Multiplex serum cytokine monitoring as a prognostic tool in rheumatoid arthritis

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Abstract

Objective

Early optimized therapy of rheumatoid arthritis (RA) results in improved outcomes. The initiation of optimized therapy is hindered by the difficulty of early diagnosis and the limitations of current disease activity and therapeutic response assessment tools. Identifying patients requiring early combination DMARD/biologic therapy is currently a significant clinical challenge given the lack of definitive prognostic criteria. Since cytokines are soluble intracellular signaling molecules that modulate disease pathology in RA, we tested the recent conjecture that en mass *serum cytokine measurement and monitoring will provide a useful tool for effective therapeutic management in RA.*

Methods

We assayed the levels of 16 serum cytokines in 18 RA patients treated prospectively with methotrexate and from 18 unaffected controls. Specific mechanistic aspects of inflammatory pathology in the periphery could be discerned on a patient-specific basis from patients' serum cytokine profiles, information that may aid in the design of anti-cytokine biologic therapy. A serum Cytokine Activity Index (CAI) was also created using multi-variant analysis methods.

Results

Distinct cytokines were significantly elevated in RA patients relative to controls, and three distinct clusters with correlations to disease activity were identified. The Cytokine Activity Index correlated well with the therapeutic response; responders and non-responders in this cohort were distinguishable as early as one month post initiation of methotrexate therapy, well before clinical assessments of response are commonly completed.

Conclusion

Clinical assessment tools could be derived from this approach that may provide a means to continually track patients, allowing intervention strategies to be better evaluated on a patient-specific basis and to identify residual cytokine activity that could be used to guide combination therapy.

Key words

Rheumatoid arthritis, cytokines, therapeutic response.

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory arthropathy characterized by heterogeneity in its clinical manifestations, pathological features, response to therapy and, ultimately, outcome (1-4). The severity of RA ranges from self-limited swelling in a limited number of joints to chronic progressive destruction of multiple joints. Although combination therapy with traditional disease-modifying antirheumatic drugs (DMARDs) and a growing list of rationally designed biological agents that target specific molecular and cellular disease mediators have improved outcomes, substantial disease activity persists in many patients (5, 6). Early aggressive treatment can minimize disease progression and pathologic changes that occur in many patients, whereas a delay could squander the narrow window of opportunity during which aggressive therapy might alter the long-term course of the disease (7-9).

Recent clinical trial data has demonstrated that radiographic erosions continue to progress in a subset of RA patients treated with Methotrexate alone, while erosive progression slowed in the majority of patients when a biologic agent that inhibits the cytokine TNF-α is added to the treatment regime (10, 11). Because of its adverse implications and complications, however, combination therapy is currently reserved for those patients thought most likely to benefit: patients with severe disease and a high risk of erosions that is not adequately controlled by DMARD therapy alone (12, 13).

Severe disease is usually distinguished in clinical practice using clinical signs and symptoms, and non-specific laboratory tests of inflammation including the C-reactive protein titer (CRP) and the erythrocyte sedimentation rate (ESR). The presence of high-titer rheumatoid factor (RF), antibodies against cyclic citrulinated peptide (CCP), and shared epitopes in the HLA-DRB1 alleles have also shown some prognostic potential (14, 15). However, no definitive criteria or tests have been established to identify the subset of patients likely to require combination therapy, leaving the community without a clear framework for its application (16, 17).

The number and targeting strategies of biological agents indicated for RA is rapidly increasing (11, 18-21). With the current development of novel biologic targeted therapies, the ability to control multiple cellular and molecular mediators will provide an unprecedented opportunity for controlling disease activity, one that will be concomitant with the increased complexity of treatment design. The limits of current disease assessment tools, disease heterogeneity, the increasing numbers of therapeutic options available for RA, and the chronic, progressive nature of the disease make it well-suited for a more evidenced-based medical approach: an approach in which individually tailored treatments are directed by direct measurements of disease regulators and effectors.

The cytokine network in RA is a complex and dynamic system in which cellular and humoral cytokines, chemokines, and growth factors regulate the initiation and perpetuation of inflammation (3, 22, 23). Our understanding of the role of cytokines in RA is largely limited to analyses of individual cytokines. However, it is clear that functional redundancy, synergy, pleiotropy, and concomitant regulation result in a dynamic cytokine network that is dependent upon the complex interplay of multiple factors, rather than the isolated effects of single signaling molecules or pathways. Previously, we have shown that serum cytokine levels when screened *en masse* are useful biomarkers for the diagnosis and prognosis of early inflammatory arthritis (24). Herein we performed "multiplex serum cytokine profiling" of RA patients in which the levels of 16 cytokines were measured in parallel in a small volume of patient serum. Levels of these cytokines were found to be correlated with disease activity and clinical outcome in a preliminary investigation of their prognostic power for the assessment of MTX efficacy. The results of these preliminary studies suggest that multiplex serum cytokine profiling provides a sensitive and quantitative measure of disease activity and a mechanistic monitor of therapeutic efficacy.

Materials and methods

Patients and controls

The study population consisted of 18 adult patients (4 men and 14 women; mean age 51.44 yrs, 95% CI: 41.29–59.01) with active RA who fulfilled the ACR 1987 criteria (25), of whom 7 were followed during the course of their treatment with methotrexate (MTX). To ensure that only early patients with a high risk prognosis for erosive disease were enrolled, the required criteria included: recent-onset disease (duration < 3 years), MTX-naïve patients, at least 6 swollen joints and at least 6 tender joints (based on a 28-joint count), at least 3 radiographic bony erosions or a positive serum test for rheumatoid factor, and an ESR of at least 28 mm per hour or a serum C-reactive protein concentration of at least 20 mg per liter. Stable doses of nonsteroidal anti-inflammatory drugs and prednisone (≤ 10 mg daily) were allowed.

Laboratory assessments were conducted both before and during MTX treatment and included routine hematology, a comprehensive metabolic panel, ESR, CRP, antinuclear antibody (ANA) and rheumatoid factor (RF). The Stanford Health Assessment Questionnaire (HAQ) (26) was the primary outcome measure of efficacy and the Disease Activity Score (DAS28) (27) was calculated at each time point as a secondary measure of efficacy. The cohort studied also included 18 normal healthy controls matched for age and sex (4 men and 14 women; mean age 46.71 yrs, 95% CI: 41.29–52.91).

The study was approved by the Institutional Review Boards of the University of Oklahoma Health Sciences Center and the Oklahoma Medical Research Foundation, and blood samples were obtained from both patients and controls after they had given their informed consent, and all samples were treated anonymously throughout the analysis.

Serum samples

Blood was collected in endotoxin-free silicone coated tubes without additive. The blood samples were allowed to clot at room temperature for 30 min before centrifugation (3000 rpm., 4°C, 10 min) and the serum was removed and stored at -80°C until analyzed.

Biometric multiplex assay

Analytes measured include IL-1β, IL-2, IL-7, IL-12p40, IL-17, TNF-α, G-CSF, GM-CSF, IL-4, IL-5, IL-6, IL-10, IL-13, CCL2 (MCP-1), CCL3 (MIP-1α) and CXCL8 (IL-8). A multiplex sandwich immunoassay from the Bioplex protein array system (Bio-Rad Inc.), which contains dyed microspheres con-jugated with a monoclonal antibody specific for a target protein, was used. Serum samples were thawed and run in duplicates. Antibody-coupled beads were incubated with the serum sample (antigen), after which they were incubated with biotinylated detection antibody before finally being incubated with streptavidin-phycoerythrin. A broad range of standards (Bio-Rad Inc.) from 1.95 to 32,000 pg/ml were used to enable the quantitation of a wide and dynamic range of cytokine concentrations while still providing high sensitivity. Bound molecules were then read using a Bio-Plex array reader, which is based on Luminex fluorescent-bead-based technology with a flow-based dual laser detector and real time digital signal processing to facilitate the analysis of up to 100 different families of color-coded polystyrene beads and allow multiple measurements of the sample, resulting in the effective quantitation of cytokines.

Statistical analysis

Analyte concentrations were quantified by fitting using a calibration or standard curve. A 5-parameter logistic regression analysis was performed to derive an equation that allowed concentrations of unknown samples to be predicted. Statistical differences in the measured values were assessed by a Wilcoxon rank-sums test. *P* values less than 0.05 were considered to be statistically significant.

Correlational clustering

Commonality among patient profiles was determined using correlational cluster analysis, which is based on the calculation of a value denoted as "connectivity", here defined as the number of patients whose cytokine expression levels and their changes with respect to time correlated with that observed in another individual (28-30). Samples were considered to be related if the Pearson correlation coefficient (ρ) was greater than 0.7. Statistical significance was determined by bootstrapping the data set; therefore the re-sampled (empirical) distribution was used to select the correlation (ρ), above which level casual associations have a $p < 0.05$ chance of occurring. Once created, the clusters were re-sorted by connectivity and cluster membership. Then a mosaic representation of the correlation coefficients was graphed using SigmaPlot v 8.02a (SPSS Inc., Chicago, Ill). Patients with positive correlations were represented in graded shades of red and negative correlations in graded shades of blue, whereas the absence of correlation was indicated in green.

Discriminant function analysis (DFA)

A forward step-wise DFA was used to select the set of analytes that maximally discriminated among the groups studied (30-32, in press). Specifically, at each step, all variables were reviewed to determine which ones maximally discriminated among the groups. This variable was then included in a discriminative function, denoted a root, which is an equation consisting of a linear combination of changes in the analytes used for the prediction of group membership. The discriminatory potential of the final equation was observed as a line plot of the root values obtained for each group.

This approach identifies groups of analytes whose changes in concentration levels can be used to delineate profiles, and to diagnose and assess therapeutic efficacy. The DFA model also creates an arbitrary score by which new subjects can be classified as either "healthy" or "diseased". To facilitate the use of this score by the medical community, it was re-scaled so that a value of 0 indicated a healthy individual and scores greater than 0 indicated increasing disease activity. This new scoring system has been denoted the Cytokine Activity Index (CAI).

Results

Pair-wise comparison of serum cytokine profiles between patients and controls

We performed broad-based serum cytokine profiling of a small cohort of RA patients and unaffected healthy controls. The cytokines assayed included key modulators of inflammation, cellular and humoral immunity, and leukocyte trafficking. The levels of 16 cytokines were assessed in the serum of 18 early RA patients and 18 age- and sex matched unaffected controls.

Twelve of the 16 cytokines were significantly upregulated on average in the peripheral blood of RA patients when compared to healthy controls (Fig. 1). Significantly upregulated cytokines include: TNF-α (p = 0.0009), IL-6 (p = 0.026), IL-1β (p = 0.0095), GM-CSF (p = 0.009), IL-7 (p = 0.03), IL-12p40 (p = 0.047), IL-4 (p = 0.002), IL-10 (p = 0.007), IL-5 (p = 0.005), IL-13 (p = 0.017), CXCL8 (IL-8) (p = 0.02), and CCL2 (MCP-1) (p = 0.049). These cytokines fall into several broad functional classes including: pro-cell-mediated immunity (*e.g.* IL-1β, IL-2, IL-7, IL-12p40, IL-17, TNF-α, G-CSF, GM-CSF), pro-humoral immunity (*e.g.* IL-4, IL-5, IL-6, IL-10, IL-13), and chemokines (*e.g.*, CCL2 (MCP-1), CCL4 (MIP-1β) and CXCL8 (IL-8). Although serum cytokines do not necessarily reflect the nature of joint pathology, these results suggest that early RA involves a highly complex interplay of multiple mediators of adaptive and innate immunity. No cytokines were decreased in this RA cohort relative to the cohort of unaffected individuals.

The serum cytokine profiles observed appear to be RA-specific, in that they were distinct from those of patients with Sjögren's syndrome, systemic lupus erythromatosis, ankylosing spondylitis, or hepatitis C virus infection studied by us earlier (30, 33-36).

Serum cytokine profiles correlate with disease activity

The issue of disease hetereogeneity in RA is not well understood at a mechanistic level. If RA patients fall into groups of independent or overlapping disease subsets, and these subsets

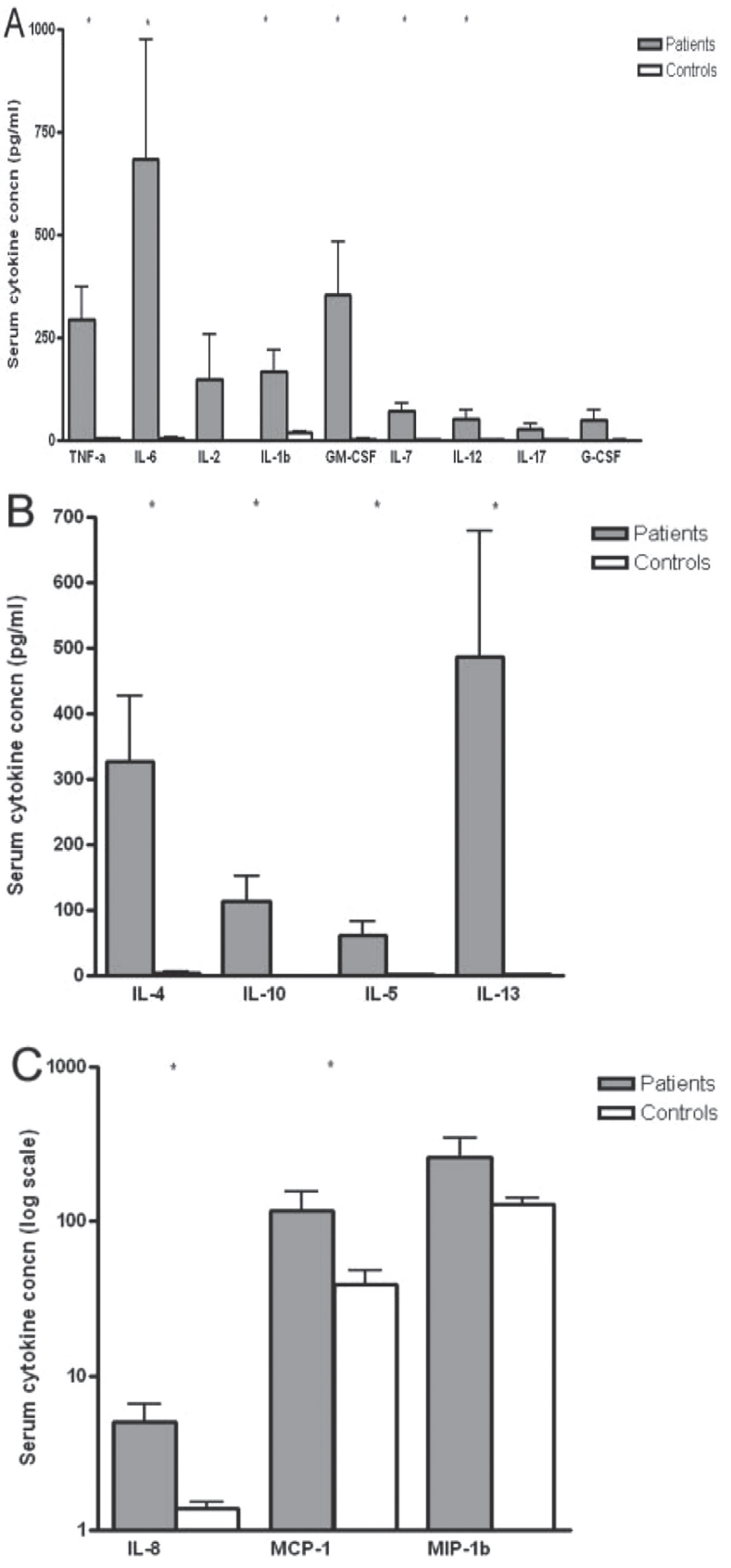

Fig. 1. Pair-wise comparison of serum cytokine profiles between RA patients and unaffected controls. Serum levels of cytokines assessed in 18 early RA patients and unaffected controls, sorted into three functional groups. (**A**) 'Cellular cytokines'; (**B**) 'humoral cytokines'; (**C**) 'chemokines'. Cytokines with significantly elevated levels in RA patients relative to controls are denoted with an asterisk.

have differential responses to therapy, characterizing these subsets may be of significant clinical importance. Correlational clustering, an unsupervised clustering method, was used to identify patient subsets based on the grouping of individuals with similar cytokine levels. In this analysis, the similarity of cytokine profiles was utilized to distinguish between classes and not simply between the levels of single cytokines. Given the co-regulatory activity of these cytokines, serum cytokine profiles are at least somewhat representative of the underlying regulatory networks. This method therefore may facilitate both the identification and the subsequent functional characterization of disease subsets.

The results of these analyses were translated into graphs (denoted mosaics) in which groups of patients with statistically significant similarities in their cytokine profiles were represented as clusters. Three major clusters were identified (Fig. 2A). The clinical, autoantibody and cytokine profile characteristics of the individuals within the clusters were compared. Interestingly, the principal differences among these clusters were the relative levels of cytokines as opposed to gross changes in the classes of cytokines (Fig. 2B). These differences correlated with disease activity and laboratory assessments, suggesting that our cohort was made up of patients with relatively homogeneous disease that varied in severity, but did not involve functionally distinct disease subsets.

Cluster 1 was comprised of 4 RA patients with serum cytokine levels similar to the controls, and was the only patient cluster that also contained unaffected controls. Patients in this cluster had the lowest cytokine profiles overall within the cohort and, correspondingly, the lowest values for several laboratory and disease activity parameters including CRP, RF and HAQ (Fig. 2b). Cluster 2 contained 7 RA patients, all of whom showed the most significant elevations in cytokine levels in the cohort (Fig. 2b). Correlation with laboratory and disease activity parameters was also observed, as the patients in this cluster had the highest CRP and HAQ values. Cluster 3 contained 3 patients with intermediate levels for both cytokines and the clinical and laboratory indices (CRP, RF, and HAQ) relative to the patients in Cluster 1 and Cluster 2 (Fig. 2). Four patients were grouped in 'minor' clusters that were not associated with any of the major clusters, further highlighting the heterogenic nature of the disease profile.

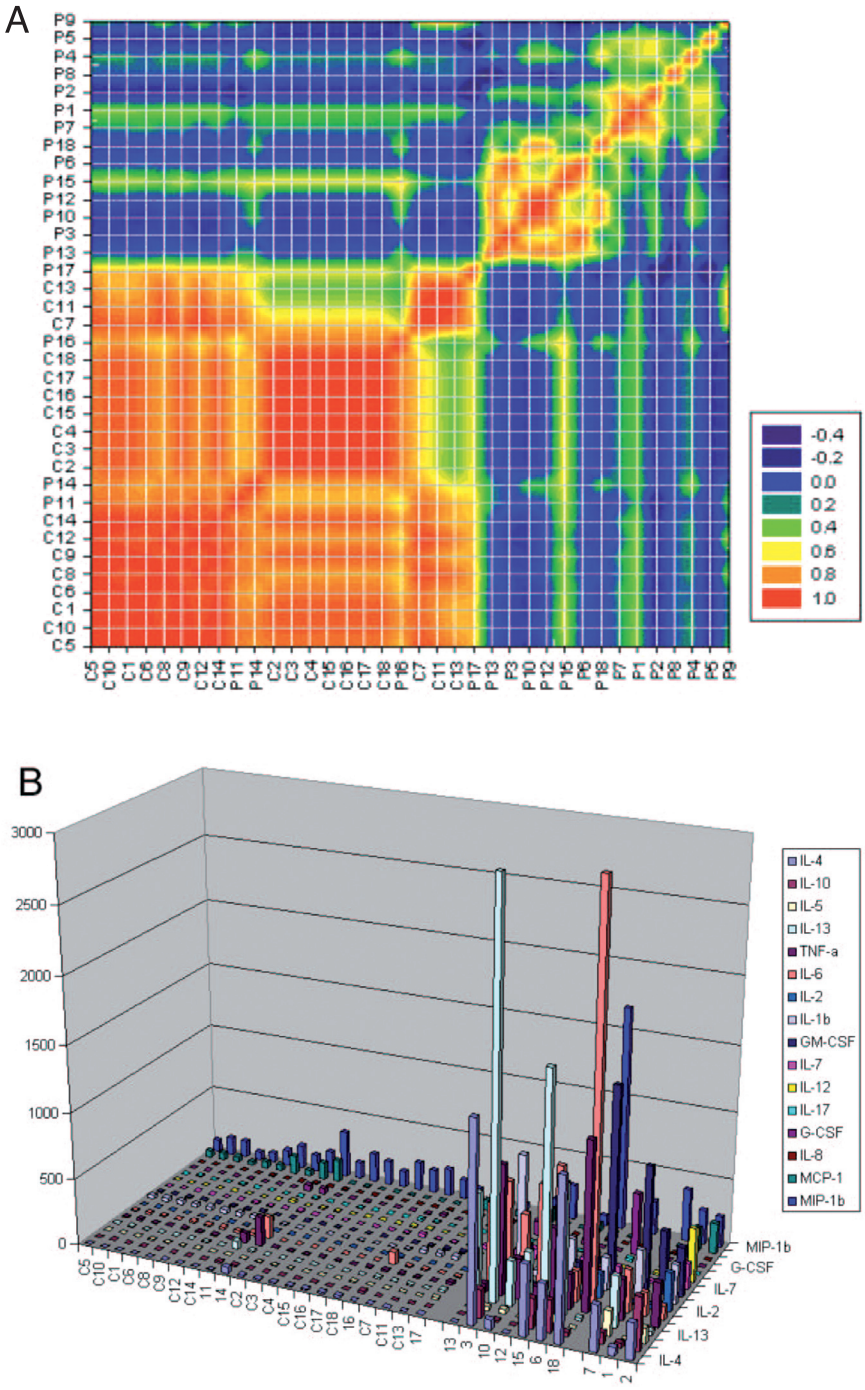

Fig. 2. Correlational clustering of serum cytokine profiles. Patients and controls with similar cytokine profiles were identified using correlational cluster analysis. (**A**) A cluster mosaic is presented in which color mapping was used to represent correlation levels. Positive correlations among study subjects are represented in graded shades of red, negative correlations in graded shades of blue, and the absence of correlation in green. Three distinct clusters of study subjects were observed: Cluster 1 consisted of 4 RA patients and all of the healthy controls, Cluster 2 comprised 7 RA patients, and Cluster 3 included 3 RA patients. (**B**) Individual cytokine profiles of the study subjects from each of the three clusters are shown.

Quantitative assessment of the clinical response to MTX treatment using cytokine-based biomarkers

Clinical assessments, laboratory values, and the levels of 16 serum cytokines were studied prospectively in 7 patients prior to and during MTX treatment. Five responders and 2 non-responders to MTX were identified, being those patients with a change in the DAS 28 score of 1.2 units after at least 8 weeks of treatment (37). When pre-treatment serum cytokine levels were compared to levels at the end of therapy, MTX-responsive patients were clearly distinguishable from non-responsive patients (Fig. 4). Responders had statistically significant reductions in 10 cytokines, including TNF-α, IL-6, GM-CSF, IL-2, IL-17, IL-4, IL-13, CCL2 (MCP-1), CXCL8 (IL-8) and G-CSF, with levels progressively decreasing during the course of treatment (Fig. 3A). Changes in serum cytokine levels correlated with changes in both the HAQ and DAS28 scores (Fig. 3C). No MTX-responsive patients achieved full remission.

Interestingly, the levels of three cytokines that were upregulated in these patients prior to treatment remained unchanged during therapy despite clinical improvement including: IL-1β, IL-5 and IL-10, suggesting that the incomplete response to MTX is driven at least in part by these known mediators of inflammation and joint erosion (Fig. 3B). These data suggest that multiplex serum cytokine profiling can identify biomarkers of residual immune system activity in the periphery of partially responsive patients, who represent the vast majority of RA patients receiving MTX treatment, information that could then be used to rationally design second-line combination therapies.

Changes in serum cytokine levels also correlated with disease indices in non-responsive patients (patients with minimal or no clinical improvement in their HAQ and DAS28 scores). In these patients, the levels of most cytokines remained unchanged during treatment, with the exception of two: G-CSF, which progressively decreased, and CCL4 (MIP-1β), which progressively increased (Fig. 3A and 3B).

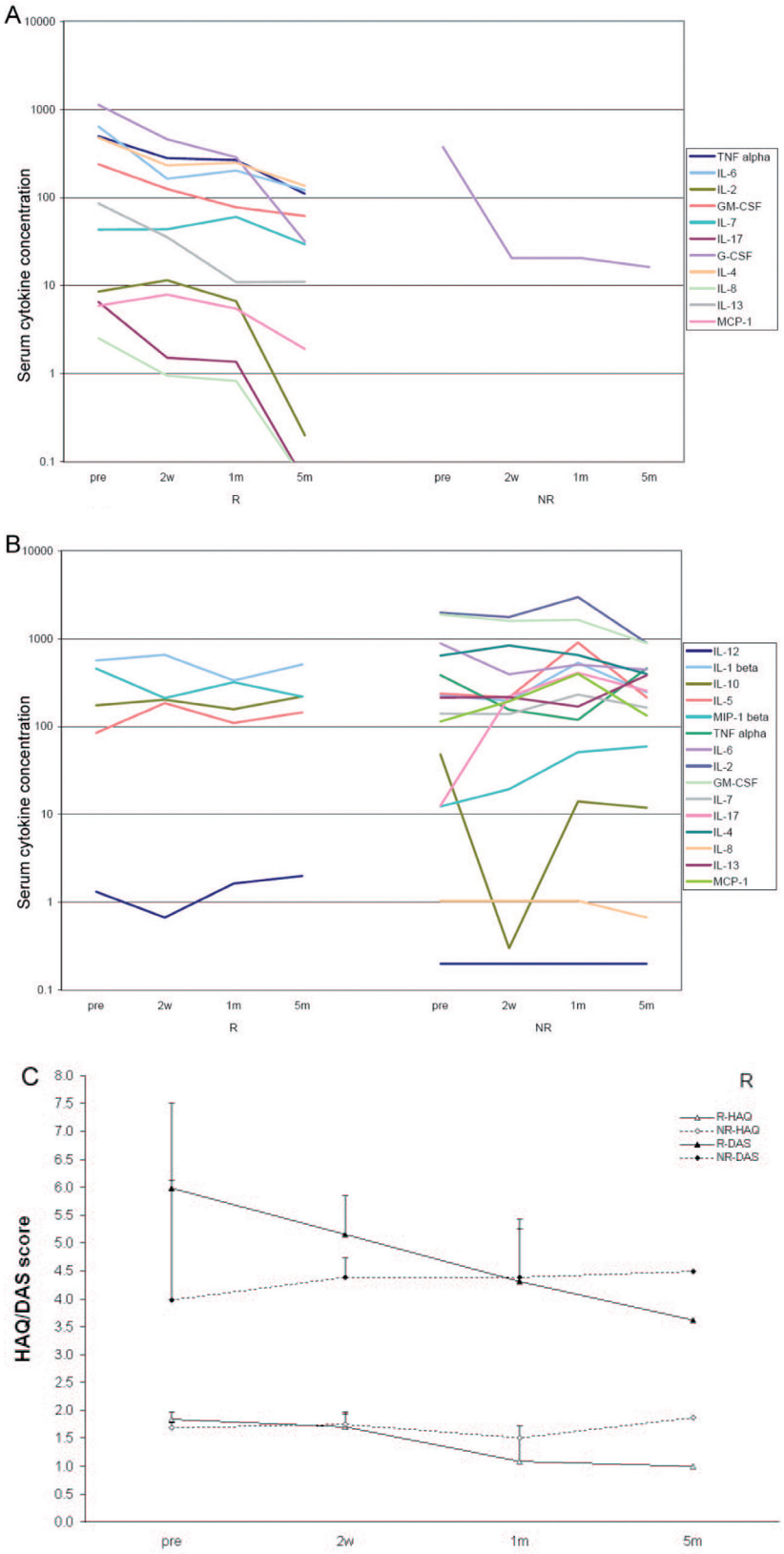

Fig. 3. Changes in the serum cytokine profiles and clinical response during MTX treatment. Serum cytokines, and HAQ, and DAS28 scores were measured in 7 patients prior to and during MTX treatment. (**A**) Decreases in serum levels of cytokines following treatment in both responders (R) and non-responders (NR); (**B**) cytokine levels that remained unchanged or increased following MTX treatment over 2 weeks (2w), 1 month (1m) and 5 months (5m); (**C**) efficacy measures of clinical response, HAQ and DAS28 scores for both responders (R) and non-responders (NR).

Developing a multiplex cytokine-based index of response

Discriminant Function Analysis (DFA) is a multivariate class distinction algorithm that was used here to identify the cytokines that best discriminated between patients and controls. DFA allows one to construct a mathematical model, denoted a "root", consisting of a linear combination of cytokines that are used to define group membership. Of the 16 cytokines, TNF-α, IL-4, IL-6, CXCL8 (IL-8), IL-13 and CCL2 (MCP-1) were identified by DFA as having the highest power for class discrimination between patients and controls, suggesting that these cytokines play a significant role in pathology in this RA cohort. Indeed, agents that block the action of TNF-α and IL-6 significantly ameliorate disease in the majority of patients.

We previously utilized the power of DFA's graphic output to monitor therapeutic response and develop prognostic predictive response criteria (32). Changes in root values represent changes in the levels of key disease mediators. Changes in the root values of individual patients under treatment can be plotted relative to healthy controls and tracked over time. Results demonstrated trends in therapy-induced changes early in the course of treatment. The root values for all 18 RA patients prior to treatment fell into a distinct cluster that was well separated (the difference being statistically significant) from the root values of unaffected controls, suggesting that the cytokine profiles in early DMARD-naïve RA have significant diagnostic power. As the patients responded to MTX therapy, their cytokine profiles became increasingly like those of the unaffected controls. The movement of a given patient's values from the "pre-treatment" cluster towards the healthy controls was proportional to the clinical response to MTX, with fully responsive patients moving closer to the healthy controls than partially responsive patients. The movement of non-responsive patients was clearly distinct from the responders and these differences were observable early in the treatment course. An animated presentation of these response data is available at: http://www.omrf.ouhsc.edu/centola/RAmovie

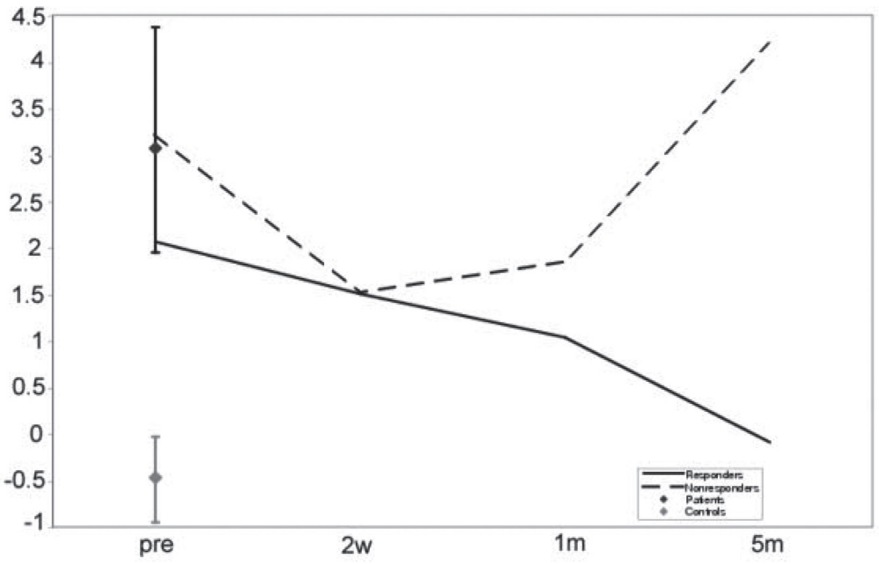

Fig. 4. The Cytokine Activity Index. An index of cytokine levels, denoted the cytokine activity index (CAI), was created using the results of a discriminate function analysis. These values represent an aggregate of cytokine measurements. Data was scaled so that the maximal value for the controls was 0. A graph of CAI values measured during treatment shows that all pre-treatment patient values were elevated relative to the unaffected controls. The average CAI of the responsive patients is shown (unbroken line). Values decreased towards normalcy. The average CAI of non-responsive patients (dashed line) changed during treatment, but remained principally in the range of the patients prior to treatment.

This pharmacogenomic analysis provides a means of developing a clinical assay that can monitor the patient's response early in the course of treatment with MTX. To obtain results that can be readily interpreted in a clinical context, a Cytokine Activity Index (CAI) was created using data from the DFA analysis. To create this index, a single DFA root that best discriminated patients from controls was re-scaled so that a value of 0 characterized the maximal value of the healthy controls. This value was plotted over time during treatment to assess the patient response. In a preliminary assessment, results demonstrated that changes in the CAI correlated with the clinical response and values obtained after 4 weeks of therapy were predictive of the MTX response (Fig. 4). Additionally, we assessed the ability of the CAI to detect disease activity in RA patients. Interestingly, all of the RA patients in this cohort had statistically significantly increased CAI values relative to the normal range of unaffected controls (sensitivity > 0.98). The normal CAI range was calculated in a standard manner (38), *i.e.* the normal range was considered to be the 25-75% interquartile range of unaffected control values. The sensitivity of this instrument in detecting disease activity was higher than that of ESR (sensitivity = 0.76) and CRP (sensitivity = 0.44) for this cohort of early active RA patients.

Discussion

Previously, we have shown that serum cytokine levels are useful biomarkers for determining the diagnosis and potential prognosis of early inflammatory arthritis (24). Herein we demonstrate that broad-spectrum serum cytokine profiling may be also be a useful surrogate for disease activity in RA, with the potential to characterize immune and inflammatory modulation, subclassify the severity of disease, and provide a mechanistic characterization of both residual disease activity and treatment efficacy.

The serum cytokine profiles of RA patients were readily distinguished from those of healthy individuals. As previously characterized (24), patients had increased levels of TNF-α, IL-6, IL-1β, GM-CSF, IL-4, IL-10, IL-5, IL-13, CXCL8 (IL-8), and CCL2 (MCP-1). These findings support the hypothesis that RA is a complex immune-inflammatory disorder involving the dysregulation of cellular, humoral and innate

immunity, with a significant systemic signature.

Identifying disease subsets with a differential response to a given therapy could aid in treatment design. We therefore used a statistical clustering method that groups individuals with similar cytokine profiles to determine whether distinct subclasses of RA patients exist in this cohort. While some differences were noted, the three subgroups principally shared a common set of cytokines that were elevated above the levels for normal controls with relatively high, moderate, and low levels observed, respectively. Clinical activity, as measured by several indices (*e.g.*, the CRP, RF and HAQ scores) and compared to clinical responses, were well correlated with the overall cytokine values, suggesting that serum cytokine levels may be useful biomarkers of disease activity and therapeutic response.

Clinical improvement with MTX in rheumatoid synovitis has been associated with a significant decrease in the number of T cells infiltrating the synovial membrane (44, 45). MTX suppresses chemokine expression, the subsequent recruitment of immune cells into the inflammatory sites in RA, and interferes with synovial fibroblast and T cell cross-talk by decreasing heterotypic cell adhesion. In the results reported above, the serum levels of several T cell modulating cytokines remained elevated in MTX-responsive patients, above the levels for unaffected controls, suggesting that T cell suppression by MTX is incomplete, at least in the periphery. These data suggest that further inhibition of T cell signaling is likely to be beneficial. In support of this, a biologic agent recently approved by the FDA that suppresses T cell co-activation signals (abatacept) has been proven to be an effective adjunctive therapy for MTX (20).

The effects of MTX treatment on humoral immunity in responsive patients are mixed. MTX suppresses activated B cells, and causes the down-regulation of IL-4 and IL-13 (46). However, MTX and the B cell-depleting agent, rituximab, act synergistically to decrease disease activity, suggesting that further suppression of humoral immunity coulde improve clinical outcomes (15). IL-1β is a key mediator of cartilage and bone destruction, which plays an important role in the activation of matrix metalloproteases and prostaglandin release by synovial fibroblasts. Previous observations of the effect of methotrexate on IL-1β have been controversial, with both decreased and increased levels reported (47, 48). Interestingly, recent studies suggest that IL-1β in MTX responsive patients is released at higher levels than in non-responsive patients, and this level is unaffected by MTX (33). In our study MTX treatment did not affect serum IL-1β levels, supporting molecular and clinical observations that the MTX response can be enhanced when the agent is used in combination with anti-IL-1 therapy, such as anakinra (10, 11).

In addition to providing insights into the types of second-line biologics likely to be of use in a given MTX-treated patient, serum cytokine profiling also provides data that could reduce the time required to identify candidates for these agents. As the patients in this cohort responded to MTX therapy, their cytokine profiles became more like those of healthy controls. We therefore developed an index of cytokine activity to monitor the therapeutic response, denoted the Cytokine Activity Index (CAI). The change in CAI was proportional to the clinical response of these patients, with fully responsive patients moving closer to the healthy controls than partially or non-responsive patients. Changes in the CAI were highly correlated over time to changes in the DAS28 ($R = 0.839$). Correlation values for changes in the ESR and CRP were not well correlated to changes in the DAS28 ($R = 0.21$ and $R = 0.59$ respectively). Importantly, changes in the cytokine response could be identified within 4 weeks of the initiation of treatment. These preliminary results suggest that CAI tracking can expedite assessment of the MTX response. All the patients in this cohort had a CAI that was above the range for the healthy controls and was not influenced by the disease duration. The sensitivity of this aggregate index for the detection of disease activity was therefore greater than 98%. This was significantly higher than the sensitivity of the standard biochemical measures of inflammation – ESR (76%) and CRP (44%) –measured in this same cohort.

Serum cytokines can be easily measured in a real-world clinical setting, are stable during shipping, and can be measured in a highly parallel manner. Given the limitations of the standard measures of inflammation (ESR and CRP), the CAI or a similar serum cytokine index could offer a practical adjunct to clinical assessment tools designed to help optimize therapy and improve patient outcomes. The results of this report are preliminary and require verification in larger outcome studies. However, it is clear even here that "personalized" and "evidence-based" approaches such as these will become increasingly useful and important as the number and target specificity of biologic agents for RA increases, allowing intervention strategies to be better evaluated on a patient-by-patient basis.

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