

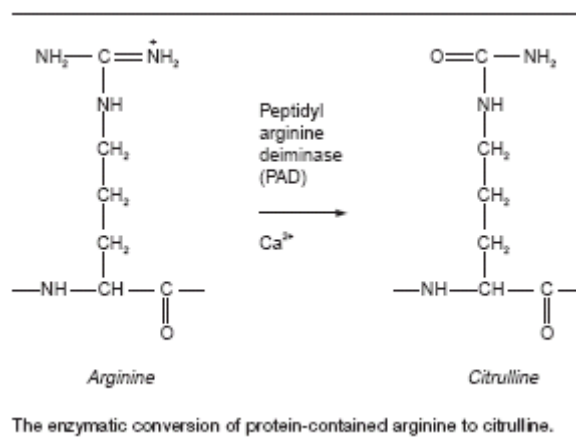
## **The Role of Anti-CCP in the Laboratory Diagnosis of Rheumatoid Arthritis**

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Rheumatoid Arthritis (RA) is one of the most common systemic autoimmune diseases, affecting approximately 0.5–1.0% of the world population.<sup>1</sup> The American Rheumatism Association criteria for the classification of RA includes: 1) morning stiffness, 2) arthritis of 3 or more joint areas, 3) arthritis of hand joints, 4) symmetric arthritis, 5) rheumatoid nodules, 6) serum rheumatoid factor (RF), and 7) radiographic changes.<sup>2</sup> A patient should have four of the seven criteria to be diagnosed with RA and the first four criteria should be present for at least six weeks. Until recently, the only serological test routinely performed for the detection of RA was for the presence of IgM RF. RF is found in approximately 50%–90% of these patients, but it is also found in patients with infections, other autoimmune diseases, and some healthy individuals with increasing frequency in older age groups, thus limiting its specificity for RA.<sup>1,3</sup>

Several studies have shown that anti-perinuclear autoantibodies, otherwise known as anti-keratin autoantibodies, are found in patients with RA.<sup>4</sup> It has been discovered that these antibodies recognize an epitope that contains the deamidated form of arginine called citrulline. Enzyme-Linked Immunosorbent Assay (ELISA) testing for these autoantibodies directed against anti-cyclic citrullinated peptide (anti-CCP) is reasonably sensitive (68%) and highly specific (98%) in patients with RA.<sup>5</sup> The pathogenesis of anti-CCP antibodies in rheumatoid arthritis has been shown to be attributable to the body's humoral response to citrulline. Citrullination is the post-translational conversion of arginine to citrulline by an enzyme called peptidylarginine deiminase (PAD). See figure 1.<sup>6,7</sup> PAD activation is assisted by calcium ions. PAD is normally present as inactive intracellular enzymes. During programmed cell death (apoptosis) in the synovial joints of patients with rheumatoid arthritis, PAD may leak out of the dying cells. Once activated, PAD will cause citrullination of extracellular arginine. In the synovium, the citrulline acts as an antigenic stimulant to induce anticitrullinated protein antibodies (ACPA) locally produced by plasma cells.<sup>7</sup> The ELISA that detects these autoantibodies uses synthetic cyclical citrulline peptides.



The original ELISA for the anti-CCP sequence was not broadly marketed due to low sensitivity and technical complexity. However, the second generation anti-CCP test (often referred to in the literature as CCP-2/CCP2) shows superior performance compared to the original peptide.<sup>8</sup> The vast majority of the laboratories that offer this test utilize the second-generation CCP assay.

In 2005, a third generation of anti-cyclic citrullinated peptide (CCP3) was made available for the laboratory diagnosis of RA. These assays have been reported to recognize additional citrulline epitopes that are not identifiable with the second-generation CCP assays. The CCP3 assays have had reported results of up to 5% increased sensitivity compared to the CCP2 assays.<sup>9, 10</sup> To the contrary, however, several publications have shown similar diagnostic performance between the CCP3 and CCP2 assays.<sup>11, 12</sup>

Recently, Nishimura et al. performed a meta-analysis of published studies regarding the diagnostic accuracy of anti-CCP and RF for rheumatoid arthritis.<sup>13</sup> Their results showed a positive likelihood ratio of 12.46 and a negative likelihood ratio of 0.36 for anti-CCP antibody in patients with RA. The same study showed a positive likelihood ratio of 4.86 and a negative likelihood ratio of 0.38 for RF. These results indicate that anti-CCP positivity alone is more specific than IgM RF for the diagnosis of RA.

In addition to diagnostic value, several studies have shown that anti-CCP may also add prognostic significance in the determination of development of erosive disease in RA.<sup>14, 15</sup> Kroot, EJ et al showed that anti-CCP positive patients developed significantly more severe radiologic damage than those patients who were anti-CCP negative.<sup>14</sup>

Although the presence of anti-CCP is not currently required for the diagnosis of RA, future classification criteria will most likely incorporate its use as an adjunct to IgM RF as a laboratory diagnostic tool. Additionally, RA patients with positive CCP status may benefit from its prognostic value by receiving earlier customized treatment regimens that could potentially delay the development of erosive disease.

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