

Biomarkers Predict Radiographic Progression in Early Rheumatoid Arthritis and Perform Well Compared With Traditional Markers

Steven Young-Min,¹ Tim Cawston,² Nicola Marshall,³ David Coady,³ Stephan Christgau,⁴ Tore Saxne,⁵ Simon Robins,⁶ and Ian Griffiths³

Objective. To evaluate the performance of biochemical and traditional markers in predicting radiographic progression in rheumatoid arthritis (RA).

Methods. One hundred thirty-two patients with early RA were treated with nonbiologic therapies for 2 years and studied longitudinally. Genomic DNA was analyzed for presence of the shared epitope. Levels of matrix metalloproteinases (matrix metalloproteinase 1 [MMP-1], MMP-13, and MMP-3), tissue inhibitor of metalloproteinases 1 (TIMP-1), and cartilage oligomeric matrix protein (COMP) were assessed in serially obtained serum samples. The presence of pyridinoline (Pyr), deoxypyridinoline, glycosylated Pyr (Glc-Gal-Pyr), and C-telopeptide of type II collagen (CTX-II) was assessed in urine samples. Radiographs obtained at entry and at 2 years were evaluated using the modified Larsen score.

Results. Baseline and 2-year radiographs were available from 118 patients. Larsen scores worsened

during the 2 years in 50 patients, while 68 patients had no radiographic progression. Levels of a variety of biochemical markers, i.e., MMP-3, CTX-II, COMP, TIMP-1, Pyr, and Glc-Gal-Pyr, correlated significantly with radiographic progression at entry and longitudinally as assessed by area under the curve (AUC). By multivariate analysis, a model including MMP-3 and CTX-II was identified as providing the best prediction of radiographic progression at entry (predictive accuracy by receiver operating characteristic [ROC] AUC = 0.76 [95% confidence interval 0.66–0.85]), while a combination of MMP-3, CTX-II, and swollen joint count formed the best longitudinal AUC model (predictive accuracy by ROC AUC = 0.81 [95% confidence interval 0.73–0.89]). Patient-reported measures (Health Assessment Questionnaire, pain scores) were of limited use. In a subset of 50 patients who were treated with methotrexate (MTX) during the followup period, median serum MMP-3 levels decreased after the initiation of MTX therapy ($P = 0.0003$).

Conclusion. These results indicate that biochemical markers are useful predictors of radiographic progression in RA and that serum MMP-3 levels decrease significantly with MTX therapy. Multivariate models that include MMP-3 and CTX-II perform better than existing traditional markers in predicting radiographic outcome in RA.

Disease-modifying antirheumatic drugs (DMARDs) and biologic agents are standard therapies for rheumatoid arthritis (RA), but these treatments have potentially toxic side effects and require carefully targeted application. It is therefore important to identify patients with progressive, destructive disease and monitor whether their disease is responding to therapy, and to identify those with milder disease for whom aggres-

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¹Steven Young-Min, PhD, Queen Alexandra Hospital, Portsmouth, UK; ²Tim Cawston, PhD, University of Newcastle-upon-Tyne, Newcastle-upon-Tyne, UK; ³Nicola Marshall, SRN, David Coady, MD, Ian Griffiths, FRCP: Freeman Hospital, Newcastle-upon-Tyne, UK; ⁴Stephan Christgau, PhD, Nordic Bioscience, Herlev, Denmark (current address: Osteologix A/S, Copenhagen, Denmark); ⁵Tore Saxne, MD, PhD: Lund University Hospital, Lund, Sweden; ⁶Simon Robins, PhD, Rowett Research Institute, Aberdeen, UK.

Dr. Christgau is the inventor on Nordic Bioscience patents for the CTX-II assays used in this study. Dr. Saxne is a cofounder of AnaMar Medical and holds stock or stock options in the company.

Address correspondence and reprint requests to Tim Cawston, PhD, Rheumatology, The Medical School, University of Newcastle-upon-Tyne, Cookson Building, Framlington Place, Newcastle-upon-Tyne NE2 4HH, UK. E-mail: T.E.Cawston@ncl.ac.uk.

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sive therapy may be less appropriate. Despite some improvements over time, methods of objectively assessing, quantifying, and predicting joint damage in RA remain inadequate (1). Imaging provides a largely historical view of damage that has already occurred, genetic and antibody markers are not dynamic, and serologic measures such as erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP) are not specific to joint disease.

Despite the theoretical advantages, the practical use of "novel" biochemical markers to predict tissue destruction and inform treatment of arthritis in clinical practice has been limited. This is due in part to the fact that their superiority over traditional markers has not been demonstrated in longitudinal prospective studies. In the present study, we investigated both biochemical and traditional markers of radiographic outcome to determine what additional contribution novel markers may make to the prediction of radiographic progression in early RA. We selected a range of biomarkers to reflect destructive enzymatic processes, breakdown products from collagenous and noncollagenous components of cartilage, and inflammatory processes within bone and synovium.

PATIENTS AND METHODS

Patients with early RA. Consecutive patients with early RA were recruited between April 1998 and April 2000 from general rheumatology clinics and from among patients referred for early synovitis and were followed up prospectively. Each patient fulfilled the American College of Rheumatology (formerly, the American Rheumatism Association) 1987 criteria for RA (2) and had had onset of persistent symptoms <2 years prior to enrollment.

Patients were assessed at study entry and at 2 months, 6 months, 12 months, 18 months, and 24 months. At each visit, the following measures were recorded: number of swollen joints (using 28- and 44-joint counts), number of tender joints (28-joint count), Ritchie Articular Index (RAI) (3), clinician's global assessment on a visual analog scale (VAS), clinician's global assessment using a 1–5 score, patient's assessment of disease activity on a VAS, patient's assessment of pain on a VAS, and score on the Stanford Health Assessment Questionnaire (HAQ) (4). Serum and urine samples were obtained at each visit. Serum samples were obtained from 20 ml of clotted whole venous blood, which was centrifuged at 1,400g for 10 minutes and then stored at -80°C . Urine samples were collected as "second morning specimens"; patients were instructed to empty their bladders completely upon waking, and then collect a sample of the next urine produced that morning. Urine samples were kept in black plastic bags to shield them from sunlight and then frozen at -80°C . Prior to freezing, serum and urine samples were aliquoted to minimize any subsequent freeze-thaw cycles. Rheumatoid factor (RF) titers

were determined (Serodia-RA; Fuji Rebio, Tokyo, Japan), and samples of whole venous blood were obtained for shared epitope (SE) analysis. Disease Activity Scores (DAS) were calculated according to an unmodified formula with 4 variables: RAI, swollen joint count (44 joints), erythrocyte sedimentation rate (ESR), and patient assessment of global activity on a VAS (5,6). A modified DAS was also calculated, with assessment of swollen and tender joints using 28-joint counts (7). Standard radiographs of the hands and feet were obtained at entry and after 24 months of followup.

Of the 132 patients recruited, 124 completed the study, of whom 118 patients had complete sets of radiographs. Patients were treated according to standardized local practice, which, at the time of the study (1998–2002), consisted largely of sequential DMARD monotherapy, i.e., sulfasalazine, methotrexate (MTX), and adjunctive corticosteroids. Some of the patients (14%) later received combination DMARD therapy. These patients with early disease were not eligible for anti-tumor necrosis factor therapy, and the study was completed prior to the more widespread availability of biologic therapy in the UK.

Scoring of radiographs. Two observers (DC and SY-M) were trained to apply the modified Larsen score (8,9) using a set of training radiographs. The modified Larsen score was selected because it is suited to longitudinal studies (9) and is more sensitive to change than other scoring systems (10). On completion of training, reliability and agreement were assessed using an intraclass correlation coefficient (ICC) (11); the mean ICC for intraobserver agreement was 0.92, and that for interobserver agreement was 0.91. The 2 observers then scored the 118 sets of radiographs, with blinding regarding the patient's identity but not regarding the sequence of films. The mean score from the 2 observers was recorded for each set of films unless there was disagreement as to whether progression had occurred, in which case films were reviewed, discussed, and a consensus score determined.

Division of the cohort based on radiographic progression. At the end of the assessment period, the study cohort was divided into 2 groups: those whose disease was radiographically stable and those in whom it had progressed. In 68 patients (57.6%), radiographic scores had not changed during the 2-year study period, while the scores of 50 patients (42.4%) had deteriorated. These patient groups were compared with regard to demographic characteristics, clinical characteristics at entry, and treatment, using the chi-square test, Student's *t*-test, or Mann-Whitney U test, as appropriate.

SE analysis. HLA-DRB1 alleles associated with RA carry the shared epitope sequence QKRAA, QRRRA, or RRRRAA at positions 70–74. These include the DRB1* alleles 0101, 0102, 0401, 0404, 0405, 0408, 1001, and 1402. Examination of HLA haplotype frequencies in the British Bone Marrow Registry revealed low frequencies of DR10- and DR14-containing haplotypes (0.6% and 2%, respectively) compared with DR1- and DR4-containing haplotypes (10% and 19%, respectively). We therefore identified individuals with the SE by initially screening the study population for HLA-DR1 and DR4 positivity. The screening method entailed knowledge of DR1 subtypes in the local population, and the rarity of DRB1*0104 in our study population allowed us to assume that individuals identified as being DR1 positive would be SE

Table 1. Biochemical markers examined in the study

Marker type and name*	Reason for interest
Serum MMPs and TIMPs	
MMP-1 (collagenase 1)	Digests fibrillar collagen
MMP-13 (collagenase 3)	Digests fibrillar collagen
MMP-3 (stromelysin 1)	Role in activating MMPs; broad activity against extracellular matrix components
TIMP-1	Local inhibitor of MMPs
Urinary type II collagen telopeptide	
CTX-II/creatinine	Specific marker of type II collagen cleavage in cartilage
Urinary collagen crosslinks	
Pyr/creatinine	Marker of type I and type II collagen breakdown
D-Pyr/creatinine	Specific marker of type I bone collagen breakdown
Glc-Gal-Pyr/creatinine	Chemically similar to Pyr; proposed as a marker of synovial metabolism
Noncollagenous cartilage breakdown marker	
COMP	General marker of cartilage breakdown; noncollagenous protein at high levels in cartilage matrix, but also in tendon, meniscus, and synovium

* MMPs = matrix metalloproteinases; TIMPs = tissue inhibitors of metalloproteinases; CTX-II = C-telopeptide of type II collagen; Pyr = pyridinoline; D-Pyr = deoxypyridinoline; Glc-Gal-Pyr = glycosylated Pyr; COMP = cartilage oligomeric matrix protein.

positive; for DR4-positive individuals, however, further subtype analysis was performed.

Genomic DNA was obtained from venous blood samples, using a BACCII kit (Nucleon Biosciences, Glasgow, UK). HLA-DR1 and DR4 typing was performed using a polymerase chain reaction technique with sequence-specific oligonucleotide primers. HLA-DRB1*01 (DR1) was genotyped using the primers 5'-TTG-TGG-CAG-CTT-AAG-TTT-GAA-T-3' and 5'-CCG-CCT-CTG-CTC-CAG-GAG-3'. These identify the DRB1* alleles 0101, 01021, 01022, and 0104. Similarly, HLA-DRB1*04 (DR4) was genotyped with the primers 5'-GTT-TCT-TGG-AGC-AGG-TTA-AACA-3', 5'-CTG-CAC-TGT-GAA-GCT-CTC-AC-3', and 5'-CTG-CAC-TGT-GAA-GCT-CTC-CA-3'. These identify the DRB1*alleles 04011-0408, 0410-0412, 0415-0417, 0421, 0422, 0424, 0425, 1122, 1410, 0419, 0420, and 0423. Subtype analysis of DR4-positive samples was then performed using the SSP DRB1*04 Subtyping Kit (Olerup SSP, Saltsjöbaden, Sweden).

Assay of biochemical markers. After collection, samples were frozen to -80°C . Samples were coded so that they remained anonymous. All samples were defrosted and assays performed at the end of the study to minimize interassay variability. Serum and second morning urine samples were analyzed without prior knowledge of the patient's clinical or radiographic characteristics. Biochemical markers assayed are shown in Table 1.

Serum matrix metalloproteinase 1 (MMP-1) levels were measured by sandwich enzyme-linked immunosorbent assay (ELISA). The assay measures proenzyme, active enzyme, and enzyme complexed with tissue inhibitor of metalloproteinases (TIMP). The methods used were similar to those described elsewhere (12,13), although recombinant human MMP-1 is now utilized as a standard in the assay. Samples were initially run in duplicate at a 1:8 dilution in an assay with standards ranging between 0.25 ng/ml and 10 ng/ml (intraassay coefficient of variation [CV] 3.4-17.9%, interassay CV 0.05-15.0%). If findings for a sample fell above the highest standard,

it was rerun in an assay with a range of 5-90 ng/ml (intraassay CV 2.2-9.0%, interassay CV 1.6-5.5%). When the CV for duplicate assays was $>10\%$, assays were repeated, and when necessary, samples were run at different dilutions.

Serum MMP-13 was assayed using a sandwich ELISA (Biotrak; Amersham Pharmacia Biotech, Buckinghamshire, UK). This assay detects pro and active MMP-13, and its use has been described elsewhere (14). The intraassay and interassay CVs for this assay are 3.0-5.4% and 2.0-5.7%, respectively. The assay was performed according to the manufacturer's instructions, and attempts were also made to improve the lower limit of detection of the assay to <0.094 ng/ml, by the addition of extra standards.

Serum MMP-3 was measured with a one-step sandwich ELISA (Daiichi Fine Chemical, Toyama, Japan). This assay (15) detects proenzyme, active enzyme, and enzyme complexed with TIMP and has intraassay and interassay CVs of 4.5-7.3% and 6.0-8.0%, respectively. Samples were not diluted and were analyzed in duplicate. When the CV for duplicate assays was $>15\%$, assays were repeated, and when necessary, samples were run at different dilutions.

Serum TIMP-1 levels were measured with a sandwich ELISA that detects total TIMP-1, including free TIMP-1 and TIMP-1 complexed with MMPs. The methods used were similar to those described elsewhere (13,16), although recombinant human TIMP-1 is now used as a standard in the assay. The intra- and interassay CVs, respectively, were 0.9-4.6% and 0.07-1.5%. Samples were initially assayed at a 50-fold dilution, and assays were performed in duplicate. When the CV for duplicate assays was $>15\%$, assays were repeated, and when necessary, samples were run at different dilutions.

Levels of cartilage oligomeric matrix protein (COMP) were measured in frozen serum samples sent to AnaMar Medical (Lund, Sweden). Serum COMP levels were determined by sandwich ELISA, using 2 monoclonal antibodies against separate antigenic determinants of the human COMP

Table 2. Characteristics of the patients whose RA remained radiographically stable and the patients who had radiographic progression*

	Radiographically stable (n = 68)	Radiographic progression (n = 50)	P†
Female, no. (%)	45 (66)	33 (66)	NS‡
Age at entry, mean ± SD years	52.5 ± 12.3	55.7 ± 13.3	NS§
Disease duration at entry, days	240 (42–730)	258.5 (42–730)	NS
RF positive, no. (%)	45 (66)	39 (78)	NS†
ESR at entry, mm/hour	19.5 (2–73)	33.5 (1–100)	0.013
DAS at entry	3.38 (0.72–6.24)	3.66 (0.58–6.78)	NS
HAQ score at entry	1.31 (0–3)	1.56 (0–3)	NS
SE positive, no. (%)			
1 SE	34 (50)	31 (62)	NS‡
2 SE	7 (10)	7 (14)	
Larsen score at entry	0 (0–19)	1.5 (0–17)	0.001
Change in Larsen score over 2 years	0 (–1.5 to 0)	+5 (+1 to +33)	<0.0001
Cumulative oral/IM prednisolone dose over 2 years, mg	150 (0–5,045)	160 (0–4,700)	NS
No. of different DMARDs taken over 2 years	1 (0–3)	2 (0–4)	0.01
Taken MTX, no. (%)	32 (47)	35 (70)	0.02‡

* Except where indicated otherwise, values are the median (range). RA = rheumatoid arthritis; NS = not significant; RF = rheumatoid factor; ESR = erythrocyte sedimentation rate; DAS = Disease Activity Score; HAQ = Health Assessment Questionnaire; SE = shared epitope; IM = intramuscular; DMARDs = disease-modifying antirheumatic drugs; MTX = methotrexate.

† Except where indicated otherwise, determined by Mann-Whitney U test.

‡ By Fisher's 2-sided exact test.

§ By Student's *t*-test.

molecule. The detection limit of the assay is 0.1 unit/liter, and intra- and interassay CVs are <5%.

Frozen urine samples were sent to one of the authors (SR) at the Rowett Research Institute, and there urinary total pyridinoline (Pyr), total deoxypyridinoline (D-Pyr), and glycosylated Pyr (Glc-Gal-Pyr) were quantified, under blinded conditions. The analysis was performed using high-performance liquid chromatography, as previously described (17). Urinary creatinine levels were measured with a Kone autoanalyzer, using the Jaffe method (18).

Urine levels of C-telopeptide of type II collagen (CTX-II) were measured, in a blinded manner, by competitive ELISA, at the laboratories of Nordic Bioscience. The intra- and interassay CVs for this assay are <8% and <10%, respectively. As with collagen crosslinks, all urine measurements were corrected for urinary creatinine concentration.

Statistical analysis. Analysis of our data revealed that most of the clinical, radiographic, and laboratory results were nonparametric. Attempts to transform the data did not result in a normal distribution, and we therefore avoided parametric analysis and linear regression methods. Plots of longitudinal data were constructed, and time-integrated areas under the curve (AUCs) selected to summarize longitudinal data (19).

Results of clinical assessments and laboratory assays were considered initially as entry variables, and subsequently as longitudinal data. The outcome variable, radiographic progression, was considered both as a continuous variable, (i.e., change in Larsen score, assessed using Spearman's rank correlation) and as a binary variable (i.e., stable versus progressive, assessed using univariate logistic regression). To determine the contribution of each potential predictor, both entry and longitudinal variables were dichotomized into high and

low values for use in binary logistic regression. For many of the traditional variables normal ranges were used, but for the novel biochemical markers, established "normal" ranges in patients with nonprogressive RA are not known. We therefore attempted to assess optimal cutoff levels using receiver operating characteristic (ROC) curve analysis (20). However, the nature of the ROC curves produced made selection of a decision level somewhat arbitrary. Therefore, for all of the novel biochemical markers and some of the more traditional markers such as joint counts, DAS, or HAQ score, results were separated into high or low values according to the median value obtained in this study. Positive and negative predictive values were then calculated, and univariate logistic regression analysis performed on each of the baseline and longitudinal variables.

In order to investigate the relative contributions of novel and traditional markers and produce the best prognostic model for radiographic progression, all variables found to be predictive on univariate analysis, including all known predictors of radiographic progression, were entered into a multivariate binomial logistic regression for either entry or longitudinal data. Care was taken not to enter directly related variables into the same model, and the results of univariate analysis were used to select the better predictor to be entered. The entry variables selected were Larsen score, CTX-II, Pyr, Glc-Gal-Pyr, COMP, MMP-1, MMP-3, TIMP-1, patient-assessed pain, patient-assessed global status, clinician-assessed global status, swollen joint count (44 joints), tender joint count (28 joints), ESR, DAS, HAQ, RF, and SE. The longitudinal variables selected were CTX-II, Pyr, COMP, MMP-3, TIMP-1, clinician-assessed global status, swollen joint count (44 joints), CRP, and DAS. We attempted to identify the best predictive model using

a stepwise forward conditional method with an entry probability of 0.05 and removal probability of 0.1. The logistic regression models obtained were also assessed for predictive accuracy using the area of the ROC curve produced by the model. Data were analyzed using SPSS 11.0 for Windows (SPSS, Chicago, IL).

Following completion of the study, a retrospective analysis was performed on patients in whom MTX treatment had been instituted during the study. These patients either had not been previously treated with a DMARD or had been treated with sulfasalazine. The usual initial dosage of MTX was 10 mg/week, with folic acid (5 mg/week) administered 3 days after the MTX administration. The MTX dosage was then usually increased based on response. Levels of biochemical markers and serum CRP in these patients before initiation of MTX treatment versus after institution of treatment were compared.

RESULTS

Table 2 shows characteristics at baseline, and some features during the 2 years of study, in the group of patients whose RA remained radiographically stable and the group who had radiographic progression. There were no significant differences in demographic characteristics, RF status, SE status, baseline DAS, or baseline HAQ score. The patients who had radiographic progres-

sion, however, did have a higher baseline ESR and baseline Larsen score; in addition, they received a greater number of DMARDs during the course of the study, and a higher proportion received MTX.

The traditional measures that were found to differ significantly between the group with and the group without radiographic progression are shown in Table 3. Levels of both MMP-3 and TIMP-1 differed significantly between the 2 groups, but no difference in MMP-1 levels was seen (Figure 1A). MMP-13 was undetectable in all baseline and final serum samples (259 samples from 132 patients) (data not shown). Figure 1B demonstrates that serum levels of COMP and urine levels of Pyr and CTX-II each differed significantly between the 2 groups at baseline and by AUC analysis. The results of D-Pyr and Glc-Gal-Pyr assays did not differ at baseline and were significantly different only by AUC analysis (D-Pyr/creatinine AUC median 232 (nmoles/mole) × months [range 122–498] in the 68 patients whose disease remained radiographically stable versus median 276 (nmoles/mole) × months [range 143–690] in the 50 patients with radiographic progression [$P = 0.013$ by Mann-Whitney U test]; Glc-Gal-Pyr/creatinine AUC median 1,533 (nmoles/mole) × months [range 592–

Table 3. Traditional markers with significantly different values in the patients whose RA remained radiographically stable and the patients who had radiographic progression*

	Radiographically stable (n = 68)	Radiographic progression (n = 50)	P^\dagger
Clinician's global assessment			
Baseline on VAS, mm	28.0 (0–88)	46.5 (0–86)	0.03
AUC on VAS, mm	580 (24–1,482)	970 (174–1,656)	<0.0001
AUC score	51 (30–86)	65 (42–97)	<0.0001
Joint counts			
AUC 44-joint swollen joint count	81 (0–308)	157 (11–538)	<0.0001
AUC 28-joint swollen joint count	78 (0–280)	136 (1–438)	<0.0001
AUC RAI	197 (18–560)	262 (69–657)	0.004
AUC 28-joint tender joint count	211 (18–460)	276 (33–574)	0.017
Inflammation markers			
Baseline CRP, mg/liter	8 (2.5–77)	18.5 (2.5–107)	0.017
AUC CRP, mg/liter	167 (60–906)	375 (60–1716)	<0.0001
Baseline ESR, mm/hour	19.5 (2–73)	33.5 (1–100)	0.013
AUC ESR, mm/hour	340 (57–1,265)	604 (88–1,582)	<0.0001
Others			
AUC DAS	67 (28–118)	88 (37–131)	<0.0001
AUC 28-joint DAS	100 (46–157)	132 (48–172)	<0.0001
Baseline RF titer	1:160 (0–1:640)	1:640 (0–1:640)	0.015
Baseline Larsen score	0 (0–19)	1.5 (0–17)	0.0009
SE status (–/–+/++), no.	40/27/1	20/26/4	0.019‡

* Except where indicated otherwise, values are the median (range); all area under the curve (AUC) values are time-integrated (times number of months). VAS = visual analog scale; RAI = Ritchie Articular Index; CRP = C-reactive protein (see Table 2 for other definitions).

† Except where indicated otherwise, determined by Mann-Whitney U test.

‡ By chi-square test for trend.

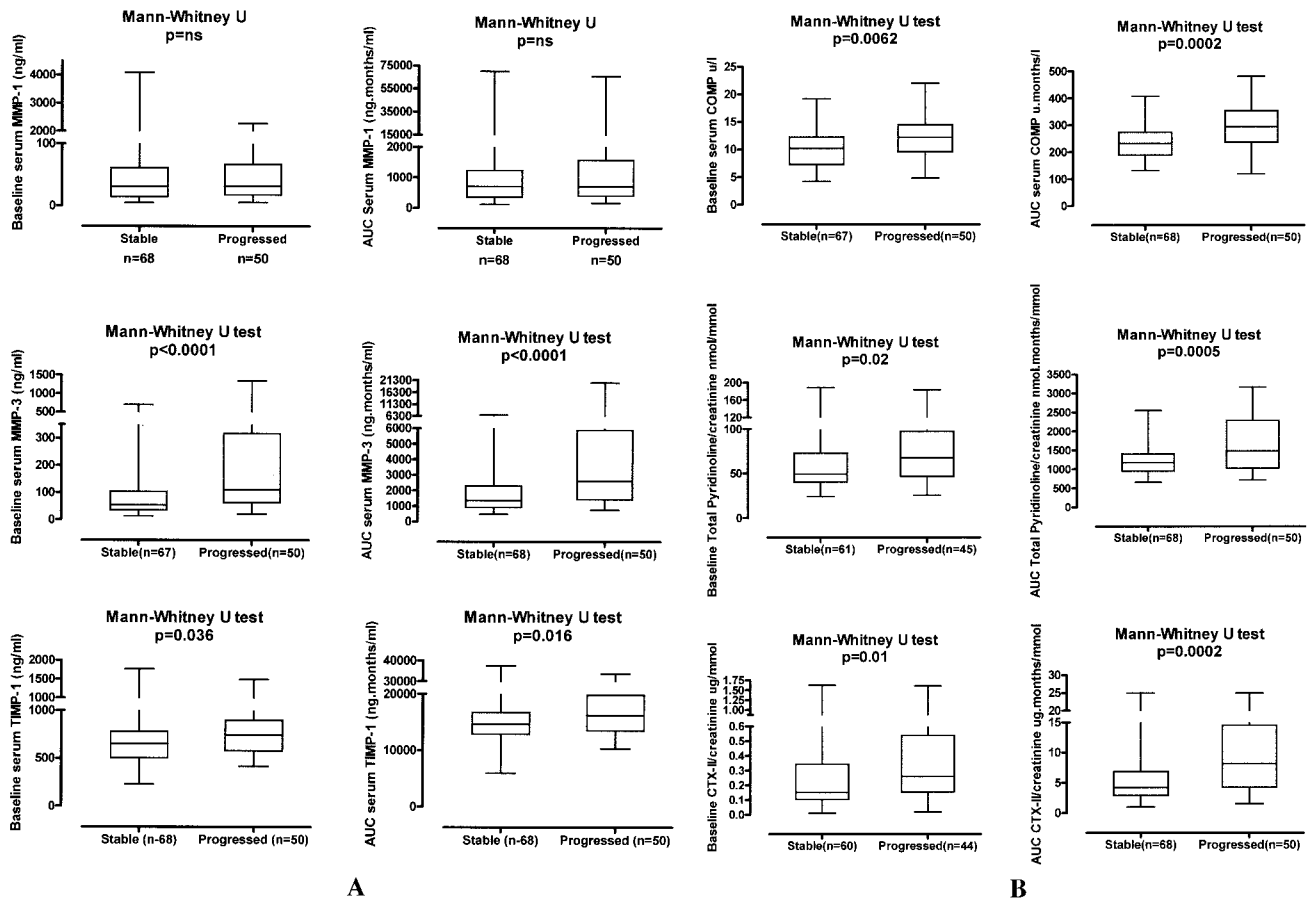


Figure 1. Baseline and longitudinal (assessed by area under the curve [AUC]) serum levels of matrix metalloproteinase 1 (MMP-1), MMP-3, and tissue inhibitor of metalloproteinases 1 (TIMP-1) (A), and serum levels of cartilage oligomeric matrix protein (COMP) and urine levels of pyridinoline and C-telopeptide of type II collagen (CTX-II) (B), in patients with early rheumatoid arthritis whose disease remained radiographically stable and those who had radiographic progression. Data are presented as box plots, where the boxes represent the 25th to 75th percentiles, the lines within the boxes represent the median, and the lines outside the boxes represent the highest and lowest values. NS = not significant.

6,788] in the radiographically stable group versus median 2,155 (nmoles/mmoles) × months [range 602–7,032] in the group with radiographic progression [*P* = 0.0099 by Mann-Whitney U test]).

Table 4 shows the Spearman’s rank correlation coefficients for entry and AUC values versus change in Larsen score over the 2-year followup period, for traditional and biochemical markers. The correlations are listed in order of descending *r* value, and only significant correlations are shown. The biochemical markers MMP-3, CTX-II, and COMP correlated with radiographic progression, and their performance compared well with that of traditional markers such as ESR, CRP, and baseline Larsen score.

Variables were dichotomized, and Table 5 shows

the results of univariate analysis of the variables at baseline and longitudinally. By subsequent multivariate logistic analysis, elevated MMP-3 and elevated urinary CTX-II were identified as the only 2 independent baseline factors that predicted radiographic progression. The performance of these 2 factors was superior to that of traditional markers, and addition of further variables did not enhance their predictive value. A test of the baseline model yielded statistically significant results ($\chi^2 = 23.08$, 2 df, *P* < 0.001; *n* = 103), with a positive predictive value of 68.8% and a negative predictive value of 69.0%. The predictive accuracy of this model as assessed by ROC was good (AUC = 0.76 [95% confidence interval 0.66–0.85]).

By multivariate analysis of the longitudinal vari-

Table 4. Spearman's rank correlation coefficients for traditional and biochemical markers, at baseline and assessed longitudinally by AUC, versus change in Larsen score at 2 years*

	r	P
Baseline markers		
MMP-3	0.4007	<0.0001
Larsen score	0.3249	0.0003
CTX-II	0.2852	0.0033
COMP	0.2658	0.0038
RF titer	0.2551	0.0053
ESR	0.2274	0.013
CRP	0.2248	0.014
TIMP-1	0.2238	0.015
Clinician's global assessment on VAS	0.2016	0.029
Pyr	0.2005	0.039
Glc-Gal-Pyr	0.2005	0.0393
AUC markers		
CRP	0.4224	<0.0001
MMP-3	0.4080	<0.0001
CTX-II	0.4063	<0.0001
44-joint swollen joint count	0.3958	<0.0001
28-joint swollen joint count	0.3799	<0.0001
COMP	0.3796	<0.0001
ESR	0.3675	<0.0001
Clinician's global assessment on VAS	0.3602	<0.0001
28-joint DAS	0.3525	<0.0001
Clinician's global assessment, score	0.3418	0.0002
DAS	0.3386	0.0002
Pyr	0.3210	0.0004
Glc-Gal Pyr	0.2904	0.0014
TIMP-1	0.2516	0.006
Pyr/D-Pyr	0.2327	0.0112
D-Pyr	0.2259	0.0139
RAI	0.1817	0.049

* Markers are listed in order of descending r value. Only significant correlations are shown. AUC = area under the curve; MMP-3 = matrix metalloproteinase 3; CTX-II = C-telopeptide of type II collagen; COMP = cartilage oligomeric matrix protein; RF = rheumatoid factor; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein; TIMP-1 = tissue inhibitor of metalloproteinases 1; VAS = visual analog scale; Pyr = pyridinoline; Glc-Gal-Pyr = glycosylated Pyr; DAS = Disease Activity Score; D-Pyr = deoxypyridinoline; RAI = Ritchie Articular Index.

ables assessed by AUC analysis, urinary CTX-II, serum MMP-3, and swollen joint count (44 joints) were identified as independent longitudinal predictors of radiographic progression. A test of the model consisting of these 3 longitudinal AUC variables yielded statistically significant results ($\chi^2 = 36.39$, 3 df, $P < 0.001$; $n = 118$), with a positive predictive value of 72.3% and a negative predictive value of 77.5%. The predictive accuracy of this model as assessed by ROC was good (AUC = 0.81 [95% confidence interval 0.73–0.89]). We then investigated whether use of the 28-joint swollen joint count instead of the 44-joint swollen joint count would have altered the results, and the AUC multivariate analysis was repeated using the 28-joint count. In this analysis, urinary CTX-II, serum MMP-3, and 28-joint swollen

joint count were identified as the best predictors of radiographic progression. The performance of this model was similar to that of the previous model ($\chi^2 = 37.77$, 3 df, $P < 0.001$, positive predictive value 73.9%, negative predictive value 77.8%, ROC AUC = 0.81 [95% confidence interval 0.73–0.89]).

Serum samples obtained prior to and following the institution of MTX treatment were available from 50 patients. Serum MMP-3 levels decreased significantly after the initiation of MTX (from a median of 88 ng/ml [range 21–1,145] to a median of 62 ng/ml [range 23–654]; $P = 0.0003$ by Wilcoxon matched pairs test). None of the other novel markers changed significantly in this group of patients. Serum CRP levels decreased following the initiation of MTX (from a median of 12 mg/liter to a median of 8 mg/liter; $P = 0.03$). No significant change in ESR occurred. A significant correlation was observed between the change in MMP-3 levels and the change in CRP levels ($r = 0.39$, $P = 0.006$). No correlation was found between changes in MMP-3 levels in response to MTX and changes in radiographic scores over the 2-year period of the study.

DISCUSSION

A number of conclusions about RA outcome variables, the use of longitudinal data, and the utility of biochemical markers can be drawn from our data. The demographic characteristics of our study patients (Table 2) are consistent with reports in the existing literature. The differences between the group with radiographically stable disease and the group with radiographic progression in terms of ESR, baseline Larsen score, number of DMARDs prescribed, and frequency of MTX use are as would be expected and have a limited impact on the interpretation of our results, which are largely based on the relationship between radiographic progression and the biomarkers measured. In accordance with the results of other studies (21,22), Tables 3 and 4 demonstrate that a wide variety of traditional markers differ between the group with radiographically stable disease and the group with radiographic progression and correlate with changes in the Larsen score.

It is apparent that patient-reported measures, such as patient-assessed disease activity, patient-assessed pain, and function as assessed by the HAQ, neither predict nor correlate with radiographic outcome (with the exception of the DAS, which includes a small patient-reported component). This highlights the need for clinicians to avoid reliance on patient-reported as-

Table 5. Performance of biochemical and traditional markers, at baseline and assessed longitudinally by AUC, as predictors of 2-year radiographic progression in univariate analysis*

	PPV, %	NPV, %	P	OR (95% CI)
Baseline†				
Biochemical markers				
MMP-3 (>71.6 ng/ml [median])‡	62.1	76.3	<0.001	5.260 (2.36–11.71)
CTX-II (>0.19 µg/mmol [median])‡	57.7	73.1	0.002	3.701 (1.62–8.43)
COMP (>10.76 units/liter [median])	55.2	69.5	0.008	2.803 (1.31–5.98)
Pyr (>54.7 nmoles/mmol [median])	52.8	67.9	0.032	2.371 (1.08–5.22)
Glc-Gal-Pyr (8.048 nmoles/mmol [median])	50.9	66.0	NS	
TIMP-1 (>663.8 ng/ml [median])	49.2	64.4	NS	
Traditional markers				
Larsen score (>0 [normal])	57.6	72.9	0.001	3.655 (1.69–7.91)
ESR (>20 mm/hour [normal])	53.8	71.7	0.006	2.956 (1.37–6.39)
SE positive (heterozygous or homozygous)	51.7	66.7	0.045	0.467 (0.22–0.98)
CRP (>5 mg/liter [normal])	48.7	69.0	NS	
Serum RF (titer >1:40 [normal])	46.4	67.6	NS	
Longitudinal§				
Biochemical markers				
MMP-3 (1,837 ng/ml)‡	59.3	74.6	<0.001	4.278 (1.96–9.36)
CTX-II (5.311 µg/mmol)‡	55.9	71.2	0.003	3.136 (1.46–6.72)
COMP	54.2	69.5	0.01	2.700 (1.27–5.74)
Total Pyr/creatinine	52.5	67.8	0.03	2.330 (1.10–4.92)
Glc-Gal-Pyr/creatinine	52.5	67.8	0.03	2.330 (1.10–4.92)
Traditional markers				
44-joint swollen joint count (115)‡	62.7	78.0	<0.001	5.951 (2.65–13.39)
CRP	61.0	76.3	<0.001	5.031 (2.27–11.15)
Clinician's global assessment	61.0	76.3	<0.001	4.278 (1.96–9.36)
ESR	59.3	74.6	<0.001	4.278 (1.96–9.36)
DAS	59.3	74.6	<0.001	4.278 (1.96–9.36)

* Markers are listed in order of descending odds ratio (OR). PPV = positive predictive value; NPV = negative predictive value; 95% CI = 95% confidence interval; NS = not significant; SE = shared epitope (see Table 4 for other definitions).

† Values in parentheses are those used in dichotomization.

‡ Markers selected by multivariate logistic regression using all variables in separate baseline and longitudinal AUC models.

§ Values in parentheses are the cutoff levels (time-integrated [times number of months]) shown to have significant predictive value among the variables found to be the best predictors in the longitudinal AUC model, by univariate analysis.

assessments when making treatment decisions aimed at preventing structural damage.

Although cross-sectional studies are often easier to perform, we believe that the true utility of biochemical markers may be revealed only by longitudinal, prospective studies. However, such studies take longer to perform, and the collection and analysis of longitudinal data are more complex. Comparisons of longitudinal results with baseline values in our study (Tables 3 and 4 and Figures 1A and B) reveal that for all observer- and laboratory-based parameters, correlation with and prediction of radiographic progression improves with the use of longitudinal data. Moreover, for a number of markers, such as swollen/tender joint counts, DAS, and D-Pyr, a relationship is apparent only from the longitudinal data.

Serum levels of MMP-3 predicted and correlated with radiographic progression, while serum MMP-1 levels did not (Figure 1A). We also found that MMP-3 levels correlated with markers of inflammation (ESR

and CRP) and levels of breakdown products (CTX-II, Pyr, and COMP) (data not shown). These results support the notion that MMP-3 has a significant role in the pathogenesis of RA, whereas MMP-1 does not. This might be explained by the suggestion that it is MMP-3-mediated MMP activation, rather than simple MMP-1 levels, that determines eventual collagenolytic activity. However, these findings directly contradict a previous report by Cunnane et al that levels of MMP-1, and not MMP-3, correlated with radiographic progression (23). Our work differs from the study by Cunnane et al in a number of respects: they examined a smaller number of patients (n = 64) over a shorter period of time (18 months), there were differences in the radiographic scoring methods used, and they measured proMMP-3 only, whereas we measured the pro, active, and TIMP-bound forms of MMP-3.

Serum TIMP-1 levels were significantly higher in the patients with radiographic progression in our cohort compared with those who had radiographically stable

disease. These findings differ from those of other studies, in which no association between TIMP-1 levels and radiographic outcome was found (23–25). Although it is counterintuitive that levels of an enzyme inhibitor would be increased in patients with more destructive disease, it may be that TIMP-1 is produced in response to the presence of increased levels of destructive enzymes, and increased MMP-3:TIMP-1 ratios in our patients with radiographic progression (data not shown) support the suggestion that despite increased TIMP-1 levels, the balance of enzymes and inhibitors in patients' joints favored net destruction.

We were unable to detect MMP-13 in any of the 259 RA serum samples tested, and other investigators detected MMP-13 in only 9 of 56 RA sera (14). In studies of patients with juvenile idiopathic arthritis, MMP-13 was also undetectable in serum and synovial fluid (26), while other authors have detected MMP-13 in the serum of patients with osteoarthritis (27,28). It has been suggested that levels of MMP-13 in RA patients may be low due to active removal and degradation by chondrocytes and synoviocytes via a specific surface MMP-13 receptor (29–31).

As seen in Figure 1B and Table 4, urine levels of CTX-II and Pyr and serum levels of COMP were significantly elevated in patients whose RA progressed radiographically compared with those whose disease remained radiographically stable, and levels correlated with radiographic progression. This finding with regard to CTX-II is consistent with previous reports that levels are elevated in patients with destructive RA (32) and are predictive of radiographic progression (33,34). A relationship with radiographic change has not, however, been previously demonstrated for urinary Pyr, although it is known that Pyr levels are elevated in patients with RA (35–37). Previous studies of serum COMP levels in patients with early RA, using the same assay that we used in the present investigation, have shown that serum COMP levels are predictive of radiographic damage (38,39). It should be noted, however, that earlier studies using a different COMP assay failed to demonstrate any relationship between COMP levels and radiographic destruction (40,41). Levels of “bone-specific” D-Pyr and “synovial” Glc-Gal-Pyr were also increased in patients with radiographic progression, although this was apparent only from longitudinal data. Such a relationship has not been previously reported for D-Pyr. Glc-Gal-Pyr, however, has been reported to be associated with progressive joint destruction in RA (42), although the present results indicated that the correlation was less marked than that for urinary Pyr.

Further evidence of a relationship between a marker and radiographic destruction would be a change in marker levels after the initiation of therapy known to reduce joint damage. In the present study, this was observed with levels of serum MMP-3, and although it is disappointing that we did not demonstrate changes in levels of other biochemical markers with MTX therapy, the study was not designed to address this question and the number of patients receiving MTX was limited. Our findings regarding serum MMP-3 levels in patients treated with MTX are consistent with the results of other studies that have shown a decrease in circulating MMP-3 levels with conventional DMARD therapy (43,44) or biologic therapy (45–47).

Methods of correlation do not readily allow comparison of the performance of different markers, nor do they indicate which combinations of markers independently predict outcome. To address these issues we performed multivariate binary stepwise logistic regression analysis. By this process, a model of baseline variables consisting of MMP-3 and CTX-II and a model of longitudinal AUC measures consisting of MMP-3, CTX-II, and swollen joint count (44- or 28-joint) were generated. These models had good predictive accuracy (baseline model ROC AUC = 0.76 [95% confidence interval 0.66–0.85]; longitudinal model ROC AUC = 0.81 [95% confidence interval 0.73–0.89]). The combinations of variables included in these models provide the best prediction of radiographic progression from all of the traditional and biochemical markers examined, and our results suggest that the variables included are independent predictors of radiographic outcome. The combination of these markers at baseline or longitudinally performed better than traditional markers such as baseline radiographic score and CRP, and adding such elements to the model did not enhance predictive value.

It is tempting to suggest from the above findings that biochemical markers such as MMP-3 and CTX-II are superior to traditional markers in predicting radiographic progression in RA. However, further testing of these models should ideally be performed on a different population, and although the statistical methods used were applied in an unbiased manner, the results must be interpreted with caution. The multivariate logistic regression method requires study of large numbers of patients in order for conclusions to be valid, and the results will vary depending on the cutoff levels chosen. Unfortunately, our cohort size of 118 patients, though reasonable, was probably not large enough for rigorous analysis of the large number of markers tested. Moreover, findings for the tested markers should not be

highly correlated with each other, but despite our efforts to exclude obviously related variables, many correlations remained. Nonetheless, the identification of MMP-3 and CTX-II as independent variables in multivariate analysis, without inclusion of markers of inflammation such as CRP, suggests that MMP-3 and CTX-II reflect destructive processes better than does CRP. This lends indirect support to the notion that destruction and inflammation are distinct processes that do not necessarily occur together but may, as has been previously suggested, become “uncoupled” (48).

Some authors suggest that levels of MMP-3 simply respond as markers of inflammation, rather than as markers of joint destruction (49–51). However, the results of our multivariate modeling indicate that MMP-3 levels are intimately related to destructive processes that result in radiographic progression. Moreover, MMP-3 levels correlate with erosive disease in RA patients despite the finding of normal CRP levels (52), and associations between MMP-3 gene promoter polymorphisms and radiographic outcome in RA have been described (53–55). Studies of conditions that are traditionally considered noninflammatory, such as osteoarthritis, have also suggested that MMP-3 levels can predict radiographic joint space narrowing (56). The ability of MMP-3 to act as a proteinase with a broad substrate profile and its ability to activate collagenase support the notion that it plays a complex role in pathologic processes. Moreover, the high serum levels of MMP-3 observed in this and other studies suggest that it may have an additional biologic role that is not yet understood.

In conclusion, we have found that patient-reported assessments are of limited value in predicting radiographic progression among patients with early RA, that summarized longitudinal measures perform better than single baseline readings, and that a number of biochemical markers, including MMP-3, TIMP-1, CTX-II, COMP, and Pyr, predict radiographic outcome. Multivariate analysis identifies serum MMP-3 and urine CTX-II as better predictors of radiographic progression compared with traditional markers, including CRP. Serum MMP-3 levels also decline following the initiation of MTX therapy. These findings are relevant with regard to the design of future longitudinal studies and highlight the unique nature and utility of information provided by biochemical markers. While these markers do not yet have a role in routine clinical practice, the costs of the assays continue to fall, and they may be used in the future to assess an individual’s response to treatment and provide a simple, direct, and more cost-effective

alternative to traditional disease activity assessment and imaging methods.

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AUTHOR CONTRIBUTIONS

Dr. Cawston had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study design. Young-Min, Cawston, Saxne, Griffiths.

Acquisition of data. Young-Min, Marshall, Coady, Christgau, Robins, Griffiths.

Analysis and interpretation of data. Young-Min, Cawston, Marshall, Coady, Christgau, Saxne, Robins, Griffiths.

Manuscript preparation. Young-Min, Cawston, Saxne, Robins, Griffiths.

Statistical analysis. Young-Min.

Coordination. Young-Min, Cawston.

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