Anti-Citrullinated Peptide Antibodies is More than an Accurate Tool for Diagnosis of Rheumatoid Arthritis

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ABSTRACT: Anti-citrullinated peptide antibodies (ACPA) are detected in the sera of rheumatoid arthritis (RA) patients and have a profound role in diagnosis of the disease. In this review we discuss the different cohorts of RA patients in whom the presence, sensitivity and specificity of ACPA were evaluated. The significance of ACPA in the pathogenesis and prognosis RA is also interpreted. Recent advances in the understanding of molecular pathways involved in the pathogenesis of RA have led to the identification of novel biologic agents that are now widely used in patients with RA

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Rheumatoid arthritis affects nearly 1% of the adult population. It is characterized by chronic joint inflammation, pain and swelling, resulting in progressive joint destruction and disability [1,2]. Acording to the 2005 census data from the U.S. Centers for Disease Control, RA affects approximately 1.5 million adults in the United States [3]. Although modern medicine has begun to overcome many of the disease signs and symptoms, the pathogenesis of the disease remains obscure. The shared epitope hypothesis, put forth by Gregersen et al. [4] in 1987, uncovered the significant role that genetics plays in the development of RA. Their study was the first of many to point out the association between RA and HLA class II alleles. Although not clear at the time, it has since been found that these alleles play a prominent role in the formation of anti-citrullinated peptide antibodies [5]. Hill and colleagues [6] provided further evidence for the close association between the shared epitope, citrullinated proteins and the development of RA, when they induced rheumatoid arthritis in DR4-IE transgenic mice simply by exposing them to citrullinated fibrinogen.

Citrullination is a post-translational modification of a protein. This process is catalyzed by peptidylargininedeiminase (PAD), an intracellular enzyme. Cigarette smoking triggers the formation of autoantibodies against citrullinated proteins

RA = rheumatoid arthritis

In accordance with the 2010 rheumatoid arthritis classification criteria, ACPA detection is now used as a means of diagnosing RA [7]. The biochemical mechanism of citrullination, therefore, is worth mentioning. Citrullination is a post-translational modification of a protein. More specifically, it is the replacement of a charged arginine with a neutral citrulline. This process is catalyzed by peptidylargininedeiminase, an intracellular enzyme that requires calcium for activation [8]. While a cell's membrane is intact, intracellular calcium is maintained at low levels and PAD remains inactive. During inflammation and cell death, however, the cell membrane is compromised, thereby allowing both the leakage of calcium into the cell and PAD out of the cell [9]. The exposure of citrullinated proteins to the humoral immune system in those who are genetically, or otherwise, predisposed leads to the formation of ACPA and in some cases the progression to an inflammatory disease of the joints, i.e., RA.

ACPA DETECTION

Once an association between ACPA and RA was established, the focus shifted toward creating the most accurate and reproducible ways for detecting these antibodies in an individual's serum. ACPA titers made a major diagnostic contribution to the 2010 RA classification criteria, in contrast to the previous 1987 criteria where rheumatoid factor was the sole serological marker. This reflects our new confidence in obtaining reproducible ACPA titer results [7,10].

The cyclic citrullinated peptide-2 assay, first used in 2002, remains the gold standard for measuring the presence of ACPA in serum. The CCP2 assay has the advantage of being more sensitive than some of the newer tests; i.e., by acting as an antigen (a citrullinated protein) it detects a wide range of ACPA. To draw a comparison, Pruijn and team [11] compiled data from 154 studies in order to evaluate the statistical performance of the CCP2 test. When these values were compared to those of other ACPA detection tests (CCP3, anti-

ACPA = anti-citrullinated peptide antibodies
PAD = peptidylargininedeiminase
CCP = citrullinated peptide

mutated citrullinated vimentin and RF), the CCP2 assay was found to have the greatest sensitivity at a stratified specificity [11]. Mjaavatten et al. [12] found that anti-CCP and immunoglobulin M rheumatoid factor together have an added predictive value. They also concluded that the higher these levels appear in serum the greater the likelihood for developing RA. A recent systematic review of 151 studies has demonstrated considerable heterogeneity in sensitivity (range 12%–93%) and specificity (63%–100%). In cohort studies that investigated second-generation anti-CCP2 antibodies in patients with early rheumatoid arthritis (less than 2 years), summary sensitivity and specificity were 57% (95% confidence interval 51%– 63%) and 96% (CI 93%– 97%), respectively. Anti-CCP2 had greater specificity than rheumatoid factor (96% vs. 86%), with similar sensitivity. Evidence was insufficient to ascertain whether the combination of anti-CCP2 and rheumatoid factor provides additional benefit over anti-CCP2 alone [13].

When we refer to ACPA we are in fact speaking of a group of antibodies, each with its own citrullinated protein target. Of great interest is pursuing the prognostic significance of the different ACPA. We know that many of the citrullinated peptides that play a role in RA are matrix proteins (filaggrin, keratin, fibrinogen, α-enolase, vimentin), and research continues in attempts to identify the significance of each in the disease process [14]. The anti-Sa antibodies, first described in 1994, were known to be present in RA patients but further details were lacking [15]. Ten years later, it was shown that these antibodies are ACPA targeted specifically against citrullinated vimentin [16]. Much work has been done since, both in elucidating the significance of citrullinated vimentin as the initiator of ACPA formation and in evaluating the ability of anti-Sa measurements to diagnose RA.

Modern ACPA tests have high specificity levels reaching 96% in the diagnosis of early RA

A review published by Van Steendam et al. [17] provided three strong arguments for the importance of citrullinated vimentin in RA: Firstly, shared epitope alleles (proven to be a risk factor for developing RA) are closely tied with antibodies against citrullinated vimentin; secondly, citrullinated vimentin is present in the affected joints of RA patients; and thirdly, citrullinated vimentin peptides bind to HLA-DR better than non-citrullinated vimentin. With regard to its potential use as a prognostic factor, these authors concluded that anti-Sa antibodies are a better predictor of RA severity as compared to RF, anti-CCP, and SE. Although there is little doubt that citrullinated vimentin plays a major role in the initiation and progression of RA, the major drawback of the anti-Sa test, according to many studies, is that its sensitivity is significantly lower than the CCP2, reaching 43% in a study by Lopez-Longo and co-researchers [18], leading to an understanding that the anti-CCP2 assay is best used as a diagnostic test, while the anti-Sa test has more prognostic value [19].

RF = rheumatoid factor
CI = confidence interval

The anti-mutated citrullinated vimentin (anti-MCV) test is another detection tool that may have a unique ability to diagnose RA. Antibodies targeted against mutated vimentin (vimentin with glycine residues replaced by arginine) have been identified in the serum of many RA patients [20]. These mutated proteins are not necessarily citrullinated, leading many to believe that the anti-MCV test should be able to detect antibodies that the CCP2 assay cannot. If this were the case, the sensitivity of previous RA diagnostic tests could be improved. The study by Bang et al. [20] showed that this is indeed true. However, another study on RA patients from Oman failed to corroborate these results [21]. In fact this group concluded that anti-MCV is an inferior diagnostic tool when compared to the CCP2 test [21]. Furthermore, a similar conclusion was reached in a large cohort study performed by an Italian group. They determined not only that the sensitivity of the anti-MCV test is lower than anti-CCP but that anti-MCV is not specific to RA (it was also found in patients with infectious diseases) [22].

PATHOGENIC AND PROGNOSTIC FACTORS OF ACPA

Several groups reported that ACPA can be detected years before the onset of disease and are associated with a more aggressive disease course [23,24]. ACPA in the inflamed synovium has been shown to be associated with citrullinated antigens to form immune complexes, resulting in progression of the inflammatory process [25]. Yet, the pathologic relevance and the potential influence on tissue injury mediated by these antibodies remain unclear.

ACPA-derived sera derived from the murine model of collagen-induced arthritis (specific for the citrullinated proteins filaggrin and fibrinogen) were shown to bind sections of rat esophagus [26]. Transfer of monoclonal ACPA was insufficient to induce arthritis in DBA/1 mice. However, aggravation in anti-collagen antibody-induced arthritis was demonstrated following co-administration of monoclonal antibodies specific to citrullinated fibrinogen antibodies together with an arthrogenic anti-CII monoclonal antibody cocktail [26]. This result suggests that there is no target injury in the absence of citrullinated target antigens, but when these antigens are present ACPA can greatly amplify inflammation and damage.

Ex vivo studies with human macrophages and mast cells have clearly indicated that ACPA-related immune complexes can stimulate pro-inflammatory cytokine production [27]. The effect of ACPA on immune cells in experimental models is an important step for understanding the involvement of ACPA in the RA process.

The question arises as to whether the quantity of ACPA in the serum has any prognostic relevance. A study by Ursum and collaborators [28] suggests this not to be the case. They assert that although ACPA positivity does reflect more aggressive disease, the quantity of ACPA in serum is of no relevance. Laki et

MCV = mutated citrullinated vimentin

al. [29] reached a similar conclusion. They found no difference in disease activity in those with moderate amounts of ACPA versus those with very high levels.

Because of the availability and reproducibility of ACPA detection tests in diagnosing RA, it became relevant to evaluate whether ACPA positivity has any correlation with response to treatment. da Mota et al. [30] followed a cohort of 40 RA patients for 3 years as they received standard treatment. The group found no statistically significant difference between ACPA-positive and negative patients with regard to remission after treatment. Other groups reported superior responses to infliximab in patients who are ACPA positive but could not find a strong enough correlation for use in predicting the response in individual patients [31].

ACPA MONITORING THROUGHOUT DISEASE

Ideally, we would like to have a marker that allows for both the monitoring of RA disease activity and for evaluation of a patient's response to therapy. Van der Woude and team [32] investigated the progression of ACPA throughout the normal course of RA. They found that as the undifferentiated arthritis phase progresses toward RA, ACPA levels do increase in detectable amounts. Once enough criteria for RA have been met and the diagnosis is established, ACPA levels remain stable. The stability of ACPA levels has been shown in numerous other studies, providing strong evidence for the ineffectiveness of its use as a marker of disease progression [33]. Kolarz et al. [34] evaluated the potential value of using ACPA levels to measure disease regression in RA patients being treated with infliximab. They found that although the disease ameliorates with treatment (evidenced by a decrease in both symptoms and inflammatory factors), ACPA levels remain unchanged. This study provides further evidence that quantifying ACPA in RA patients is an inappropriate means of monitoring the efficacy of infliximab treatment. Of note, the group did find a correlation between disease regression and IgM RF, which they claim can be an effective measure to monitor success of treatment. However, in a recent study Bohler et al. [35] demonstrated that ACPA and RF levels decreased significantly after 6 months of biological therapy. The reductions of both autoantibodies were closely related to the reduction of concomitant disease activity.

ACPA positivity reflects more aggressive and erosive disease in RA patients

RA RISK FACTORS

As mentioned previously, the etiology of RA remains unclear, yet new risk factors and biochemical explanations for old risk factors continue to be uncovered. An awareness of the interplay between genetics and the environment is integral when broaching this subject. Genetically, it was previously recognized that HLA-DR4 alleles, known as shared epitopes, are a risk factor for developing RA. Kapitany et al. [36], in an article published in *IMAJ*, sought to find the biochemical explanation for this association. When comparing 53 patients with RA, this group found a significant correlation between HLA-DR4 and anti-CCP levels. Thus, they came to the conclusion that HLA-DR4 allele positivity poses an increased risk to developing RA because of its association with higher serum ACPA levels.

In an attempt to uncover those features that provoke genetically predisposed people toward developing RA, Klareskog and associates [37] evaluated the role of environmental factors in disease onset. It was shown that in HLA-DR-positive patients, cigarette smoking triggers the formation of autoantibodies against citrullinated proteins. More recently, it was shown that HLA-DRB1 SE, PTPN22 polymorphism (another known RA risk factor), and smoking, together are associated more significantly with ACPA reactivities than with anti-CCP levels. This study and others showed the complexities of the relationship between genetics and environment. They assert that further genetic analysis will allow for separation of RA into subgroups that each have specific risk factors and disease course [38,39].

Interestingly, socioeconomic status was found to have an impact on the phenotype of RA as well. In a study carried out in the UK, it was shown that a low socioeconomic status predicted rheumatoid factor positivity but not ACPA positivity, a result that was independent of smoking [40]. This group took into account a risk factor that has not been extensively studied, and the correlation should direct our attention toward ways that we can provide care and information for these at-risk groups.

CONCLUSIONS

RA, which not long ago caused significantly greater morbidity, is now managed considerably better thanks in large part to advancements in diagnostics and pharmacotherapy. Our growing knowledge of the relationship between ACPA and RA has been and continues to be an integral part of this progress. In addition to enhancing our understanding of the etiology of this disease it has been fundamental in improving our ability to diagnose RA at a pre-disease phase. It is clear that the more we uncover about ACPA, the better able we become at decreasing disease-associated morbidity. Much continues to be learned of this newly discovered group of antibodies and we continue to find ways to use it to our advantage. In a broad sense, the future of ACPA research seems to lie in expansion of our ability to separate RA patients into subgroups, thus allowing for treatments better tailored to the individual.

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IgM = immunoglobulin M

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