Rheumatoid Arthritis: Novel Serologic Markers

Introduction

Rheumatoid arthritis (RA), a chronic systemic autoimmune disease and the most common form of inflammatory polyarthritis, affects approximately 0.5% or 1.5 million people in the United States.[1] Without appropriate treatment, the persistent inflammation of RA causes a progressive erosive arthropathy that leads to severe joint damage, deformity, and disability.

Therapeutic Window of Opportunity

Identifying RA in its earlier stages allows for early intervention during a "therapeutic window of opportunity" when prompt initiation of disease modifying anti-rheumatic drugs (DMARDs) may be more effective than in later stages, reaping both short-term and long-term benefits.[1,2] Early therapy may slow or avert the erosive arthropathy, allowing better disease activity responses and preventing irreversible damage. It may also alter the long-term course of RA, modifying disease to a milder course, resulting in sustained long-term benefits in radiographic and functional outcomes.[2]

Challenges of RA diagnosis

Early recognition of RA at disease onset remains challenging due to variability in clinical presentations where RA may be difficult to distinguish from undifferentiated inflammatory arthritis (UA).[1] Despite the diagnostic contribution of anti-CCP (cyclic citrullinated peptide) antibody and RF (rheumatoid factor) as classified by the 2010 American College of Rheumatology/ European League Against Rheumatism (ACR/EULAR) RA criteria, approximately one third of patients with RA are considered "seronegative."[3]

New serologic testing may be used with RF, anti-CCP, clinical finding and imaging to help recognize RA as early as possible and identify early RA with worse prognosis.

Autoantibodies to Citrullinated and Carbamylated proteins— Implications for RA diagnosis, prognosis & disease activity monitoring

Citrullination and carbamylation are post-translational modifications that generate citrulline and homocitrulline from amino acids arginine and lysine, respectively.[4] Autoantibodies against citrullinated and carbamylated proteins have been identified in RA patients and may play a pathogenic role.[4]

Anti-citrullinated protein antibodies (ACPA) include anti-CCP (cyclic citrullinated peptide), anti-Sa (directed against citrullinated vimentin) and anti-CEP-1 (citrullinated α-enolase peptide 1) antibodies. These different ACPA are not equivalent.[5,6]

Newer, third generation enzyme-linked immunosorbent assay (ELISA) with cyclic citrullinated peptide (version 3.1) detects anti-CCP antibodies with 98% specificity for RA and with higher sensitivity (70%) than earlier versions by detecting both IgG and IgA to CCP.[7] Since a synthetic peptide is used as the capture molecule of anti-CCP assays, the test cannot elucidate endogenous citrullinated proteins responsible for triggering a patient's immune response.[5] In contrast, the endogenous targets of anti-Sa and anti-CEP-1 antibodies are known, and while there may be some cross-reactivity, anti-CEP-1 and anti-Sa have been shown to be distinct from anti-CCP.[5]

Clinical Usefulness

- Anti-Sa positivity predicts more severe disease and poor prognosis.[6]
- Anti-Sa antibody titers have been shown to correlate with higher disease activity.[8]
- Anti-CEP-1 is an early marker that can predict the onset of symptoms in pre-clinical RA years before onset.[9]
- Anti-CarP antibodies may also be present years before the onset of symptoms in RA.[10]
- Anti-CarP is associated with more severe clinical and radiographic disease.[11]
- Anti-Sa may identify an additional 8.7% of RA that is anti-CCP-negative[5]
- Anti-CEP-1 was detected in 12.5% of RA patients who test negative for anti-CCP.[12]
- Anti-CarP and 14-3-3 eta may identify an additional 10% and 15% respectively, of early RA patients who are RF- and anti-CCP-negative.[11,13]

labcorp

Anti-Sa (citrullinated vimentin) antibodies target the citrullinated form of vimentin, a filament protein that is part of the cytoskeleton.[8] Anti-Sa has nearly 100% specificity for RA and sensitivities of 20-25% in early RA and up 47% in established RA.[14,15] Anti-Sa testing may identify an additional 8.7% of RA patients who are anti-CCP-negative.[5] Anti-Sa antibodies are associated with joint erosions,[5] and anti-Sa titers have been shown to correlate with RA disease activity.[8] Most importantly, Anti-Sa positivity, in recent onset or early disease, is a strong prognosticator of a more aggressive rapid disease progression.[6]

Anti-CEP-1 (citrullinated α-enolase 1) antibodies are directed against citrullinated α-enolase, an enzyme involved in glycolysis.[12] Anti-CEP-1 is an early marker that can predict the onset of symptoms in pre-clinical RA years before onset.[9] Anti-CEP-1 identifies RA with a specificity of 98% and sensitivity of 37-62% and may be detected in 12.5% of RA patients who test negative for anti-CCP.[5]

Anti-CarP (carbamylated protein antibodies) are novel autoantibodies that predict the development of RA independently of anti-CCP.[3] A meta-analysis of 7 studies including 1898 RA patients showed diagnostic sensitivity of anti-CarP antibodies ranging from 36.2% to 47.7% and specificity from 92.9% to 97.0%.[3] Furthermore, anti-CarP antibodies are present years before the onset of symptoms in RA.[10] In pre-symptomatic RA (median 5.3 years before symptoms), the sensitivity of anti-CarP was 13.9% with higher sensitivity of 21.8% when within 2 years of the time of symptom onset.[10] In seronegative RA (RF-negative, anti-CCP-negative) RA, anti-CarP may confer a 10% incremental benefit in identifying early RA.[11] Anti-CarP is associated with more active disease and higher risk of developing joint erosions.[11]

14-3-3 eta protein is a joint-derived, proinflammatory mediator that is implicated in the joint erosion process and pathogenesis of RA.[16] Serum 14-3-3 eta is highly specific for RA and is elevated in both established RA (sensitivity 77%) and early RA (sensitivity 59-64%).[16,17] It may provide a 15% incremental benefit in identifying early RA in RF-negative and anti-CCP Ab-negative patients.[13] Positive serum 14-3-3 eta levels are associated with higher rates of joint damage as measured by radiographic assessments.[13,18]

Test Name	Test No.
14.3.3 eta, Rheumatoid Arthritis	**504550**
Anti-CCP (Cyclic Citrullinated Peptide) Antibodies, IgG and IgA (RDL)	**520008**
Anti-CEP-1 Ab, IgG (RDL)	**520133**
Anti-Sa Ab, IgG (RDL)	**520081**
Anti-Carbamylated Protein (CarP) Antibody	**520311**
SeroNeg RAdx4 Profile	**520305**
RAdx6 Profile	**520304**

References

1. Taylor PC. Update on the diagnosis and management of early rheumatoid arthritis. *Clin Med (Lond).* 2020 Nov;20(6):561-564.
2. Finckh A, Liang MH, van Herckenrode CM, de Pablo P. Long-Term Impact of Early Treatment on Radiographic Progression in Rheumatoid Arthritis: A Meta-Analysis. *Arthritis Rheum.* 2006 Dec 15:55(6):864-872.
3. Li L, Deng C, Chen S, et al. Meta-Analysis: Diagnostic Accuracy of Anti-Carbamylated Protein Antibody for Rheumatoid Arthritis. *PLoS One.* 2016 Jul 20;11(7):e0159000.
4. Pruijn GJM. Citrullination and carbamylation in the pathophysiology of rheumatoid arthritis. *Front Immunol*. 2015 Apr 27:6:192.
5. Montes A, Perez-Pampin E, Calaza M, Gomez-Reino JJ, Gonzalez A. Association of Anti-Citrullinated Vimentin and Anti-Citrullinated a-Enolase Antibodies with Subsets of Rheumatoid Arthritis. *Arthritis Rheum*. 2012 Oct;64(10):3102-3110.
6. Boire G, Cossette P, de Brum-Fernandes AJ, et al. Anti-Sa antibodies and antibodies against cyclic citrullinated peptide are not equivalent as predictors of severe outcomes in patients with recent-onset polyarthritis. *Arthritis Res Ther.* 2005;7(3):R592-603.
7. QUANTA Lite CCP3.1 IGG/IgA ELISA [directional insert]. INOVA Diagnostics, Inc. October 2019. Revision 5.
8. Bang H, Egerer K, Gauliard A, et al. Mutation and citrullination modifies vimentin to a novel autoantigen for rheumatoid arthritis. *Arthritis Rheum*. 2007 Aug;56(8):2503-2511.
9. Brink M, Hansson M, Mathsson L, et al. Multiplex Analyses of Antibodies Against Citrullinated Peptides in Individuals Prior to Development of Rheumatoid Arthritis. *Arthritis Rheum*. 2013 Apr;65(4):899-910.
10. Brink M, Verheul MK, Rönnelid J, et al. Anti-carbamylated protein antibodies in the pre-symptomatic phase of rheumatoid arthritis, their relationship with multiple anti-citrulline peptide antibodies and association with radiological damage. *Arthritis Res Ther*. 2015 Feb 7;17(1):25.
11. Truchetet ME, Dublanc S, Barnetche T, et al. Association of the Presence of Anti–Carbamylated Protein Antibodies in Early Arthritis With a Poorer Clinical and Radiologic Outcome. *Arthritis Rheum*. 2017 Dec;69(12):2292-2302.
12. Montes A, Dieguez-Gonzalez R, Perez-Pampin E, et al. Particular Association of Clinical and Genetic Features with Autoimmunity to Citrullinated a-Enolase in Rheumatoid Arthritis. *Arthritis Rheum*. 2011 Mar;63(3):654-661.
13. Carrier N, Marotta A, de Brum-Fernandes AJ, et al. Serum levels of 14-3-3 eta protein supplement C-reactive protein and rheumatoid arthritis-associated antibodies to predict clinical and radiographic outcomes in a prospective cohort of patients with recent-onset inflammatory polyarthritis. *Arthritis Res Ther.* 2016 Feb 1;18:37.
14. Menard HA, Lapointe E, Rochdi MD, Zhou ZJ. Insights into rheumatoid arthritis derived from the Sa immune system. *Arthritis Res*. 2000;2(6):429-432.
15. El-Gabalawy HS, Wilkins JA. Anti-Sa antibodies: prognostic and pathogenetic significance to rheumatoid arthritis. *Arthritis Res Ther*. 2004;6(2):86-89.
16. Maksymowych WP, Boire G, van Schaardenburg D, et al. 14-3-3 η Autoantibodies: Diagnostic Use in Early Rheumatoid Arthritis. *J Rheumatol.* 2015 Sep;42(9):1587-1594.
17. Maksymowych WP, Naides SJ, Bykerk V, et al. Serum 14-3-3 eta is a Novel marker that Complements Current Serological Measurements to Enhance Detection of Patients with Rheumatoid Arthritis. *J Rheumatol.* 2014 Nov;41(11):2104-2113.
18. Maksymowych WP, van der Heijde D, Allaart CF, et al. 14-3-3 eta is a novel mediator associated with the pathogenesis of rheumatoid arthritis and joint damage. *Arthritis Res Ther.* 2014 Apr 21;16(2):R99.