Changes in Biomarkers of Inflammation and Bone Turnover and Associations with Clinical Efficacy Following Infliximab plus Methotrexate Therapy in Patients with Early Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** To determine if changes in biomarkers of inflammation and bone turnover in response to treatment with infliximab plus methotrexate (MTX) versus MTX alone are associated with improvement in clinical measures of signs, symptoms, and structural damage in early rheumatoid arthritis.

Methods. Sera were collected from patients in the ASPIRE study who received 3 mg/kg (n = 48) or 6 mg/kg infliximab plus MTX (n = 55), or MTX alone (n = 41). Several baseline biomarker levels correlated with changes in median percentage of American College of Rheumatology improvement (ACR-N), 50% improvement in ACR response (ACR50), and van der Heijde-modified Sharp score (vdHSS) at Week 54.

Results. Infliximab plus MTX treatment resulted in more rapid decreases in levels of matrix metalloproteinase-3 (MMP-3), intercellular cell adhesion molecule-1, interleukin 8 (IL-8), and tumor necrosis factor-α than treatment with MTX alone. Baseline levels and decreases from baseline to Weeks 6 and 54 in MMP-3 correlated with improvement in ACR-N response at Week 54. An increase in IL-8 levels from baseline to Week 54 correlated with worsening in vdHSS at Week 54 in the MTX-alone group. Regression analysis of markers at baseline showed that MMP-3 was the only variable associated with ACR50 response and less worsening in vdHSS at Week 54.

Conclusion. Treatment with infliximab plus MTX resulted in a rapid decrease in inflammation markers. MMP-3 levels at different timepoints were consistently associated with clinical improvements at Week 54 in the infliximab plus MTX group, while increases in IL-8 levels correlated with a worsening in vdHSS at Week 54 in the MTX-alone group. (First Release June 1 2007; J Rheumatol 2007;34:1465-74)

Key Indexing Terms:
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INFLAMMATION BONE TURNOVER

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The strong, persistent inflammatory response in rheumatoid arthritis (RA) is mediated by the production of specific cytokines, which play key roles in the pathogenesis of joint inflammation and damage[1,2]. Among the proinflammatory cytokines, tumor necrosis factor-α (TNF-α), interleukin 1ß (IL-1ß), granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), and IL-8 are expressed at high levels in the rheumatoid joint and are central to the mechanisms of the inflammatory response[3-5]. TNF-α and IL-1ß upregulate endothelial expression of the intercellular cell adhesion molecule-1 (ICAM-1), and activate various cells to produce other inflammatory cytokines and mediators. Chemokines such as IL-8 may contribute to the inflammatory process by promoting the migration of lymphocytes and macrophages[1]. Activated macrophages secrete a variety of matrix metalloproteinases (MMP), including MMP-1 and MMP-3, that are associated

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with cartilage erosion in RA[6,7]. Further, increased levels of MMP-3 have been shown to correlate in early RA with the acute phase response[6]. Anti-TNF-α therapies have been shown to reduce serum MMP levels in patients with RA[8].

TNF-α and IL-1ß contribute to the mechanisms of reduced cartilage turnover and repair[9], which can be assessed using biomarkers of bone and cartilage turnover. In RA, type II collagen is extensively cleaved and degraded by the activity of collagenases. The type II collagen fragments COL 2-3/4C long neoepitope and C telopeptide II (CTX-II) have been found at increased levels in sera from patients with RA[10-12]. The COL 2-3/4C long neoepitope fragment, when occurring at increased levels in serum, serves as a marker for increased collagenase cleavage and MMP activity[6]. Recently, elevated levels of collagen type I and II fragments have been observed in patients with RA that have rapid radiographic progression, and COL 2-3/4C long neoepitope was shown to be a predictor of joint space narrowing in these patients[13]. Increased levels of C telopeptide I (CTX-I), a fragment of type I collagen, have been associated with increased bone resorption in RA patients with joint destruction[14]. Baseline levels of CTX-I and CTX-II have also been associated with an increased risk of disease progression in RA as measured over 1 year by the van der Heijde-modified Sharp score (vdHSS)[15,16]. A recent study showed that baseline CTX-II could be used as a predictor of radiographic progression in patients with established RA[17].

Treatment of patients with early RA with infliximab in combination with methotrexate (MTX) [the Active-Controlled Study of Patients Receiving Infliximab for the Treatment of Rheumatoid Arthritis (ASPIRE) trial][18] has been shown to be effective in reducing the signs and symptoms of RA, as assessed by measures of disease activity, and prevention of radiographic progression, as assessed by the vdHSS. In the ASPIRE study, a variety of biomarkers shown to be important in chronic inflammatory processes and cartilage destruction in RA were selected for evaluation. The aims of this post-hoc analysis of the ASPIRE study were to determine whether treatment with infliximab plus MTX in early RA modulates specific biomarkers differently than treatment with MTX alone, and to evaluate possible associations between baseline levels or changes in these biomarkers and major clinical endpoints [signs and symptoms of RA as measured by the American College of Rheumatology (ACR) scores and vdHSS] in the study.

MATERIALS AND METHODS

Study protocol. The ASPIRE study design has been described in detail[18]. Patients from 27 sites in the United States were randomized in a 4:5:5 ratio to receive placebo, infliximab 3 mg/kg, or infliximab 6 mg/kg at Weeks 0, 2 and 6, and then every 8 weeks through Week 46 and evaluated in a biomarker substudy. All patients were naive to MTX for at least 4 weeks prior to treatment with an oral MTX dosage of 7.5 mg/week at Week 0; MTX doses were increased by 2.5 mg every 1 to 2 weeks to 15 mg/week by Week 4 and 20 mg/week by Week 8. After Week 8, stable doses of MTX were administered to all patients who could tolerate the medication through Week 54. Patients in the ASPIRE substudy were selected by order of enrollment and availability of specific visits from patients in the ASPIRE main study.

Laboratory analyses. Sera from treated patients in the 3 mg/kg infliximab plus MTX group (n = 48) and 6 mg/kg infliximab plus MTX group (n = 55) and the placebo plus MTX group (n = 41) were collected prior to treatment at Weeks 0, 2, 6, 14, 30, and 54. Aliquoted samples were stored at –20°C and shipped to the central laboratory (Quintiles Laboratories, Atlanta, GA, USA), where they were stored at –70°C until sent to Centocor for biomarker testing. The biomarkers tested were COL 2-3/4C long neoepitope, CTX-I, MMP-3, TNF-α, IL-1ß, IL-8, and ICAM-1. ELISA kits were utilized for COL 2-3/4C long neoepitope, CTX-I (Nordic Bioscience, Herlev, Denmark), ICAM-1, and MMP-3 (R&D Systems, Minneapolis, MN, USA). TNF-α, IL-1ß, and IL-8 were tested using a Bio-plex™ bead-based sandwich enzyme immunoassay technique (Bio-Rad Laboratories, Hercules, CA, USA). All assays were validated by Centocor prior to use. Duplicate determinations were performed in a blinded fashion. The lower limit of quantification was determined for COL 2-3/4C long neoepitope (24.1 ng/ml), CTX-I (0.1 ng/ml), MMP-3 (1.6 ng/ml), TNF-α (3.2 pg/ml), IL-1ß (3.2 pg/ml), IL-8 (9.2 pg/ml), and ICAM-1 (24.7 ng/ml) (data not shown).

Clinical endpoints. Disease activity for each patient was assessed using the primary clinical measure in the ASPIRE study[18] and ACR-N criteria. The ACR-N is determined by calculating the smallest degree of improvement from baseline in the following criteria: number of tender joints, number of swollen joints, and the median of the 5 remaining measures of disease activity[19]. Improvement in ACR-N and ACR50 response was measured at Week 54. Radiographs of the hands and feet were taken prior to treatment, at Weeks 30 and 54[18]. Joint damage was assessed using the vdHSS[20].

Statistical analysis. Due to small sample sizes, all analyses were conducted on the combined 3 and 6 mg/kg infliximab plus MTX group and the placebo plus MTX group. This study is post-hoc and exploratory; therefore, the statistics are descriptive only and are not adjusted for multiplicity. Descriptive statistics (mean, median, range) were calculated for all 3 treatment groups.

The median percentage change from baseline for each biomarker was determined at Weeks 0, 2, 6, 14, 30, and 54. Statistical comparisons were made between the placebo plus MTX and infliximab plus MTX groups using analysis of variance on the van der Waerden scores. For TNF-α, IL-8, and IL-1ß, data for patients with sample baseline values that were less than the lower limit of quantification were excluded from analyses of data representing the changes in biomarker levels over time (Figures 1 and 2).

Univariate Spearman rank correlations were performed for COL 2-3/4C long neoepitope, MMP-3, ICAM-1, IL-8, CTX-I, TNF-α, IL-1ß, and C-reactive protein (CRP) on biomarker data for all patients. Correlations between percentage changes from baseline in individual biomarker levels after treatment were determined at Week 54. Correlation analyses were evaluated between clinical response measures (ACR-N, tender joint count and swollen joint count; vdHSS, joint space narrowing and erosion score; and CRP) from baseline to Week 54, and the biomarkers COL 2-3/4C long neoepitope, MMP-3, ICAM-1, IL-8, and CTX-I at baseline and changes from baseline to Week 6 and Week 54.

A multiple logistic regression analysis was performed on biomarker data for all patients to explore whether baseline levels of COL 2-3/4C long neoepitope, MMP-3, ICAM-1, IL-8, and CTX-I could serve as potential indicators of ACR50 response or vdHSS (> 0 vs ≤ 0) at Week 54 for each treatment group. The distribution MMP-3 levels was based on categories. ACR-N response and change in the vdHSS from baseline to Week 54 were compared between the infliximab plus MTX groups and the placebo plus MTX group.

Statistical analyses were performed using the SAS® system (SAS Institute, Cary, NC, USA). No multiplicity adjustments were made in the statistical tests, and 2-sided p values < 0.05 were considered significant.

RESULTS

Study population and baseline biomarker levels. Overall, the patients' baseline characteristics were comparable between

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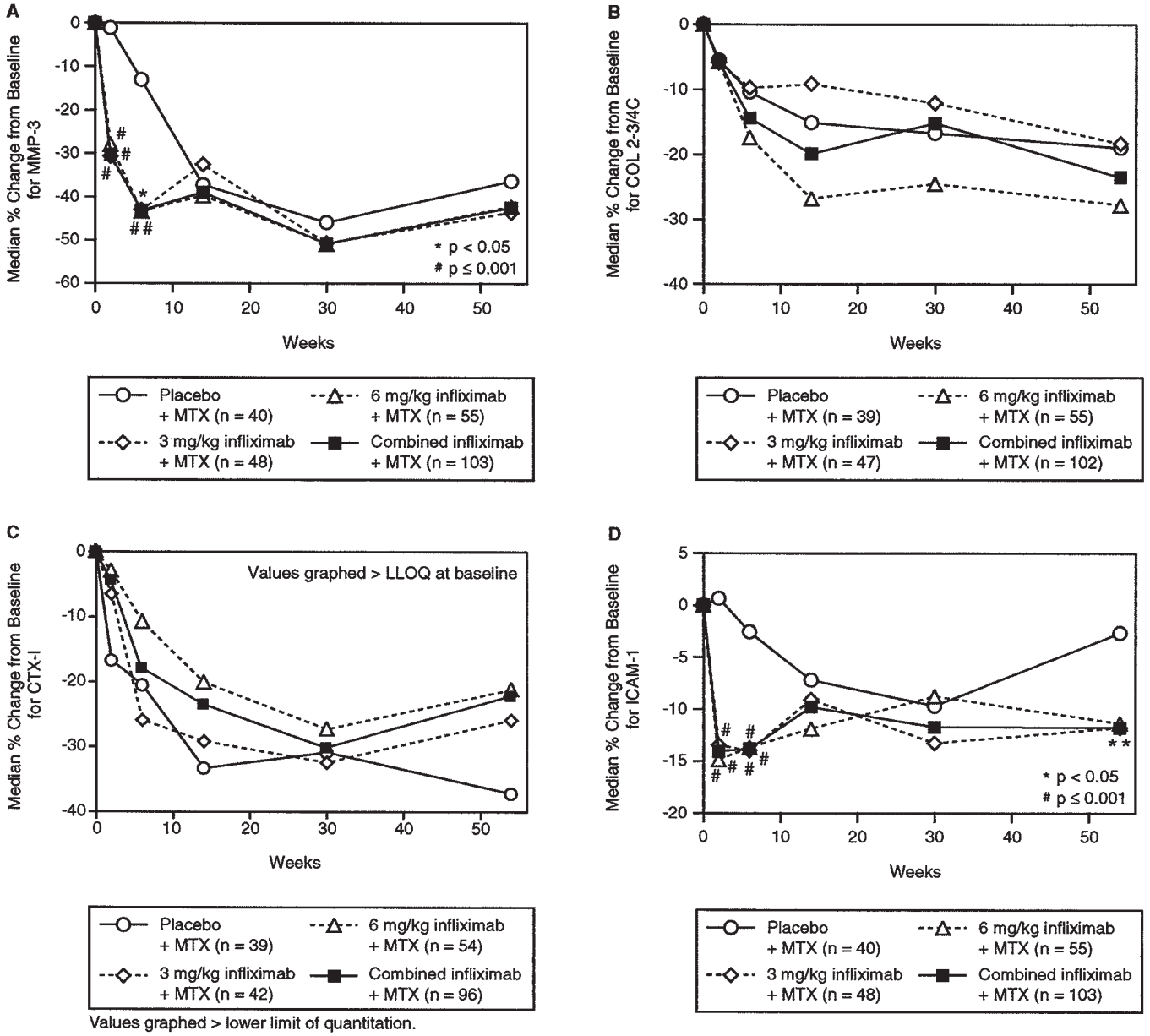

Figure 1. Change in biomarker levels over time following treatment with infliximab plus MTX or placebo plus MTX. (A) MMP-3, (B) COL 2-3/4C long neoepitope, (C) CTX-I, (D) ICAM-1. *p < 0.05; #p ≤ 0.001. MMP-3: matrix metalloproteinase-3; CTX-I: C-telopeptide I; ICAM-1: intracellular adhesion molecule-1.

the ASPIRE main trial[18] and this substudy. The baseline characteristics were similar between the combined infliximab plus MTX group and the placebo plus MTX group in the ASPIRE substudy; however, there were some differences in disease state that deserve discussion. There were no statistically significant differences between the combined infliximab plus MTX and placebo plus MTX groups for any evaluated markers at baseline, with the exception of COL 2-3/4C long neoepitope, which was significantly higher in the combined infliximab plus MTX group than in the placebo plus MTX group (p = 0.002; Table 1). Patients in the placebo plus MTX group had a higher median CRP level than patients in the infliximab plus MTX group, but this difference was not significant (Table 1). Median baseline values for MMP-3 were 1.7-fold lower in the combined infliximab plus MTX group (38.2 ng/ml) than those in the placebo plus MTX group (63.2 ng/ml) (Table 1). Also, the median baseline values of IL-8 were slightly lower for the combined infliximab plus MTX group (9.7 pg/ml) compared with the levels for the placebo plus MTX group (14.2 pg/ml).

Correlations between baseline biomarker levels. In order to determine any associations among specific baseline levels of

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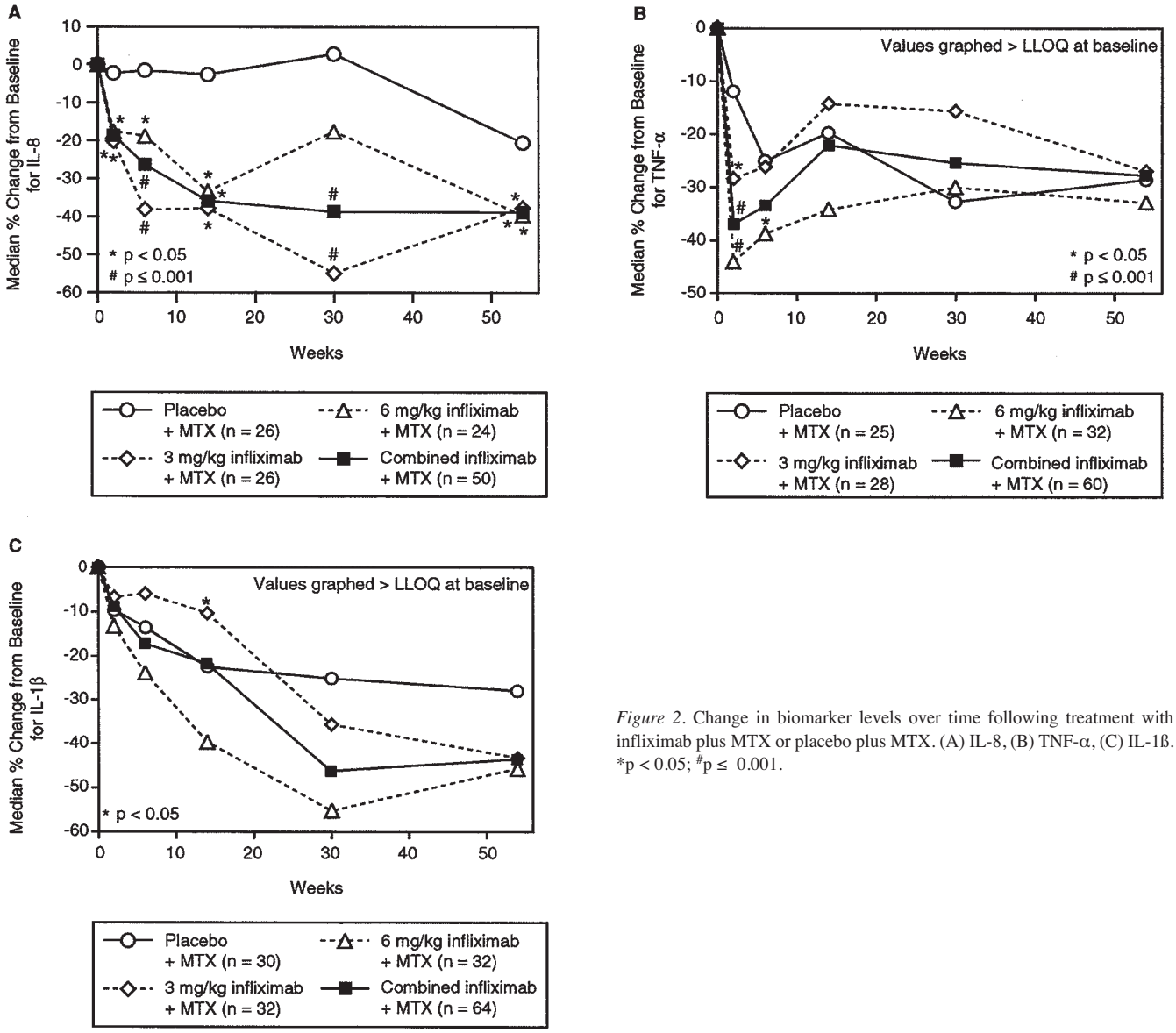

Figure 2. Change in biomarker levels over time following treatment with infliximab plus MTX or placebo plus MTX. (A) IL-8, (B) TNF-α, (C) IL-1ß. *p < 0.05; #p ≤ 0.001.

MMP-3, ICAM-1, IL-8, COL 2-3/4C long neoepitope, CTX-I, IL-1ß, and TNF-α, intercorrelations were determined for all patient samples tested (Table 2). Significant correlations at baseline were observed between COL 2-3/4C long neoepitope and ICAM-1, CTX-I, IL-1ß, and TNF-α; MMP-3 with IL-8 and IL-1ß, and CRP; ICAM-1 with CTX-I, IL-1ß, TNF-α, and CRP; CTX-I with IL-1ß, TNF-α, and CRP; and IL-1ß with TNF-α (Table 2). Of note, the strongest correlation was observed between baseline MMP-3 and CRP ($r = 0.601$, $p < 0.001$).

Correlations between changes from baseline to Week 54 in biomarker levels in the combined infliximab plus MTX group and the placebo plus MTX group. Correlations among biomarkers at Week 54 were also evaluated. In the combined infliximab group, the most significant correlation was between the change in MMP-3 and CRP from baseline to Week 54 ($r = 0.429$, $p < 0.001$; data not shown). In the placebo plus MTX group, the most significant correlation was between the change in COL 2-3/4C long neoepitope and CTX-I from baseline to Week 54 ($r = 0.549$, $p < 0.001$; data not shown). Two sets of markers, MMP-3 and ICAM-1 ($r = 0.200$, $p < 0.05$), and TNF-α and CRP ($r = 0.295$, $p < 0.001$), were significantly correlated with each other at Week 54 in the combined infliximab plus MTX group, but not at baseline (data not shown). In the placebo plus MTX group, COL 2-3/4C long neoepitope and MMP-3 ($r = 0.336$, $p = 0.037$), and IL-8 and IL-1ß ($r = 0.437$, $p = 0.006$), were significantly correlated with each other at Week 54, but not at baseline (data not shown). In addition, a number of markers were correlated at both baseline and at Week 54 in each treatment group (data not shown).

Reductions in biomarker levels following treatment with

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Table 1. Baseline characteristics for patients in the ASPIRE substudy.

Assessment	Placebo + MTX	Combined Infliximab + MTX
Patients treated, N	41	103
Age		
Mean ± SD, yrs	53.9 ± 10.5	51.4 ± 11.9
Median, yrs	56	50
p		0.231
Women, %	70.7	75.7
p		0.536
Disease characteristics		
Tender joint count		
Mean ± SD	36.9 ± 15.3	35.4 ± 16.8
Median	37	33
p		0.685
Swollen joint count		
Mean ± SD	25.1 ± 11.6	23.3 ± 11.0
Median	24	21
p		0.344
C-reactive protein, mg/dl		
Mean ± SD	2.7 ± 3.5	2.2 ± 2.8
Median	1.6	0.7
p		0.206
Patients receiving oral corticosteroids, %	51.2	45.6
p		0.544
Patients rheumatoid factor positive, %	80.5	74.8
p		0.465
Biomarker levels		
MMP-3, ng/ml		
N	41	103
Mean ± SD	70.0 ± 60.4	59.5 ± 66.3
Median	63.2	38.2
p		0.300
COL 2-3/4C long neoepitope, ng/ml		
N	40	103
Mean ± SD	221.1 ± 163.9	297.3 ± 202.2
Median	167.4	215.9
p		0.002
CTX-I, ng/ml		
N	40	103
Mean ± SD	0.64 ± 0.63	0.76 ± 2.11
Median	0.38	0.39
p		0.086
IL-8, pg/ml		
N	41	103
Mean ± SD	187.1 ± 900.8	127.7 ± 471.7
Median	14.2	9.7
p		0.210
ICAM-1, ng/ml		
N	41	103
Mean ± SD	384.5 ± 165.4	414.6 ± 244.6
Median	321.2	328.6
p		0.589
TNF-α, pg/ml		
N	41	103
Mean ± SD	7.92 ± 12.52	8.50 ± 15.47
Median	4.43	4.01
p		0.871
IL-1ß, pg/ml		
N	39	103
Mean ± SD	11.84 ± 15.15	16.35 ± 42.04
Median	5.38	5.11
p		0.603

Table 1. Continued.

Number of patients (%) with values greater than the lower limit of quantification for inflammatory biomarkers at baseline

Biomarker	Placebo + MTX, N (%)	Infliximab + MTX, N (%)
MMP-3	41 (100)	103 (100)
COL 2–3/4C long neoepitope	41 (100)	102 (99.0)
CTX-I	40 (100)	96 (93.2)
IL-8	27 (65.9)	50 (48.5)
ICAM-1	41 (100)	103 (100)
TNF-α	26 (63.4)	61 (59.2)
IL-1ß	32 (80)	65 (63.1)

MMP-3: matrix metalloproteinase-3, ICAM-1: intracellular adhesion molecule-1, TNF-α: tumor necrosis factor-α, IL-1ß: interleukin 1ß, CTX-I: C-telopeptide I.

infliximab plus MTX compared with placebo plus MTX. Decreases in markers of cartilage turnover (MMP-3 and COL 2-3/4C long neoepitope) and inflammation (ICAM-1 and IL-8) were observed at early and later timepoints for patients treated with infliximab plus MTX compared with those of patients treated with placebo plus MTX. For MMP-3, COL 2-3/4C long neoepitope, and ICAM-1 measurements, the majority of patients at baseline had values greater than the lower limit of quantitation for the assay. However, for IL-8, only limited numbers of patients had baseline samples greater than the limit of quantitation of the assay (9.2 pg/ml).

The median percentage changes of MMP-3, ICAM-1, and IL-8 were significantly reduced from baseline at the induction period (Week 2 and Week 6, or both weeks) in the combined infliximab plus MTX group, compared with the reduction from baseline in the placebo plus MTX group (Figures 1A, 1D, and 2A). TNF-α levels were significantly reduced from baseline to Week 2 in the combined infliximab plus MTX group (37%; $p < 0.001$) compared with the placebo plus MTX group (11.9%; Figure 2B). In general, no significant differences in levels of COL 2-3/4C long neoepitope (Figure 1B) or CTX-I (Figure 1C) were detected for the combined infliximab plus MTX group compared with the placebo plus MTX group throughout the 54 weeks.

A significant reduction of IL-8 was observed from baseline to Week 14 (35.9%; $p = 0.005$) and Week 30 (38.7%; $p < 0.001$) in the combined infliximab plus MTX group, but not in the placebo plus MTX group; however, these findings are based on only a subset of patients with elevated IL-8 levels at baseline (Figure 2A). By contrast, TNF-α levels were reduced from baseline in both the combined infliximab plus MTX group and the placebo plus MTX group at Week 30 (25.4% vs 32.7%, respectively; Figure 2B). Similarly, greater reductions from baseline in the levels of IL-1ß were observed in both the combined infliximab plus MTX group and the placebo plus MTX group at Week 6 (17.5% vs 13.6%, respectively) and Week 30 (46.6% vs 25.1%, respectively; Figure 2C).

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Table 2. Correlations between biomarker levels at baseline in all patients.

	COL 2–3/4C long neoepitope	MMP-3	IL-8	ICAM-1	CTX-I	IL-1ß	TNF-α	CRP
COL 2–3/4C long neoepitope		0.044	–0.127	0.341§	0.463§	0.511§	0.344§	0.066
MMP-3			0.185†	0.153	0.128	0.193†	0.083	0.601§
IL-8				0.004	–0.141	0.018	0.028	0.141
ICAM-1					0.312§	0.377§	0.263§	0.270§
CTX-I						0.497§	0.374§	0.201††
IL-1ß							0.496§	0.101
TNF-α								0.079

Values are Spearman's rank correlation coefficients (r values). † $p < 0.05$; †† $p < 0.01$; § $p = 0.001$.

At Week 54, a significant reduction from baseline in the median levels of ICAM-1 (11.8%; p = 0.019) and IL-8 (38.9%, p = 0.013) was observed in the combined infliximab plus MTX group compared with that in the placebo plus MTX group (Figures 1D, 2A). Greater reductions from baseline in the levels of IL-1ß were observed in both the combined infliximab plus MTX group and the placebo plus MTX group at Week 54 (43.9% vs 28%, respectively; Figure 2C). TNF-α levels were also reduced from baseline in both the combined infliximab plus MTX group and the placebo plus MTX group at Week 54 (27.8% vs 28.6%, respectively); however, this was not significant (Figure 2B). No significant differences were observed in the levels of MMP-3 or COL 2-3/4C long neoepitope from baseline to Week 54 (Figures 1A, 1B) between the combined infliximab plus MTX group and the placebo plus MTX group.

Correlations between biomarker changes and clinical endpoints after treatment with infliximab plus MTX. In the substudy, patients treated with infliximab plus MTX showed a significant improvement from baseline in ACR-N and ACR50 response at Week 54 compared to patients treated with placebo plus MTX (Table 3). However, there were no significant differences in changes from baseline to Week 54 in vdHSS scores between the combined infliximab plus MTX group and the placebo plus MTX group.

ACR-N and ACR50 responses. In the combined infliximab plus MTX group, a significant correlation (r = 0.319) was observed between baseline MMP-3 levels and ACR-N at Week 54: the higher the baseline MMP-3 level, the greater the improvement in ACR-N score at Week 54 (Table 4). Significant correlations were also observed between both the change in MMP-3 levels from baseline to Week 6 (r = –0.257) and from baseline to Week 54 (r = –0.247), and the change in ACR-N score from baseline to Week 54: the greater the decrease from baseline to Week 54 in MMP-3 levels, the greater the improvement in ACR-N scores from baseline to Week 54 in the combined infliximab plus MTX group (Table 4). In addition, the change from baseline to Week 54 in the levels of COL 2-3/4C long neoepitope (r = –0.212) and ICAM-1 (r = –0.247) significantly correlated with change in ACR-N score from baseline to Week 54 in the combined infliximab plus MTX group. In the placebo plus MTX group,

Table 3. Summary of van der Heijde-modified Sharp score (vdHSS), ACR-N, and ACR50 by treatment groups.

Assessment	Placebo + MTX	Combined Infliximab + MTX
vdHSS		
Change from baseline to Week 54		
N	41	103
Mean ± SD	1.85 ± 5.5	0.57 ± 5.3
Median	0.00	0.00
p		0.116
ACR-N		
Percentage improvement from baseline to Week 54		
N	41	103
Mean ± SD	0.1 ± 117.2	33.1 ± 71.6
Median	17.5	41.7
p		0.033
ACR50		
N	41	102
Patients with ACR50 response at Week 54, n (%)	12 (29.3)	48 (47.1)
p		0.051

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Table 4. Correlations between biomarker baseline levels and changes at Week 6 and Week 54 with ACR-N and change of van der Heijde-modified Sharp score observed at Week 54*. Two categories were created for MMP-3 based on the median level at baseline in all treatment groups (< 39 ng/ml vs ≥ 39 ng/ml).

Biomarker	ACR-N		van der Heijde-Modified Sharp Score	
	Placebo + MTX, n = 40[a]	Combined Infliximab + MTX, n = 99[b]	Placebo + MTX, n = 40	Combined Infliximab + MTX, n = 99
Baseline				
MMP-3	–0.019	0.319††	0.091	0.092
COL 2–3/4C long neoepitope	0.011	0.023	0.122	–0.083
CTX–I	–0.062	–0.023	–0.020	0.009
IL-8	–0.200	0.047	–0.198	–0.103
ICAM-1	–0.133	0.020	0.207	0.087
Change from baseline to Week 6				
MMP-3	–0.255	–0.257††	0.107	0.035
COL 2–3/4C long neoepitope	–0.167	–0.114	0.034	0.111
CTX–I	–0.280	–0.135	0.064	0.034
IL-8	0.110	–0.066	0.135	0.087
ICAM-1	0.247	–0.188	0.076	–0.175
Change from baseline to Week 54				
MMP-3	–0.173	–0.247††	0.074	–0.024
COL 2–3/4C long neoepitope	–0.260	–0.212†	0.074	0.074
CTX–I	–0.243	–0.149	0.090	–0.050
IL-8	0.075	0.015	0.380†	0.069
ICAM-1	0.016	–0.247††	0.136	–0.060

* Values are Spearman's rank correlation coefficients (r values). [a] Except for IL-8, where n = 26 (greater than lower limit of quantitation at baseline); [b] except for IL-8, where n = 48 (greater than lower limit of quantitation at baseline). † $p \leq 0.05$; †† $p \leq 0.01$.

there was no significant correlation between any of the biomarkers and ACR-N score.

In the combined infliximab plus MTX group, results of the logistic regression analysis of ACR50 responses at Week 54 with baseline levels of COL 2-3/4C long neoepitope, MMP-3, ICAM-1, IL-8, and CTX-I showed that MMP-3 was the only significant (p = 0.003) independent variable. An odds ratio of 1.017 indicated that increases in baseline MMP-3 levels were significantly associated with ACR50 response at Week 54 in the combined infliximab plus MTX group (Table 5). The results in Table 5 show the large variation in the confidence intervals for CTX-I in both the infliximab plus MTX group (0.627 to 1.368) and the placebo plus MTX group (0.662 to 10.201). Meanwhile, the confidence intervals for the other markers showed less variation. In comparison, no significant associations between baseline levels of these 5 biomarkers and ACR50 response at Week 54 were found in a similar logistic regression analysis for the placebo plus MTX group (Table 5).

Higher baseline levels of MMP-3 were also significantly

Table 5. Multiple logistic regression analysis of the associations between baseline biomarker levels and ACR50 responses or van der Heijde-modified Sharp scores at Week 54. Values are expressed as odds ratio (95% confidence interval). Multiple logistic regression analysis for baseline levels of biomarkers as potential indicators of ACR50 response and van der Heijde-modified Sharp Score (> 0 vs ≤ 0) at Week 54. The van der Heijde-modified Sharp score was used as a binary variable as described[21].

Biomarker	ACR50		van der Heijde-Modified Sharp Score	
	Placebo + MTX, n = 40	Combined Infliximab + MTX, n = 101	Placebo + MTX, n = 40	Combined Infliximab + MTX, n = 103
MMP-3	0.999 (0.986–1.011)	1.017† (1.006–1.028)	1.007 (0.995–1.019)	1.008†† (1.000–1.015)
COL 2–3/4C long neoepitope	1.002 (0.997–1.007)	1.000 (0.998–1.003)	1.002 (0.997–1.007)	1.001 (0.998–1.003)
CTX-I	2.599 (0.662–10.201)	0.926 (0.627–1.368)	0.578 (0.144–2.316)	0.803 (0.412–1.566)
IL-8	1.000 (0.997–1.002)	1.001 (1.000–1.002)	0.997 (0.988–1.007)	0.998 (0.994–1.001)
ICAM-1	0.997 (0.991–1.003)	0.998 (0.996–1.001)	1.003 (0.997–1.008)	1.000 (0.998–1.002)

† p = 0.003; †† p = 0.05.

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correlated with improvement in ACR-N at Week 54 for patients receiving infliximab plus MTX, but not for patients receiving placebo plus MTX. Biomarker subgroup analyses demonstrated that patients with baseline MMP-3 levels ≥ 39 ng/ml showed a significant improvement in ACR-N scores at Week 54 in the combined infliximab plus MTX group compared with patients in the placebo plus MTX group ($p = 0.003$; data not shown). In contrast, patients with baseline MMP-3 levels < 39 ng/ml did not show a difference in the ACR-N scores at Week 54 between the combined infliximab plus MTX group (N = 56) and the placebo plus MTX group (N = 16) (data not shown).

Van der Heijde-modified Sharp score. In this ASPIRE substudy, 42.8% of all patients had worsening in structural damage as measured by a change in the vdHSS > 0 [compared with 41.3% of the infliximab plus MTX-treated population in the ASPIRE main trial (Centocor, Inc.; data on file)]. There was no significant correlation between change in vdHSS at Week 54 and baseline levels of MMP-3, COL 2-3/4C long neoepitope, CTX-I, IL-8, and ICAM-1. Changes from baseline to Week 6 and to Week 54 in these biomarkers in the infliximab plus MTX group also did not correlate with worsening of vdHSS at Week 54 (Table 4). However, the change in IL-8 levels from baseline to Week 54 was significantly correlated ($r = 0.380$) with change in vdHSS at Week 54 in the placebo plus MTX group (Table 4). Specifically, an increase in IL-8 levels from baseline to Week 54 correlated with an increase in vdHSS from baseline to Week 54 in the placebo plus MTX group.

In the combined infliximab plus MTX group, the results of logistic regression analysis of the vdHSS (> 0 vs ≤ 0, as described[21]) at Week 54 with baseline levels of COL 2-3/4C long neoepitope, MMP-3, ICAM-1, IL-8, and CTX-I showed that MMP-3 was the only significant ($p < 0.05$) independent variable. An odds ratio of 1.008 indicated that elevations of baseline MMP-3 levels were significantly associated with lower vdHSS at Week 54 (Table 5). Similar to the comparison with ACR50, the results in Table 5 show the large variation in the confidence intervals for CTX-I in both the infliximab plus MTX group (0.412 to 1.566) and the placebo plus MTX group (0.144 to 2.316). In comparison, no significant associations were observed in the placebo plus MTX group between baseline levels of the 5 different biomarkers and the vdHSS at Week 54 (Table 5).

Components of ACR50 and vdHSS. In the combined infliximab plus MTX group, a significant correlation ($r = 0.246$, $p = 0.0127$) was observed between baseline MMP-3 levels and change in the tender joint count to Week 54: higher baseline levels of MMP-3 correlated with improvement in tender joint count from baseline to Week 54 in the combined infliximab plus MTX group (data not shown). Further, a decrease in MMP-3 levels observed at Week 6 correlated with improvement in joint space narrowing scores from baseline to Week 54 ($r = 0.255$, $p = 0.0098$) in the combined infliximab plus MTX group (data not shown). In contrast, in the placebo group, baseline levels of ICAM-1 correlated with a change from baseline to Week 54 in joint space narrowing scores ($r = 0.309$, $p = 0.0493$). The higher baseline levels of ICAM-1 correlated with a worsening in joint space narrowing scores. Similarly, an increase in IL-8 from baseline to Week 54 correlated with a worsening in joint space narrowing scores from baseline to Week 54 ($r = 0.348$, $p = 0.0279$; data not shown). Interestingly, in the combined infliximab group, a decrease from baseline in levels of COL 2-3/4C long neoepitope ($r = –0.35$, $p = 0.0004$) and ICAM-1 ($r = 0.31$, $p = 0.0021$), but not MMP-3 ($r = 0.015$, $p = 0.8835$), correlated significantly with a decrease in CRP levels from baseline to Week 54.

DISCUSSION

The results of this post-hoc analysis of the primary ASPIRE trial[18] show that MMP-3 levels may be linked to improvement in both signs and symptoms and structural damage associated with RA. It has been shown that patients with early RA have high serum concentrations of metalloproteinases, such as MMP-3[22]. A statistically significant association was shown between baseline levels of MMP-3 and improvement in ACR-N and ACR50 at 1 year after treatment with infliximab plus MTX. The results of this substudy also showed that baseline levels of MMP-3 had a statistically significant association with lower vdHSS at 1 year in the combined infliximab plus MTX group. The improvement in components of the ACR50 and vdHSS (tender joint count and joint space narrowing) measured at Week 54 was correlated with baseline levels of MMP-3 from baseline to Week 54 in the infliximab plus MTX group. MMP-3 levels were consistently associated at baseline and at different timepoints (change from baseline to Week 6 and to Week 54) with improvement in clinical measures in patients treated with infliximab plus MTX. These results support the role of MMP-3 in both primary (inflammation) and secondary (cartilage erosion) disease processes that occur in early RA. Of note, a recent study of 75 patients with severe and long-standing RA has shown that MMP (MMP-1 and MMP-3), along with autoantibodies and markers of bone turnover, were not predictive of clinical response to treatment with infliximab plus MTX[23]. This suggests that MMP-3 plays a more important role in the response of patients with early RA to infliximab plus MTX therapy.

Biomarkers may be useful in monitoring changes in clinical response to treatment in patients with early RA. Because RA involves inflammatory processes that if unabated will eventually lead to bone and cartilage damage, we examined biomarkers associated with inflammation (TNF-α, IL-1ß, ICAM-1, IL-8, MMP-3), bone turnover (CTX-I), and cartilage erosion (COL 2-3/4C long neoepitope, MMP-3). Our findings demonstrated from this post-hoc analysis that significant reductions from baseline occur in TNF-α, MMP-3, ICAM-1, and IL-8 at early timepoints (Week 2 and Week 6) after treatment with infliximab plus MTX, but not after treat-

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ment with placebo plus MTX. The decreases in ICAM-1 from baseline to Week 54 were significant and sustained in patients treated with infliximab plus MTX compared with patients treated with placebo plus MTX. The results suggest that these biomarkers are modulated early after initiation of infliximab plus MTX treatment, and that the effect of infliximab plus MTX is sustained. Even after 54 weeks, the significant correlation between MMP-3 and CRP observed at baseline was maintained in the infliximab plus MTX group, but not in the placebo plus MTX group (data not shown).

This study also showed that treatment with MTX alone modulated biomarkers associated with inflammation (MMP-3, ICAM-1) and bone turnover (CTX-I) in patients with early RA, although this effect occurred at later timepoints. There was a significant correlation between COL 2-3/4C long neoepitope and CTX-I at baseline in all patients and also at 54 weeks in the placebo plus MTX group, suggesting that treatment with MTX alone did not affect the relationship between these 2 markers. However, treatment with MTX alone did result in a significant correlation between MMP-3 and COL 2-3/4C long neoepitope at Week 54.

In terms of clinical significance, the reductions from baseline to Week 6 and from baseline to Week 54 in COL 2-3/4C long neoepitope, MMP-3, and ICAM-1 correlated with significant improvement in ACR-N at 1 year after treatment with infliximab plus MTX therapy, but not after treatment with MTX alone. These results suggest that MMP-3, ICAM-1, and COL 2-3/4C long neoepitope might be useful surrogate markers to monitor improvement in the signs and symptoms of RA after initiation of treatment with infliximab plus MTX therapy. Further, an increase from baseline in IL-8 levels may be useful as a biomarker to identify those patients treated with MTX alone who will likely develop structural damage as measured by the change in vdHSS from baseline to Week 54.

This substudy has several limitations. First, it was not designed to test a specific hypothesis related to biomarkers. Rather, the questions addressed here were exploratory, and therefore any results will need to be confirmed in further studies. Second, the patients in both treatment groups were a subset of the patients from the ASPIRE main study. Third, components of the ACR50 or ACR-N were used to generate the correlation analysis, which may not be the best method to determine clinical endpoints. Fourth, it is possible that the low sensitivity of the multiplex assay for TNF-α, IL-1ß, and IL-8 limited our ability to detect patients with low levels of these cytokines at baseline, thereby providing only a minimal dataset for these markers. Lastly, a more intensive sampling of blood for biomarker analyses may have minimized the variability in serum levels. These limitations may have resulted in inability to detect potentially significant associations between biomarker changes and improvement in clinical signs and symptoms or radiographic progression in the placebo plus MTX group or the combined infliximab plus MTX group.

This exploratory study suggests that MMP-3 may be a useful surrogate marker of clinical response to infliximab plus MTX therapy in patients with early RA. In contrast, IL-8 may be a useful surrogate marker of structural damage in response to treatment with MTX alone in patients with early RA. Baseline levels of MMP-3 may predict improvement in signs and symptoms (as measured by improvement in ACR-N and ACR50) and improvement in structural damage (as measured by vdHSS) after 1 year of infliximab plus MTX therapy, with further study to substantiate these findings. Because signs and symptoms and radiographic progression develop over many years in patients with RA, baseline levels and early changes in specific serum biomarkers may be useful tools to determine which patients would benefit most from infliximab plus MTX therapy. Additional studies should be conducted to explore these relationships more thoroughly.

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