Rheumatoid Factor and Anti-CCP Autoantibodies in Rheumatoid Arthritis: A Review

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For many years, laboratory diagnosis of rheumatoid arthritis has relied on the detection of rheumatoid factor. A new assay that detects antibodies to citrullinated peptides, called the anti-CCP assay, has demonstrated a comparable sensitivity but a much higher specificity than the RF test. This paper reviews RF and anti-CCP in rheumatoid arthritis and examines the usefulness of each autoantibody in RA testing.

ABBREVIATIONS: AFA = antifilaggrin autoantibodies; AKA = antikeratin antibodies; APF = antiperinuclear factor; CCP = cyclic citrullinated peptide; CRP = C-reactive protein; DMARDS = disease-modifying anti-rheumatic drugs; ELISA = enzyme-linked immunosorbent assay; ESR = erythrocyte sedimentation rate; Ig = immunoglobulin; MTX = methotrexate; NSAIDS = non-steroidal anti-inflammatory drugs; PAD = peptidylarginine deiminase; RA = rheumatoid arthritis; RF = rheumatoid factor.

INDEX TERMS: anti-CCP; rheumatoid arthritis; rheumatoid factor.

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Rheumatoid arthritis (RA) is a systemic autoimmune disease of unknown etiology that is characterized by chronically inflamed synovial joints and subsequent destruction of cartilage and bone. RA is found in about one percent of the population, making it one of the most common autoimmune diseases in the United States.[1] RA is marked by several key characteristics, including synovitis occurring in a symmetrical fashion, polyarthritis, morning stiffness lasting over an hour, periods of disease flare-ups followed by periods of disease remission, and the development of subcutaneous rheumatoid nodules.[2] The disease does not affect all patients the same way, and may range from a mild form to one that is very debilitating. RA can present with many symptoms, including pain, swelling, stiffness, joint deformity, and loss of movement. It can have a serious impact on a patient's quality of life, and early intervention is key to minimizing the damaging effects of the disease.[1] The standard therapies for RA include analgesic drugs, non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids or prednisone, and disease-modifying anti-rheumatic drugs (DMARDs).[2]

RA can be difficult to diagnose, especially in the early stages of the disease. In 1987, the American College of Rheumatology established seven criteria for the diagnosis of RA, four of which must be met for diagnosis.[3] The only criterion based upon laboratory testing is the detection of abnormal amounts of serum rheumatoid factor (RF). Testing for RF in the diagnosis of RA has been performed for over fifty years. Unfortunately, the RF test does not yield a high specificity for RA.[1] Research over the past several years has focused on developing other tests that have increased diagnostic specificity over the RF test. Such research led to the development of the anti-cyclic citrullinated peptide (anti-CCP) assay, which detects the presence of anti-citrullinated peptide autoantibodies found in the serum of many patients with RA. The anti-CCP assay has proven to be as sensitive as the RF test with a much higher specificity.[4] Studies have also indicated that the anti-CCP assay has higher disease predictability[5,6,7] and prognostic value[8,9] than the RF test, while RF appears to be a better marker for patient response to treatment than anti-CCP.[10]

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BACKGROUND

Rheumatoid factor

The first autoantibody detected in patients with RA was RF. It was discovered in the early twentieth century when researchers noticed that sera from patients with RA agglutinated sheep red blood cells that had been sensitized with rabbit antibody. In the 1940s, the term "rheumatoid factor" was coined after researchers confirmed that the factor inducing agglutination correlated with the presence of RA. RF antibodies may be of the IgM, IgG, or IgA classes, and they target patient IgG. Following the discovery of RF, the RF test became the primary laboratory test used in the diagnosis of RA.[11]

Many methods of RF detection have been developed. Particle agglutination tests employ latex, charcoal, or human erythrocytes as carrier molecules to which human or rabbit IgG is bound.[12] Agglutination tests detecting IgM-RF are the most common methods used in laboratory diagnosis of RA.[11] Nephelometry is another method used for detecting RF. In nephelometry, latex particles are coated with human IgG that captures RF. Complexes formed between the IgG and RF are detected by light scattering. The degree of light scatter is dependent upon the concentration of immune complexes formed, making this a quantitative test.[12] A third method of RF detection is an enzyme-linked immunosorbent assay (ELISA). It is a solid phase assay that detects IgM- and IgA-RF using human IgG Fc as the substrate, and detects IgM-, IgG-, and IgA-RF if rabbit IgG is used as the substrate.[11]

Anti-CCP

In 1964, researchers described antibodies to perinuclear granules in the cytoplasm of human buccal cells, called antiperinuclear factor (APF), that had high specificity for RA. In 1979, antibodies to cytokeratin of *stratum corneum* of rat esophagus epithelium, called antikeratin antibodies (AKA), were discovered and also found to be highly specific for RA. Years later, APF and AKA were both found to be directed against filaggrin or its precursor, profilaggrin, and were grouped into a family of antibodies called antifilaggrin autoantibodies (AFA). Researchers discovered that AFA recognize epitopes that are created by citrullination of the targeted proteins.[13] Citrullination is the posttranslational deimination of arginine residues by peptidylarginine deiminase (PAD), which hydrolyzes the NH_2 group of arginine to a neutral oxygen group and results in the formation of an atypical amino acid called citrulline. The neutral oxygen group of the citrulline residue is the part that is recognized by the autoantibodies.[4] Research has demonstrated that AFA do not target filaggrin or profilaggrin in patients with RA, but instead are directed towards other citrullinated proteins. Vincent and others recently discovered that filaggrin variants are not found in the synovial tissue and proposed that (pro)filaggrin is recognized by AFA due to cross-reactivity. AFA are actually directed against citrullinated forms of the α- and β-chains of fibrin, and represent only one group of autoantibodies to citrullinated proteins. Fibrin deposits are a characteristic of rheumatoid synovial tissues, and autoantibodies to citrullinated human fibrin are secreted locally in the synovial tissue interstitium.[13]

Original tests for the detection of autoantibodies to citrullinated proteins detected APF or AKA in patient sera by utilizing filaggrin antigens and indirect immunofluorescence. The tests did not become widely used, likely due to technical difficulties associated with the assays. The development of citrullinated peptides paved the way for a new laboratory test for RA – the first-generation anti-CCP (CCP1) assay. The anti-CCP assay utilizes synthetic peptides containing citrulline and detects the presence of autoantibodies to citrullinated peptides.[14] The peptides were made cyclic because the three-dimensional structure optimizes the sensitivity of the test and allows the antigenic group of the peptides to be recognized by a heterogeneous population of RA autoantibodies. The CCP1 assay yielded an excellent specificity (97%) and a decent sensitivity (68%). A second-generation anti-CCP (CCP2) assay was soon developed that employed other citrullinated peptides and yielded a better sensitivity (75%-80%) than the CCP1 assay.[4] Recently, a third-generation anti-CCP (CCP3) assay was developed that demonstrated a sensitivity about five percent greater than that of the CCP2 assay.[15]

COMPARISON OF RF AND ANTI-CCP

Sensitivity and specificity

Since its discovery, RF has become the primary laboratory test used in the diagnosis of RA. RF is found in the sera of up to 85% of patients with RA;[16] however, it is also found in many other diseases, including Sjögren's syndrome, systemic lupus erythematosus, and mixed connective tissue disease.[11] In addition, RF is found in the sera of five percent to ten percent of apparently healthy individuals.[14] The presence of RF in so many other conditions decreases the diagnostic specificity of the RF test, resulting in the search for a more specific test for the diagnosis of RA. The CCP2 assay was found to have a sensitivity comparable to that of

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the RF test; however, the specificity proved to be superior.[4] Riedemann and others performed a comprehensive review of CCP2 studies and found that the specificity ranges from 88.9 – 100%, depending upon the diseases included in each study. Anti-CCP autoantibodies are also found in diseases other than RA, although at a lower frequency than RF.[17]

Predictive value

Early treatment of RA is important for providing the patient with the best outcome and quality of life. It is therefore essential that a diagnosis be made as early into the course of the disease as possible. Anti-CCP and RF autoantibodies can both be used as predictors of RA in some patients. Studies have shown that autoantibodies can be detected as early as ten years prior to the onset of RA. In a study by Nielen and others, frozen serum from RA patients who had donated blood prior to developing RA was tested for the presence of anti-CCP antibodies and IgM-RF. Of the 79 patients who were included in the study, 39 patients, or 49%, were positive for anti-CCP antibodies, IgM-RF, or both. These patients were positive for autoantibodies a median of 4.5 years prior to the onset of symptoms. Of 2,138 matched healthy control subjects, 0.6% tested positive for anti-CCP antibodies and 1.1% tested positive for IgM-RF.[5] The researchers determined that the chance of developing RA five years after the detection of autoantibodies was 69.4% with anti-CCP and 37.7% with RF. Therefore, anti-CCP shows higher disease predictability than RF. The presence of both markers increases the risk to 100%. This study demonstrates that autoantibody testing may be useful for predicting RA development in individuals in high-risk populations, such as those possessing the genetic marker HLA-DR4. Patients with autoantibodies prior to developing RA also tended to be younger and suffered a more aggressive disease than those who tested negative for autoantibodies before the onset of RA.[6] Rantapää-Dahlqvist and others found similar results in two Swedish cohorts of 83 patients. The prevalence of antibodies more than 1.5 years prior to disease onset was 33.7% with anti-CCP, 19.3% with IgM-RF, 33.7% with IgA-RF, and 16.9% with IgG-RF. These results were all highly significant compared to matched controls. The prevalence of each autoantibody was even higher when measured less than 1.5 years prior to disease onset, with anti-CCP demonstrating the highest prevalence (52%), followed by IgA-RF (39%). This study demonstrated that anti-CCP and IgA-RF are significant predictors of RA, with anti-CCP exhibiting a higher predictive value.[7]

Prognostic value

The presence of anti-CCP[8] and RF[11] autoantibodies has been associated with a less favorable prognosis than the absence of them. Meyer and others reported that the percentage of patients with significant progression of joint destruction five years after the onset of disease was higher in patients who were positive for anti-CCP antibodies (67%) than patients who were negative (44%). The presence of anti-CCP in RF negative patients was also associated with more severe joint damage than in patients positive for RF and negative for anti-CCP.[8] In a study by Vallbracht and others, the presence of anti-CCP autoantibodies was the most predictive marker for severe joint damage when compared with all RF isotypes. Of 295 patients with RA, 109 presented with minimal joint damage, 115 with moderate joint damage, and 71 with severe joint damage. The prevalence of each autoantibody in patients with severe damage was 80.3% with anti-CCP, 67.6% with IgM-RF, 48.3% with IgA-RF, and 47.9% with IgG-RF. By comparison, the prevalence of each autoantibody in patients with minimal damage was 54.1% with anti-CCP, 67.0% with IgM-RF, 45.3% with IgA-RF, and 40.4% with IgG-RF. These results demonstrate a higher incidence of anti-CCP in RA with severe damage than any RF isotype, and a higher incidence of IgM-RF than anti-CCP in RA with minimal damage. Patients with severe joint damage were also more likely to present as negative for RF and positive for anti-CCP than patients with minimal joint damage.[9]

Effects of drug therapy

Many studies have researched the effects of DMARDs, especially methotrexate (MTX) with or without infliximab, on autoantibodies in RA. Most researchers have found that the titers of RF decrease when patients use DMARDs, while the drugs induce no significant changes in the titers of anti-CCP. De Rycke and others studied the effects of treatment with DMARDs on levels of autoantibodies in patients with RA. Among the 62 patients in the study, treatment with MTX and infliximab resulted in a significant decrease in the titer of RF after 30 weeks of treatment. In contrast, titers of anti-CCP showed no significant changes. The researchers also tested the predictability of the autoantibodies with regard to patient response to treatment. Baseline IgM-RF correlated inversely with changes in the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels during treatment, with higher baseline IgM-RF demonstrating a smaller decrease in ESR and CRP levels than lower baseline IgM-RF. These results show that IgM-RF levels may be used to predict the response of the patient

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to therapy with MTX and infliximab. Baseline anti-CCP titers did not demonstrate any significant changes in acute phase reactants, and therefore would not be a good predictor of patient response to the chosen drug therapy. Though most studies found similar results, one study did find a significant decrease in anti-CCP titers after treatment with MTX and infliximab.[18]

CONCLUSION

Although the diagnosis of RA relies primarily upon clinical symptoms,[2] laboratory tests that detect autoantibodies, such as RF and anti-CCP, are helpful aids in diagnosis. The usefulness of these autoantibodies in established RA has been demonstrated, but more research is needed to determine the value of these tests in detection of early RA, disease prognosis, and disease monitoring. In addition, the ability of these tests to predict disease development may allow for their use as screening tools in at-risk populations. Because early treatment is essential to reduce or reverse morbidity in patients with RA,[7] it is important to distinguish between RA and other rheumatic diseases. This can be achieved for many patients via detection of anti-CCP autoantibodies.[9] An advantage of RF is that the different isotypes can give a better idea of how the disease will progress. Patients with IgM-RF tend to have a more severe disease, those with IgG-RF tend to have vasculitis, and those with IgA-RF tend to have a more erosive disease with extra-articular manifestations. There are advantages and disadvantages to the RF and anti-CCP tests, but both tests together may provide a very useful tool in early and accurate diagnosis of RA.[11]

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