E-selectin, Interleukin 18, Serum Amyloid A, and Matrix Metalloproteinase 9 Are Associated with Clinical Response to Golimumab plus Methotrexate in Patients with Active Rheumatoid Arthritis Despite Methotrexate Therapy

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ABSTRACT. ***Objective.*** To assess the effect of golimumab (human monoclonal antibody to tumor necrosis factor-α) plus methotrexate (MTX) on selected inflammatory biomarkers, and to determine if these effects predict clinical response in rheumatoid arthritis (RA).

Methods. Sera from adults with active RA despite MTX therapy, who received subcutaneous injections of placebo + MTX (MTX alone, n = 34) or golimumab 50 or 100 mg every 2 or 4 weeks + MTX (n = 137), were analyzed for levels of C-reactive protein (CRP), serum amyloid A (SAA), interleukin 18 (IL-18), E-selectin, matrix metalloproteinase 9 (MMP-9), and tissue inhibitor of matrix metalloproteinase 1 (TIMP-1).

Results. Golimumab + MTX treatment significantly decreased serum CRP, SAA, IL-18, E-selectin, TIMP-1, and MMP-9 levels (median percent changes of –4.1% to –74.3% across treatment groups) versus MTX alone (–5.8% to 9.7%) when first measured at Week 4; decreases were sustained through Week 16. Larger magnitudes of decrease in all biomarkers were observed for clinical responders versus nonresponders. For golimumab + MTX, regression analyses including all biomarkers and select clinical measures showed that reductions in levels of several markers (SAA, E-selectin, MMP-9) as early as Week 4 correlated significantly with improvement in swollen joint count (SJC) at Week 16, as did reductions in E-selectin with improvement in tender joint count at Week 16. After accounting for the biomarkers, however, treatment group was no longer significant for SJC.

Conclusion. Significant decreases in several inflammatory biomarkers were associated with golimumab + MTX therapy. Decreases in serum levels of SAA, E-selectin, and MMP-9 at Week 4 may be useful in predicting clinical response at Week 16. (J Rheumatol First Release June 1 2009; doi:10.3899/jrheum.080755)

Key Indexing Terms:
GOLIMUMAB RHEUMATOID ARTHRITIS BIOMARKERS
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Rheumatoid arthritis (RA) is characterized by a strong and persistent inflammatory response that is mediated by the production of specific cytokines. These cytokines play a significant role in the destruction of articular cartilage and bone[1,2]. Specifically, elevated expression of interleukin 18 (IL-18) and tumor necrosis factor-α (TNF-α) has been associated with macrophage infiltration in the synovial tissue of patients with RA[3]. The activated macrophages involved in the chronic inflammatory response in RA secrete several matrix metalloproteinases (MMP) that contribute to articu-

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lar tissue damage. Serum amyloid A (SAA) is an acute-phase reactant that is elevated in serum from patients with autoimmune diseases, including RA[4]. Additionally, elevated serum levels of E-selectin are associated with increased C-reactive protein (CRP) levels and erythrocyte sedimentation rates (ESR) in patients with RA, and these levels can be modulated by disease modifying antirheumatic drug (DMARD) therapy[5,6].

Treatment of RA patients with anti-TNF-α therapies reduces localized and systemic expression of markers associated with inflammation, as well as those linked with bone and cartilage turnover. Specifically, infliximab reduces the synthesis of TNF-α, IL-1α, and IL-1ß in rheumatoid synovium within 2 weeks of treatment[7]. Also, patients with RA who were treated with infliximab have decreased serum levels of TNF receptor 1 (TNFR1); TNFR2; IL-1R antagonist; IL-6; the acute-phase proteins SAA, haptoglobin, and fibrinogen[8]; IL-18[9,10]; the chemokine growth-related oncogene (GRO)-α[11]; IL-8[12]; chemokine ligand (CXCL)16[13]; and MMP-1, MMP-3[14], and MMP-9[15].

Golimumab, a novel human monoclonal antibody to TNF-α, has high affinity and specificity for human TNF-α *in vivo*, and effectively neutralizes TNF-α bioactivity *in vitro*[16]. In a Phase II study, golimumab plus methotrexate (MTX) reduced RA signs and symptoms and was generally well tolerated among patients who had an inadequate response to MTX alone. Sixty-two percent of patients in the golimumab plus MTX group achieved a clinical response based on the American College of Rheumatology 20% improvement (ACR20) criteria at Week 16, compared with 37% of patients who received MTX alone (p = 0.008). Through Week 20, 8.0% of patients who received golimumab plus MTX and 5.9% of patients who received MTX alone reported serious adverse events[17].

Selected biomarkers, known to be important in the chronic inflammatory processes associated with RA, were also evaluated in this Phase II study[17]. As a secondary endpoint to this study, we evaluated the effect of adding golimumab to MTX on specific inflammatory biomarkers in patients who were responding inadequately to MTX. We also assessed whether baseline biomarker levels or changes from baseline in these biomarkers correlated with improvement in clinical measures, i.e., ACR50 response or changes in swollen joint count (SJC) or tender joint count (TJC).

MATERIALS AND METHODS

Study protocol. Details regarding the study design and patient selection criteria have been described[17]. Briefly, this multicenter, randomized, double-blind, placebo-controlled, 5-arm, dose-ranging study assessed the efficacy, safety, and pharmacology of golimumab plus MTX compared with placebo plus MTX ("MTX alone") in patients with active RA despite treatment with MTX. Adult patients with a diagnosis of RA, as defined by ACR criteria for at least 3 months before screening, were eligible for the study. Patients were considered to have active RA if they demonstrated persistent disease activity despite receiving stable doses of MTX of at least 10 mg/week. The study was conducted in accordance with the Declaration of Helsinki and good clinical practices. The protocol was reviewed and approved by each site's institutional review board or ethics committee. All patients provided written informed consent before undergoing any study-related procedure.

Study treatment. Patients were randomly assigned in approximately equal proportions to 1 of 5 treatment groups: placebo, golimumab 50 mg every 4 weeks, golimumab 50 mg every 2 weeks, golimumab 100 mg every 4 weeks, or golimumab 100 mg every 2 weeks. Randomization was stratified by investigational site. Study medication was administered by subcutaneous injection every 2 weeks from Week 0 to Week 18. All patients continued to receive stable doses of MTX (at least 10 mg/wk) throughout the study. The primary study endpoint was the proportion of patients achieving an ACR20 response at Week 16.

Biomarker assessments. Serum samples were collected at Weeks 0, 4, and 16 from 171 patients who received subcutaneous injections of placebo every 2 weeks plus MTX (MTX alone, n = 34) or golimumab 50 or 100 mg every 2 or 4 weeks plus MTX (golimumab plus MTX group, n = 137) for evaluation of levels of CRP and other selected inflammatory biomarkers. Aliquoted samples were stored at –20°C and then shipped to the central laboratory (Quintiles Laboratories, Atlanta, GA, USA), where they were stored at –70°C. SAA, IL-18, E-selectin, MMP-9, and tissue inhibitor of matrix metalloproteinase 1 (TIMP-1) were tested using the Pierce Searchlight multiplex platform (Pathway Diagnostics, Malibu, CA, USA). Quintiles Laboratories performed CRP testing using the Roche Tinaquant assay (Roche Diagnostics, Indianapolis, IN, USA).

Statistical analyses. The percentage change from baseline for each biomarker was determined at Weeks 4 and 16. Statistical comparisons were made between the MTX-alone and the combined golimumab plus MTX groups using analysis of variance (ANOVA) on the van der Waerden normal scores.

Univariate Spearman rank correlations were performed for the biomarkers (SAA, IL-18, E-selectin, MMP-9, TIMP-1, and CRP) between the combined golimumab plus MTX and MTX-alone treatment groups at various timepoints. Correlations between baseline biomarker levels and percentage changes from baseline to Week 16 in individual biomarker levels were determined, as were correlations between select clinical characteristics [i.e., CRP, ESR, rheumatoid factor (RF) status, TJC, SJC, disease activity score using 28-joint count and CRP (DAS28-CRP), and DAS28-ESR] at baseline and biomarker levels at both baseline and Week 4. Correlation analyses were also performed between changes from baseline to Week 16 in TJC, SJC, DAS28-CRP, and DAS28-ESR and the absolute and percentage changes in biomarkers IL-18, SAA, E-selectin, MMP-9, and CRP from baseline to Weeks 4 and 16. Using ANOVA on the van der Waerden scores, baseline levels of biomarkers were also compared between ACR50 responders and nonresponders. To test the predictive power of the change in biomarkers from baseline to Week 4 on the change in SJC or TJC from baseline to Week 16, a regression analysis with treatment group and the biomarkers was used with a backward selection method on the biomarkers. Treatment group was forced into the model[18]. 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 less than 0.05 were considered significant.

RESULTS

Study population and baseline biomarker levels and clinical measures. Baseline characteristics and inflammatory biomarker levels for the study population are summarized in Table 1. The baseline characteristics of the study population have been described[17]. Overall, differences between the baseline biomarker levels in the combined golimumab plus MTX and MTX-alone groups were not significant, with the exception of E-selectin levels, which were significantly

Table 1. Baseline characteristics and biomarker levels.

Assessment	Placebo+ MTX	Golimumab + MTX 50 mg every 4 wks	50 mg every 2 wks	100 mg every 4 wks	100 mg every 2 wks
Patients treated	34	37	32	33	35
Sex, n (%) women	26 (74.3)	30 (85.7)	23 (67.6)	26 (76.5)	27 (79.4)
Age, median (IQR), yrs	52.0 (46.0, 66.0)	57.0 (50.0, 64.0)	48.0 (41.0, 63.0)	57.5 (47.0, 66.0)	53.5 (45.0, 65.0)
No. swollen joints (0–66), median (IQR)	13 (10, 18)	14 (10, 21)	14 (7, 26)	20 (12, 26)	14 (11, 21)
No. tender joints (0-68), median (IQR)	22 (16, 38)	28 (18, 40)	28 (9, 42)	32 (21, 44)	22 (16, 32)
Biomarker levels					
IL-18, median (range), pg/ml	12.1 (5.9, 87.1)	13.6 (4.3, 78.0)	11.7 7.0 (48.5)	13.6 (6.8, 60.3)	10.3 (4.6, 31.9)
E-selectin, median (range), pg/ml	44,077 (13,132, 80,537)	46,291 (12,293, 270,911)	46,129 (13,457, 125,653)	57,276 (12,639, 140,479)	44,705 (12,437, 148,044)
MMP-9 median (range), pg/ml	252,001 (32,093, 962,822)	262,242 (23,455, 1.63 10^6)	316,407 (75,197, 1.86 10^6)	279,090 31,817, 1.12 10^6)	291,702 (71,693, 886,152)
TIMP-1, median (range), pg/ml	307,049 (111,035, 498,369)	247,559 (74,840, 524,029)	257,727 (94,055, 601,486)	212,751 (1200.0, 469,205)	265,650 (35261, 751,407)
SAA, median (range), pg/ml	48,765 (1779.8, 3.56 10^6)	57,682 (2717.8, 2 10^6)	40,947 (122.0, 1.58 10^6)	29,787 (122.0, 359,932)	33,677 (3298.8, 3.31 10^6)
CRP, mg/dl	2.0 (1.3, 3.4)	2.1 (1.2, 3.4)	1.6 (0.9, 2.7)	1.4 (0.9, 2.7)	1.6 (1.0, 3.0)

CRP: C-reactive protein; IL-18: interleukin-18; IQR: interquartile range; MMP-9: matrix metalloproteinase 9; MTX: methotrexate; SAA: serum amyloid A; TIMP-1: tissue inhibitor of matrix metalloproteinase 1.

higher in the combined golimumab plus MTX group (median of 49,207 pg/ml) than in the MTX-alone group (median of 44,077 pg/ml; p = 0.0272).

Among the biomarkers evaluated at baseline in terms of correlations with select baseline clinical measures, SAA levels were significantly correlated with select clinical characteristics, including CRP (r = 0.656, p < 0.001), ESR (r = 0.270, p < 0.001), and DAS28-CRP (r = 0.179, p = 0.0217). There were also significant correlations between baseline MMP-9 and CRP levels (r = 0.216, p = 0.0054) and between baseline TIMP-1 and both CRP (r = 0.242, p = 0.0018) and ESR (r = 0.163, p = 0.037) levels.

Further assessment of correlations between baseline clinical measures and percentage changes in biomarker levels at Week 4 indicated only 1 significant relationship: changes from baseline to Week 4 in SAA levels were significantly correlated with baseline TJC (r = 0.216, p = 0.015) for the combined golimumab plus MTX group.

Correlation between biomarker levels at baseline and at Week 16. Significant correlations between several of the biomarkers were observed at baseline (Table 2A). The most significant of these correlations was between SAA and CRP, both acute-phase reactants (r = 0.656, p < 0.0001). Statistically significant but weaker correlations were also observed between MMP-9 and TIMP-1 (r = 0.289, p = 0.0002), MMP-9 and E-selectin (r = 0.269, p = 0.0005), TIMP-1 and E-selectin (r = 0.248, p = 0.0014), and TIMP-1 and CRP (r = 0.242, p = 0.0018).

At Week 16, following treatment with golimumab plus MTX, newly significant correlations were observed between E-selectin and CRP (r = 0.262, p = 0.0048), as well as between E-selectin and SAA (r = 0.247, p = 0.0077) (Table 2B). Further, the significant baseline correlations between SAA and CRP and between MMP-9 and TIMP-1 were maintained at Week 16 after treatment with golimumab plus MTX (r = 0.628, p < 0.0001; r = 0.208, p = 0.0256, respectively), but were not present after treatment with MTX alone only for SAA and CRP (r = 0.610, p < 0.0009).

Changes in biomarker levels over time by dose. There was greater magnitude of decrease from baseline to Week 4 in serum levels of IL-18, E-selectin, MMP-9, TIMP-1, and SAA in the golimumab plus MTX treatment groups compared with the MTX-alone group (Figure 1, Panels A-E). Significant decreases from baseline to Week 4 in SAA and E-selectin levels were observed across all golimumab plus MTX dose groups versus MTX alone. A similar reduction was observed at Week 16 (except for the 50 mg every 4 wks group for E-selectin). A significant reduction from baseline was also observed for MMP-9, TIMP-1, and IL-18 levels in the golimumab 50 mg q 2 weeks group versus MTX alone.

Treatment with golimumab plus MTX also resulted in reductions in CRP levels at Week 4. The reduction in CRP levels at Week 4 was more pronounced in the groups receiving 50 mg of golimumab plus MTX when compared with those receiving the 100 mg dose. Significant differences in the median percentage change from baseline in CRP levels

Table 2A. Correlations between biomarkers at baseline (Week 0).

Biomarker	IL-18	MMP-9	TIMP-1	SAA	E-selectin
MMP-9	NS				
TIMP-1	r = 0.219, p = 0.0049	r = 0.289, p = 0.0002			
SAA	NS	r = 0.201, p = 0.0100	r = 0.198, p = 0.0110		
E-selectin	r = 0.177, p = 0.0237	r = 0.269, p = 0.0005	r = 0.248, p = 0.0014	NS	
CRP	NS	r = 0.217, p = 0.0054	r = 0.242, p = 0.0018	r = 0.656, p < 0.0001	NS

CRP: C-reactive protein; IL-18: interleukin-18; MMP-9: matrix metalloproteinase 9; NS: not significant; SAA: serum amyloid A; TIMP-1: tissue inhibitor of matrix metalloproteinase 1.

Table 2B. Biomarker correlations at Week 16 by treatment group. Results for golimumab plus MTX are in bold type and below the diagonal; those for MTX alone are above the diagonal.

Biomarker	IL-18	MMP-9	TIMP-1	SAA	E-selectin	CRP
IL-18		NS	NS	NS	NS	NS
MMP-9	**NS**		NS	NS	NS	NS
TIMP-1	**r = 0.310, p = 0.0007**	**r = 0.208, p = 0.0256**		NS	NS	NS
SAA	**NS**	**NS**	**NS**		NS	(r = 0.610, p < 0.0009)
E-selectin	**r = 0.229, p = 0.0138**	**NS**	**r = 0.197, p = 0.0348**	**r = 0.247, p = 0.0077**		
CRP	**r = 0.183, p = 0.0518**	**NS**	**NS**	**r = 0.628, p < 0.0001**	**r = 0.262, p = 0.0048**	

CRP: C-reactive protein; IL-18: interleukin-18; MMP-9: matrix metalloproteinase 9; MTX: methotrexate; NS: not significant; SAA: serum amyloid A; TIMP-1: tissue inhibitor of matrix metalloproteinase 1.

were observed in the golimumab 100 mg every 4 weeks and every 2 weeks dose groups at Week 16 versus MTX alone (Figure 1, Panel F).

Comparison of biomarker changes in ACR50 responders versus nonresponders at Week 16. The baseline median levels of select clinical characteristics for ACR50 responders and nonresponders were similar for CRP (1.9 and 1.6 mg/dl, respectively), ESR (42 and 36 mm/h, respectively), SJC (11 and 9, respectively), TJC (15 and 13, respectively), DAS28-CRP score (5.43 and 5.11, respectively), and DAS28-ESR score (6.76 and 6.24, respectively).

In the combined golimumab plus MTX group, E-selectin and CRP levels were significantly decreased at Week 16 in ACR50 responders, but not in ACR50 nonresponders. Moreover, a similar trend was observed for other markers evaluated in the combined golimumab plus MTX group (Table 3). While individual baseline biomarker levels did not generally predict response to golimumab plus MTX treatment, the patients who achieved an ACR50 response tended to have higher levels of baseline inflammatory biomarkers, with the exceptions of TIMP-1 in the golimumab plus MTX group and MMP-9 and TIMP-1 in the MTX-alone group.

Correlations and associations between changes in biomarker levels and improvement in DAS28-CRP scores and swollen/tender joint counts. In the combined golimumab plus MTX group, individual reductions from baseline in SAA, MMP-9 (both at Week 4) and E-selectin (at Weeks 4 and 16) correlated significantly with improvement in DAS28-CRP at Week 16 (Table 4A). While correlations between change in CRP at Weeks 4 and 16 and improvement in DAS28-CRP at Week 16 were also significant, these findings were deemed invalid since CRP was a component of the DAS28-CRP score for this analysis. However, baseline levels and changes from baseline to Week 16 in CRP levels were significantly correlated with DAS28-ESR scores, with lower levels of CRP being associated with improvement in DAS-28 ESR scores. In the combined golimumab plus MTX group, baseline levels of IL-18 correlated significantly with improvement in SJC, and individual reductions from baseline in SAA (Week 16), E-selectin (Weeks 4 and 16), and CRP (Week 16) levels correlated significantly with improvement in SJC at Week 16, as did individual reductions from baseline in SAA and E-selectin levels at Week 16 with improvement in DAS28-ESR at Week 16 (Table 4A). Reductions from baseline in E-selectin (Weeks 4 and 16) and CRP (Week 4) levels also correlated significantly with improvement in TJC at Week 16. In the patients treated with MTX alone, changes from baseline to Week 16 in IL-18 levels correlated significantly with worsening in TJC and DAS28-ESR scores, reductions from baseline to Week 4 in CRP levels correlated significantly with improvement in SJC, and baseline levels of SAA correlated significantly with improvement in DAS28-CRP at Week 16.

Among ACR50 responders (n = 42) in the combined golimumab plus MTX group, reductions in IL-18 correlated significantly with improvement in TJC (r = –0.396, p = 0.0095) and SJC (r = –0.436, p = 0.0039) at Week 16. Only 1 patient treated with MTX alone was an ACR50 responder at Week 16; thus, no correlations could be determined in this group.

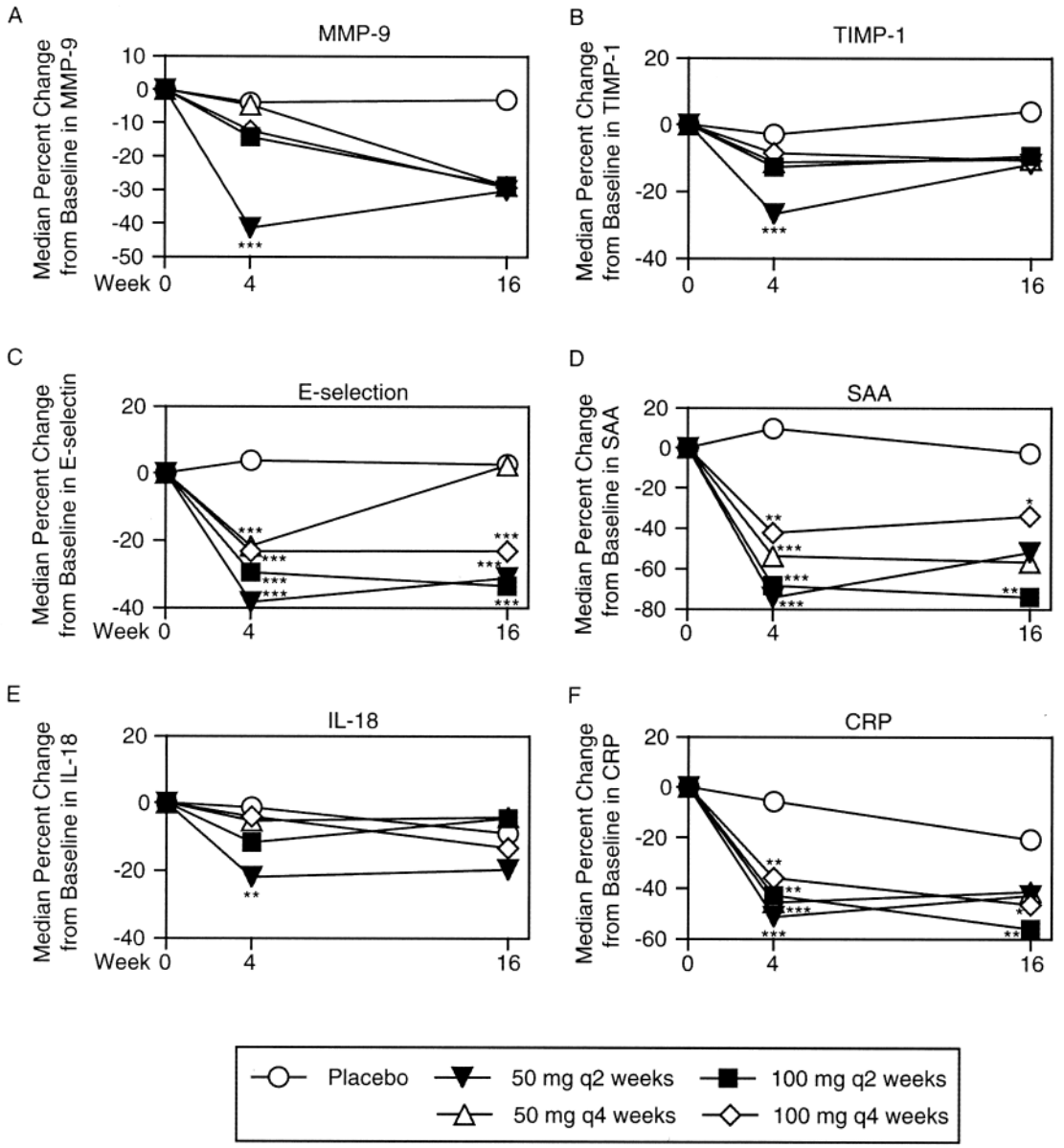

Figure 1. Changes from baseline in biomarker levels. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, for comparisons with the placebo group. All treatment groups also received methotrexate. MMP-9: matrix metalloproteinase 9; TIMP-1: tissue inhibitor of proteinase l; SAA: serum amyloid A; CRP: c-reactive protein; IL-18: interleukin 18.

In a regression analysis including all of the above biomarkers (Table 4B), decreases from baseline in E-selectin ($p = 0.045$), SAA ($p < 0.0001$), and MMP-9 ($p = 0.042$) at Week 4 were significantly associated with reductions in SJC at Week 16 (Table 4B). The decrease from baseline in SAA at Week 4 was also significantly associated with a reduction in TJC at Week 16. Moreover, in a separate analysis, decreases in TJC were significantly affected by treatment group (golimumab vs placebo; $p < 0.0001$). In an analysis including both changes in biomarkers at Week 4 and treatment group, the decreases in E-selectin, SAA, and MMP-9 remained significant, while treatment group was not significant for SJC. However, both SAA and treatment group were significant for TJC (data not shown).

DISCUSSION

As previously reported, golimumab, a new anti-TNF-α antibody administered subcutaneously in combination with MTX, effectively reduced the signs and symptoms of RA in patients inadequately responsive to MTX[17]. In this multicenter, randomized, double-blind, placebo-controlled, 5-arm, dose ranging study, we compared early changes in inflammatory biomarkers between patients receiving golimumab plus MTX and those receiving MTX alone and eval-

Table 3. Comparison of biomarker changes in ACR50 responders and nonresponders.

Biomarker	Treatment Group	Visit	ACR50 Responders (n = 43) median	ACR50 Nonresponders (n = 75) median	p
SAA, pg/ml	Combined golimumab plus MTX	Baseline value	44,237	27,947	NS
		Percent change at wk 16	–67.1	–44.1	NS
	Placebo plus MTX	Baseline value	48,847	46,723	NS
		Percent change at wk 16	–18.0	–2.6	NS
E-selectin, pg/ml	Combined golimumab plus MTX	Baseline value	51,504	46,129	NS
		Percent change at wk 16	–36.6	–19.0	< 0.001
	Placebo plus MTX	Baseline value	56,291	35,116	NS
		Percent change at wk 16	–13.2	3.4	NS
CRP, mg/dl	Combined golimumab plus MTX	Baseline value	1.9	1.4	NS
		Percent change at wk 16	60.0	44.4	0.042
	Placebo plus MTX	Baseline value	3.2	2.0	NS
		Percent change at wk 16	–1.3	20.8	NS
IL-18, pg/ml	Combined golimumab plus MTX	Baseline value	12.7	11.4	NS
		Percent change at wk 16	–11.4	–7.0	NS
	Placebo plus MTX	Baseline value	15.2	11.9	NS
		Percent change at wk 16	3.4	–9.1	NS
MMP-9,	Combined golimumab plus MTX	Baseline value	304,188	278,280	NS
		Percent change at wk 16	–32.4	–24.1	NS
	Placebo plus MTX	Baseline value	178,248	249,387	NS
		Percent change at wk 16	–17.5	6.4	NS
TIMP-1, pg/ml	Combined golimumab plus MTX	Baseline value	248,852	271,179	NS
		Percent change at wk 16	–10.7	–10.2	NS
	Placebo plus MTX	Baseline value	290,442	308,858	NS
		Percent change at wk 16	8.7	4.0	NS

ACR50: at least 50% improvement in American College of Rheumatology response criteria; CRP: C-reactive protein; IL-18: interleukin-18; MMP-9: matrix metalloproteinase 9; MTX: methotrexate; NS: not significant; SAA: serum amyloid A; TIMP-1: tissue inhibitor of matrix metalloproteinase 1.

uated the correlation of those changes with clinical responses. The findings from the current analysis are limited by the fact that controlled data were only available through Week 16 since patients in the MTX plus placebo group were given infliximab after this timepoint.

As demonstrated for clinical efficacy[17], treatment with golimumab plus MTX resulted in greater and more rapid reductions in serum levels of select markers associated with the disease processes of RA (IL-18, MMP-9, TIMP-1, SAA, E-selectin, and CRP) as early as Week 4 and continuing through Week 16, than did treatment with MTX alone. Since patients with active RA have higher circulating levels of these markers[3-6], these reductions more likely reflect inhibition by golimumab plus MTX treatment of the acute-phase response (SAA and CRP), inflammatory processes (IL-18 and E-selectin), and collagen breakdown (MMP-9 and TIMP-1) associated with the progression of RA. More specifically, IL-18 has been shown to have a direct effect on macrophages and to upregulate expression of TNF-α, IL-1, and IL-6, while E-selectin is an adhesion molecule that is upregulated during inflammation. Correlations between SAA and CRP, as well as between MMP-9 and TIMP-1, were maintained during treatment with golimumab plus MTX, suggesting that the improvement in the levels of these serum markers is more likely an indication of changes in the disease processes by golimumab plus MTX treatment rather than selective and specific modulation of any of these markers by golimumab. The weak correlations observed between serum IL-18, E-selectin, and TIMP-1 levels at baseline and slightly stronger correlations at Week 4 suggest that treatment with golimumab plus MTX did not affect these associations.

As expected, regardless of the treatment regimen, larger decreases from baseline in most of the inflammatory biomarkers studied were observed in ACR50 responders relative to ACR50 nonresponders. Interestingly, the combination of golimumab plus MTX treatment led to larger decreases in essentially all biomarkers studied compared with MTX-alone treatment in both ACR50 responders and nonresponders, although these differences did not reach statistical significance. Levels of IL-18 and TIMP-1 actually increased among patients treated with MTX alone, even among ACR50 responders. Among ACR50 responders, lower baseline levels of SAA were associated with greater improvement in SJC and TJC; among ACR50 nonresponders, higher baseline levels of IL-18 were associated with less improvement in SJC and TJC in the combined golimumab plus MTX group. Reductions in levels of several markers (SAA, E-selectin, and MMP-9) as early as Week 4 correlated significantly with improvement in SJC at Week

Table 4A. Correlations between percentage changes in biomarker levels and improvement in swollen and tender joint counts and DAS28-CRP at Week 16.

Biomarker	Treatment Group	Visit	Swollen Joint Count	Tender Joint Count	DAS28-CRP	DAS28-ESR
IL-18	Golimumab plus MTX	Baseline	r = –0.178, p = 0.0410	NS	NS	NS
		Wk 4	NS	NS	NS	NA
		Wk 16	NS	NS	NS	NS
	Placebo plus MTX	Baseline	NS	NS	NS	NS
		Wk 4	NS	NS	NS	NA
		Wk 16	NS	r = +0.465, p = 0.0126	NS	r = –0.467, p = 0.0186
SAA	Golimumab plus MTX	Baseline	NS	NS	NS	NS
		Wk 4	NS	NS	r = +0.203, p = 0.0240	NA
		Wk 16	r = –0.205, p = 0.0296	NS	NS	r = +0.258 p = 0.0059
	Placebo plus MTX	Baseline	NS	NS	r = +0.374, p = 0.0348	NS
		Wk 4	NS	NS	NS	NA
		Wk 16	NS	NS	NS	NS
E-selectin	Golimumab plus MTX	Baseline	NS	NS	NS	NS
		Wk 4	r = –0.212, p = 0.0177	r = –0.192, p = 0.0317	r = +0.317, p = 0.0003	NA
		Wk 16	r = –0.216, p = 0.0218	r = –0.230, p = 0.0144	r = +0.302, p = 0.0012	r = +0.301, p = 0.0012
	Placebo plus MTX	Baseline	NS	NS	NS	NS
		Wk 4	NS	NS	NS	NA
		Wk 16	NS	NS	NS	NS
CRP	Golimumab plus MTX	Baseline	NS	NS	NA	r = +0.273, p = 0.0012
		Wk 4	NS	r = –0.198, p = 0.0240	NA	NA
		Wk 16	r = –0.204, p = 0.0272	NS	NA	r = –0.257, p = 0.0056
	Placebo plus MTX	Baseline	NS	NS	NA	NS
		Wk 4	r = –0.354, p = 0.0432	NS	NA	NA
		Wk 16	NS	NS	NA	NS
MMP-9	Golimumab plus MTX	Baseline	NS	NS	NS	NS
		Wk 4	NS	NS	r = +0.206, p = 0.0220	NA
		Wk 16	NS	NS	NS	NS
	Placebo plus MTX	Baseline	NS	NS	NS	NS
		Wk 4	NS	NS	NS	NA
		Wk 16	NS	NS	NS	NS

CRP: C-reactive protein; DAS28-CRP/ESR: disease activity score based on 28-joint count and incorporating CRP/ESR; IL-18: interleukin-18; MMP-9: matrix metalloproteinase 9; MTX: methotrexate; NA: not applicable/invalid because DAS28-CRP includes CRP in its calculation or because DAS28-ESR was not determined at Wk 4; NS: not significant; SAA: serum amyloid A.

16, as did reductions in SAA with improvement in TJC at Week 16. However, after accounting for the biomarkers, treatment group was no longer significant for SJC. This is noteworthy in that the overall results showed that treatment affected the biomarkers at Week 4, which in turn affected SJC at Week 16. These data imply that the decreases in SAA, E-selectin, and MMP-9 levels at Week 4 can be used to predict improvement in SJC at Week 16 for patients treated with golimumab plus MTX. Similarly, decreases in SAA and E-selectin correlated significantly with improvement in DAS28-CRP scores in golimumab plus MTX-treated patients. These results suggest that measuring levels of select serum proteins as early as Week 4 after initiation of golimumab plus MTX treatment could be useful in determining if a patient will have an improvement in specific clinical measures at Week 16. Thus, this type of analysis would allow early identification of responders and nonresponders to golimumab plus MTX treatment.

The majority of published data describing the pharmacodynamic effects of anti-TNF-α therapies have been derived

Table 4B. Regression analysis of improvements in swollen and tender joint counts at Week 16.

Measure	Tender Joint Count		Swollen Joint Count	
	Regression Coefficient*	p	Regression Coefficient*	p
Intercept	–9.97	NS	–44.01	< 0.0001
Treatment**	–45.17	< 0.0001	–7.53	NS
SAA	6.99×10^{-4}	0.016	0.0018	< 0.0001
E-selectin	—	NS	0.30	0.045
MMP-9	—	NS	0.13	0.042

* Treatment group was required to be in the final regression model. All biomarkers were included in the regression model, but only significant ones are displayed. Coefficients for measures not retained in the final model are not shown. ** Placebo plus MTX or golimumab plus methotrexate. SAA: serum amyloid A; MMP-9; matrix metalloproteinase 9; NS: nonsignificant.

from patients with RA treated with infliximab. We have previously shown that infliximab plus MTX therapy results in the more rapid decrease of select inflammatory markers, including MMP-3, compared to treatment with MTX alone. In addition, MMP-3 levels at baseline and decreases in MMP-3 levels at Week 6 and Week 54 correlated significantly with clinical improvement in the infliximab-treated group, as measured by the ACR Index of Improvement (ACR-N) response at Week 54[12]. In our current study, levels of serum markers associated with the disease processes of RA decreased in response to golimumab plus MTX treatment. Further, these changes suggest that several of these proteins would serve as markers of improvement in clinical response to this novel anti-TNF agent. Reductions in serum levels of SAA[19], MMP-9[15], and IL-18[9] in patients with RA have been described following infliximab treatment; however, associations between these changes and improvement in clinical measures were not determined in the previous studies. In contrast, in a small study of 10 patients with RA treated with infliximab, no significant differences were observed in plasma levels of intercellular cell adhesion molecule-1, vascular cell adhesion molecule-1, vascular endothelial growth factor, or E-selectin prior to and after each infliximab infusion[20].

Ours is the first report of the effects of golimumab, a new human monoclonal antibody to TNF-α, on biomarker levels in patients with active RA despite MTX treatment. The addition of golimumab to MTX, in patients inadequately responsive to MTX alone, resulted in significant decreases in serum levels of select markers associated with the disease processes of RA as early as 4 weeks (the first evaluation after initiating golimumab treatment), consistent with the effect of golimumab therapy on signs and symptoms. In addition, decreases in E-selectin, IL-18, SAA, and MMP-9 correlated with improvement in several clinical measures in patients with RA treated with golimumab plus MTX. Larger studies are needed to confirm our findings and determine if these proteins could be utilized as markers of clinical response to the combination of golimumab and MTX in patients with active RA.

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Correction

E-selectin, interleukin 18, serum amyloid A, and matrix metalloproteinase 9 are associated with clinical response to golimumab plus methotrexate in patients with active rheumatoid arthritis despite methotrexate therapy. Visvanathan S, Wagner C, Rojas J, Kay J, Dasgupta B, Matteson EL, Mack M, Baker DG, Rahman MU. J Rheumatol 2009;36:1371-9.

Page 1373, left column, the first paragraph should read: "Among the biomarkers evaluated at baseline in terms of correlations with select baseline clinical measures, SAA levels were significantly correlated with select clinical characteristics, including CRP ($r = 0.656$, $p < 0.001$), ESR ($r = 0.270$, $p < 0.001$), and DAS28-CRP ($r = 0.226$, $p = 0.0036$)."

Page 1374, left column, the second section should read: "The baseline median levels of select clinical characteristics for ACR50 responders and nonresponders were similar for CRP (1.9 and 1.6 mg/dl, respectively), ESR (42 and 36 mm/h, respectively), SJC (11 and 9, respectively), TJC (15 and 13, respectively), DAS28-CRP score (5.85 and 5.82, respectively), and DAS28-ESR score (6.76 and 6.24, respectively)."

Page 1374, bottom of the left column, the sentence should read: "In the combined golimumab plus MTX group, individual reductions from baseline in MMP-9 (at Week 4) and SAA and E-selectin (both at Weeks 4 and 16) correlated significantly with improvement in DAS28-CRP at Week 16 (Table 4A)."

Table 4A should read as given below; we regret the errors.

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Table 4A. Correlations between percentage changes in biomarker levels and improvement in swollen and tender joint counts and DAS28-CRP at Week 16.

Biomarker	Treatment Group	Visit	Swollen Joint Count	Tender Joint Count	DAS28-CRP	DAS28-ESR
IL-18	Golimumab plus MTX	Baseline	$r = –0.178$, $p = 0.0410$	NS	NS	NS
		Wk 4	NS	NS	NS	NA
		Wk 16	NS	NS	NS	NS
	Placebo plus MTX	Baseline	NS	NS	NS	NS
		Wk 4	NS	NS	NS	NA
		Wk 16	NS	$r = +0.465$, $p = 0.0126$	NS	$r = –0.467$, $p = 0.0186$
SAA	Golimumab plus MTX	Baseline	NS	NS	$r = +0.185$, $p = 0.0335$	NS
		Wk 4	NS	NS	$r = +0.247$, $p = 0.0057$	NA
		Wk 16	$r = –0.205$, $p = 0.0296$	NS	$r = +0.219$, $p = 0.0206$	$r = +0.258$ $p = 0.0059$
	Placebo plus MTX	Baseline	NS	NS	$r = +0.417$, $p = 0.0176$	NS
		Wk 4	NS	NS	NS	NA
		Wk 16	NS	NS	NS	NS
E-selectin	Golimumab plus MTX	Baseline	NS	NS	NS	NS
		Wk 4	$r = –0.212$, $p = 0.0177$	$r = –0.192$, $p = 0.0317$	$r = +0.341$, $p = 0.0001$	NA
		Wk 16	$r = –0.216$, $p = 0.0218$	$r = –0.230$, $p = 0.0144$	$r = +0.306$, $p = 0.0010$	$r = +0.301$, $p = 0.0012$
	Placebo plus MTX	Baseline	NS	NS	NS	NS
		Wk 4	NS	NS	NS	NA
		Wk 16	NS	NS	NS	NS
CRP	Golimumab plus MTX	Baseline	NS	NS	NA	$r = +0.273$, $p = 0.0012$
		Wk 4	NS	$r = –0.198$, $p = 0.0240$	NA	NA
		Wk 16	$r = –0.204$, $p = 0.0272$	NS	NA	$r = –0.257$, $p = 0.0056$
	Placebo plus MTX	Baseline	NS	NS	NA	NS
		Wk 4	$r = –0.354$, $p = 0.0432$	NS	NA	NA
		Wk 16	NS	NS	NA	NS
MMP-9	Golimumab plus MTX	Baseline	NS	NS	NS	NS
		Wk 4	NS	NS	$r = +0.203$, $p = 0.0236$	NA
		Wk 16	NS	NS	NS	NS
	Placebo plus MTX	Baseline	NS	NS	NS	NS
		Wk 4	NS	NS	NS	NA
		Wk 16	NS	NS	NS	NS

CRP: C-reactive protein; DAS28-CRP/ESR: disease activity score based on 28-joint count and incorporating CRP/ESR; IL-18: interleukin-18; MMP-9: matrix metalloproteinase 9; MTX: methotrexate; NA: not applicable/invalid because DAS28-CRP includes CRP in its calculation or because DAS28-ESR was not determined at Wk 4; NS: not significant; SAA: serum amyloid A.