non-specific inflammatory marker.

Some studies have reported that the serum MMP-3 level decreases in patients who respond to DMARDs (20,21). We therefore explored the possibility that serum MMP-3 levels during non-biological DMARDs treatment reflect the effectiveness of the treatment and could be a useful marker for predicting joint destruction in RA.

Although MMP-3 is recognized to represent synovitis, a specific inflammation constricted to the synovium, there are few studies that have evaluated the predictive value of MMP-3 for joint destruction in RA (21,22,23). In clinical practice, markers should be easily obtainable and yield objective measurements. Our aim in this retrospective study was to evaluate the prognostic value of MMP-3 for early to moderate stage RA patients (stage I or II in the Steinbrocker classification) who are already being treated with non-biological DMARDs.

METHODS

Patient selection:

This is a retrospective study of 58 RA patients who were referred to the Department of Clinical Pathology and Immunology, Kobe University Hospital between May 2002 and April 2009. Diagnosis of RA was made according to the 1987 RA criteria proposed by the American Collage of Rheumatology (24). Candidate patients fulfilled the following criteria: (1) they were available for first and second posteroanterior radiographs of hands and anteroposterior radiographs of feet over a time span for more than 6 months; (2) they were Stage I or II on the Steinbrocker radiological stage at entry; (3) they had been treated with non-biological DMARDs for more than 3 months before entry; and (4) they were not treated with biological DMARDs throughout the study.

Clinical observation items:

The following parameters were assessed for all patients at entry: age, gender, disease duration, DMARDs use, and steroid use.

Biomarkers:

ESR, CRP, IgM-RF, and MMP-3 were obtained at entry (i.e. at the time of the first radiograph). When data were not available at that time, we used data of biomarkers within one month prior or after the optimal time. ACPA data were not available at entry for many patients so that we used data outside of the time scale mentioned because it has been reported that ACPA seroconversion (i.e. change from positive to negative or negative to positive) is unusual (25), and the data can therefore be used as baseline data, for which we used second-generation ACPA. Serum MMP-3 was measured with a one-step sandwich ELISA.

Radiographic evaluation and definition of progression:

Joint space narrowing and erosions were scored with the van der Heijde-modified Sharp Score (vdHSS) method, which has a maximum score of 448 (26). Two trained rheumatologists who are familiar with this scoring method independently evaluated the degree of joint destruction. The two radiologists read all radiographs in pairs in a known time sequence, but were blinded to the patients' names and clinical information.

We defined radiographic progression as incremental for a vdHSS score of more than 1 in one year by comparing the second with the first radiograph (i.e. second vdHSS - first vdHSS > 1/year). The definition of radiographic progression varies in each study and we used the definition which is close to the study by Syversen's group (27). The degree of change in vdHSS score is defined as $\triangle$Sharp in this paper.

Statistical analysis:

For assessment of the relationship between the ratio of patients with elevated biomarkers at entry and radiographic progression, we used Fisher's exact probability method. To assess the relationship between the levels of biomarkers and radiographic progression, we converted the laboratory data to log scale according to the distributions of the biomarkers. We then used student's or Welch's t-test for comparisons between the progressive and non-progressive group. To investigate the predictive value of the biomarkers, we assessed the relationship between each of the four biomarkers (i.e. MMP-3, RF, CRP, and ESR) at entry and progression of joint destruction, as well as the relationship between ACPA and progression of joint destruction to determine whether the correlation between the ratio of patients with an elevated biomarker and the presence of radiographic progression was significant. We set the cutoff value for any given biomarker as the value determined by ROC analysis and an elevated biomarker was defined as one with a value greater than the cutoff level. We used ROC analysis to determine the cutoff value instead of the upper limit of healthy control, because the values of healthy control were not intended to identify the risk of joint destruction. Some studies have used the maximum value of healthy control as cutoff value, but since our study was intended to distinguish high-risk patients from low-risk patients, we considered using the ROC analysis better than using the value of healthy control because the latter is not intended to be used for this purpose.

We used multiple logistic regression analysis to identify the factors which effectively influenced the progression of joint destruction. For this investigation, age and disease duration were adjusted for statistical comparison of the two groups. Age, gender, disease duration, treatment status (i.e. use of MTX, DMARDs other than MTX, and steroid), and

biomarkers (i.e. MMP-3, RF, ACPA, CRP, and ESR) were used as the covariates. "DMARDs other than MTX" denotes sulfasalazine, bucillamine, auranofin, actarit, cyclosporine A, sodium aurothiomate, and tacrolimus. We also performed logistic regression analysis with stepwise selection of the aforementioned covariates. All statistical analyses were performed by R version 2.92 (R foundation for Statistical Computing, Vienna, Austria) and statistical significance was defined as $p<0.05$.

Table I. Baseline characteristics of the 58 RA patients

	Non-progressive group ⊿Sharp score≦1/year (n=26)	Progressive group ⊿Sharp score>1/year (n=32)	p value
Female	20 (77%)	27 (84%)	ns
Age (y)	59 ± 16	51 ± 15	ns
Disease duration (y)	4 (1～24) ± 5	5 (1～10)±3	ns
Onset age (y)	55 (14～80) ± 18	47 (20～71)±16	ns
DMARDs except MTX^{+}	20 (77%)	19 (59%)	ns
MTX	15 (58%)	18 (56%)	ns
Steroid	13 (50%)	19 (59%)	ns
Elevated MMP-3	13/25$^{\#}$	23/32	ns*
MMP-3 level (ng/ml)	103.5 ± 71.0$^{\#}$	180.7 ± 179.4	0.17**
Elevated RF	12/26	25/32	0.015*
RF level (IU/ml)	39.0 ± 70.8	233.3 ± 437.8	0.0005**
Elevated ACPA	12/25$^{\#}$	24/31$^{\#}$	0.028*
ACPA level (U/ml)	117.6 ± 312.8$^{\#}$	247.8 ± 40$^{\#}$	0.048**
Elevated CRP	8/26	18/32	0.067*
CRP level (mg/dl)	0.68 ± 1.63	1.26 ± 1.82	ns**
Elevated ESR	14/25$^{\#}$	25/32	0.092*
ESR level (mm/hr)	28.2 ± 25.7$^{\#}$	35.8 ± 26.0	ns**

MTX: methotrexate, ACPA: anti-citrullinated peptide antibody, MMP-3: matrix metalloproteinase 3, CRP: C-reactive protein, RF: rheumatoid factor, ESR: erythrocyte sedimentation rate
* : Fisher exact test, ** : Student's t test with log-transformation
#: no data available for 1 patient
cutoff value: MMP-3<121 ng/ml for male, <59.7 ng/ml for female, RF<15 IU/ml,
ACPA< 4.5 U/ml, CRP<0.3 mg/dl, ESR<10 mm/hr for male, <15 mm/hr for female
+: sulfasalazine, bucillamine, auranofin, actarit, cyclosporine A, sodium aurothiomate, tacrolimus

RESULTS

Baseline characteristics of progressive and non-progressive groups are given in Table I. There were no significant differences in demographic status such as age at entry and onset, gender, disease duration, and treatment status (i.e. MTX use, DMARDs use except MTX, and steroid use) between the two groups.

RF ($p = 0.005$), and ACPA ($p = 0.048$) level was significantly higher in the progressive group. MMP-3 level was not significant ($p = 0.17$). Ratios of positive patients for RF ($p = 0.015$) and ACPA ($p = 0.028$) were also significantly higher in the progressive group, but no significance was seen for MMP-3. In regards of CRP and ESR, no statistical significance was found between the two groups.

Table II. Biomarker positivity rate between progressive and non-progressive group

	Non-progressive group ⊿Sharp score≦1/year (n=26)	Progressive group ⊿Sharp score>1/year (n=32)	p value
MMP-3 (> 62 ng/ml)	56%[#]	78%	0.092
RF (> 35 IU/ml)	23%	72%	0.001
ACPA (> 112 U/ml)	17%[#]	45%	0.052
CRP (> 0.3 mg/dl)	31%	59%	0.056
ESR (> 20 mm/hr)	48%[#]	75%	0.069

MMP-3: matrix metalloproteinase 3, ACPA: anti-citrullinated peptide antibody,
CRP: C-reactive protein, RF: rheumatoid factor, ESR: erythrocyte sedimentation rate
Cutoff level of each biomarker is determined by ROC analysis.
P values are determined by Fisher's exact test, #: no data available for 1 patient

Table III. Performance of biochemical markers at baseline as predictor of radiographic progression in multivariate analysis

	Odds ratio (95% CI)	p value
Male	0.96 (0.08-14.14)	0.97
DMARDs use except MTX*	0.03 (0.00-0.31)	0.011
MTX use	0.04 (0.00-0.41)	0.016
Steroid use	0.21 (0.01-2.13)	0.22
MMP-3 (> 62 ng/ml)	16.79 (1.34-414.19)	0.046
RF (> 35 IU/ml)	9.15 (1.65-73.92)	0.019
ACPA (> 112 U/ml)	7.30 (1.02-78.14)	0.065
CRP (> 0.3 mg/dl)	9.37 (0.72-234.06)	0.11
ESR (> 20 mm/hr)	0.31 (0.03-2.85)	0.31

95% CI: 95% confidence interval
MTX: methotrexate, DMARDs: disease modifying anti-rheumatic drugs,
ACPA: anti-citrullinated peptide antibody, MMP-3: matrix metalloproteinase 3,
CRP: C-reactive protein, RF: rheumatoid factor, ESR: erythrocyte sedimentation rate,
*:sulfasalazine, bucillamine, auranofin, actarit, cyclosporine A,sodium aurothiomate, tacrolimus

Determination of cut off value and evaluation of laboratory data:

Using ROC analysis, we defined the cut off value of each biomarker. The cutoff value of MMP-3, RF, ACPA, CRP, and ESR were 62 ng/ml, 35 IU/ml, 112 U/ml, 0.3 mg/dl, and 20 mm/hr respectively. Table II show the positivity rate of each biomarker between progressive and non-progressive groups, using the cutoff value. Positivity rate of RF were significantly higher in the progressive group ($p = 0.001$). There were no significant differences in MMP-3 ($p = 0.092$), ACPA ($p = 0.052$), CRP ($p = 0.056$), and ESR ($p = 0.069$) at baseline, however there were trend toward higher positivity rate in the progressive group for each biomarker.

In a multiple logistic regression analysis with full covariates RF positive and MMP-3 positive were significant factor for RA radiographic progression (OR for RF 9.15[95%CI: 1.65-73.92], for MMP-3 16.79[1.34-414.19], Table III). The results of logistic regression analysis after stepwise showed the same tendency (Table IV). Diagnostic performances of the two logistic regression models evaluated by AUC of ROC analyses were 0.89 for full covariates model (Figure 1) and 0.87 for stepwise model (Figure 2).

Table IV. Performance of biochemical markers at baseline as predictor of radiographic progression in stepwise multivariate analysis

	Odds ratio (95% CI)	p value
DMARDs use except MTX*	0.07 (0.01-0.55)	0.019
MTX use	0.05 (0.00-0.38)	0.010
MMP-3 (> 62 ng/ml)	9.33 (1.72-76.44)	0.018
RF (> 35 IU/ml)	8.89 (1.87-58. 28)	0.011
ACPA (> 112 U/ml)	5.23 (0.83-44.31)	0.096

95% CI: 95% confidence interval
MTX: methotrexate, DMARDs: disease modifying anti-rheumatic drugs,
MMP-3: matrix metalloproteinase 3, RF: rheumatoid factor,
ACPA: anti-citrullinated peptide antibody
*:sulfasalazine, bucillamine, auranofin, actarit, cyclosporine A, sodium aurothiomate, tacrolimus

Figure 1. Receiver operating characteristic (ROC) curve of multiple logistic regression analysis with full covariate model.

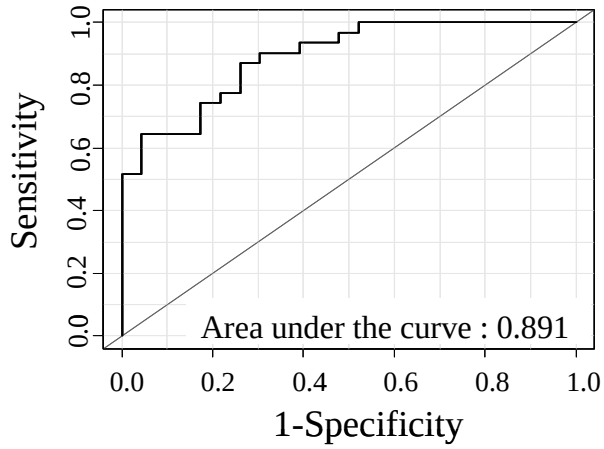
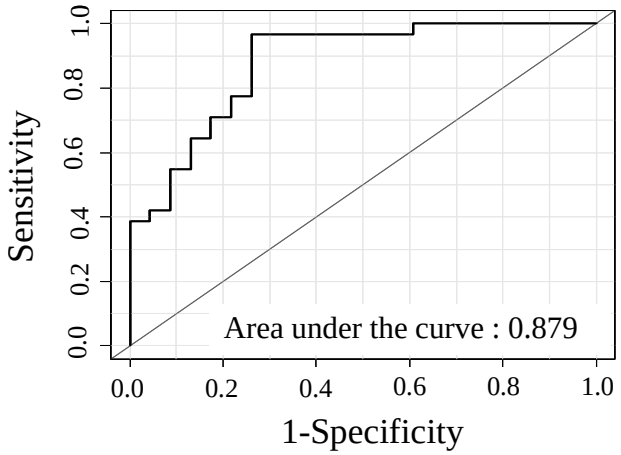


Figure 2. Receiver operating characteristic (ROC) curve of multiple logistic regression analysis in a stepwise covariate model.



DISCUSSION

We demonstrated that MMP-3 is a useful marker for predicting joint destruction. There are many studies that have evaluated the prognostic value of biomarkers (such as RF, ACPA, CRP, and ESR). As for MMP-3, we found four reports on the value of baseline data as predictors of joint destruction (21,22,23,28).

Although Green's study (23) concluded that baseline MMP-3 can predict radiographic progression, which is the same conclusion as ours, their result should be interpreted with caution since they used pretreatment MMP-3 to predict joint destructions, which obviously does not reflect the efficacy of treatment.

In Hashimoto's study (28), baseline MMP-3 was not found to be predictive of radiographic progression. However, there are some important differences between this study and ours (28). We used Steinbrocker's radiologic stage (regardless of disease duration) for patient selection and did not take disease activity into consideration, whereas Hashimoto's group studied RA patients with disease duration of less than 5 years and with an active disease. The patients in our study thus had a longer disease duration (5 years vs. 2.4 years) and lower level of disease activity in terms of inflammatory marker levels (for example, the mean CRP was 0.98 mg/dl in our study, and 4.9 mg/dl in Hashimoto's). The patients in Hashimoto's study had a higher level of disease activity presumably because they were taking part in a study of tocilizumab, a biological DMARDs, and therefore, these patients were probably candidates for aggressive treatment. On the other hand the purpose of our study was to distinguish the aggressive disease from milder forms of RA.

Yamanaka's study (22) and Young-Min's study (21) showed the predictive value of MMP-3 for joint destruction, and are thus consistent with our finding, with our study confirming the results of the two previous studies but for a different patient group. These studies are both prospective, and in that sense provide more robust evidence than our study. However, Yamanaka's study evaluated MMP-3 as a single marker for prediction of joint destruction, while Young-Min's study did not include ACPA. Although our study is retrospective, it is the first to include ACPA, which is said to be one of the best prognostic markers, for the evaluation of MMP-3 as a prognostic marker.

The performance of RF was comparable to that of MMP-3 in our study. Although RF is not regarded as an inflammatory marker such as MMP-3, there are reports that RF decreases significantly only in responders under RA treatment (29,30,31,32,33), and that therefore RF levels reflect treatment response. On the other hand, MMP-3 is a marker that reflects inflammation of the synovium (18,19), and is thus directly linked to disease activity. Since MMP-3 level is known to decrease when treatment is effective, it also reflects treatment response. Since the multivariate analysis in our study showed that both RF and MMP-3 were significantly higher in the progressive group, they could be regarded as independent markers, so that it may be useful to measure both RF and MMP-3 even though they both reflect treatment response.

Our study has several limitations. First, this is not a controlled study and treatment may have been decided based on the clinical situation of each individual patient. Second, MMP-3 showed a better performance than ACPA for predicting joint destruction in our study but the ACPA level that we used was not the one at entry point so that this made the study design less precise. Third, we performed multiple logistic regression analysis using the same data as those used to estimate the optimal cutoff values of the biomarkers. These data might overestimate the performance of the logistic regression analysis. We therefore believe that further studies with a large population are needed.

In conclusion, we demonstrated that MMP-3 could predict joint destruction for RA being treated with non-biological DMARDs by reflecting response to the non-biological DMARDs.

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