Biomarkers of outcome in rheumatoid arthritis

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Introduction

Rheumatoid arthritis (RA) is the most common autoimmune inflammatory arthritis, affecting approximately 1% of the population. This systemic disease is marked by chronic inflammation that predominantly affects the synovial membrane of diarthrodal joints. Its etiology is unknown, but it is presumed to be an immunological disease with contributing genetic and environmental factors. Evidence suggests that RA develops in 3 phases: an asymptomatic period of genetic risk, a pre-clinical period in which RA-related antibodies can be detected, and a clinical phase with acute signs and symptoms of inflammatory arthritis.

Evidence suggests that the sooner RA patients are treated, the better is their prognosis. The availability of markers that could help to identify patients with more aggressive, rapidly progressive RA with poorer prognosis would offer a rational basis for early and aggressive treatment. In that way it may be possible to avoid many irreversible clinical complications. The number of disease modifying anti-rheumatic drugs (DMARD) available has increased in recent years. The effectiveness, cost and toxicity of the new agents vary widely and the natural history of RA itself includes spontaneous remission, remission with medical treatment or continuously progressive disease despite medication. Hence, there is a clear need to identify efficient diagnostic and prognostic indicators of disease to assist clinicians and help them in making initial treatment choices.

This article is a literature review of current biomarkers to aid in the diagnosis of RA and to compare risks between studies. Our aim was to review the literature in an inclusive manner.

Biochemical markers

For a long time, the diagnosis of RA was mainly based on clinical manifestations. However, it is often difficult to diagnose RA in the very early phases of the disease and in many cases irreversible damage had occurred by the time the diagnosis was confirmed. Therefore, laboratory tests which are sensitive and specific early in the disease course are desirable to allow earlier diagnosis and intervention.

A number of such biomarkers have been investigated focusing on analytes found in the different cellular compartments including biomarkers involved in the synthesis and degradation of bone/cartilage, inflammation or autoimmune processes in order to identify those that could be clinically useful.

Genetic markers

Genetic markers in RA have been widely reviewed elsewhere. At least 12 genetic loci have been confidently identified as associated with susceptibility to RA but each locus confers a relatively modest effect and, even in combination, cannot be used to diagnose the condition as variants are common in the healthy population (Table 1). Only 2 show evidence of association with severity or outcome of disease. Firstly, variants within the human leucocyte antigen (HLA)-DRB1 gene show the strongest association with RA. In particular alleles encoding a shared amino acid motif are collectively called the shared epitope (SE) and not only increase the risk of RA but are also associated with more severe disease. These alleles have been also reported to be associated with anti-citrullinated protein antibodies (ACPAs) as measured by anti-cyclic citrullinated peptide (anti-CCP) antibodies. More severe disease with an increased rate of joint destruction has been found in patients with both ACPA antibodies and SE alleles. As anti-CCP antibodies are cheaper and easier to measure, testing for SE alleles carriage is not used clinically. Secondly, variants within the TRAF1/C5 susceptibility locus have been reported in two studies to be associated with erosive change independently of the SE. However, the risk conferred by carriage at the susceptibility variants is only in the order of 1.5 and so not clinically useful at the current time.

Bone markers

Receptor activator of nuclear factor κB ligand/osteoprotegerin

The receptor activator of nuclear factor κB ligand (RANKL) is a member of the TNF superfamily; it is expressed on various cell populations and also exists as a soluble molecule. It is a key factor in the stimulation of osteoclast formation and activation. In turn, osteoclasts play a central role in the mechanism of joint destruction in RA. It has been demonstrated...
that T cells express RANKL and that there is overexpression of RANKL mRNA in the synovium of RA patients at the site of the bone resorption. The soluble receptor-like molecule osteoprotegerin (OPG), a decoy receptor, is a natural inhibitor of RANKL. Both RANKL and OPG can be measured in serum and the ratio RANKL/OPG is thought to regulate bone resorption. The COBRA trial showed that a baseline value of a low ratio RANKL/OPG predicted a higher 5-year radiographic progression of joint damage. An independent study found that baseline levels of the RANKL/OPG-ratio in patients with early, active, untreated RA are strong independent predictors of rapid and persistent damage progression over 11 years follow up. Interestingly, another study found that increased levels of OPG are effective in compensating for the action of soluble RANKL, but do not directly prevent bone degradation. A fourth study found that baseline levels of OPG and RANKL were not associated with radiographic progression at five or ten years. This discrepancy with the COBRA study might be related to differences in patient characteristics. Dysregulation of the RANK/RANKL/OPG system has been implicated in the pathophysiology of multiple bone remodeling disorders including osteoporosis, glucocorticoid-induced bone loss, multiple myeloma and rheumatoid arthritis. Therefore RANK/RANKL/OPG may eventually be used as markers of bone metabolism, though the broad role of RANKL signaling in the immune system may limit its specificity.

Collagen cross-linked C-telopeptide of type I collagen

Collagen cross-linked C-telopeptide (CTX-I) is a degradation product of C-terminal cross-linking telopeptide of type I collagen which has been suggested to be a candidate biomarker for joint destruction. A prospective study of patients with early RA showed that high baseline levels of urinary CTX-I predicted increased risk of radiological progression and correlation with more rapid progression of joint destruction whilst another long-term follow-up study noted correlation of serum CTX-I levels and subsequent joint destruction. These results suggest that CTX-I might be useful for identifying patients with a high risk of joint damage progression before damage can be detected radiographically.

Bone sialoprotein

Bone contains non-collagenous proteins such as bone sialoprotein (BSP), which is released during bone damage and can reflect the rate of bone turnover. Serum levels of bone sialoprotein were measured in the chronic and destructive arthritis induced in dark agouti (DA) rats after immunization with autologous rat native collagen type II in Freund’s incomplete adjuvant. Increased serum levels BSP were seen on day 21 after immunization and even higher levels were observed on day 28 at termination of the experiment, paralleling increases in the clinical joint score and histopathological signs of cartilage and bone erosions suggesting that it may be a marker of joint damage. In a study by Mansson et al. of 42 patients, BSP was quantified by enzyme linked immunosorben (ELISA) assays in longitudinally collected knee joint synovial fluid (SF) samples of RA patients rapidly developing destruction in knees or hips, the “destructive” group (n=18), and in patients slowly developing destruction, the “non-destructive” group (n=25). The destructive group was characterized by rising BSP concentrations in synovial fluid with time compared with the initial levels (P<0.001). The authors conclude that this molecule contributes to the assessment of extent of tissue destruction and may help in the early identification of patients at risk of rapidly progressing destruction.

Cathepsin K

Cathepsin K is expressed by osteoclasts and synovial fibroblasts to degrade type I collagen during bone resorption and is hypothesized to play a role in the pathology of RA. Some studies suggest that it may be interesting as a biomarker of bone resorption. For example, a study of 100 patients with active, longstanding RA found that the radiological destruction of

Table 1. Previously known rheumatoid arthritis risk associated SNPs in Europeans. Listed are the chromosome, SNP ID, and candidate gene(s) in the region. The table includes susceptibility loci with a P<10\(^{-8}\).

<table>
<thead>
<tr>
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related with the levels of cathepsin K. However, this has not yet been validated so further studies will be required to investigate the role of cathepsin K as a marker of bone resorption in RA.

**Osteocalcin**

Osteocalcin is a large peptide that is synthesized by osteoblasts, odontoblasts and some chondrocytes. Circulating osteocalcin and its fragments reflect both bone formation and resorption. In an experiment to study the changes in markers of bone metabolism, including osteocalcin, during anti-TNF treatment, it was found that the levels of osteocalcin were appreciably increased at 14 weeks compared with baseline, but not at 30 and 46 weeks, and no significant association was found between changes in disease activity and levels of osteocalcin at any of the time intervals. However, another study reported that osteocalcin fragments are released during osteoclastic bone resorption and that the quantification of specific age-modified osteocalcin fragments can provide an index of bone resorption suggesting that it may be a marker of joint erosion rather than disease activity.

In summary, although many bone markers have been tested as potential biomarkers, the evidence, to date, suggests that the RANKL/OPG ratio is most reliable in predicting bone erosions. However, further larger studies are needed to confirm this and to assess the clinical utility.

**Synovial and cartilage markers**

**Cartilage oligomeric matrix protein**

Cartilage oligomeric matrix protein (COMP) is an extracellular matrix glycoprotein member of the thrombospondin family of calcium-binding proteins. Several studies indicate that changes in COMP serum concentration are related to processes in cartilage. In a prospective study of 183 patients with early RA, it was found that early determination of serum COMP was one of the most abundant proteins of the cartilage matrix, current cartilage turnover markers are based mainly on immunological reagents detecting their synthesis and degradation. In one study, aggrecan fragments are increased in the synovial fluid of RA patients and the levels have been related to disease severity. In one study, aggrecan was quantified by ELISA in longituidally collected knee joint synovial fluid samples of both patients rapidly developing destruction in knee or hip joints and in patients slowly developing destruction and it was found that the aggrecan concentrations were initially highest in the group developing destruction (P<0.001). In a more recent study, it was found that levels of aggrecan fragments in human synovial fluid are increased in RA, OA and after knee injury. Conversely, the data from a third study strongly suggests that total aggrecan levels are lower in RA patients than in healthy controls, and that RA patients have at least one specific subpopulation of aggrecan fragments, namely argasanase generated ARGSVI fragments which, if confirmed, could help in differentiating RA from other types of arthritis.

**Chondroitin sulphate epitope of aggrecan**

The chondroitin sulphate epitope of aggrecan recognized by the monoclonal antibody 846 (CS846-epitope) is present in hardly detectable amounts in the normal adult cartilage. Preliminary *in vitro* studies indicate that the level of the epitope in cartilage is directly correlated with aggrecan biosynthesis. The available evidence suggests that fragments of aggrecan containing the 846 epitope reflect the degradation of newly synthesized aggrecan molecules. Serum concentration of CS846-epitope was measured in two patient groups with early RA but with distinctly different disease outcomes. The results showed that concentrations of CS846-epitope in patients with slow joint destruction were increased, whilst low levels were observed in patients with rapid joint destruction, concomitant with elevated levels of C-propeptide of type II procollagen (CPII), suggesting a selective increase in collagen synthesis. Further clinical studies are needed to investigate the potential of aggrecan as a biochemical marker in destructive joint diseases.

**C-terminal crosslinking telopeptide of type II collagen**

Urinary C-terminal crosslinking telopeptide of type II collagen (CTX-II) is a marker of cartilage destruction. A prospective study of a cohort comprising 116 patients with early RA investigated the relationship between levels of urinary Glucosyl-Galactosyl-Pyridinoline (Glc-Gal-Pyd), urinary CTX-II and serum matrix metalloproteinase (MMP)-3 and the progression of joint destruction. The levels of Glc-Gal-Pyd, CTX-II and MMP-3 were elevated compared with the levels in 76 healthy controls and high baseline levels of Glc-Gal-Pyd, CTX-II, and MMP-3 were associated with increased risk of progression of joint destruction over one year in early RA. Later, the same group investigated the relationship between baseline levels of urinary CTX-I and CTX-II and the mean annual progression of joint destruction over a median of four years and concluded that high baseline levels of urinary CTX-I and CTX-II independently predict an increased risk of radiological progression over four years in patients with early RA, especially those without radiological joint damage at onset. Another longitudinal analysis found that cartilage degradation as measured by urinary CTX-II and, to a lesser extent, bone degradation as measured by CTX-I closely follow indices of RA activity. Clinically perceptible arthritis is responsible for immediate damage which will become visible on plain X-rays only much later. In experiments using experimental arthritis models (collagen-induced arthritis (CIA) model and monooiodoacetate-induced arthritis (MIA) model) in rats, markedly increased levels of CTX-II were detected in the synovial fluid and the serum and both showed strong correlations with the microscopic severity scores of joint lesions at day 22 post-induction in the CIA model (synovial fluid CTX-II, r=0.76; P<0.0001; serum CTX-II, r=0.85; P<0.0001). In the MIA model, CTX-II concentration in the synovial fluid (r=0.53; P<0.0001), but not in the serum, correlated with the microscopic severity score. In a recent prospective study of 66 RA patients and 76 healthy controls, in which measurements of urinary Glc-Gal-Pyd and CTX-II were performed at baseline and at one year, it was found that baseline levels of Glc-Gal-Pyd and CTX-II were increased in patients with RA and correlated with progression of joint damage.

Further support for CTX-II as a biomarker of joint progression was provided by a study of 148 RA patients receiving conventional DMARDs who participated in the SAMURAI trial. The 28 joint count disease activity score (DAS28), clinical improvement in signs and symptoms of RA, tender joint count, swollen joint count, and modified health assessment questionnaire (MHAQ) were assessed at baseline and 26 biological markers were measured. Among of a panel of 40 different variables, the investigators identified baseline joint damage, urinary CTX-II, the PPD/DPD (total pyridinoline/urinary total deoxypyridinoline) ratio and...
Body Mass Index (BMI) as strong and independent predictors of radiological progression in patients with RA receiving conventional DMARDs. Therefore, there are several lines of evidence to suggest that urinal CTX-II levels could be useful for detecting patients who are at high risk of joint damage progression very early in the disease before abnormalities can be detected radiographically.

Matrix metalloproteinases

In RA, degradation of articular cartilage is caused by proteinases derived from both the inflamed synovium and stimulated chondrocytes. In RA matrix metalloproteinase (MMP)-1, MMP-3, MMP-9 and membrane type 1 (MT1)-MMP are over-produced. These MMPs are secreted into the synovium and attack the cartilage immersed with synovial fluid. Interestingly, MMP-3 levels in rheumatoid synovial fluid are higher than other MMPs and in some studies have been shown to be predictive of joint destruction.44,45 For example, in a longitudinal study of 132 patients with early RA, levels of MMP-1, MMP-13, MMP-3, TIMP-1, and COMP were assessed in serially obtained serum samples. Levels of MMP-3, CTX-II, COMP and TIMP-1 correlated with radiographic progression at entry and longitudinally as assessed by area under the curve (AUC). By multivariate analysis, a model including MMP-3 and CTX-II was identified as providing the best prediction of radiographic progression at baseline and a combination of MMP-3, CTX-II, and swollen joint count formed the best longitudinal AUC model.46 In another experiment involving patients with RA and systemic lupus erythematosus (SLE), it was found that levels of MMP-2 and MMP-9 were higher than controls in both groups of patients, supporting the involvement of MMP proteins in these autoimmune disorders.47 Baseline serum levels of 2 elements of the metalloproteinase network, MMP-1 and TIMP-1, reflected subsequent joint destruction.48 All these data support the important potential role of MMP proteins as biomarkers in RA.

Col2-3/4Clong mono and Col2-3/4Cshort

Type II collagen (CII) is the main collagen of articular cartilage and is highly degraded in RA due to its cleavage by collagenases, exposing a neoepitope Col2-3/4Clong mono (C2C), which can be detected by immunoblot. Two studies have found that C2C as a measure of CII synthesis may be useful as a clinical prognostic biomarker for disease onset and activity in RA and as a predictor for joint space narrowing and radiographic damage progression.49,50 By contrast, a third study showed no association between baseline serum levels of C2C and radiographic progression.51 A related neoepitope Col2-3/4Cshort (C1,2C) is a marker for degradation for type I collagen and type II collagen in cartilage,52 and is related to specific joint space narrowing and erosion in RA.79 Thus, these biomarkers may be of value when studying progression of joint damage in RA patients but large, definitive studies are required.

Glycosyl-Galactosyl-Pyridinoline

Glycosyl-Galactosyl-Pyridinoline (Glc-Gal-PYD) is a recently described marker of synovial tissue (ST) destruction, which correlates with joint damage in RA.53 These findings were confirmed in a prospective study of 66 RA patients treated with infliximab and metotrexate. It was found that baseline urinary levels of Glc-Gal-PYD were elevated compared with the healthy controls and were associated with erosive changes.54 Measurement of this specific molecular marker may thus be useful in identifying patients with early RA at high risk of rapid progression of joint damage.

Hyaluronic acid

Soluble fragments of Hyaluronic Acid (HA) in sera of RA patients can be elevated from 10 to 20-fold compared with healthy controls and correlate with disease activity.55 However, HA levels vary significantly during the daytime and peak levels correspond to times of physical activity so the diagnostic utility of HA could be questioned. In a prospective study, 62 patients were followed for five years with radiographic examinations at baseline and at one, two and five years. It was found that HA at baseline correlated with the one, two and five year radiographic score. Interestingly, it was found that HA also correlated with other markers including ESR and CRP. An experiment including RA and OA patients showed that the highest concentration of HA was found in RA SF compared with asymptomatic donor and OA SF samples and that HA correlated with osteogenic protein 1 (OP-1) which, in turn, is correlated with cartilage degeneration.56 Of the synovial and cartilage markers, therefore, COMP, Aggrecan, CTX-II and MMPs show promise as possible biomarkers of rapidly progressive joint damage in RA patients. However, further studies are needed to confirm the associations and to determine whether a single or a combination of markers provides most accuracy in prediction.

Autoantibodies

Rheumatoid factor

Rheumatoid factor (RF) consists of antibodies directed against the Fc region of human IgG immunoglobulin. They are the hallmark of RA and can be detected in 60-80% of RA patients in hospital series.57 RF was included in the 1987 laboratory criteria by the American Rheumatism Association (ARA).58 However, RF is not specific for RA and is found in other rheumatic diseases, other chronic inflammatory disorders, infections and even in healthy people.59 The sensitivity for rheumatoid factor in RA varies from 19-53% and the variation in specificity is from 91.7-98.6%.60 RF is widely used for diagnosis and prognosis in RA and several studies have shown that it correlates with radiographic outcome.61-63 Its predictive value is dependent on the stage of the disease.64,65,66 Thus, in the early stages of RA, RF positivity is associated with more active disease and the development of erosions. However, in later phases of the disease, it is less predictive of subsequent joint progression.67 The RF assay is used as a marker for RA diagnosis, since its presence is associated with an increased risk of developing RA.68

Anti-citrullinated protein/peptide antibodies

Anti-citrullinated protein/peptide antibodies (ACPA) are antibodies to peptides or proteins containing citrulline, a modified form of the amino acid arginine. Several citrullinated antigens have been found.

Antibodies against cyclic citrullinated peptide

Perhaps even more predictive of disease progression in RA is the presence of antibodies against cyclic citrullinated peptide (anti-CCP). In recent years, several studies have demonstrated that the anti-CCP antibodies are predictive for RA and that they are associated with joint destruction.69-72 Anti-CCP can be detected years before RA onset and its specificity is higher than RF.72,73-75 Reported diagnostic sensitivities and specificities ranged from 39-94% and 81-100%, respectively.75 In 2007, anti-CCP antibodies were included in the European League Against Rheumatism (EULAR) guidelines for the diagnosis of early RA and in the American College Rheumatology (ACR) Criteria for RA classification.76 Anti-CCP positive RA shows a higher rate of local joint destruction and is associated with HLA-DRB1 alleles containing the shared epitope, while anti-CCP negative RA is associated with HLA-DR3,77,78,79,80-82 The anti-CCP test also enables clinicians to distinguish RA patients from other arthritic diseases in cases where the RF is not useful since the anti-CCP test is highly specific for RA.

Antiperinuclear factor/antikeratin antibodies

Antiperinuclear factor (APF) and antikeratin antibodies (AKA) have been found to be specific for RA as RF.83 The APF are present in 49-91% of RA patients with specificity from 73-99%. Antikeratin antibodies have been identified in the serum of RA patients with an estimated incidence of positive AKAs reactions in RA sera from 36-59%.84 The presence of APF and AKA is independent of the disease duration. Both of them appear early and are sometimes present before the onset of RA.85-87 Thus, APF and AKA may help to identify RA to enable early intervention with treatment. These antibodies are correlated with each other, with the presence of RF and with the activity and severity of RA.88 Other studies have confirmed that
measurement of anti-CCP, AKA, APF, and individual isotypes of RFs are useful for prediction of structural damage early in the disease course but it is not clear which is most useful. For example, it was reported that AKA and anti-CCP were similarly useful for diagnosing RA and were better than APF and the authors suggested that rheumatologists can choose either AKA or anti-CCP to test for and to help with the diagnosis of RA. The major issue is that the test for APF is difficult to perform and not routinely available.

**Anti-mutated citrullinated vimentin**

Mutated citrullinated vimentin (MCV) has been described as an important autoantigen expressed in synovial tissue and was formerly known as Sa. Anti-Sa antibodies have a high specificity for the diagnosis of RA (98%) but a moderate sensitivity from 20-40%. Anti-Sa also have a high predictive value for severe joint involvement and extraarticular disease in RA.

Recently, a new ELISA test has been developed for anti-MCV testing, and can be used for the diagnosis of RA with similar specificity and sensitivity to anti-CCP antibodies. In a two year follow-up study of 210 patients with early RA, antibodies against MCV, CCP type 2 and 3 (both of IgG isotype) and 3.1 (of both IgG and IgA isotype) were analyzed at baseline and disease activity was evaluated at baseline and regularly for 24 months. Radiographs of the hands and feet were graded using the Larsen score. This study showed that anti-MCV antibodies were associated with more severe disease, as measured by the DAS28, ESR and swollen joint count over time, compared with anti-CCP2, CCP3 and CCP3.1 tests. Interestingly, it was found that radiological progression was predicted equally by all 4 tests.

In another study, where anti-MCV antibody levels were measured at baseline and at one year and two year follow up in 162 patients with early arthritis, it was found that anti-MCV had a specificity of 92.3% and a sensitivity of 59.3% when using the recommended cut-off of 20 U/mL and that anti-MCV-positive patients had a higher Sharp-van der Heijde score, ESR and CRP levels than did anti-MCV-negative patients at all time points. Recently, a number of publications have concluded that anti-MCV antibodies are a specific and sensitive marker for the diagnosis of RA and could provide an alternative to anti-CCP testing.

**Antip68**

Antibodies to stress protein immunoglobulin heavy-chain binding protein or chaperone (anti-BIP or antip68) are found in the sera of more than 60% of RA patients and are also found in experimental animal models of arthritis. Moreover, BIP is over-expressed in the rheumatoid joint and is present in early and pre-disease sera. These findings suggest that BIP may be an important autoantigen in RA but its role as a potential biomarker has yet to be fully evaluated.

**C-reactive protein**

Human C-reactive protein (CRP) is one of the most responsive acute-phase serum reactants and its levels in patients with RA has been correlated with clinical disease activity, radiological progression and response to therapy. As with ESR, CRP has been incorporated into several disease activity scoring systems, but its measurements are influenced by confounding factors including age, sex, fibrinogen levels, RF, hypergammaglobulinemia, and anemia. Nonetheless, the ESR level has been incorporated into a risk prediction model for rapid radiographic progression (RRP) that in the future could be used to predict the risk of joint damage progression in RA patients.

**Selectin**

Selectin, Thioredoxin, intracellular adhesion molecule (ICAM)-1 and vascu lar cell adhesion molecule (V-CAM)1 have been found to correlate with disease activity in RA but there is little evidence to suggest that additional information is gained over and above measurement of ESR and CRP. Thus, ESR and CRP remain the two biomarkers used routinely in clinical practice.

**Markers of inflammation**

**Erythrocyte sedimentation rate**

A number of studies have suggested that high erythrocyte sedimentation rate (ESR) levels at onset of early RA predicts long-term radiological progression. It is, together with CRP, the most frequently used laboratory measure reflecting disease activity. ESR measurements tend to reflect disease activity of the previous weeks and has been incorporated in several disease activity scoring systems, but its measurements are influenced by confounding factors including age, sex, fibrinogen levels, RF, hypergammaglobulinemia, and anemia. Nonetheless, the ESR level has been incorporated into a risk prediction model for rapid radiographic progression (RRP) that in the future could be used to predict the risk of joint damage progression in RA patients.

**SAA**

Serum amyloid-associated protein (SAA) is a precursor for Amyloid A and, similar to CRP, correlates with several clinical parameters in inflammatory disease but SAA levels are generally higher and vary more among individuals. One study reported that SAA is an inducer of MMPs in the synovial tissue fibroblasts of RA patients. Therefore, SAA may play a significant role in the degradation of extracellular matrix in joint tissues of RA patients. However, SAA measurements correlate significantly with CRP levels in sera of patients with RA, but not in healthy subjects suggesting that they do not provide additional information. More recently, another study showed that the incorporation of SAA together with anti-CCP in a RA diagnosis model, increased the sensitivity compared with the anti-CCP alone, but decreased the selectivity of the model.

**Others markers of inflammation**

Such as E-Selectin, Thioredoxin, intracellular adhesion molecule (ICAM)-1 and vascu lar cell adhesion molecule (V-CAM)1 have been found to correlate with disease activity in RA but there is little evidence to suggest that additional information is gained over and above measurement of ESR and CRP. Thus, ESR and CRP remain the two biomarkers used routinely in clinical practice.

**Cytokines**

In RA, the balance between pro- and anti-inflammatory cytokines is tilted toward continued inflammation. The normally low levels of pro-inflammatory cytokines become chronically increased causing prolonged inflammation. Therefore, inappropriate activity of the main inflammatory cytokines and their receptors could potentially have an important role in the RA pathogenesis. A broad array of macrophage and fibroblast cytokines, including interleukin (IL)-1, IL-6, IL-15, IL-18, tumor necrosis factor (TNF)-α, granulocyte macrophage-colony stimulating factor (GM-CSF), various chemokines and many others are produced by the rheumatoid synovium. Many of them, such as IL-1α and β, IL-6, M-CSF, IL-15, IL-17 and TNF-α, enhance osteoclast formation, activity and survival. Pro-inflammatory cytokines such as IL-6, TNF-α and IL-1 are present at higher levels in the SF of RA patients compared with other arthritic disorders or normal patients. Levels of both TNF-α and IL-6 are elevated in serum and joints during active RA and correlated with joint damage measures in addition to laboratory and clinical markers of inflammation. This has been supported by the findings of a recent study in which calprotectin was an independent predictor of clinical and radiographic joint damage after ten years, suggesting that calprotectin may be a prognostic biomarker for erosive disease in patients with RA.  

**Other biomarkers**

The most frequently used laboratory measure reflecting disease activity is the Erythrocyte sedimentation rate (ESR). An increased ESR level is typically associated with severe disease activity and survival. Pro-inflammatory cytokines and their receptors could potentially have an important role in the RA pathogenesis. A broad array of macrophage and fibroblast cytokines, including interleukin (IL)-1, IL-6, IL-15, IL-18, tumor necrosis factor (TNF)-α, granulocyte macrophage-colony stimulating factor (GM-CSF), various chemokines and many others are produced by the rheumatoid synovium. Many of them, such as IL-1α and β, IL-6, M-CSF, IL-15, IL-17 and TNF-α, enhance osteoclast formation, activity and survival. Pro-inflammatory cytokines such as IL-6, TNF-α and IL-1 are present at higher levels in the SF of RA patients compared with other arthritic disorders or normal patients. Levels of both TNF-α and IL-6 are elevated in serum and joints during active RA and correlated with joint damage measures in addition to laboratory and clinical markers of inflammation. This has been supported by the findings of a recent study in which calprotectin was an independent predictor of clinical and radiographic joint damage after ten years, suggesting that calprotectin may be a prognostic biomarker for erosive disease in patients with RA.
Table 2. Biomarkers of outcome in rheumatoid arthritis.

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<th>Biomarker</th>
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<th>Outcome</th>
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<tr>
<td>RF</td>
<td>Blood</td>
<td>Severity and radiological progression</td>
<td>Autoantibody against the Fc portion of IgG. RF and IgG join to form immune complexes which contribute to the disease.</td>
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<td>AKA label the stratum corneum of various cornified epithelia; AFA label the keratohyalin granules of human buccal mucosa epithelium; AFA are autoantibodies to filaggrins, an important class of the intermediate filament-associated proteins which interact with keratin intermediate filaments.</td>
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<td>Citrulline is an amino acid that is incorporated into proteins during inflammation.</td>
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<td>MCV</td>
<td>Blood</td>
<td>Disease activity and radiological progress</td>
<td>Antibodies against mutated citrullinated vimentin.</td>
<td>105-110</td>
</tr>
<tr>
<td><strong>Inflammatory markers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP/ESR</td>
<td>Blood</td>
<td>Disease activity and progression of radiological damage</td>
<td>Acute-phase reactants; CRP binds to phosphocholine on microbes, to assist in complement binding to foreign and damaged cells and enhances phagocytosis by macrophages. Innate immunity.</td>
<td>38,115,116,118-121</td>
</tr>
<tr>
<td>A-SAA</td>
<td>Blood and synovial tissue</td>
<td>Disease activity and cartilage degradation</td>
<td>Transport of cholesterol, recruitment of immune cells to inflammatory sites, and the induction of enzymes that degrade extracellular matrix.</td>
<td>127,128</td>
</tr>
<tr>
<td>Cytokines/inhibitors (e.g., TNF-α, IL-1, IL-6, IL-8, IL-18)</td>
<td>Blood and synovial fluid and membrane</td>
<td>Disease activity, inflammation, therapeutic response and radiographic progression</td>
<td>T-cell differentiation, expansion and survival, NK-cell activation, T-cell and NK-cell cytotoxicity, B-cell activation and proliferation, Th1-cell proliferation and maturation, Th17-cell expansion and stabilization. T-cell chemokinesis and memory/maintenance, synovial fibroblast activation, macrophage activation/suppression, adhesion molecule expression and oxidative burst. Increase of macrophage cytokine release, T-cell cytokine release and hepatic acute-phase response. Growth and differentiation of mesenchymal, epithelial and neuroectodermal cells. Angiogenesis. Cytokines, chemokines, MMP, iNOS and PG release. Antibody-mediated immunity. Control of parasitic infections. Cell-mediated immunity</td>
<td>86,138-150,153-156</td>
</tr>
<tr>
<td>Calprotectin</td>
<td>Blood and synovial fluid</td>
<td>Inflammation and radiographic damage</td>
<td>Calcium and zinc binding protein; bacteriostatic and fungistatic immunomodulating and antiproliferative effects; chemotactic factor for neutrophils.</td>
<td>123-126</td>
</tr>
<tr>
<td>E-selectin</td>
<td>Blood</td>
<td>Severity and disease activity</td>
<td>Recruitment of leukocytes; leukocyte adhesion to the wall of the vascular endothelium.</td>
<td>130,131</td>
</tr>
<tr>
<td>sVCAM, ICAM</td>
<td>Blood</td>
<td>Cardiovascular risk</td>
<td>VCAM1: adhesion of lymphocytes, monocytes, eosinophils, and basophils to vascular endothelium; leukocyte-endothelial cell signal transduction. ICAM1: stabilizing cell-cell interactions and facilitating leukocyte endothelial transmigration.</td>
<td>130,135,136</td>
</tr>
<tr>
<td>Thioredoxin</td>
<td>Blood</td>
<td>Disease activity</td>
<td>Antioxidant by facilitating the reduction of other proteins by cysteine thiol-disulfide exchange.</td>
<td>132,133</td>
</tr>
<tr>
<td><strong>Synovium/cartilage markers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyaluronic acid</td>
<td>Blood</td>
<td>Activity and duration of disease. Erosive status</td>
<td>Contributes to tissue hydrodynamics, movement and proliferation of cells and participates in a number of cell surface receptor interactions.</td>
<td>47,72</td>
</tr>
<tr>
<td>COMP</td>
<td>Blood</td>
<td>Radiographic progression, disease severity</td>
<td>Involved in cell adhesion, platelet aggregation, cell proliferation, angiogenesis, tumor metastasis, and tissue repair.</td>
<td>36-38,43-47</td>
</tr>
<tr>
<td>Aggrecan/CS846-epitope</td>
<td>Blood</td>
<td>Cartilage erosion/joint damage</td>
<td>Aggrecan mediates chondrocyte-chondrocyte and chondrocyte-matrix interactions through its ability to bind hyaluronan; Cell adhesion; chondrocyte apoptosis; cartilage structure.</td>
<td>37,38,46,51-54</td>
</tr>
<tr>
<td>Glc-Gal-PYD</td>
<td>Urine</td>
<td>Progression of joint damage</td>
<td>Cartilage structure.</td>
<td>55,58</td>
</tr>
<tr>
<td>CTX-II</td>
<td>Urine</td>
<td>Cartilage destruction and radiographic progression</td>
<td>Cartilage structure.</td>
<td>33,34,55-58,70</td>
</tr>
<tr>
<td>MMPs</td>
<td>Blood</td>
<td>Radiographic progression and disease activity</td>
<td>Cleave extracellular matrix proteins; cartilage destruction.</td>
<td>60,61,65,66,68</td>
</tr>
<tr>
<td>C2c, C1,2c</td>
<td>Blood</td>
<td>Radiographic damage</td>
<td>Cartilage structure.</td>
<td>33,70</td>
</tr>
</tbody>
</table>

Continued in the next page
Table 2. Continued from previous page.

<table>
<thead>
<tr>
<th>Bone markers</th>
<th>Blood and synovial fluid</th>
<th>Bone destruction and radiographic progression</th>
<th>Bone destruction</th>
<th>Bone destruction and calcification; bone mineralization and calcium ion homeostasis.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cathepsin K</td>
<td>Blood and synovial fluid</td>
<td>Bone destruction</td>
<td>Bone remodeling and resorption</td>
<td>32,38,39</td>
</tr>
<tr>
<td>BSP</td>
<td>Blood and synovial fluid</td>
<td>Bone destruction</td>
<td>Properties of a nucleator, associated with mineral crystal formation and angiogenesis; protection from complement-mediated cell lysis.</td>
<td>35,37,46</td>
</tr>
<tr>
<td>CTX-I</td>
<td>Urine and blood</td>
<td>Bone destruction and radiographic progression</td>
<td>Bone structure.</td>
<td>33,34</td>
</tr>
<tr>
<td>RANKL and OPG ratio</td>
<td>Blood and synovial fluid</td>
<td>Bone destruction</td>
<td>RANKL is involved in stimulation of osteoclast formation and activation; OPG inhibits the differentiation of osteoclast precursors into osteoclasts and also regulates the resorption of osteoclasts.</td>
<td>28-30,32</td>
</tr>
</tbody>
</table>

**Immunological markers**

| Regulatory T cells            | Blood and synovial fluid | Disease development and activity | Suppress activation of the immune system and prevent pathological self-reactivity. | 157,162,163,165-167 |
| Cytokines/Inhibitors (e.g., IL-1, IL-6, IL-8, IL-16) | Blood and synovial fluid and membrane | Disease activity, inflammation, therapeutic response and radiographic progression | T-cell differentiation, expansion and survival, NK-cell activation, T-cell and NK-cell cytotoxicity, B-cell activation and proliferation, Th1-cell proliferation and maturation, Th17-cell expansion and stabilization, T-cell chemokinesis and memory maintenance, synovial fibroblast activation, macrophage activation/suppression, adsorption molecule expression and oxidative burst. Increase of macrophage cytokine release, T-cell cytokine release and hepatic acute-phase response. Growth and differentiation of mesenchymal, epithelial and neuroectodermal cells, Angiogenesis. Cytokines, chemokines, MMP, NOS and PG release; Antibody-mediated immunity; Control of parasitic infections; Cell-mediated immunity. | 86,138-159,153-156 |

Table 3. Biomarkers in rheumatoid arthritis vs. other diseases.

<table>
<thead>
<tr>
<th>Biomarker in RA</th>
<th>Other diseases</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autoantibodies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RF</td>
<td>Sjogren’s syndrome, crioiglobulinemia, SLE</td>
<td>76,77,81,83</td>
</tr>
<tr>
<td>AKA, AFA, APF</td>
<td>ReA</td>
<td>100</td>
</tr>
<tr>
<td>Anti-CCP</td>
<td>AS, ReA, PsA, SLE, CREST, systemic sclerosis, Sjogren’s syndrome</td>
<td>18,82,83,86,90,93,94</td>
</tr>
<tr>
<td>MCV</td>
<td>OA, UA, PsA, SLE, Sjogren’s syndrome, scleroderma, AS, systemic sclerosis, viral hepatitis B, tuberculosis, PMR</td>
<td>107-110</td>
</tr>
<tr>
<td>Inflammatory markers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytokines/Inhibitors (e.g., TNF-α, IL-1, IL-6, IL-8, IL-16)</td>
<td>OA, GOUT, PMR, ReA, acute crystal arthritis</td>
<td>141-143,150</td>
</tr>
<tr>
<td>Calprotectin</td>
<td>OA</td>
<td>123,124</td>
</tr>
<tr>
<td>E-selectin</td>
<td>OA</td>
<td>130,137</td>
</tr>
<tr>
<td>sVCAM, ICAM</td>
<td>OA, IA, SLE</td>
<td>130,134,135,137</td>
</tr>
<tr>
<td>Thioredoxin</td>
<td>OA, GOUT, ReA</td>
<td>133</td>
</tr>
<tr>
<td>Synovium/cartilage markers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyaluronic acid</td>
<td>OA</td>
<td>73</td>
</tr>
<tr>
<td>COMP</td>
<td>JCA, OA, SLE, ReA, PsA, Scleroderma, vasculitis, Sjogren’s syndrome, Raynaud’s syndrome</td>
<td>43,49</td>
</tr>
<tr>
<td>Aggrecan/CS846-epitope</td>
<td>OA</td>
<td>50</td>
</tr>
<tr>
<td>Glc-Gal-PYD</td>
<td>Paget’s disease</td>
<td>71</td>
</tr>
<tr>
<td>MMPs</td>
<td>IA, OA, SLE</td>
<td>62,64,67</td>
</tr>
<tr>
<td>C2C, C1C</td>
<td>AO, PsA</td>
<td>69</td>
</tr>
<tr>
<td>Bone markers</td>
<td>PsA</td>
<td>35</td>
</tr>
<tr>
<td>Immunological markers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regulatory T cells</td>
<td>PsA, JIA, spondyloarthopathies, ReA</td>
<td>162,165</td>
</tr>
</tbody>
</table>

**References**

RF: rheumatoid factor; AKA: antikeratin antibodies; AFA: antifilaggrin antibodies; APF: antiperinuclear factor; MCV: antimutated citrullinated vimentin; CRP: C-reactive protein/ESR: erythrocyte sedimentation rate; A-SAA: acute serum amyloid protein; COMP: cartilage oligomeric protein; Glc-Gal-PYD: urinary glucosyl-galactosyl-pyridinoline; CTX-II: C-terminal crosslinking telopeptide type II; MMPs: matrix metalloproteinases; BSP: bone sialoprotein; CTX-I: C-terminal crosslinking telopeptide type I; RANKL: receptor activator for nuclear factor □B ligand; OPG: osteoprotegerin.
are thought to contribute to the joint destruction in RA.\textsuperscript{141,142} IL-6 is the most abundant cytokine in the serum and SF of patients with RA and its levels have been shown to correlate with disease activity in both fluids. However, reports are conflicting about whether IL-6 levels correlate with joint destruction with some suggesting they do\textsuperscript{144} but others showing no association with progression.\textsuperscript{150,156} Moreover, baseline IL-6 concentrations may vary almost 100-fold between different individuals, can increase with exercise\textsuperscript{147} and are elevated in several other diseases characterized by inflammation. In a multivariate analysis of biomarkers in RA, it was found that the combination of anti-CCP with IL-6 had the greatest discrimination for the diagnosis of established RA\textsuperscript{157} but whether it is a useful biomarker for outcome remains questionable.

Soluble TNF receptor (sTNFR)-II parallels TNF-α levels and is a surrogate marker for inflammation. One study suggested that levels of sTNFRII can be elevated up to 12 years prior to the development of RA and so it may be a potential biomarker of future disease development.\textsuperscript{164} Similarly, in an extensive analysis of 16 cytokines or cytokine-related markers, 7 of the analytes (IL-1α, IL-1β, IL-1 receptor antagonist (RA), IL-4, IL-10, TNF-α and sTNFR) were found to be significantly elevated at five years before the diagnosis of RA.\textsuperscript{168} In another study, a panel of 23 cytokines and chemokines was measured in SF from patients with early synovitis, who where subsequently followed up. It was reported that patients who develop RA had a distinct but transient SF cytokine profile. The levels of cytokines such as IL-2, IL-4, IL-13, IL-17, IL-15, basic fibroblast growth factor (bFGF) and endothelial growth factor (EGF), were significantly elevated in these patients three months after symptom onset, when compared with early arthritis patients who did not develop RA.\textsuperscript{169} In addition, this profile was no longer present in established RA. Other cytokines such as IL-4, interferon (IFN)-γ, IL-13 and IL-18 inhibit osteoclastogenesis, whereas suppressive cytokines like transforming growth factor (TGF)-β and IL-1Ra, as well as the suppressor of cytokines signaling-3 (SOCS3), are expressed in RA synovium but at levels that are inadequate to block synovitis.\textsuperscript{170}

Production of IL-17 is now believed to play a crucial role in the development of joint lesions in RA as IL-17 can induce the production of several chemokines such as monocyte chemo-attractant protein (MCP)-1, macrophage inflammatory protein (MIP)1α, RANTES, MCP-4, pulmonary and activation-related chemokine (PARC), MIP-3α, growth-related oncogene (GRO), IL-8, monocyte induced by IFN-γ (Mig), interferon-γ-inducible protein (IP)-10 and stromal cell-derived factor (SDF)-1.\textsuperscript{171} A recent study found that some of these chemokines (MIP-3, transforming growth factor (TGF), CXCL13, monocyte colony stimulating factor (M-CSF)) were predictive of RA disease activity.\textsuperscript{172} These chemokines are involved in RA pathogenesis via the recruitment and retention of leukocytes in the joints. However, a number of chemokines have been shown to have other biological functions; such as release of mediators of inflammation, cell proliferation and angiogenesis. Therefore, cytokines and cytokine-related molecules are key molecules in the pathogenesis of RA.

Further prospective studies are needed to understand their role in the disease and their possible use as biomarkers in RA.

The traditional markers, ESR and CRP, remain the most reliable inflammatory markers in the clinical setting. Due to the complexity of the cytokine network and the instability of these cytokine markers it is not clear whether they can be used in a clinical setting as biomarkers. However, further studies including families of cytokines, chemokines and adipokines are necessary to assess the value of more complex models as predictors of outcome in RA.

**Immunological markers**

In RA, the prominent T-cell infiltrate suggests that the rheumatoid synovium contains large number of CD4+ T cells. An accepted model is that the chronic inflammation in established RA is mainly driven by interactions between T cells, macrophages and fibroblasts in an abnormal environment.\textsuperscript{173} Several types of Treg cells have been described. Thymically-derived CD4+CD25+ Treg cells play a role in the maintenance of self tolerance and prevention of autoimmune disease.\textsuperscript{174} Treg cells can be divided into subpopulations such as T helper 3 (Th3) cells, which play an important role in mediating tolerance.\textsuperscript{175} These cells might be insufficiently effective or even defective in RA.\textsuperscript{176,177} A number of studies have reported that CD4+CD25+ Tregs are increased in the inflamed joint and present a more powerful suppressor activity than peripheral CD4+CD25+ Tregs.\textsuperscript{178-180} However, RA is not suppressed despite this increased suppressor activity. The opinion from the authors is that the effector cells might be less sensitive for the suppression of CD4+CD25+ Tregs. Several studies have compared CD4+CD25+ Tregs in patients with RA and other types of inflammatory arthritis\textsuperscript{181,182},\textsuperscript{183,184} and a number have reported an enrichment of CD4+CD25+ Tregs in SF of patients with RA and other types of inflammatory arthritis. This enrichment is independent of disease duration and severity. In addition SF CD4+CD25+ Tregs showed suppressive activity in terms of cytokine production and proliferation. The role of Tregs as potential biomarkers of disease state or outcome remains controversial, however, as different studies have reported similar,\textsuperscript{185,186} increased\textsuperscript{187} or decreased\textsuperscript{188,189} frequencies of CD4+CD25+ Tregs in SF when compared to normal controls.

**Conclusions**

During recent years, a huge number of potential biomarkers of prognosis (Table 2) and diagnosis (Table 3) in RA have been investigated but only the autoantibody tests (RF and anti-CCP) and inflammatory markers (ESR and CRP) are currently tested routinely in clinical practice. The best marker for the development of erosions remains the anti-CCP2 antibody test but this lacks sensitivity in early disease. Correlation of outcome with other biomarkers, including CTX-I, CTX-II, COMP, Glic-Gal-Pyd, MMP3 and C2C in urine or serum samples have been reported in more than one study or patient group (Table 2). Whether these markers correlate with each other or add any information over and above that provided by autoantibodies and inflammatory markers is not known but it would seem reasonable to investigate these as a panel of biomarkers may provide more information than the single testing largely attempted to date.

The prospective cohort studies provide an opportunity to find new and better biomarkers, improving prognosis and diagnosis of the disease, making it possible to distinguish patients who will develop more aggressive or rapidly progressive disease from the patients who will develop a milder disease who could be spared potentially toxic medications. Prospective cohort studies also permit the study of the effects of different therapies throughout time and to assess the evolution of the disease in each case. New complex models involving several biomarkers and clinical markers may result in a biomarker signature capable of predicting and monitoring a variety of outcomes.
important to patients. In turn, this has the potential to allow tailoring of treatment regimens to groups of patients according to their biomarker signature and would truly represent personalized medicine.

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