Beneficial Effects of Adalimumab on Biomarkers Reflecting Structural Damage in Patients with Ankylosing Spondylitis

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ABSTRACT. ***Objective.*** We analyzed the effects of adalimumab on biomarkers predictive of structural damage in inflammatory arthritis.

Methods. In a 24-week randomized controlled trial, patients with active ankylosing spondylitis (AS) received adalimumab 40 mg or placebo every other week. Efficacy measures included ASsessment in Ankylosing Spondylitis International Working Group response, Bath AS Disease Activity Index (BASDAI), Total Back Pain, Bath AS Functional Index, C-reactive protein (CRP), and patient's global assessment of disease activity. Urinary type II collagen C-telopeptides (CTX-II), serum type I collagen N-telopeptides (NTX), and serum metalloproteinase-3 (MMP-3) were assessed using ELISA for treatment-group differences at baseline, 12, and 24 weeks. We determined correlations between changes in biomarkers and AS efficacy outcomes.

Results. A total of 82 patients (38 adalimumab, 44 placebo) enrolled. At 12 and 24 weeks, significant reductions in urinary CTX-II and MMP-3, but not NTX concentrations, were observed for adalimumab versus placebo ($p < 0.001$). Significant baseline correlations were noted between CRP and CTX-II ($r = 0.71$), MMP-3 ($r = 0.45$), and NTX ($r = 0.37$) ($p \leq 0.001$), as well as between CTX-II and NTX ($r = 0.49$; $p < 0.0001$). Changes in CTX-II and MMP-3 at 12 weeks correlated significantly with changes in BASDAI ($r = 0.31$ and 0.33), and CRP ($r = 0.40$ and 0.43) ($p \leq 0.005$). Change in CTX-II at 12 weeks also correlated significantly with change in MMP-3 ($r = 0.41$; $p < 0.0001$).

Conclusion. Adalimumab suppresses biomarkers that reflect matrix turnover in patients with AS. (First Release Sept 1 2008; J Rheumatol 2008;35:2030–7)

Key Indexing Terms:
ANKYLOSING SPONDYLITIS ADALIMUMAB RANDOMIZED CONTROLLED TRIAL
METALLOPROTEINASE 3 URINARY TYPE II COLLAGEN C-TELOPEPTIDE

Ankylosing spondylitis (AS) is a common form of arthritis. AS is associated with spinal pain and stiffness that leads to ankylosis of spinal joints and functional disability comparable to that of rheumatoid arthritis (RA)[1]. The advent of tumor necrosis factor (TNF) antagonists for AS represents a major advance for the management of symptoms and functional disability. To date, no treatment has been shown to halt the progression of radiographic damage manifested primarily as spinal ankylosis. A major limitation to further evaluation of these agents as potential disease modifying therapies is the current measurement instrument of choice for radiographic progression, the modified Stoke AS Spinal Score, or mSASSS[2]. This instrument scores damage according to radiographic features in the anterior corners of the cervical and lumbar spine, such as erosions, sclerosis, squaring, syndesmophytes, and bridging ankylosis. The mSASSS does not readily reveal changes, and 2 years are required to reliably detect demonstrable changes, and even then progression in the cervical and lumbar spine can be observed in only about half of all patients using this instrument[3]. Consequently, trials evaluating disease modification as a primary endpoint need to be placebo-controlled for more than 2 years, to allow detection of statistically significant changes. However, conducting a 2-year, placebo-controlled trial is neither feasible nor ethical. Data from 2 studies evaluating infliximab and etanercept suggest that anti-TNF

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agents may not prevent radiographic progression in AS[4,5]. Conclusions from these studies, however, are premature as the 2 actively treated patient groups were compared with the same historical cohort of patients with AS. Therefore, there is considerable interest in the effects of therapeutic agents on surrogates, such as soluble biomarkers, that might predict the development of radiographic damage in AS.

Several biomarkers have been shown to predict radiographic damage in patients with RA[6]. These include serum metalloproteinase 3 (MMP-3), which recently has been shown to predict radiographic progression in patients with AS[7]. This enzyme is produced by a variety of cells within joint synovia and cartilage[8]. The C-telopeptide fragment of type II collagen (CTX-II) is measurable in urine and reflects degradation of cartilage[9]. Independent of disease activity measures and baseline radiographic damage, CTX-II is one of the strongest predictors of radiographic damage in patients with RA[10,11]. A preliminary report has shown that it may also predict radiographic damage in AS[12].

Osteoclast activation is a prominent feature of AS, and markers of bone resorption are increased in active disease[13]. Moreover, TNF is a major regulator of osteoclast activity, and anti-TNF therapies suppress markers of bone resorption[14,15].

Studies evaluating the effects of anti-TNF therapies on soluble biomarkers in AS have been mostly open-label and focused mainly on MMP-3. Whereas most have shown that infliximab reduces MMP-3[16-18], it has been reported that this decrease is observed only in patients with concomitant peripheral arthritis[16], and a small randomized, placebo-controlled study showed no significant effect of etanercept on MMP-3[19]. It is not known whether this reflects the small sample size or real differences among anti-TNF agents. Anti-TNF agents also have effects on other inflammatory markers that may predict radiographic damage, such as C-reactive protein (CRP), and on magnetic resonance imaging (MRI), and it is unclear to what degree measurement of soluble biomarkers adds new information or merely reflects change in other potential prognostic markers.

Adalimumab is a fully human, anti-TNF monoclonal antibody of proven efficacy and safety in the treatment of the signs, symptoms, and functional disability of AS[20]. It substantially reduces inflammatory lesions, as evident on MRI, although its effects on progression of radiographic damage are presently unknown[21]. Our subanalysis had 3 objectives: (1) to examine the effects of adalimumab on MMP-3, urinary CTX-II, and serum N-telopeptide of type I collagen (NTX), a key bone resorption marker, in a randomized, placebo-controlled trial; (2) to examine the effects of treatment in those with and without peripheral arthritis; and (3) to examine the degree to which changes in biomarkers reflect changes in other variables of disease activity.

MATERIALS AND METHODS

Patients. Patients were adults (≥ 18 yrs of age) who had a diagnosis of AS as defined by the modified New York criteria and who were not responsive to or were intolerant of at least 1 nonsteroidal antiinflammatory drug (NSAID)[22]. Patients who had failed 1 or more disease modifying antirheumatic drugs (DMARD, such as methotrexate and sulfasalazine) were also allowed to enroll. Active AS at baseline was defined by fulfillment of at least 2 of the following 3 criteria: a Bath Ankylosing Spondylitis Disease Activity Index (BASDAI)[23] score ≥ 40; total back pain visual analog scale (VAS) score ≥ 40; and morning stiffness ≥ 1 hour. Patients could continue sulfasalazine (≤ 3 g/day), methotrexate (≤ 25 mg/wk), hydroxychloroquine (≤ 400 mg/day), prednisone and/or prednisone equivalents (≤ 10 mg/day), and/or NSAID as long as dosages had remained stable for at least 4 weeks prior to baseline. Patients who had previously received TNF antagonist therapy or ≥ 1 intraarticular joint injections with corticosteroids 4 weeks or less before baseline visit were excluded. Intraarticular corticosteroids were not allowed during the first 24 weeks of treatment. Patients with radiologic evidence of total spinal ankylosis (bamboo spine) were excluded.

The study was approved by an independent ethics committee at 7 of the 11 study centers; the other 4 study centers used a central independent ethics committee. The study was performed in accord with the ethical principles originating from the Declaration of Helsinki. Written informed consent was obtained from each patient before any study related procedures (including withholding of any medication).

Study design. This was a subanalysis of a randomized, multicenter, double-blind, placebo-controlled comparison of adalimumab with placebo for the treatment of patients with active AS. Patients were randomized 1:1 to receive either adalimumab 40 mg every other week (eow) or placebo during the initial 24-week double-blind period. Study visits occurred at baseline, Week 2, Week 4, every 4 weeks through Week 24, and then every 6 weeks through Week 52. Patients who failed to achieve an ASsessment in Ankylosing Spondylitis International Working Group 20% response (ASAS20)[24] at Weeks 12, 16, or 20 could receive open-label adalimumab 40 mg eow (early escape option). Prefilled syringes for subcutaneous injection, containing either adalimumab 40 mg or matching placebo, were provided by Abbott Laboratories (Abbott Park, IL, USA).

Clinical assessments of disease activity. Disease activity was measured at each visit using the BASDAI, a 6-item patient self-administered questionnaire. Each item was scored on a 0–100 mm VAS. Duration of morning stiffness was measured as the sixth item of the BASDAI. Additional clinical measures of disease activity included total and nocturnal back pain (0–100 mm VAS), and the patient's global assessment of disease activity (0–100 mm VAS). Other assessments included the Bath AS Functional Index (BASFI)[25], a 10-item (0–100 mm VAS) self-administered questionnaire that assesses functional disability; the Bath and Edmonton AS metrology indices (BASMI, EDASMI)[26,27]; the 44 swollen joint count; and the Maastricht AS Enthesitis Score (MASES)[28] and the Spondyloarthritis Research Consortium of Canada (SPARCC) Enthesitis Index[29]. CRP concentrations were measured at baseline and at every clinic visit using an ultrasensitive CRP assay (normal range 0.007–0.494 mg/dl).

Magnetic resonance imaging. For each patient, an MRI of the spine and sacroiliac (SI) joints was performed at baseline, Week 12, and Week 52 using appropriate surface coils for systems operating at 1.0–1.5 Tesla. The methodological and scoring approaches have been described[30,31], and are available at www.arthritisdoctor.ca. In brief, all study sites were instructed to acquire short-tau inversion recovery (STIR) sequences using the following parameters: repetition time 2720–3170 ms, echo time 38–61 ms, and time to inversion 140 ms. Spinal STIR sequences were obtained in sagittal orientation, with twelve to fifteen 4-mm slices acquired. Imaging of the spine was in 2 parts: (1) the entire cervical spine and most of the thoracic spine and (2) the lower portion of the thoracic spine and the entire lumbar spine. STIR sequences of SI joints were obtained in a coronal plane tilted parallel to the long axis of the SI joint, with twelve 4-mm slices acquired.

T1 spin-echo images of the entire spine and SI joints were also obtained for use as anatomical references. MRI lesions in the spine and SI joints were scored using the SPARCC method, as described.

Biomarker assays. Serum samples were obtained at baseline, and at 12 and 24 weeks, and stored at –20°C until assays were performed. Serum MMP-3 was measured by ELISA (Icon Central Laboratories, Farmingdale, NY, USA). The MMP-3 assay employed recognizes natural total human MMP-3 but not MMP-3 bound to α_2-macroglobulin. The range of the assay is 3.75–120 ng/ml, with sensitivity of 0.01 ng/ml. Individual samples were diluted 1:10 and assayed in duplicate.

Urinary CTX-II was measured by ELISA (Urine CartiLaps, Pacific Biometrics, Seattle, WA, USA). The second morning void urine specimens were obtained and frozen at –20°C until analysis. Values were corrected for creatinine concentration. The detection limit of the assay is 0.04 ng/ml. Individual samples were assayed in duplicate.

Serum NTX was measured by ELISA (Osteomark NTx, Icon Central Laboratories). Serum samples were diluted 1:5, and individual samples assayed in duplicate. The range of the assay is 3.2–40 nM.

Statistical analysis. The primary analysis was a between-treatment group comparison of serum concentrations for individual biomarkers, conducted using a Wilcoxon's matched-pairs, signed-ranks test (2-tailed). A last observation carried forward (LOCF) approach to the analysis of Week 24 data was used if a patient received early escape, open-label therapy prior to Week 24. The analysis was also stratified according to the presence or absence of concomitant peripheral arthritis. Pearson's correlation coefficient was used to analyze the baseline relationship between concentrations of individual biomarkers with clinical and laboratory indicators of disease activity. The same analysis was performed for the baseline to 12-week change data. Cumulative probability plots were drawn to illustrate the changes in biomarker concentrations from baseline to 12 weeks according to treatment allocation.

RESULTS

From December 22, 2003, to April 25, 2004, a total of 82 patients with active AS were enrolled in the study. Baseline demographics and clinical disease characteristics for patients randomized to receive adalimumab (n = 38) or placebo (n = 44) were similar and consistent with a typical population of patients with AS (Tables 1 and 2). Two placebo-treated patients withdrew from the study because of lack of efficacy. No patients withdrew because of adverse events.

Statistically significant improvements were observed in the signs and symptoms of AS in adalimumab-treated patients compared with those who received placebo, as determined by changes in BASDAI, patient's global assessment, total back pain, nocturnal back pain, and spinal stiffness scores at 12 and 24 weeks (Table 2). Significant improvements in function (BASFI), mobility (EDASMI), and SPARCC MRI scores for spinal inflammation, as well as CRP concentrations, were also observed for adalimumab-treated patients. Concentrations of the biomarkers MMP-3 and CTX-II decreased statistically significantly in adalimumab-treated patients, while no change was noted for NTX (Table 2, Figure 1). Cumulative probability plots show that nearly 70% of adalimumab-treated patients had a reduction in CTX-II concentrations from baseline to 12 weeks, compared with 30% of placebo patients (Figure 2). At baseline, MMP-3 concentrations were numerically but not statistically significantly greater in those patients with concomitant peripheral arthritis [mean 59.5 (SD 125.4) ng/ml] versus those with axial disease alone [mean 26.9 (SD 14.9) ng/ml]. Analysis according to the presence or absence of concomitant peripheral arthritis (defined as having at least 1 swollen joint) at baseline demonstrated that MMP-3 concentrations were significantly lower in adalimumab-treated patients (with or without peripheral arthritis) after 12 weeks (Table 3). In addition, concentrations had increased in placebo-treated patients at 12 weeks, particularly in those with concomitant peripheral arthritis. Similarly, adalimumab treatment significantly reduced CTX-II concentrations in patients with and without peripheral arthritis, while some increase was evident in placebo-treated patients at 12 weeks (primarily in those with peripheral arthritis).

At baseline, all 3 biomarkers tested correlated statistically significantly with CRP. MMP-3 and CTX-II correlated significantly with each other and with NTX (Table 3). No significant correlations were evident with clinical or MRI measures of inflammation. When correlations were analyzed for change from baseline to 12 weeks, MMP-3 and CTX-II correlated significantly with each other and also with CRP (Table 4). Moreover, significant although weak correlations were evident between change in both MMP-3 and CTX-II and clinical measures of inflammation. No such correlation was evident with MRI measures of inflammation (Table 5). Changes in NTX correlated neither with change in other biomarkers, nor with changes in clinical or MRI measures of inflammation. Analysis stratified by treatment group indicated that these correlations were evident only in adalimumab-treated patients (data not shown).

Table 1. Demographic and baseline characteristics of patients with AS randomized to adalimumab or placebo.

Variable	Placebo, n = 44	Adalimumab 40 mg eow, n = 38
Age, yrs, mean ± SD	40.0 ± 10.87	41.9 ± 11.14
Male, n (%)	36 (81.8)	29 (76.3)
Duration of AS, yrs, mean ± SD	12.1 ± 8.65	14.5 ± 9.02
Concomitant DMARD at baseline*, n (%)	9 (20.5)	6 (15.8)
Methotrexate	4 (9.1)	4 (10.5)
Sulfasalazine	5 (11.4)	3 (7.9)
Corticosteroids at baseline, n (%)	7 (15.9)	5 (13.2)
Concomitant NSAID at baseline, n (%)	40 (90.9)	34 (89.5)
HLA-B27+, n (%)	36 (81.8)	33 (86.8)
Uveitis, n (%)	15 (34.1)	17 (44.7)
Inflammatory bowel disease, n (%)	5 (11.4)	4 (10.5)
Crohn's disease	3 (6.8)	2 (5.3)
Ulcerative colitis	2 (4.5)	2 (5.3)
Psoriasis, n (%)	8 (18.2)	3 (7.9)
Reactive arthritis, n (%)	3 (6.8)	2 (5.3)

* At least 1 DMARD. AS: ankylosing spondylitis; EOW: every other week; DMARD: disease modifying antirheumatic drugs; NSAID: nonsteroidal antiinflammatory drugs.

Table 2. Mean (SD) pre- and post-treatment scores for clinical and laboratory measures of patients with AS randomized to placebo or adalimumab for 12 weeks (intention-to-treat).

Measure	Placebo, n = 44			Adalimumab, n = 38		
	Baseline	12 weeks	24 weeks*	Baseline	12 weeks	24 weeks*
BASDAI	6.5 (1.6)	5.9 (2.1)	6.1 (2.4)	6.2 (1.7)	4.2 (3.0)†	4.2 (3.2)†
Inflammation**	7.3 (1.9)	6.3 (2.4)	6.5 (2.5)	6.7 (2.2)	4.3 (3.4)†	4.4 (3.6)†
Total back pain	71.7 (14.8)	63.7 (21.3)	64.3 (25.6)	67.2 (16.7)	42.4 (32.4)†	46.1 (33.5)††
Nocturnal back pain	64.3 (20.8)	57.8 (22.4)	58.6 (26.9)	56.3 (25.0)	38.7 (31.5)†	42.5 (32.4)†
Patient global	67.8 (19.1)	60.0 (23.7)	62.8 (25.2)	66.1 (18.9)	42.4 (31.6)†	44.9 (33.1)
BASFI	5.6 (2.2)	5.3 (2.3)	5.5 (2.5)	5.3 (2.0)	4.0 (3.0)††	4.1 (3.1)††
Swollen joint score	1.8 (3.4)	3.0 (5.4)	3.0 (5.4)	2.1 (3.8)	2.8 (4.9)	2.7 (5.0)
BASMI	3.6 (2.1)	3.7 (2.2)	3.7 (2.1)	4.0 (2.2)	3.7 (2.3)	3.7 (2.3)
EDASMI	9.4 (3.8)	9.2 (3.9)	9.1 (4.0)	10.6 (3.4)	9.3 (3.0)††	9.4 (2.8)
SPARCC spinal MRI score	19.9 (19.8)	18.6 (17.5)	NC	16.0 (15.6)	6.7 (8.5)§	NC
CRP, mg/dl	2.3 (2.6)	2.4 (3.6)	2.6 (3.6)	1.8 (1.7)	0.4 (0.7)§	0.4 (0.9)§
MMP-3, ng/ml	57.1 (116.4)	73.2 (153.1)	73.3 (153.1)	25.3 (26.6)	17.0 (10.7)§	17.8 (10.8)§
Urinary CTX-II, ng/ml	388.2 (413.4)	426.3 (421.8)	430.2 (410.4)	324.8 (365.6)	254.3 (259.8)§	266.1 (286.0)§
NTX, nM	9.8 (3.5)	10.5 (4.0)	10.6 (4.0)	10.6 (4.7)	11.3 (5.5)	11.6 (6.4)

* Last observation carried forward adjusted means. ** Mean of items 5 and 6 of the BASDAI. † $p < 0.01$; †† $p < 0.05$; § $p < 0.001$. BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; BASFI: Bath Ankylosing Spondylitis Functional Index; BASMI: Bath Ankylosing Spondylitis Metrology Index; EDASMI: Edmonton AS Metrology Index; SPARCC: Spondyloarthritis Research Consortium of Canada; MMP-3: metalloproteinase 3; CTX-II: C-telopeptide fragment of type II collagen; NTX: serum N-telopeptide of type I collagen; NC: not conducted.

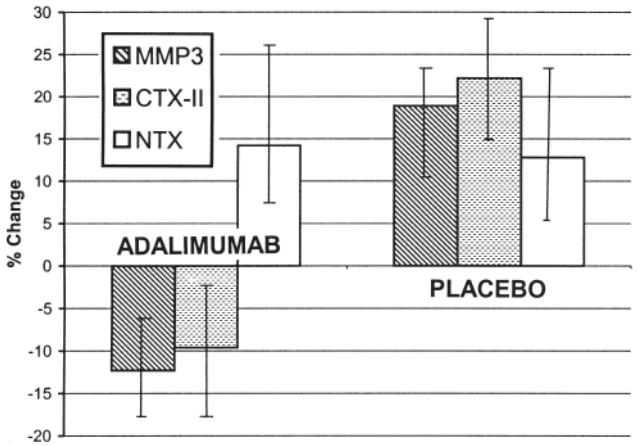


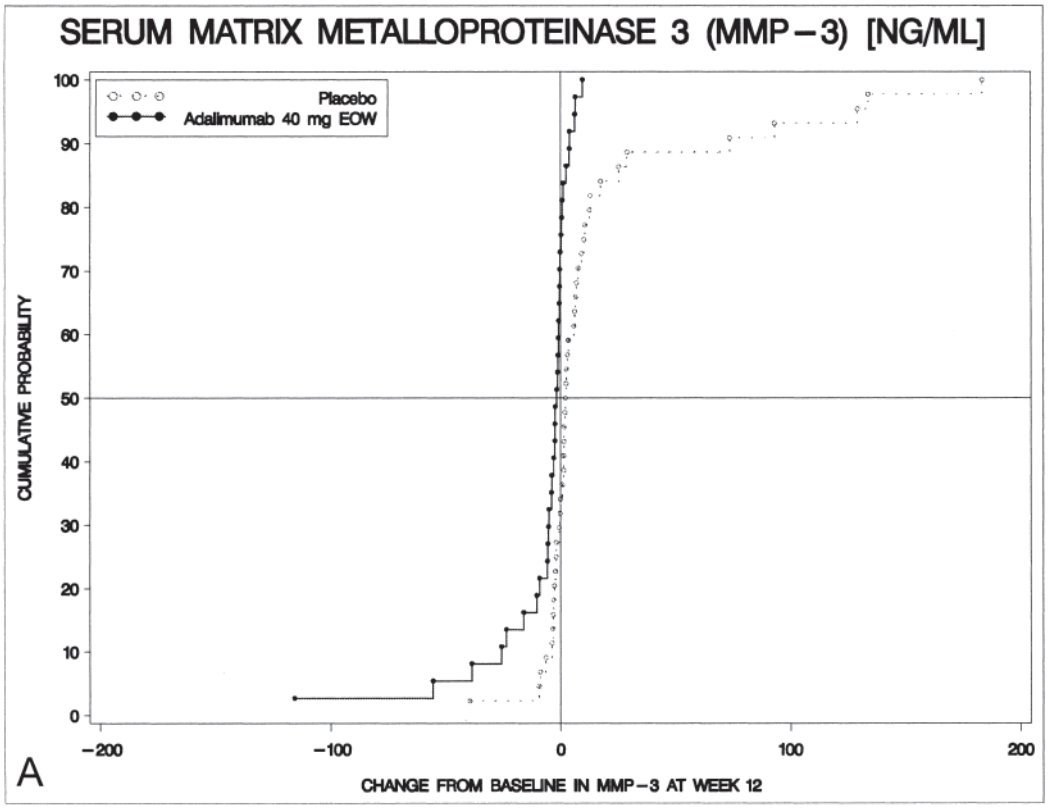
Figure 1. Percentage changes from baseline to 12 weeks in MMP-3, CTX-II, and NTX concentrations in patients, with patients randomized to placebo (n = 44) or adalimumab (n = 38). MMP-3: metalloproteinase 3, CTX-II: C-telopeptide fragment of type II collagen, NTX: serum N-telopeptide of type I collagen.

DISCUSSION

This subanalysis of patients with AS in a randomized, placebo-controlled trial demonstrated that adalimumab effectively suppresses serum concentrations of MMP-3 and urinary concentrations of CTX-II, both biomarkers shown to be associated with radiographic damage and progression in AS. This suppression was observed regardless of the presence or absence of peripheral arthritis. Both these biomarkers correlated significantly with CRP. Weak correlations were noted with clinical measures of disease activity, and no correlations were observed with MRI changes. We observed no effect of treatment on a biomarker of bone resorption, NTX.

The increased serum concentration of MMP-3 in patients with AS with concomitant peripheral arthritis and its reduction following treatment with an anti-TNF agent, infliximab, was documented[16-18,32]. However, our work now shows that adalimumab reduces MMP-3 concentrations, regardless of the presence or absence of peripheral arthritis. The further increase in MMP-3 over 12 weeks in patients who received placebo points to the persistence or even worsening of peripheral inflammation, compared with the suppression of MMP-3 observed in adalimumab-treated patients. This possibility is reinforced by the observation that both CRP and CTX-II concentrations also increased over 12 weeks in placebo-treated patients with peripheral arthritis. These observations emphasize the necessity for stratifying patients according to disease phenotype when conducting biomarker analyses.

Relative changes in MMP-3 and CTX-II were similar in adalimumab-treated patients, although baseline concentrations of MMP-3 and the absolute change in concentrations after anti-TNF therapy were less than those reported in patients with RA[33]. This is because the primary source of MMP-3 is peripheral joint synovial fibroblasts and macrophages, and most of these AS patients had limited peripheral joint involvement (Table 1). MMP-3 was one of 3 genes differentially expressed in spondyloarthropathic peripheral joint tissue compared to peripheral blood



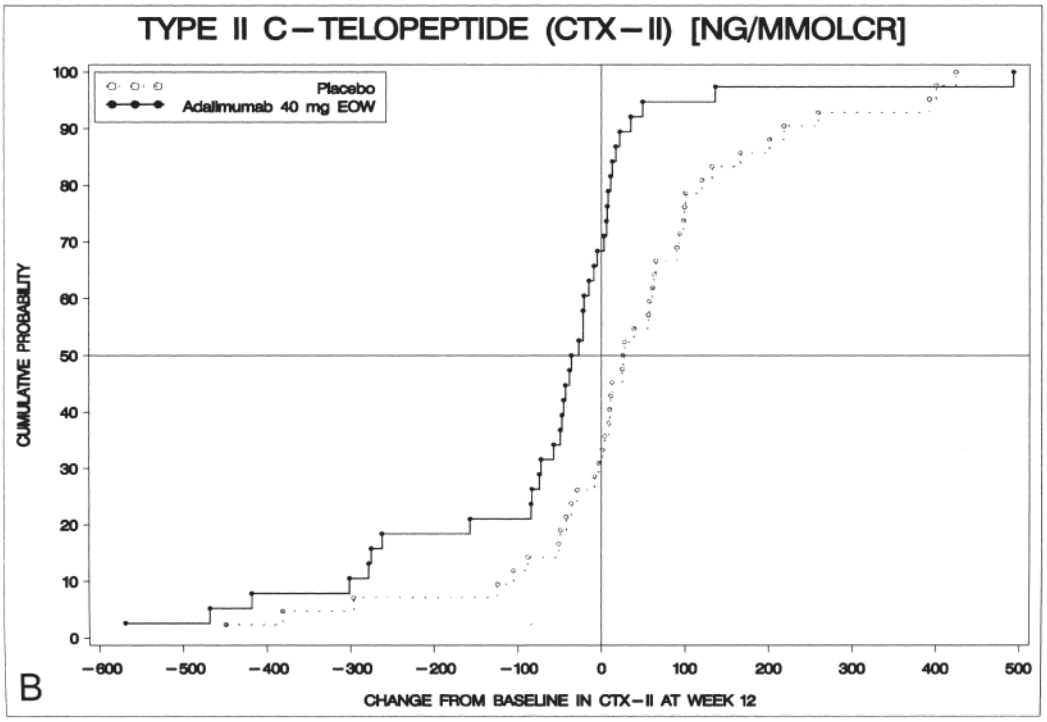


Figure 2. Cumulative probability plots for changes in (A) MMP-3 and (B) urinary CTX-II concentrations in patients randomized to adalimumab or placebo. MMP-3: metalloproteinase 3, CTX-II: C-telopeptide fragment of type II collagen.

Table 3. Biomarker concentrations at baseline in patients with AS according to peripheral joint inflammation (defined as having at least one swollen peripheral joint), All values are expressed as mean (SD).

	With Peripheral Arthritis, n = 39						Without Peripheral Arthritis, n = 43					
	Baseline		Week 12		Week 24***		Baseline		Week 12		Week 24***	
Variable	ADA	PBO	ADA	PBO	ADA	PBO	ADA	PBO	ADA	PBO	ADA	PBO
CRP	2.0 (1.4)	2.2 (3.3)	0.4 (0.5)*	2.8 (4.8)	0.6 (1.1)	0.6 (0.9)	1.6 (1.9)	2.3 (1.8)	0.4 (0.9)**	2.1 (1.8)	0.2 (0.5)	0.5 (0.6)
MMP-3	23.6 (33.1)	90.3 (163.6)	14.6 (6.4)†	122.2 (213.1)	15.7 (6.6)	44.0 (79.1)	27.0 (19.3)	26.8 (10.6)	19.3 (13.3)†	28.4 (12.7)	21.6 (11.7)	21 (10.3)
CTX-II	292.1 (243.0)	341.7 (397.7)	223.1* (137.5)	393.0 (458.8)	227.1 (173.0)	357.8 (288.0)	354.2 (453.3)	426.6 (430.9)	282.5§ (335.8)	425.7 (388.4)	340.8 (367.9)	333.3 (252.8)
NTX	11.0 (5.6)	9.8 (3.7)	10.9 (5.5)	11.0 (4.9)	10.9 (7.7)	12.6 (6.2)	10.2 (3.8)	9.8 (3.4)	11.7 (6.0)	10.1 (3.0)	11.0 (4.5)	10.3 (3.1)

* $p < 0.001$; ** $p = 0.003$; † $p = 0.01$; § $p = 0.02$; *** Week 24 data represent observed data. ADA: adalimumab; PBO: placebo; MMP-3: metalloproteinase 3; CTX-II: C-telopeptide fragment of type II collagen; NTX: serum N-telopeptide of type I collagen.

Table 4. Pearson correlation matrix of baseline concentrations of biomarkers with clinical, laboratory, and imaging variables reflecting disease activity at baseline in 82 patients randomized to adalimumab:placebo (1:1).

	MMP-3	CTX-II	NTX	CRP	BASDAI	Total Back Pain	Nocturnal Back Pain	Morning Stiffness	BASFI	MRI Spinal Score
MMP-3	—	0.27 (0.02)	0.24 (0.03)	0.45 (< 0.0001)	0.06	–0.02	0.05	0.00	0.15	–0.08
CTX-II	—	—	0.49 (< 0.0001)	0.71 (< 0.0001)	0.08	0.09	0.04	0.08	0.14	0.1
NTX	—	—	—	0.37 (< 0.05)	0.05	0.08	0.14	–0.09	0.21	0.05
CRP	—	—	—	—	0.11	0.12	0.1	0.10	0.31 (0.005)	0.36 (0.0008)

p values are in parentheses. MMP-3: metalloproteinase 3; CTX-II: C-telopeptide fragment of type II collagen; NTX: serum N-telopeptide of type I collagen; CRP: C-reactive protein; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; BASFI: Bath Ankylosing Spondylitis Functional Index; MRI: magnetic resonance imaging.

Table 5. Pearson correlation matrix of baseline to 12-week change in biomarkers with changes in clinical, laboratory, and imaging variables reflecting disease activity in 82 patients randomized to adalimumab:placebo (1:1).

	MMP-3	CTX-II	NTX	CRP	BASDAI	Total Back Pain	Nocturnal Back Pain	Morning Stiffness	BASFI	MRI Spinal Score
MMP-3	—	0.41 (0.0002)	0.16	0.45 (< 0.0001)	0.33 (0.003)	0.24 (0.03)	0.20	0.32 (0.003)	0.24 (0.03)	0.14
CTX-II	—	—	0.17	0.40 (0.0002)	0.31 (0.005)	0.35 (0.002)	0.11	0.29 (0.008)	0.29 (0.009)	0.15
NTX	—	—	—	0.07	0.13	0.17	0.22 (0.05)	0.16	0.18	0.18
CRP	—	—	—	—	0.45 (< 0.0001)	0.38 (0.0004)	0.31 (0.005)	0.32 (0.003)	0.38 (0.0004)	0.5 (< 0.0001)

p values are in parentheses. Definitions as in Table 4.

mononuclear cells from healthy controls in a 1176-gene array study[17]. Moreover, synovial fluid concentrations were 4-fold greater than serum concentrations in one study of symptomatic AS patients from China, where the majority of patients (63%) had peripheral synovitis[17]. Results from another study revealed that MMP-3 expression in synovia of AS patients with concomitant peripheral synovitis was equal to that observed in the synovia of RA patients and correlated well with cellular infiltration, vascularization, and cartilage degradation[16].

The finding that adalimumab reduces urinary CTX-II and that this reduction correlates with changes in clinical and laboratory measures of inflammation suggests that adalimumab reduces cartilage turnover through amelioration of inflammation. Because type II collagen is found in peripheral hyaline cartilage, it is not surprising that this biomarker not only is an independent predictor of damage progression in osteoarthritis but also has predictive validity for development of erosions in RA[10,11]. However, type II collagen also constitutes the most abundant protein in the nucleus pulposus of the intervertebral disc, and spondylodiscitis is a common feature observed on MRI. It is also found in the annu-

lus fibrosus. Further, a cross-sectional study of postmenopausal women demonstrated that CTX-II was independently associated with degenerative disc disease[34]. On the other hand, we observed no correlation between CTX-II and MRI measures of spinal inflammation.

The lack of effect of adalimumab on NTX, a marker of bone resorption, is consistent with results in an abstract report describing a lack of effect of infliximab in a Phase III placebo-controlled trial of patients with AS[35]. This appears to be inconsistent with a primary role for TNF in regulating osteoclast activity[36]. However, baseline NTX concentrations were relatively low at 9.77 nM for the placebo group and 10.59 nM for the adalimumab group; the mean normal concentration for men is 14.8 nM (range 5.4–24.2). This may reflect the longstanding nature of the disease prevalent for typical patients with AS recruited into Phase III trials, as some studies have shown that bone resorption markers may decrease with increasing disease duration[37]. Recent work has shown that the Wnt signaling pathway, specifically the regulatory molecule Dickkopf-1 (DKK-1), appears to play a major role in joint remodeling in RA and osteoarthritis[38]. Signaling through the Wnt pathway leads to new bone formation, while DKK-1 is a Wnt antagonist and thereby leads to increased bone resorption. Preliminary data in patients with AS show that DKK-1 concentrations are lower than those in patients with RA, and are even lower than concentrations in healthy controls, which may account for the low concentrations of NTX observed in our study.

Open-label studies have reported variable correlations between changes in MMP-3 and changes in acute-phase reactants, but associations with clinical measures have been inconsistent[16-18,32]. We observed a range of correlations for both baseline and change scores between the measured biomarkers and CRP. Particularly noteworthy was the strong correlation between CTX-II and CRP, which likely reflects the close relationship between inflammation and cartilage turnover, particularly in peripheral joints. The observation that the correlation between MMP-3 and CRP was less evident may reflect the possibility that, in some patients, the primary source of MMP-3 was the axial spine and not peripheral joints. MMP-3 is substantially expressed in macrophages within the granulation tissue associated with herniated intervertebral discs and in fibroblasts of both the annulus fibrosus and chondrocytes[39]. The presence of this enzyme in chondrocytes is related to the grade of granulation tissue formation. Spondylodiscitis is an MRI feature of spinal inflammation in AS, so it appears surprising that we observed no correlation between changes in these biomarkers and changes in MRI measures of spinal inflammation. Moreover, baseline concentrations of biomarkers were not predictive of changes in MRI over 12 weeks (data not shown). This may reflect the predominance of inflamed peripheral joints as a source of these biomarkers. Alternatively, the abnormal STIR signal quantified on MRI directly reflects inflammation, while biomarkers primarily reflect the turnover of joint matrix components, which is only indirectly related to inflammation. This would also account for our observation that baseline biomarkers do not predict antiinflammatory responses to adalimumab therapy (data not shown). These biomarkers and MRI are therefore likely to measure entirely different outcome domains. Consequently, future studies should analyze both MRI and biomarkers as potential predictors of structural damage in AS.

We examined a limited number of biomarkers considered to be high priority candidates as predictors of radiographic damage in patients with AS. Previous analysis of a panel of biomarkers reflecting turnover of joint matrix showed that only MMP-3 was predictive of radiographic progression in AS[7]. Because the hallmark of radiographic progression is joint remodeling and new bone formation, further studies in AS should examine the effects of anti-TNF agents on biomarkers such as DKK-1 and other molecules influencing the Wnt signaling pathway. This in turn will require studies evaluating their predictive validity for radiographic progression in AS.

Our study demonstrated that adalimumab suppressed a biomarker that reflects cartilage turnover as well as a metalloproteinase, specifically MMP-3. This was observed in AS patients with and without peripheral arthritis. Both biomarkers previously were shown to have predictive validity for radiographic damage in AS. Changes in biomarkers correlated with changes in CRP, and less so with clinical measures associated with inflammation. Together with the absence of a correlation with MRI features of inflammation, our observations indicate that these biomarkers reflect different facets of the disease process, and we should continue to evaluate them in studies of new therapeutic agents.

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