

# Comparative utility of anti-citrullinated protein antibodies verses rheumatoid factor in the management of rheumatoid arthritis

Volume 8 Issue 4 - 2017

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## Mini review

Traditionally test for the diagnosis of rheumatoid Arthritis (RA) is Rheumatoid Factor (RF). The RF was the first autoantibody described by Waaler in 1940, as a serological biomarker for RA.<sup>1</sup> It was used with other serological markers such as total white cell counts, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and with clinical information for the diagnosis and determine the prognosis in RA patients. Unfortunately, all of these biomarkers have poor specificity for RA. The RF can be positive in several other diseases and the list includes bacterial endocarditis, systemic sclerosis, sarcoidosis, Sjogren's syndrome, interstitial pulmonary fibrosis, systemic lupus erythematosus (SLE), hepatitis b, chronic liver disease and chronic hepatitis, Dermatomyositis, Essential mixed cryoglobulinemia, Primary biliary cirrhosis, Infectious mononucleosis and any chronic viral infection, Syphilis, Leprosy, Malaria, Leukemia, visceral leishmaniasis ,tuberculosis and even after vaccination or transfusion in normal individuals. The patient can have RF without being positive for RF. The classic RF antibody identified initially was the immunoglobulin class -M (IgM) antibody against the Fc-Fraction of the immunoglobulin class -G antibody. Now three isotypes of RF(IgA), IgG and IgM of RF are recognized.

The existence of autoantibodies against citrullinated proteins was first described in the 1970s in the patients with rheumatoid arthritis. This time the biochemical basis of antibody reactivity against antigens called keratin, and filaggrin was investigated. It is recognized now that during several natural processes such as apoptosis and some inflammatory processes, an enzyme called Peptidyl arginine deiminase (PAD) enzyme is released. The enzyme PAD has five isotypes expressed in the several parts of the body in the side and outer side of various cells. This enzyme is released from the dying cells and is responsible for citrullinating (replacing arginine residue with Citrulline amino acid) the extracellular proteins that contain arginine. Citrullination is a normal physiologic process routinely occurring in the many dying cells. During the Citrullination process citrullination, the shape of the peptide/protein may so grossly change that it is not anymore recognizable by the immune system. The immune system, therefore, treats these peptide/proteins as foreign antigens and starts producing antibodies against them. Out of the five isoforms of the PAD enzyme, type two and four (PAD2, PAD4) are expressed in inflammatory leukocytes and released when cells are dying. PAD4 It is found in the cytoplasmic granules of eosinophils and nucleus of neutrophils. Pad4 is also expressed in synovial tissues. The antibodies that are produced against citrullinated protein peptides were grouped as Anti-citrullinated protein antibodies (ACPAs).

There were several antigenic proteins later recognized which were similar to Vimentin which also reacted with autoantibodies identified in RA patients. The examples of few such proteins are

fibrinogen, Deiminated Epstein-Barr Virus Nuclear Antigen 1 and vimentin.<sup>2-4</sup> Over the years, the assays using genetically modified citrullinated Vimentin antigen protein were developed to make them more standardized and efficient. These new tests include mutated citrullinated Vimentin (MCV-assay), filaggrin-derived peptides (CCP-assay) and viral citrullinated peptides (VCP-assay).<sup>5</sup> Interestingly it has same diagnostic performance as is of RF and anti-CCP, it is especially useful in the diagnosis of RF & Anti -CCP negative RA patients. It has not been studied well in the pediatric population as yet.

The 2010 ACR-EULAR classification criteria for rheumatoid arthritis included ACPA in the diagnostic criteria, and ACPA testing played a significant part in the classification system of RA also.<sup>6,7</sup> Anti-Cyclic Citrullinated Peptide (ACCP) antibodies are one of the antibodies in the ACPA group, but interestingly all antibodies in this group not necessarily cross react with each other.<sup>8</sup>

In the pediatric population where the juvenile onset idiopathic rheumatoid arthritis is the most common rheumatic diseases of childhood, there is an absence of specific biomarkers for the diagnosis. A tiny pediatric population with JIA is seropositive (RF positive and Anti-Citrullinated Protein Antibodies (ACPAs) positive). This situation takes away the opportunity of early and aggressive therapy which is known to impact long-term morbidity and outcomes. It is also the fact that biomarkers are not well researched and explored in the pediatric population as they are in adult patients. Interestingly as it is evident in the classification of JIA, it is automatically presumed that only polyarthritis patients can be rheumatoid factor positive (RF-positive and RF-negative polyarthritis). There are only a few biomarkers that are tested using standardized universally accepted tests which, so that results from different geographical areas can be compared. These biomarkers include Rheumatoid Factor (RF) /RF isotypes and Anticyclic citrullinated peptide antibodies/ anti-CCP isotopic antibodies (Anti -CCP).

Citrullinated proteins and peptides are never present in normal joints or blood unless there is autoimmune inflammation ongoing on.<sup>9</sup>

The antiperinuclear factor (APF) and anti-keratin antibodies (AKA) were the first two members of ACPA family, and they were seen to recognize citrullinated epitopes of filaggrin peptide. Later on, based on these facts the first (Anti-CCP), the second (Anti-CCP2) and now third generation anti-cyclic citrullinated peptide (anti-CCP3) antibody assays were developed.

To detect the citrullinated antibodies, various manufacturers use different citrullinated antigen substrates such as Epstein–Barr virus- or IgG-derived peptides, synthetic cyclic peptides, mutated human vimentin, and, recombinant rat filaggrin. These manufacturers though have little difference in their characteristics of analytical methods such as titrating various sensitivities for a fixed specificity, they all perform testing by enzymatic immunoassay (EIA) methods.<sup>10</sup> Research has shown that the diagnostic accuracy and analytical imprecision can be influenced significantly by the antigenic source used in the test.<sup>10</sup> The sensitivity and specificity of different manufacturers may vary which is based on their selected values of cut off. For example, at a fixed specificity of 'X,' the sensitivities ranged from 'Y' to 'Z' for different manufacturer's tests. False-positive results are common in patients who had or were recovering from viral syndromes.<sup>10</sup>

The vimentin is a relatively new but considered a very reliable and standard autoantigen. Vimentin is a naturally occurring antigen as oppose to several others artificially developed for the test. Vimentin is secreted from dying macrophages and then citrullinated also by macrophages during the process of apoptosis, or by pro-inflammatory cytokines (tumor necrosis factor- $\alpha$ : TNF- $\alpha$ ) during the inflammation process.<sup>11</sup> To improve further the accuracy and specificity a genetically modified citrullinated vimentin (MCV) is used as an antigen to identify auto antibodies.<sup>12,13</sup>

CCP antibody testing may also be ordered to help evaluate the likely development of RA in people with undifferentiated arthritis or test the possibility of RA in the relatives of RA patients the familial incidence is not very significant. In Monozygotic Twins the incidence is 15.4% while in dizygotic twins it is 3.8%. According to ACR, approximately 95% of those with a positive CCP antibody will die eventually in future will develop after a variable and uncertain period of latency. It goes without saying that early detection of RA is essential for guiding appropriate treatment decisions and can affect outcomes. Therefore, Anti-Citrullinated Protein Antibodies (ACPAS) is used for undifferentiated arthritis and early detection when clinical suspicion is high where RF is not useful.<sup>14</sup> The RF is used as a screening test, but Anti-CCP cannot be used. As mentioned before Anti -CCP can predate the RA by several years while RF does not predate the disease.

Both RF Anti-Citrullinated Protein Antibodies (ACPAS) have high positive predictive value in predicting erosive disease if they are present at onset. Anti-Citrullinated Protein Antibodies (ACPAS) has a higher positive predictive value for erosive disease as compared to RF.<sup>5,14,15</sup>

The sensitivity of RF is marginally better than Anti-Citrullinated Protein Antibodies (ACPAS) in the diagnosis of the RA (69% VS 67% in most of the meta-analysis) while the Specificity of Anti-Citrullinated Protein Antibodies (ACPAS) in the diagnosis of RA is better than that of RF.

Several immunogenic viruses are known to produce false positive reactions or even positive tests due to the production of polyclonal -B-Cell reaction such as hepatitis- C virus (HCV)-associated cryoglobulinemia. The possibility of RF being positive in these situations is higher than Anti-Citrullinated Protein Antibodies (ACPAS) being positive.

The titer of both the antibodies decreases with treatment<sup>16</sup> but never disappear (PMID). The titers of RF antibodies and associated diseases are more responsive to infliximab or any other TNF- inhibitor therapy as compared to Anti-Citrullinated Protein Antibodies (ACPAS) titers and associated disease.<sup>17-21</sup> The positive RF can be used as an early predictor of the efficacy of anti-TNF therapy.<sup>22</sup>

The HLA-DRB1 alleles carrying the shared epitope (SE) association with Anti-Citrullinated Protein Antibodies (ACPAS) autoantibodies but not with RF antibodies.<sup>23-27</sup>

The RF antibodies have the correlation with Th1/Th2 cytokines while Anti-Citrullinated Protein Antibodies (ACPAS) antibodies do not have any such relationship.<sup>23</sup>

As it is known, RF antibodies can be present in several other systemic autoimmune diseases such as in lupus, Graves' disease and Sjogren's syndrome, etc. while Anti-Citrullinated Protein Antibodies (ACPAS) is not necessarily positive in these diseases.<sup>28</sup> On the other hand, Anti-Citrullinated Protein Antibodies (ACPAS) can be positive in active systemic or pulmonary tuberculosis Positive in Tuberculosis.

Apparently, RF can be recommended as a screening test for the diagnosis of RA while ideally, Anti-Citrullinated Protein Antibodies (ACPAS) is not.

Summarizing the communication on the comparative utility of RF verses in the management of RA, clearly Anti-Citrullinated Protein Antibodies (ACPAS) show better value in making the diagnosis of RA and predicting outcomes and even selecting the therapy options based on the responsiveness. It is amazing fact that RF and Anti-Citrullinated Protein Antibodies (ACPAS) apparently are not the similar spectrum of antibodies. Both of these antibodies may be representatives of different RA diseases with specific diseases course prognosis and drug responsiveness. Therefore, RF and Anti-Citrullinated Protein Antibodies (ACPAS) cannot be used synonymously or could be used interchangeable as they represent different diseases and outcomes.

## Conflicts of Interests

There is no conflict of interest in composing this manuscript.

## Acknowledgments

None.

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