

**NEW, HIGHLY SPECIFIC AUTOANTIBODY MARKERS IN RA
PRESENT STATUS AND FUTURE PROSPECTS**

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The treatment of rheumatoid arthritis (RA) has undergone dramatic change in recent years, with impressive improvements in patient outcome both radiologically and clinically. In particular, TNF alpha blockade and IL 1 receptor antagonist treatment protocols have ushered in a molecular approach in RA therapeutics based on more precise targeting of key molecules intimately involved in RA pathogenesis. A variety of new biologic agents are currently in clinical trials and the scientific community eagerly awaits the results of these studies.

Because of these new, but costly therapeutic options and the knowledge that early treatment results in better response rates, a need for new serodiagnostic assays with improved sensitivity and specificity, particularly at the earliest stages of RA synovitis, is essential and urgently needed. .

The discovery of anti-cyclic citrullinated peptide antibodies (anti-CCP) as a highly specific marker for RA promises to revolutionize the serodiagnostic evaluation of patients with polyarthritis and represents the first commercially available assay that approaches this goal.

Traditionally, IgM rheumatoid factor (RF) has been the mainstay serologic test in evaluating patients with suspected RA. Although reasonably sensitive (75-80%), the specificity of rheumatoid factor in RA is modest (60-80%). Importantly, the absence of rheumatoid factor positivity in the majority of cases of early RA is a major void diagnostically. Furthermore, the diagnosis of seronegative RA is a common diagnostic challenge given the fact that numerous arthropathies can be considered in the evaluation of seronegative inflammatory arthritis. In addition, a substantial number of diseases may have rheumatoid factors and arthritis (SLE, parvoviral arthropathy, hepatitis C, sarcoidosis, occasional patients with pseudogout, seronegative spondyloarthropathy and PMR) creating potential diagnostic problems in differentiating these entities from RA. The development and availability of two new FDA-approved anti-CCP assays is a significant step forward in addressing the above clinical scenarios. Anti-CCP has 96% specificity and 78% sensitivity for RA and also occurs in 70% of early RA synovitis. The data available for seronegative RA suggests that about 40% are anti-CCP positive. More studies are required to assess the value of anti-CCP testing in seronegative inflammatory arthritis, but the assay appears to have substantial utility in this area. Importantly, anti-CCP is also predictive of erosive disease. Furthermore, the combined presence of rheumatoid factor positivity and anti-CCP positivity has 99.5% specificity for RA. An important question currently unresolved is the status of anti-CCP testing in juvenile rheumatoid arthritis in its various forms.

The autoantigenic targets for anti-CCP are citrullinated peptides. Citrulline results from the deimination of arginine. Importantly, recent studies have identified citrullinated peptides as the epitope recognized in antikeratin and antiperinuclear factor and antifilaggrin assays. All of these assays have high specificity for RA but standardization and other technical factors have not led to their widespread use. In addition, another seemingly unrelated but highly specific autoantibody in RA is anti-Sa. It appears to be directed at citrullinated vimentin and has a sensitivity of 43% and a specificity of 96% for RA.

Important new findings presented at the ACR meeting in New Orleans 2002 demonstrated the presence of anti-CCP and IgM rheumatoid factor well before clinical onset from stored blood bank sera in individuals who subsequently developed rheumatoid arthritis. Of 72 patients identified with RA, 47% had either anti-CCP or IgM rheumatoid factor several years before clinical onset. Interestingly, 20% of seronegative RA and 46% of seropositive RA had anti-CCP antibodies 4.8 years and 5.5 years, respectively, prior to clinical onset. IgM rheumatoid factor was detected in 37% of seropositive RA 3.3 years before onset of clinical symptoms. Another study using sera stored at a blood bank before clinical onset of disease were available in 83 individuals who developed rheumatoid arthritis. In this study, anti-CCP antibodies were present in 29 (35%) before onset. IgM rheumatoid factor and IgG rheumatoid factor were also detected in 23% and 15%, respectively, prior to disease presentation. Furthermore, the titers of anti-CCP progressively increased until clinical onset. The longest interval of anti-CCP prior to the expression of clinical disease was nine (9) years. The positive predictive value of anti-CCP for RA exceeded both IgM and IgG rheumatoid factor in this study. These two abstracts highlight the potential role of anti-CCP and rheumatoid factor, in the early pathogenesis of rheumatoid arthritis and provide an opportunity of studying the disease processes in individuals well before clinical manifestations appear.

Two papers on the role of HLA DRB1 0401 and T-cell responses to citrullinated peptide antigens were presented with important findings. Both of these abstracts demonstrated the absolute requirement of citrullinated peptides, as opposed to peptides containing arginine, for binding to the DRB1 0401 shared epitope and subsequent T-cell reactivity. When citrullinated peptides were used, T-cell proliferation and gamma interferon production occurred compared to the absence of T-cell proliferation and gamma interferon production when arginine was substituted for citrulline in these assays. Arginine is a positively charged amino acid and is converted to citrulline, a neutrally charged molecule, by peptidyl arginine deiminase of which four (4) isoforms exist. In these RA patients, the absolute requirement for the presence of HLA DR molecules was demonstrated in that anti-DR antibodies abolished T-cell reactivity.

Another autoantibody reported to be highly specific for RA is anti-BiP. BiP is a protein produced in the endoplasmic reticulum and functions as a molecular chaperone for several proteins. Under cellular stress, BiP can be expressed on the cell surface. This antibody has been found to be 63% sensitive and 96% specific for rheumatoid arthritis in

one study, which interestingly also demonstrated T-cell proliferation to BiP as well as evidence suggesting the presence of regulatory T cells that recognize this protein.

Antibodies to glucose-6-phosphate isomerase, a ubiquitously expressed molecule, has also been shown to be quite specific for RA. In addition, an animal model of inflammatory arthritis secondary to autoimmunity directed against this enzyme has been identified. Importantly, two abstracts presented at the New Orleans ACR meeting demonstrated an early autoantibody response to glucose-6-phosphate isomerase in a collagen-induced arthritis model as well as in a Pristane-induced arthritis model. Anti-glucose-6-phosphate isomerase appeared consistently before the development of Type II collagen antibodies in the collagen-induced arthritis model. In the Pristane-induced arthritis animals, anti-glucose-6-phosphate isomerase antibodies appeared prior to the onset of arthritis and before the development of autoantibody responses to 16 different joint antigens. These two abstracts suggest that immunity to glucose-6-phosphate isomerase is an early event in RA disease pathogenesis, and clinical studies designed to look at this phenomenon may well be rewarding from a diagnostic perspective as well as pathogenetically.

There are a number of other autoantibodies that occur in RA, including anti-calpastatin, anticalreticulin and anti-RA33. Although these antibodies are not specific for RA, they can be useful diagnostically when ordered in conjunction with more specific antibodies, as described below. Two new innovative approaches to RA diagnostics are being developed which utilize simultaneous testing for multiple antibody specificities. The first approach utilizes six (6) autoantigens chemically modified or produced by recombinant technology. These antigens include: citrullinated peptides, rheumatoid factor, BiP, calpastatin, calreticulin and RA33 (A2 protein). One hundred (100) RA and 100 control patients studied showed specific patterns of autoreactivity to a number of combinations of antibodies detected. A simple example of this includes 100% specificity for a combination of three antibodies occurring together; namely, anti-BiP, anti-CCP and rheumatoid factor. In addition, there was 100% positivity when anti-BiP and anti-citrulline were positive but rheumatoid factor was negative. There were also a variety of other autoantibody combinations that include sets of four (4), five (5) and six (6) of these antibodies, some of which were positive, others negative, but the end result was that the specificity of the profile was 100% specific for RA. In this analysis, use is made of moderately specific autoantibodies (RA33, calpastatin and calreticulin), but when used in combination with highly specific autoreactivities, produces patterns that are 100% specific for RA.

A second study presented at the ACR meeting in New Orleans utilized a very innovative approach to early RA diagnosis. This system employs a synovial proteome model where 315 distinct proteins and peptides representing candidate RA autoantigens are spotted in a microarray format on a glass slide. The proteins and peptides were derived from citrulline, BiP, GP39, collagen I, collagen II, collagen III, collagen IV, collagen VI, collagen IX and collagen XI as well as overlapping peptides from Type II collagen. Further autoantigens included active and deiminated forms of fibrinogen, vimentin and filaggrin (deimination means arginine converted to citrulline), glucose-6-phosphate

isomerase, heat-shock proteins 60 and 65, heterogenous nuclear RNP proteins and other peptides. Sixty (60) RA patients versus disease controls consistently revealed autoantibody responses to citrulline-modified filaggrin and fibrin peptides but without reactivity when arginine was substituted for citrulline. Autoreactivity to BiP and glucose-6-phosphate isomerase was also seen but an occasional control demonstrated this as well. Other reactivities were also noted. This technology is currently being applied to early RA. Importantly, sera can be studied serially to identify different targeted epitopes as the disease evolves. Profiles of these autoantibody reactivities are being analyzed by statistical algorithms to determine the optimal sensitivity and specificity for RA.

These are indeed exciting times for RA, both therapeutically and diagnostically. The challenge to develop new specific markers is being met, as demonstrated by the development of novel serologic assays with high specificity being combined with other less specific autoantibodies but when used together yields test results that are virtually 100% specific for RA. An ultimate goal of this kind of approach might be the development of tolerization protocols directed against specific autoreactivities determined by this methodology.

If these new assays can be developed in a cost-effective manner with good sensitivities, utilization of such assays could reliably diagnose rheumatoid disease and allow treatment to proceed in an unambiguous fashion. For now, the only available commercial assay is anti-CCP, but it is highly likely that in the next 1-2 years we will see the evolution of such products.

SUMMARY

1. Two anti-CCP assays by ELISA are FDA approved and have excellent and comparable sensitivity and specificity (75-80% sensitivity and 96-98% specificity) for RA.
2. Anti-CCP, anti-perinuclear factor, anti-keratin antibody and anti-Sa are all antibodies with high specificity for RA. In addition, they all occur frequently in early RA and predict erosions, but only anti-CCP is commercially available at this time. Citrullinated peptide autoantigens are the targets for these 4 seemingly distinct autoantibodies.
3. Anti-CCP and both IgM and IgG rheumatoid factors occur in substantial numbers of sera, often many years prior to the clinical onset of RA.
4. T-cell reactivity to citrullinated peptide antigens in the context of the RA shared epitope, DRB1 0401, has been demonstrated.
5. New, highly specific antibody specificities for RA include anti-BiP and anti-glucose-6phosphate isomerase, not yet commercially available.

6. Testing for multiple autoantibodies simultaneously, including such antibodies as anti-CCP, anti-BiP, anti-RA33, rheumatoid factor, anti-calreticulin, anti-calpastatin, can yield combinations that are 100% for RA.
7. One would anticipate the rapid commercialization of assays as described above that have specificities approaching 100% in the near term and their availability will help to unambiguously confirm RA.

Bibliography

1. Schellekens: The Diagnostic Properties of Rheumatoid Arthritis Antibodies Recognizing a Cyclic Citrullinated Peptide. *Arthritis & Rheumatism*, 2000; 43: 144-163.
2. Information obtained from 8 clinical trials performed by Axis Shield involving 2,774 sera.
3. Eric, Jan, J.A Kroot: The Prognostic Value of Anti-Cyclic Citrullinated Peptide Antibody in Patients with Recent Onset Rheumatoid Arthritis. *Arthritis & Rheumatism*, VOL.43, No.8, Aug 2000, pgs.1831-1835.
4. Piette, A L., et al: Detection of Anti-CCP and Diagnosis of Rheumatoid Arthritis. Accepted for presentation, AMLI, 2002.
5. Visser, Hank, et al: How to Diagnose Rheumatoid Arthritis Early. *Arthritis & Rheumatism*, 2002; 46:357-365.
6. Quinn, Mark A. et al: Early RA: Anti-CCP Antibodies Predict Radiographic Progression. ACR 65th Annual Scientific Meeting, San Francisco, CA Nov.10-15, 2001.
7. Jansen, Annemarie, et al: The Prognostic Value of Anti-Cyclic Citrullinated Peptide (CCP) Antibodies in Early Polyarthritis. ACR 65th Annual Scientific Meeting, San Francisco, CA Nov.10-15, 2001.
8. Meyer, O.C., et al: Antifilaggrin and Anticitrullinated Peptide Antibody Assays in Early Rheumatoid Arthritis for Predicting 5-Year Radiographic Damage. ACR 65th Annual Scientific Meeting, San Francisco, CA Nov. 10-15, 2001.
9. Bizzaro, Nicola, et al: Diagnostic Accuracy of the Anti-Citrulline Antibody Assay for Rheumatoid Arthritis. *American Association for Clinical Chemistry*, 2001; 47:6.
10. Dahlqvist, Solbritt R., et al: Antibodies against Citrullinated Peptides (CCP) Predict the Development of Rheumatoid Arthritis. *Arthritis & Rheumatism*, Abstract Supplement, 2002 Annual Scientific Meeting; 46:9:200.
11. Nielen, Mark M.J, et al: Autoantibodies in Serum of Blood Donors Precede Symptoms of Rheumatoid Arthritis (RA) by 1 to 6 Years. *Arthritis & Rheumatism*, Abstract Supplement, 2002 Annual Scientific Meeting; 46:9:370. .

12. Hill, Jonathan A, et al: The Role of HLA-DRB1*0401 MHC Class II in Autoimmune Responses to Citrullinated Antigens. *Arthritis & Rheumatism*, Abstract Supplement, 2002 Annual Scientific Meeting;46:9:298.
13. Hill, Jonathan A, et al: Citrullinated Vimentin and Fibrinogen Peptides Activate CD4+ T Cells in HLA-DRB1*0401 Transgenic Mice. *Arthritis & Rheumatism*, Abstract Supplement, 2002 Annual Scientific Meeting; 46:9:299.
14. Bläß, Stefan, et al: The Stress Protein BiP is Overexpressed and is a Major B and T Cell Target in Rheumatoid Arthritis. *Arthritis & Rheumatism*, 2001; 44:4:761-771.
15. Morgan, Rebecca A., et al: Immune Reactivity to Glucose-6-Phosphate Isomerase in Collagen Induced Arthritis. *Arthritis & Rheumatism*, Abstract Supplement, 2002 Annual Scientific Meeting; 46:9:299.
16. Morgan, Rebecca A., et al: Pristane-Induced Arthritis is Characterized by Broad Reactivity to Joint Antigens. *Arthritis & Rheumatism*, Abstract Supplement, 2002 Annual Scientific Meeting; 46:9:299.
17. Behrens, S., et al: Autoreactivity Patterns in Rheumatoid Arthritis. *Arthritis Research*, Abstracts of the 21st European Workshop for Rheumatology Research, March 2001, 3:A1-A47; P5.
18. Hueber, Wolfgang; Genovese, Mark C.; van Venrooij, Walter J., et al: 'Synovial Proteome' Microarray Characterization of the Autoantibody Response in Rheumatoid Arthritis. *Arthritis & Rheumatism*, Abstract Supplement, 2002 Annual Scientific Meeting; 46:9: 199.