Protein Biomarkers that Predict Changes in Rheumatoid Arthritis (RA) Disease Activity

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PURPOSE

Rheumatoid arthritis is a chronic, debilitating autoimmune disease. The goal of developing proteomic biomarkers is to identify markers that correlate with disease progression and severity, and can be normalized with treatment. An ideal marker could serve both as a diagnostic tool and as a monitoring device for therapeutic drug efficacy. Biomarkers that can be measured in peripheral blood or serum offer superior clinical utility. In this study we sought to identify protein biomarkers predictive of change in RA disease activity. We also investigated how well the biomarkers were correlated to established clinical activity scores, such as the DAS28 and ACR50, which are used as endpoints in clinical trials.

METHODS

The Brigham Rheumatoid Arthritis Sequential Study (BRASS) is a longitudinal prospective registry that collects biological samples and clinical data over a 5-year period. The BRASS cohort includes 1000 patients who are diagnosed with RA based on American College of Rheumatology Criteria. Its ultimate goal is discovery of genetic, genomic and proteomic markers predictive of RA disease activity and treatment response.

Serum samples and clinical data were collected at baseline and after one year for 214 RA patients. A multiplex ELISA platform (Searchlight Array, Pierce) was used to assay 31 serum proteins (Table 1). The protein panel was selected based on literature reports of protein role in disease pathophysiology.

Disease activity was assessed using DAS28-CRP and ACR50 response criteria. Both activity scores are accepted standards in medical care for RA, and both contain components that are objective (protein measures) and subjective (patient and physician assessments).

All protein concentrations were measured in pg/mL, subsequently the data was log base2 transformed prior to analysis. Statistical analysis was done using SAS 9.1 (SAS Institutes, Cary North Carolina). Regression models were used to relate changes in disease activity to serum protein data. Models were adjusted for age, disease duration, presence of erosive disease and rheumatoid nodules, and medication use.

Table 1. Functional groups of proteins assessed in this study.

Cell adhesion molecules				
CD14	ICAM1	VCAM	Eselectin	Lselectin
Cytokines and cytokine receptors				
IL18	IL1RA	IFNg	IL7	TNFa
IL2R	IL6	TNFR2	MCP1	RANTES
MIP1a	MIP3a	TNFR1		
Other RA related serum proteins				
ASSA	OPG	OPN	CD40L	VEGF
Matrix metalloproteinases and their tissue inhibitors				
MMP1	MMP9	MMP8	MMP2	MMP3
MMP10	TIMP1	TIMP2		

RESULTS

Both DAS28-CRP and ACR response criteria indicated an overall clinical improvement in the patient cohort over one year. Mean DAS28-CRP changed by -0.58 (95% CI: -0.40, -0.77; p<0.01), with significant decreases in both tender and swollen joint counts, and CRP (each p<0.01) (Table 2).

The greatest clinical improvement based on DAS28-CRP was observed in patients using concomitant methotrexate (MTX) and anti-TNFa therapy during the entire year (N=26, mean -0.88, p<0.01). DAS28-CRP improved less in patients receiving a single DMARD: anti-TNFa -0.64 (N=33, p=0.01); MTX -0.45 (N=48, p=0.01). Overall ACR response criteria were concordant: 22% of subjects achieved an ACR20 response, 11% an ACR50, and 4% an ACR70.

Baseline levels of several serum proteins significantly predicted change in both DAS28–CRP and ACR50 after one year. The strongest DAS28-CRP effect was observed for MMP3, where a doubling in baseline level associated with a mean DAS28-CRP change of -0.33 (p<0.01). Baseline TNFRII (p=0.01), IL2R (p=0.03), RANTES (p=0.04), and IL6 (p=0.04) were also predictive of clinical improvement (Table 3). Higher baseline MMP3 was also significantly associated with ACR50 response, with an odds ratio of 2.1 (p<0.01).

Changes in serum protein levels over time also correlated with change in DAS28-CRP. A 50% decrease in MMP3 levels over one year correlated with a mean DAS28-CRP change of -0.37 (p<0.01) (Figure 1). Changes in IL6 (p<0.01) and IL1Ra (p<0.01) also correlated with DAS28-CRP improvement. Changes in MMP3 (p<0.01), ICAM-1 (p=0.01), and TNFRII (p=0.01) correlated with ACR50 response (Table 4).

Table 2. Change in Clinical Variables from Baseline to Year 1.

Variable	N	Difference	P-value
DAS28-CRP	208	-0.58	<0.01*
Anti-CCP	162	0.88	0.44
CRP (mg/mL)	209	-2.63	<0.01*
RF (IU/mL)	205	29.88	0.10
Tender Joint count	213	-2.13	<0.01*
Swollen Joint count	213	-3.05	<0.01*
Physician's Global Assessment	207	-0.92	<0.01*
Patient Overall Assessment	185	-5.54	0.01*
Pain Scale	166	-4.25	0.06

Table 3. Baseline proteins predictive of DAS28-CRP Change.

	Continuous	
Protein	Estimate	P-value
MMP3	-0.33	<0.01
TNFRII	0.46	0.01
IL2R	0.72	0.03
Rantes	-0.19	0.04
IL6	-0.13	0.04

Figure 1. Association of the change in DAS28-CRP and MMP3.

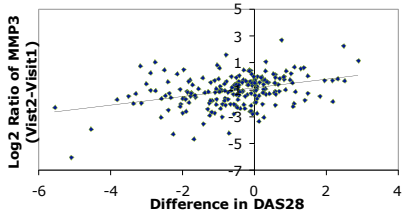

Table 4. Changes in serum proteins correlated with ACR50.

	ACR 50 Responder		ACR 50 Non -Responder		
Protein	Mean	Min, Max	Mean	Min, Max	P-value
MMP3	-1.96	(-6.00, 0.47)	-0.98	(-4.65, 2.74)	<0.01
I-CAM	-0.07	(-1.35, 1.01)	0.37	(-2.07, 5.58)	0.01
TNFRII	1.08	(-0.81, 4.57)	0.76	(-0.73, 2.90)	0.01

CONCLUSIONS

Baseline serum levels of MMP3, TNFRII, IL2R, RANTES, and IL6 are predictive of change in DAS28-CRP and/or ACR50 response over one year in a clinical practice setting. Changes over one year in MMP3, ICAM-1, TNFRII, IL6, and IL1Ra levels correlate with changes in DAS28-CRP and/or ACR50 response. Serum biomarkers that predict response in clinical settings offer new approaches to selecting treatments, identifying response, and improving clinical trial efficiency.