Targeted antibody therapy and relevant novel biomarkers for precision medicine for rheumatoid arthritis

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Abstract

Over the past two decades, the management of rheumatoid arthritis (RA) has progressed remarkably, encompassing the development of new diagnostic tools and efficacious biological agents, such as monoclonal antibodies against inflammatory cytokines and surface markers on immune cells. In addition to the significant efficacy of these biological agents, biomarkers for RA are under consideration for their potential to classify heterogeneous patients into several groups based on clinical and immunological phenotypes for the prediction of clinical course and prognosis and the facilitation of appropriate and precise treatment with the appropriate therapeutic monoclonal antibodies. Biomarkers, particularly those for the prediction and monitoring of the responses to therapeutic monoclonal antibodies for RA, are in demand, with many approaches examined in recent years. In this article, we have summarized the background research on biomarkers and introduced recent topics in the field that enable the possible clinical applications of biomarkers, especially those related to pathogenic cytokines, to guide the treatment of RA.

Keywords: biological agents, biomarker, cytokine, monoclonal antibody, rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by synovial inflammation, which results in irreversible joint destruction accompanied by physical impairment (1). A combination of genetic, environmental and hormonal factors are responsible for the development of autoimmune processes and the subsequent disease (2).

The recent development of efficacious biological agents, such as monoclonal antibodies against pivotal molecules in the pathogenesis of RA, with standardized treatment strategies has enabled clinical and radiological remission to become achievable targets of clinical practice (3). The mechanisms of action of the current therapeutic biological agents for RA interfere with the function of cytokines, cytokine receptors or cell surface molecules of T and B cells that are involved in the pathogenesis of RA. Although these biological agents have significant efficacy, the selection of the optimal treatment approach remains a complex issue.

The appropriate treatment options are selected by rheumatologists after the consideration of the pathogenesis of RA, the mechanisms of action of drugs and the classification of patients with RA into several subsets that follow different clinical courses and exhibit different responses to drugs. It has been anticipated that the classification of patients with RA can be clearly achieved through biomarkers (4, 5). Herein, we review and discuss biological agents for the therapy of RA, the currently available, potentially useful biomarkers focused on cytokines, and the factors that are relevant to cytokines, in order to predict the disease prognosis and response to biological agents of patients with RA.

Roles of cytokines in RA pathogenesis

RA affects 0.5–1.0% of the global population and is recognized as a common human disease. The pathogenesis of RA is not fully understood; however, as shown in Fig. 1, many cytokines are expressed in synovial tissues and involved in the immune processes that are associated with the pathogenesis of RA. Various cytokines from myeloid and plasmacytoid dendritic cell subsets, such as IL-1β, IL-6, IL-15, IL-18, TNF and type I interferons, facilitate the expansion and differentiation of T,1 and T,17 cells. Macrophage-derived cytokines, such as IL-1β, IL-6, TGF-β and TNF, also play an important role in T-cell differentiation. Activated T cells mediate effector functions through the release of cytokines and mesenchymal...
cell activation with the help of B cells. The synergistic effect of several pro-inflammatory cytokines leads to synovial fibroblast activation. Synovial fibroblasts express receptor activator of NF-κB ligand (RANKL), which drives the differentiation of bone-resorbing osteoclasts from haematopoietic osteoclast precursors (6). Biological agents for RA can block some of those pathways and yield a strong efficacy. The current targets are TNF, IL-1 receptor (IL-1R), IL-6R, CD80/CD86 (thereby preventing the binding to T-cell CD28), CD20 and RANKL, which are underlined in Fig. 1.

**Efficacy of biological agents**

The currently available biological agents for RA are summarized in Table 1. Most biological agents are monoclonal antibodies containing various portions of the residual mouse peptide against the pivotal inflammatory cytokine pathways and cell surface molecules; the others are fusion proteins composed of a receptor part for the target molecules and the Fc region of immunoglobulin (Fig. 2). Irrespective of the target and structure, all effective therapies achieve similar therapeutic effects, including the reduction of joint inflammation and the inhibition of the progression of joint damage; one exception to this is the anti-RANKL drug, which hardly has effect on disease activity, but almost halts erosion progression (7). Although the paradigm-shifting efficacy of these agents has resulted in the attainment of 30–50% remission or 70% improvement in patients with RA (8–20) (Fig. 3), a large variation in response is frequently observed among individual patients because of several possible factors: RA may proceed through continuous phases, which show different immunoregulatory disturbances and clinical phenotypes (21), or the dominance of different immune-mediated inflammatory pathways in each patient. Therefore, although the determination of the optimal treatment for a patient with RA is challenging, biomarkers are anticipated to assist this optimization.

**Biomarkers**

A biomarker, as defined by the National Institutes of Health Biomarkers Definitions Working Group, is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention (22). In addition, the committee defines ‘objectively’ to imply ‘reliably and accurately’ (22). Biomarkers vary based on their purpose: (i) as a diagnostic tool; (ii) for staging or classification of the extent of the disease; (iii) as an indicator for prognosis; and (iv) for the prediction and monitoring of the clinical response and toxicity of an intervention (Fig. 4). There are many types of biomarkers, including clinical, histological, imaging, genomic, proteomic or lipidomic, which arise from various sources, such as affected tissues, lymphoreticular tissues, peripheral blood, urine, saliva and organ fluids (22). The recent progress in the comprehension of RA pathogenesis and the development of efficacious treatment options has
increased the interest in the identification of useful biomarkers in different stages of the disease for early diagnosis and assistance in treatment decisions.

Cytokines as biomarkers

Higher levels of the cytokines that are involved in the pathogenesis of RA have been reported in patients with RA than in healthy controls; their serum levels are related to disease severity and poor outcomes. One study reported approximately four times higher serum levels of TNF and IL-6 and two times higher levels of IL-10 in patients with RA than in normal, healthy controls. Disease activity was correlated positively with IL-6 and TNF levels and negatively with IL-10 levels (23). Circulating IL-6 levels may help to predict the progression of erosion. A study showed that a pre-treatment level of 7.6 pg ml\(^{-1}\) IL-6 in newly diagnosed patients with RA was a cut-off for the detection of erosion progression by the sensitive modality of magnetic resonance imaging with an area under the curve (AUC) of 0.82, a sensitivity of 69% and specificity of 95%. The IL-6 levels appeared more influential than the seropositivity of rheumatoid factors or anti-cyclic citrullinated peptide (CCP) antibody, which is a well-known indicator of poor prognosis (Fig. 5A) (24). In addition, plasma IL-6 levels of 4.0 pg ml\(^{-1}\) after a 1-year treatment with methotrexate were related to tangible erosions that could be observed on X-ray photographs with a solid AUC of 0.94 (25). Patients with higher IL-6 levels may need more intensive treatment from an earlier stage to prevent joint destruction.

Other than the above-mentioned well-known cytokines, other novel potential biomarkers have been identified for RA. 14-3-3\(\eta\) is a joint-derived protein that up-regulates inflammatory cytokines and matrix metalloproteinase (MMP) expression, which may contribute to systemic and local inflammation and joint damage (26). After the diagnostic utility of RA was assessed, a cut-off of \(\geq 0.19\) ng ml\(^{-1}\) was shown to yield a sensitivity of 63–77% and a specificity of 92% in the discrimination of patients with RA from healthy control patients (27). Higher levels of the 14-3-3\(\eta\) protein also indicate poorer clinical and radiographic outcomes in patients with early RA. Patients with \(\geq 0.50\) ng ml\(^{-1}\) 14-3-3\(\eta\) levels may have twice as high risk as those with \(< 0.50\) ng ml\(^{-1}\) (28). Leucine-rich \(\alpha\)-2 glycoprotein (LRG) is a glycoprotein that contains repetitive sequences with a leucine-rich motif, is reported to be expressed by liver cells and neutrophils and is induced by cytokines such as IL-1\(\beta\) and TNF. Although its function remains unclear, serum LRG concentrations are increased in patients with bacterial infections, several types of cancer and various autoimmune or autoinflammatory diseases (29). The serum concentrations of LRG were reported to be considerably elevated in patients with active RA compared with that in healthy controls and patients with inactive RA and correlated with disease activity (30, 31). Serum LRG may contribute to the inflammatory process independently of IL-6 and reflects joint inflammation more accurately than C-reactive protein levels during tocilizumab treatment (anti-IL-6R humanized monoclonal antibody) (32).

Multi-biomarker disease activity (MBDA) is an indicator that is designed to fit composite disease activity measures and is calculated on the basis of levels of 12 different biomarkers,
including cytokines and non-cytokines: C-reactive protein, epidermal growth factor, IL-6, leptin, MMP-1, MMP-3, resistin, serum amyloid A, TNF receptor type I, vascular cell adhesion molecule 1, vascular endothelial growth factor A and human cartilage glycoprotein 39. Although the combined-marker appears to be useful as an indication of the disease activity and the prospective progression of joint destruction (33), there may yet be several limitations, including conflicting results depending on the type of drugs, stage of the disease and cost (34).

As expected, the circulating levels of the target molecules affected the efficacy of biological agents. The baseline TNF level is closely related to the requirement for higher doses of anti-TNF agents in order to achieve the maximum clinical response (35). The RISING study from Japan reported that while the clinical response to different doses of infliximab, an anti-TNF chimeric monoclonal antibody, was not significantly different among patients with TNF levels <1.65 pg ml\(^{-1}\) at baseline, the good response rates to 3, 6, 10 mg kg\(^{-1}\) infliximab in patients with 1.65 pg ml\(^{-1}\) or higher TNF levels were 14, 31 and 60% \((P = 0.025)\), respectively (Fig. 5B). Likewise, the baseline level of soluble IL-6R (sIL-6R) may be related to the clinical response to tocilizumab. A study assessed the relationship and found that 63% of patients with a sIL-6R level \(<72.6\) ng ml\(^{-1}\) achieved remission at 24 weeks after tocilizumab initiation; this was only achieved in 25% of patients with \(>72.6\) ng ml\(^{-1}\) sIL-6R (36). The cut-off of 72.6 ng ml\(^{-1}\) discriminated remission from non-remission with a sensitivity of 67% and a specificity of 72%. Serum osteopontin levels before treatment assist in the prediction of later clinical remission that is induced by tocilizumab, but not in the prediction of remission induced by infliximab (37). A cut-off osteopontin level of 17.3 ng ml\(^{-1}\) with an AUS of 0.713 also discriminated between remission and non-remission with a sensitivity of 66% and a specificity of 80% in patients treated with tocilizumab (37).

**Genomic biomarkers**

RA can be characterized serologically by the presence of auto-antibodies, such as anti-CCP antibodies (1), which have also been identified to be predictors of poor prognosis in terms of disease severity and joint damage (38, 39).
Many studies have revealed that a positivity and a high titre of anti-CCP antibodies are related to certain MHC genes, which suggests that certain HLAs are related to a more aggressive phenotype of RA (40–42). In particular, HLA-DRB1 alleles that code a shared epitope (SE), a 5-amino acid sequence motif in residues 70–74 of the HLA-DRβ chain (QKRAA, RRRAA, QRRAA), are associated with severe RA. The well-known SE-coding alleles are HLA-DRB1*0401, *0404, *0405, *0408, HLA-DRB1*0101, *0102, HLA-DRB1*1402 and HLA-DRB1*1001. The HLA-DRB1 SE was correlated to C-reactive protein levels and circulating cytokines such as TNF, which underscored the relationship of SE and the inflammatory process in RA (43). SE may also be related to the response to biologic agents. The cohort of SE-negative patients was nine times more likely to discontinue abatacept, a soluble fusion protein that consisted of the extracellular domain of CTLA-4 and the Fc portion of human IgG1, because of insufficient efficacy in SE-positive patients (44).

A considerable number of studies on the single nucleotide polymorphisms (SNPs) that affect the cytokine pathways involved in RA pathogenesis has been conducted with regard to the responses to biological agents. Patients who carry the A allele on TNF–308G/A are reportedly predisposed towards increased TNF expression: only 42% of patients with the A/A and A/G alleles responded to infliximab compared with 81% of patients with the G/G (45). Another study examined SNPs within other cytokine genes, such as those encoding IL-10, TGF-β, IL-1β, IL-1R-antagonist and their combinations, for their response to anti-TNF agents; it was found that the combinations of A2 alleles for −308 TNF1/TNF1 for TNF and −1087 G/G for IL-10 were associated with a good response to etanercept, a fusion protein of the TNF receptor and IgG1 Fc, although the response rate was only 24%, even in patients with the SNP combination (46). Some SNPs that are located within the TLR genes and NF-κB pathways have been reported to be related to good responses to anti-TNF agents (47). However, conflicting results have also been reported (48). The true relationship of SNPs and responses to biological agents is yet to be investigated.

**Gene expression**

Several studies have attempted to predict the responses to biological agents by combined gene expression analysis. Whole-blood gene expression analysis revealed considerable
changes in the gene expression. One study revealed a consistent and significant increase in transcripts related to plasma cells, B cells, MHC and ribosomal proteins and T cells, and a consistent down-regulation of transcripts in several myeloid lineages and platelets in patients who were responsive to treatment with anti-TNF agents, but not in patients who were resistant to the drugs (49), which suggested a possible valuable method for objective monitoring by gene expression modules. It also has important implications for a more dynamic understanding of the pathogenesis of RA. A whole-blood gene expression analysis identified different patterns for the predictive pre-treatment gene expression signatures that uniquely indicated the effects of biological agents on TNF-α, IL-6 and T-cell co-stimulation (50). The up-regulated gene expression patterns of the inflammasome, which is a multiprotein complex that plays a key role in the production of IL-1β and IL-18, reflected non-remission by infliximab; the expression of NK cell-related genes relevant to CD56 was higher in patients who did not respond to abatacept; and a low expression of a B-cell-related gene set relevant to CD19 was a promising predictive signature for a poor response to tocilizumab. The study showed their capability to discriminate non-responder from remission achiever with AUCs of 0.637, 0.796 and 0.768, respectively. Another study demonstrated a combination of the global gene expression and the histological and cellular analyses of the synovial tissues of patients with RA documented four major phenotypes (lymphoid, myeloid, low inflammatory and fibroid) and these patterns might be related to the responses toadalimumab, a fully human anti-TNF monoclonal antibody, and tocilizumab. The results demonstrated that the myeloid phenotype exhibited the most robust response to anti-TNF monoclonal antibody, thereby suggesting that the underlying molecular and cellular heterogeneity in RA may affect response to therapy (51).

Conclusions

Therapeutic antibodies, which are known as biological agents, have become essential treatments in the management of RA. The identification of novel biomarkers with clinical utility for RA practice, that is, to determine which patients respond to which biologic agent, remains a major topic of investigation. Although the findings that we have reviewed herein highlight the possibility of the accurate classification of patients and prediction of drug efficacy, their application in clinical practice requires more reliable and reproducible evidence than is available at present. Further efforts to identify useful, simple and cost-effective biomarkers shall direct us towards precision medicine.

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