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Editorial

Cytokine biomarkers and the promise of personalized therapy in rheumatoid arthritis

Citocinas biomarcadoras y la promesa de un tratamiento personalizado en artritis Reumatoide

John M. Davis III y Eric L. Matteson *

Division of Rheumatology, Mayo Clinic College of Medicine, Rochester, Minnesota, USA

INFORMACIÓN DEL ARTÍCULO

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To rheumatologists, the term "cytokine" signifies more than its basic definition as an intercellular signaling molecule of the immune system. Cytokines represent an increasingly diverse family of small proteins that participate in the orchestration of immune-mediated processes by communicating signals among various cell types. As discussed elegantly by Gary Firestein, cytokine signaling, in various pathways of the innate and adaptive immune systems, and relating to the functions of synovial fibroblasts and osteoclasts, is fundamental to the pathogenesis of rheumatoid arthritis (RA).[1] Yet, to rheumatologists, the term "cytokine" conveys a sense of optimism as this regards the success of several anti-cytokine therapies in revolutionizing the treatment of RA. The array of anti-cytokine therapies now includes the inhibitors of tumor necrosis factor (TNF), interleukin (IL)-1, and most recently, IL-6. Further, cytokines are often discussed optimistically in the context of the diligent search for new "biomarkers" of RA: molecules that correlate accurately with levels of disease activity and/or predict disease outcomes.[2] But are biomarkers truly needed, and is there currently sufficient evidence to bring cytokine biomarkers to the clinic in order to personalize treatment for patients with RA?

Why are biomarkers needed?

Several motivations for such biomarkers can be discussed. Firstly, as alluded to above, the therapeutic armamentarium for treatment of RA is expanding, including multiple conventional disease-modifying antirheumatic drugs (DMARDs) and an expanding list of "biologic" DMARDs, including the TNF inhibitors, anakinra (recombinant IL-1 receptor antagonist), abatacept (CTLA-4:Ig; T cell co-stimulation blocker), rituximab (monoclonal anti-CD20 antibody; B cell depletion therapy), and most recently, tocilizumab (anti-IL-6 receptor monoclonal antibody). Effective strategies for the treatment of many patients with RA are published, which emphasize early treatment with combination therapy, targeted to achieve a state of low/minimal disease activity, and the use of biologic DMARDs in patients with poor prognostic factors and/or inadequate response to conventional DMARD therapy.[3] However, it remains difficult to determine up front which RA patients will respond well, for example, to methotrexate therapy, and which will truly require combination therapy. Potentially, biomarkers may help guide this decision. Furthermore, among the group of patients who fail methotrexate therapy (and potentially combinations of oral DMARDs), there are now 5 effective biologic agents licensed for use in several countries across the world.

How do patients and their rheumatologists decide among these options? No valid head-to-head trials have been performed, and none are on the horizon at present. Cost and patient factors, such as particular infection risk factors in the context of burgeoning information about the different biologist in these settings, may eventually guide the selection among these options. However, the ability to check the level of a cytokine (or cytokine profile, often referred to as a "signature") that would inform the clinician regarding the likelihood of responding to these various agents would be invaluable.

What do we know about cytokines as potential biomarkers?

Firstly, it is important to consider some general issues with regards to measuring cytokine profiles. One must consider the fact that the most fundamental lesion of RA, synovial inflammation, occurs in the synovium locally, with release of cytokines into the peripheral blood circulation. However, peripheral blood is accessible

* Autor para correspondencia.
Correo electrónico: Matteson.eric@mayo.edu (E.L. Matteson).

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and does not require an invasive procedure to obtain, and therefore, is among the best tissue samples to use for biomarker discovery. The cytokines present in the plasma/serum are derived from numerous cell types, including those that circulate in the blood (i.e., T and B lymphocytes, monocytes, and dendritic cells) and others that are not (i.e., fibroblasts, endothelial cells).

Secondly, it is important to understand the concern about potential interference due to heterophilic antibodies, namely rheumatoid factor. Rheumatoid factor can potentially lead to non-specific binding and false positive results in enzyme immunoassays.[4] However, manufacturers of cytokine assays include methodology to block the interference of heterophilic antibodies; additionally, methods are published including the use of protein L or Heteroblock to minimize the effect of such antibodies and improve the accuracy/validity of the results.[4,5] Thus, assaying cytokine profiles in peripheral blood is an appealing option for discovery of new biomarkers of this disease.

Finally, statistical methods to analyze multi-cytokine profiling data must take into account the potential for "false-positives" due to the large number of independent comparisons. This will be discussed further in the context of a recent publication later in the article.

Current knowledge of cytokine biomarkers in RA

A dizzying number of publications have reported on the roles of cytokines in the pathogenesis and disease manifestations of RA. For the purposes of this discussion, let us consider those articles that have evaluated associations between peripheral blood cytokine profiles and disease activity and/or severity markers (Table 1). The last decade has witnessed major advances in high-throughput multiplex technology, providing the means to simultaneously measure many analytes in a single microtiter plate well. This has led to an increasing number of studies screening numerous peripheral blood proteins, in serum, plasma, or in supernatants from cultured peripheral blood immune cells, in order to discover new predictive biomarkers. Scrutiny of these articles (Table 1) generally reveals small sample sizes, and variable rigor in the definitions of the outcomes or phenotypes that are being predicted with the markers. Although much is known regarding the involvement of cytokines and chemokines in the pathogenesis of synovial inflammation, surprisingly few studies have investigated the reliability and validity of cytokines as predictive biomarkers in this disease.

Nonetheless, some progress has been made, supporting the significant promise of this approach. The chemotactic cytokines (chemokines) have particularly shown potential as candidate biomarkers, with relatively robust levels detected in peripheral blood samples of patients, and promising associations with clinical phenotypes. For example, high serum levels of CCL5 (RANTES)[6] and CXCL1 (GRO-α)[6] in contrast to low levels of CCL11 (eotaxin)[7] have been shown to predict radiographic progression among patients with active RA during one year of methotrexate therapy. Circulating levels of other chemokines, including CCL2 (MCP-1),[8] CCL4 (MIP-1β),[9] CCL23 (MPIF-1),[10] CXCL8 (IL-8),[9] CXCL10 (IP-10),[5] and CXCL13 (BLC),[10] have also been significantly associated with an important surrogate of RA severity, the presence of anti-citrullinated protein antibodies. These findings suggest that relatively "downstream" signaling mediators such as chemokines, when measured in the peripheral blood, may assist with predicting clinical outcomes for patients with RA.

Several other peripheral blood cytokines deserve mention. Cytokines that recruit and activate innate immune effectors, such as monocytes and macrophages, have shown potential as candidate predictive biomarkers. For example, GM-CSF has been shown to correlate with anti-citrullinated protein antibodies.[5,9] We have found GM-CSF to be among the strongest correlates with pain levels in patients with RA.[11] IL-7, which is involved in maintaining memory T cell populations and additionally has proinflammatory activity, has been shown to decrease in tandem with the disease activity during successful methotrexate therapy[12]; in another study, IL-7 levels remained significantly elevated in RA patients who responded inadequately to anti-TNF therapy.[13] This exemplifies another benefit of candidate biomarker investigation, which is the demonstration of relevant pathogenetic mechanisms. Van Roon et al. have suggested that IL-7 immunopathogenesis may be independent of TNF pathways among their patients who had elevated IL-7 and non-response to anti-TNF, suggesting the potential for novel treatment strategies targeting IL-7 in such patients.

A recent publication by Rioja et al.[14] demonstrates a fine example of a candidate biomarker discovery study. These investigators studied 44 patients with RA (defined by the ACR

Table 1
Notable published data regarding plasma/serum cytokines as biomarkers of disease activity or response to therapy in rheumatoid arthritis (RA)

Study	Year	Cytokine(s)	Finding
Boiardi et al.[6]	1999	CCL5 (RANTES), CXCL1 (GRO-α)	High levels of RANTES or GRO-α after six months of methotrexate predict radiographic progression at one year
Seitz et al.[23]	2003	IL-1ra, IL-1β	Ratio of IL-1ra:IL-1β secreted by patient cultured PBMC accurately predicted patients' ACR responses to methotrexate therapy.
Hitchon et al.[9]	2004	Multi-cytokine signature: IL-2, IL-6, IL-12, IL-17, GM-CSF, IFN-γ, CCL4 (MIP-1B), CXCL8 (IL-8)	'Severe' patient cluster (based on high anti-CCP) was differentiated from 'mild' patient cluster by significantly higher cytokine values
Hueber et al.[5]	2007	Multi-cytokine signature: TNF-α, IL-1α, IL-1β, IL-2, IL-4, IL-10, IL-12, IFN-γ, GM-CSF, CXCL10 (IP-10)	'High' cytokine cluster associated with higher C-reactive protein, RF titer, and anti-CCP; listed cytokines significantly associated with anti-CCP2
Fabre et al.[8]	2008	MCP-1, EGF	High serum MCP-1 and EGF associated with response to etanercept.
Knudsen et al.[24]	2008	IL-6	Decreases in plasma IL-6 associated with clinical response/remission during 24 weeks of DMARD therapy
Van Roon et al.[12,13]	2008	IL-7	Reduction of serum IL-7 upon MTX therapy correlates with suppression of disease activity indices; persistent elevation of IL-7 correlated with non-response to anti-TNF treatment.
Rioja et al.[10]	2008	Il-6, CCL23 (MPIF-1), TGF-α, CXCL13 (BLC), M-CSF, TNFRSF9	Rolling cycle amplification technology was used to screen 163 blood proteins for potential biomarkers among patients with active versus quiescent RA; 6 biomarker panel selected by multivariate discriminant analysis; ROC analysis showed this panel had overall 88.6% accuracy in predicting disease activity status.
Syversen et al.[7]	2008	CCL11 (Eotaxin)	Low levels of serum eotaxin were associated with increased radiographic progression.

criteria), including 22 with active RA and 22 with quiescent disease. Candidate plasma immune proteins, totaling 163, were investigated by rolling cycle amplification, a technique whereby the assay sensitivity is enhanced by amplification of a fluorescent circular oligonucleotide linked to the detection antibody by T7 polymerase. They selected 18 plasma proteins with significant elevations compared to controls as determined by $\geqslant$1.5-fold change and *P* for the false discovery rate $\leqslant$0.005. These included cytokine biomarkers: IL-6, IL-2, TNFRSF9, lymphotoxin receptor, CXCL13, CCL23 (MPIF-1), CCL8 (MCP-2), CXCL11 (I-TAC), lymphotactin, TGF-α, macrophage colony-stimulating factor (M-CSF) and IL-9. These investigators then proceeded with multivariate approaches, including principal components analysis and partial least-squares discriminant analysis, in order to identify biomarkers correlated with disease activity. This revealed six biomarkers, IL-6, CXCL13, CCL23, TNFRSF9, M-CSF, and TGFα, that best discriminated the two groups of patients. Each of these was confirmed elevated by separate enzyme immunoassay. A receiver-operating-characteristic (ROC) analysis demonstrated excellent test characteristics in the test sample of patients, with sensitivity, specificity, and accuracy of the model incorporating the six biomarker panel of 86.4%, 90.9%, and 88.6%, respectively. Preliminary validation of these findings was shown in an independent sample of patients initiating anti-TNF therapy.

This study illustrates a methodical approach for plasma/serum biomarker discovery that should serve as the key paradigm for this type of study. The initial step should be a screening approach with a high-throughput multiplex system. Akin to gene expression studies, the selection of candidate biomarkers using the false discovery approach to control for multiple comparisons should be used. Statistical approaches to ascertain the most informative panel, such as principal components analysis and partial least squares discriminant analysis, should be an important step. Finally, validation of the candidate biomarkers in an independent patient cohort, and utilizing a different assay approach (such as ELISA) is critical. Such a thoughtful approach to biomarker screening is likely to produce a number of potentially informative markers to test in clinical trial settings.

Biomarkers and Clinical Drug Development

Personalized medicine has been defined as "the management of the patient's disease or predisposition toward a disease by using molecular analysis to achieve the optimal medical outcome for that individual, thereby improving the quality of life and health and potentially reducing overall healthcare costs".[15] For an individual patient, personalized medicine means that when the patient is seen by the physician, factors of the patient's individual characteristics including age, gender, weight, smoking history, family history, and effects of medications as well as their molecular genetic profile will contribute to better understanding of this disease, both with respect to diagnosis and classification, as well as factors that govern response to therapy.[16,17]

At present, in addition to established severity markers such as rheumatoid factor and anti-citrullinated protein antibodies, there is better understanding of how an individual's genetic make-up governs the response to therapy, particularly how drugs used for the treatment of rheumatoid arthritis are metabolized and how the genes and gene products may be related to positive response to the drug as well as adverse reactions.[18]

Research in the past several years has uncovered genes which affect drug sensitivity, including receptor markers to cortisone, and drug toxicity, including metabolism of azathioprine, methotrexate, and mycophenolate mofetil. Studies directed at the genetic variation in drug metabolism have elucidated enzyme products of genes such as thiopurine methyltransferase, which, in patients who are homozygote deficient, is associated with an increased risk for azathioprine toxicity.[19] However, the value to an individual patient of a unidimensional test such as thiopurine methyltransferase testing to evaluate for potential toxicity does offer the prospect of safer and more effective treatment; however, its role in actually reliably predicting azathioprine toxicity, including neutropenia, is not yet established.[20]

The US Food and Drug Administration's office of Clinical Pharmacology and other agencies have stressed the importance of critical pathways for biomarkers and drug development with identification of stratification markers in the preclinical drug development phase, studies of clinical utilities of the stratification markers during clinical development in phase one, clinical validation for stratification markers in phase two, and drug labeling based on trial results in phase three, prior to FDA filing approval.

Increasingly, such information is requested and sought in the drug development process, which has resulted in ever increasing number of drugs with labeling containing pharmacogenomic information. More than 10 percent of approved drugs now have pharmacogenomic information, and this number is over 40 percent for drugs approved since 2006.[21] This labeling is still mostly pharmacokinetic, such as mentioned for thiopurine methyltransferase for azathioprine metabolism; however, the trend is for development of pharmacodynamic information as well, including receptors for tumor necrosis factor and others such as cellular receptors. These receptors play a major role in cancer treatment and treatment of some autoimmune rheumatic diseases.

Outcomes incorporating biomarkers in rheumatoid arthritis currently do not utilize combinations of biomarkers, which may be able to provide better predictive information. None of the efforts in biomarker development yet include tests for specific disease and drug metabolism pathways, nor are there any current clinical trials in rheumatoid arthritis that incorporate biomarkers in the drug development programs of agents designed to treat these diseases.[22]

Challenges in clinical trial design which use biomarkers include limiting the number of patients which can be enrolled in trials, for which reason, random variability in potential biomarkers and disease expression will be difficult to assess, and the narrowly focused design of clinical trials in rheumatoid arthritis typically focus on one composite set of clinical outcomes such as the ACR20 or the DAS.[17]

In rheumatoid arthritis research, a switch from voluntary to mandatory genomic data submission will improve the treatment and outcomes of patients with vasculitis and advance the field from the current trial and error approach to one which is based upon a better understanding of individual patient risk factors for disease, treatment response, and risk for adverse treatment events.

Summary and Future Directions

As discussed herein, cytokines are promising as candidate biomarkers and are likely to continue to emerge in the coming years as powerful predictors of disease activity and response to therapy. The technology with which to measure cytokines is likely to evolve with improved sensitivity and through-put. Information from genome-wide association studies and gene expression analysis will further compliment this data, leading to new insights into pathogenesis. Reagents to assay new cytokine targets, such as reliable antibodies to emerging key cytokines (i.e. IL-23), will become available to expand the breadth of coverage of the

immune response in RA. Future studies should assay candidate biomarkers from stored samples from randomized control trials of biologic response modifiers to determine if treatment responses might be predicted. If this can be shown, then the potential of cytokine biomarkers to inform treatment decisions in the vain of 'personalized medicine' may be truly realized.

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