THE BIOMARKERS USED FOR DIAGNOSIS AND ASSESSMENT OF RHEUMATOID ARTHRITIS

Ileana Cosmina Filipescu, Simona Rednic
Rheumatology Clinic, University of Medicine and Pharmacy, „Iuliu Hatieganu", Cluj-Napoca

Abstract

Significant progress in the pathogenesis, assessment and treatment of rheumatoid arthristis has been made after 1920, the year when the most frequently used remissive agents, methotrexate was introduced. The clinical and biological assessment is the "cornerstone" of monitoring any patient with rheumatoid arthritis. The clinical, biological or genetic parameters used individually are not enough to monitoring the disease or to predict the answer to therapy.

Keywords: rheumatoid arthritis, biomarkers, diagnosis, treatment

Rheumatoid arthritis (RA) is a chronic autoimmune disease with a variable presentation in different patient but also in the same subject in the different moment. Monitoring the therapy, the clinical and paraclinical evolution of this disease is extremely important to prevent the occurrence of disabling sequel and major adverse effects. The uniform assessment is very difficult. The biological parameters like acute phase reactants, autoantibodies, the cytokines and genetics polymorphisms are useful for diagnosis, for monitoring inflammation and assess joint damages. They are also used to assess the stage of the disease and to quantify the response to therapy. Previous research has demonstrated that clinical, biological or genetic parameters used individually are not enough to predict the answer to treatment (1,2,3).

Recent medical research in this field had as its main objective finding the best biomarker able to monitoring the therapy and to predict the response to biological treatments (4,5).

ACUTE PHASE REACTANTS

The most commonly used in current practice are erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). These markers are known to have a low diagnostic value. They can be of normal value in 35-45% of patients with arthritis, especially at the beginning of the disease (1). ESR is considered a more sensitive activity marker at the beginning of the disease; the marked and persistent growth is considered a predictive factor for severe evolution. It is less specific and may be influenced by sex, age, infection, neoplasia, anemia, hyper immunoglobulin syndromes and obesity (2).

CRP has a higher specificity and is a predictor of functional status and radiological progression. It correlates well with response to treatment (6). Its level decrease rapidly if therapy is effective. ESR and/or CRP enter into formulas for calculating composite scores, making them useful tools for monitoring the evolution of arthritis (6).

Other acute phase reactants (e.g., haptoglobin, α1-anti-trypsin, ferritin, fibrinogen, serum amyloid A, orosomucoid) were shown to correlate with disease activity in RA, but further studies are needed to characterize in a more complex manner their relationships to RA (3,7).

AUTOANTIBODIES ASSOCIATED WITH RA

The rheumatoid factor (RF) is an autoantibody to the Fc fragment of an immunoglobulin G. The most common is type IgM, but IgA and IgG may also present. RF is not specific for RA and can be found

Adresă de corespondență:
Ileana Cosmina Filipescu, University of Medicine and Pharmacy „Iuliu Hatieganu", 6 Pasteur, Cluj-Napoca
E-mail: ileana_nicoara@yahoo.com

in many other diseases (8). Approximately 70-80% of the patients with arthritis have positive RF. In the first year of the disease, RF is only present in 50% of cases. However, high titres of RF without evidence of other causes to explain his presence give further prognosis of severe disease (9). Determination of IgA isotype and dosage of RF from synovial fluid proved to bring useful information for monitoring response to treatment (10).

The recently discovered Anti-cyclic Citrullinated Peptide Antibodies (Ac anti-CCP) have been added to the diagnostic criteria of disease because of their high specificity for RA (95%) with a low prevalence in other diseases (0.4% in general population) (11). The combination of Ac anti-CCP with RF is different as follows: 70% of sero-positive RA patients and 30% of sero-negative (12). The autoantibodies occur early in the course of the disease and it was found that smoking has a pathogenetic role in their occurrence (13). Similar to the RF, the Ac anti-CCP are infaust prognosis factors, predicting erosive disease and low response to treatment (14). Emery and colleagues have shown that it is possible to obtain a suboptimal response to monotherapy with methotrexate (MTX) in patients with arthritis with high titres of Ac anti-CCP (15). Their presence is associated with a response to treatment with anti-TNFα agents, but the presence of a low titre increases the chance of a good response to the classical combined remitting therapy (Disease Modifying Anti Rheumatic Drugs-DMARD)-inhibitors TNFα (9,10). The monitoring of reducing Ac anti-CCP titre as response to treatment has been shown effective only if the disease was at its beginning (< 1 year) (10,16).

THE CYTOKINES

The cytokines are protein cell products, with low molecular weight, synthesized by the inflamed cells. Their action is exerted on the cells and at distance (17). They play a major role in the stimulation, maturation, specific differentiation and activation of other cells involved in the inflammatory process. The class of cytokines includes the interleukins, colony growth factors, tumour necrosis factor, colony stimulating factors, interferons and chemokines (18).

The characteristic inflammation in rheumatoid arthritis occurs as a consequence of the imbalance between cytokines with pro and anti-inflammatory role (17). A number of studies on the profile of cytokines and chemokines in the serum and synovial fluid on RA patients found that monokines (Il-1, IL-6, TNFα, IL-8) with a pro-inflammatory role in particular are in excess, while lymphokines (IFN-γ, IL-2, TNF-β) are deficient in these patients (18,19).

In the past years special attention has been given to identifying the role of cytokines as biomarkers of disease activity and treatment response. Hueber and his colleagues have identified 24 antibodies and chemokines capable of predicting the response to etanercept. The small number of patients and the assessment of the response to a single drug were some of the limitations of this study (20). Results of other studies have been conflicting (21,22).

TNFα and interleukin 6 (Il-6) are cytokines strongly involved in the RA pathogenesis and they also represent important therapeutic targets.

TNFα

TNFα, the pro-inflammatory cytokine produced by macrophages is one of the first cytokines secreted in RA patients and is considered the main trigger and enhancer of the inflammatory process (23).

TNFα belongs to a family of peptides mediators that contains at least 19 members. Its functions are not known but it is assumed that TNFa is involved in cell proliferation, apoptosis and inflammation (24). Due to its biological properties, TNFα plays a beneficial role in the physiological immune response, but when its output is excessive, it determines inflammation, tissue damage and organ damage. In the human body there is precursor of TNFα, a transmembranar protein with 26 kDa, expressed by several cells. The macrophages, monocytes and T cells are the major producers of TNF and other pro-inflammatory cytokines, chemokines, proteases, nitric oxide, free radicals. The active form of TNF is a protein fragment of 17 kDa, obtained after TNF converts (TACE) splits the 26 kDa precursor molecule (23,24). After their separation from the cell membrane, these soluble molecules of TNFα aggregate to TNF trimolecular complex (51 kDa-homotrimeri) which then binds to two receptors (type 1 - p55 or p60 and type 2 - p75 and p80), which are expressed on different cells (fibroblasts, leukocytes, endothelial cells).

TACE also splits the extracellular domain of the complementary TNF ligand forming the soluble receptor of TNFα (sTNFR). These circulating soluble receptors may bind the TNF trimolecular complexes, rendering them biologically inactive, thus sTNFR act as natural inhibitors of the inflammation

mediated by TNFα (23). TNFp75 receptors have high affinity for binding to TNFα, while TNFp55 receptors have a low affinity to the substrate and serve as physiological regulators of the inflammatory response by inhibiting TNF activity (24,25).

TNFα causes the induction of proinflammatory cytokines (IL-1, IL-6, and IL-8) and the stimulation of leukocyte migration (by increasing endothelial permeability and VCAM-1 expression by the endothelial cells and by leukocytes with increased influx of immune cells and production of inflammation in the joints) (24). It also produces functional activation of neutrophils and eosinophils and induction of acute phase proteins (CRP, serum amyloid A, haptoglobin, fibrinogen). TNFα stimulates the synthesis and releases the inflammatory mediators (MMP, PG, and NO), inhibits the collagen and proteoglycans causing poor achievement of reparative phenomena, produces cartilage and bone destruction (by stimulating and inhibiting the resorbtion of proteoglycans and inhibiting their synthesis) (24,25).

The role of TNFα in granuloma formation is very important. For this reason, patients whose TNFα was neutralized have a greater susceptibility to infections with microorganisms such as Mycobacterium, fungi, Listeria (26).

Although the name suggests a predominant role in host defence against tumours, TNFα is just one of the immune system elements capable of destruction the malignant cells. The TNFα molecules belong to the triad of effectors molecules (together with perforin and IFN-γ) which activate the antitumor T cells (23,24).

The chronic inflammatory process is not only due to the increased expression of TNFα but also due to the existence of an interrelation with other effectors systems (Table 1).

Since TNFα is found in increased quantity in the specific hyper proliferating synovium and the synovial fluid and since it is directly involved in the RA pathogenesis, blocking this cytokine has been hypothesized to be important in the improvement of the disease (26). The TNFα concentration in the serum and synovial fluid of RA patients is correlated with the clinical disease activity and the level of other acute phase reactants. Increased levels of soluble TNF receptors were found in serum and synovial fluid of patients with arthritis (27). The plasmatic level of the soluble TNFR (sTNFR) is increased in patients with RA but it is insufficient to neutralize the large quantity of TNFα produced and thus to prevent chronic inflammation (28). Compared with the level of TNFα, the concentration of soluble receptors is higher in synovial fluid than in serum and thus explains why the detection of active TNFα at synovial level is difficult to achieve (29). Notably the TNF β (lymphotoxin), cytokine which has its origins in LT is not present in the synovial fluid of patients with arthritis (30).

Il-6

IL-6 is a cytokine with a marked role in inflammation, hematopoiesis and immune response. It produces bone resorbtion (through RANKL system) and cartilage degradation (by stimulating the metalloproteinases) (31). It induces the formation of acute phase reactants, it is responsible for systemic effects causing anemia (by stimulating hepcidin) and hypoalbuminemia, and induces synovitis by stimulating neoangiogenesis, inflammatory cell infiltration and synovial hyperplasia. It is involved in autoimmunity by stimulating the differentiation of B lymphocytes and Th17 (31,32). The leading role of this cytokine

TABLE 1. *The effect of blocking TNFα in rheumatoid arthritis (Adapted by Wong M., Clin Immunol 2008) (24)*

Target	Mechanism of action
Cytokines	Reduce the levels of proinflammatory cytokines
Immune system cells	Reduce the number of synovial level granulocytes, neutrophils, CD3 + T cells, CD22 + and CD68 + B Reduce the production of IL-8 and MCP-1
Monocytes and macrophages	Induce apoptosis at the synovial level and in the peripheral blood
Peripheral T Cells	Induce apoptosis via caspase 3
Hematopoietic cells	Increase the level of hemoglobin Reduce the concentration of erythropoietin
Endothelial cells and atherosclerosis	Reduce the concentration of E-selectin, ICAM-1 and VEGF Increase the endothelial cell function
T cells and dendritic cells	Increase serum levels of T cell regulators Reduce the expression and prevents the maturation of dendritic cells

in the pathogenesis of RA was demonstrated by the favourable effect of tocilizumab (a monoclonal antibody blocking the IL-6 receptor) on the disease activity (32,33).

It has been shown that the serum level of IL-6 correlates with disease activity and joint damage especially in advanced forms of the disease and has been studied as a prognostic marker for the evolution of RA (34).

The reduction of the serum level of IL-6 was observed in responders, including patients treated with TNFα inhibitors agents (35). The Braun-Moscovici study observed a significant and lasting reduction in the titre of IL-6 in responders to infliximab, whereas in non-responders the plasma levels of this cytokine increased to the level prior to the administration of the biological therapy (36).

Other cytokines and chemokines

In search of an ideal biomarker for diagnosis and monitoring of disease other molecules were studied as well. Elevated levels of *monocyte chemotactic protein* 1 (MCP1) and epidermal growth factor (EGF) were associated with a good response after 3 months of treatment with etanercept (15,37). For infliximab, the serum level of apolipoprotein A-1 was proven to be a positive predictor and the presence of thrombocytic factor 4 was associated with a low response (15). The low level of *cartilage oligomeric matrix protein* (COMP) correlated with good response to treatment with adalimumab, but not with infliximab (37). RANKL low values were found in relation to the response to adalimumab and infliximab, by the same researchers (15). Recently COMP, the E-selectin and soluble adhesion molecule-1 for soluble intercellular proved their predictive role in the radiological progression of the disease (38).

In a study conducted in 2012 by Curtis et al, of the 396 biomarkers evaluated, 12 were found to be related to RA activity, among witch was the endothelial growth factor (*Vascular endothelial Growth factor* - VEGF) (39). VEGF is produced by synoviocytes, macrophages, endothelial cells, leucocytes, fibroblasts and is responsible for angiogenesis characterizing articular pannus. The VEGF concentration in the synovial fluid is increased in patients with RA compared with that of other arthritic patients and its level in serum was found in correlation with rheumatoid activity in particular the number of swollen joints (40). TNFα and IL-6 stimulate VEGF production (41). Its production is decreased by cyclosporine, anti-TNFα therapy and anti-IL-6, but it is not clear whether this effect is related to the type of medication occur to a better control of the disease which reduces synovial inflammation (42).

GENETIC FACTORS – GENETIC POLYMORPHISMS

Since the genetic constellation is stable over time and influence by previous treatment since DNA extraction is less invasive compared to synovial biopsy, there are more studies that have sought predictive genetic markers for obtaining a better control of the disease (43).

The most investigated have been the polymorphism of the genes encoding TNFα, the major histocompatibility complex, p38 network, STAT4, PTPN22, PADI4, CTLA-4 and FcγIIIa receiver (44,45).

The gene encoding TNFα synthesis has been extensively studied in relation to assessing the response to the agent blocking this cytokine. Recently, interest has been focused on single nucleotide polymorphism (SNP-*Single Nucleotide Polymorphisms*) (46). Increased allelic frequency implies a causal marker involvement in the susceptibility or severity of the disease or the presence of a pathogenic gene in linkage disequilibrium with marker alleles (association at the population level of particular marker alleles with known gene for a particular disease). The most studied was the SNP in position 308, originally identified as a predictor of response to anti-TNFα therapy in Caucasians and Koreans patients (47). It has been shown that the presence of TNF-308GG genotype is a positive predictor for a better response to etanercept, adalimumab and infliximab compared with genotype TNF-308A or-308Ag (47,48,49). Recently Pavy et collaborators rule out these hypothesis in a meta-analysis which included 1721 patients with RA and comprised the largest studies focused on the role of the polymorphism of the gene encoding TNFα as a predictive factor for the response to therapy with TNFα blockers (50).

Results were inconclusive in studies on other mononucleotide polymorphisms in position 238 and 857 of the same gene encoding TNFα as well (4).

The gene encoding the synthesis of TNFα receptor type II has been extensively studied. The TNF receptor family include a member 1α receptor encoded by the gene TNFRSF1A (TNFRI, p55) and a 1β member, encoded by the gene TNFRSF1B (TN-

FRII, p75), each with a different involvement in RA pathogenesis (51). These receptors may be linked to the cell membrane or may circulate as soluble proteins. The plasmatic levels of these receptors (sTNFR) could become a useful tool in assessing the response to TNFα blockers, and sTNFR may act as a natural inhibitor of the TNFα cytokine (29,51).

The genotype profile of TNFα and its receptor type II (TNFRII) is presumed to play an important role in the resistance to therapy with TNFα inhibitors (30,52). The gene encoding the synthesis of TNFRII is located on chromosome 1p36 and is made up of 10 exons and 9 introns (53).

The genotype TNFRII-676TG was associated with lower ACR response at 3 and 12 months of treatment with the three main TNFα blockers, when compared to the 676TT genotype (54). In another study on 234 patients by the 676TG genotype did not show an influence in obtaining a favourable response to IFX and ADA after 3 and 6 months treatment (29).

Rooryk and collaborators have argued that the existence of polymorphism 196 in the gene encoding the TNFα family of 1B receptor induces a suboptimal response to IFX (55).

In relation to activity and severity of RA and the response to anti-TNFα agents, the most studied single nucleotide polymorphism of gene TNFRSF1B is rs1061622 (located in exon 6), but the results were not concludent (51,56).

Although some results were encouraging, these genotypes can be considered prognostic factors for disease severity or radiological progression, but cannot be used as reliable tools in predicting treatment response to TNFα inhibitors, in current clinical practice (33). There is a need for new broader studies, with a more complex design including other biomarkers in order to complete the picture of a heterogeneous disease like rheumatoid arthritis.

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