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Antibodies to Citrullinated Protein Antigens (ACPAs): Clinical and Pathophysiologic Significance

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Abstract

Antibodies to citrullinated protein antigens (ACPAs) are highly specific for rheumatoid arthritis (RA) and are useful in the diagnosis of RA as well as the prediction of the course and outcomes of disease. Multiple methodologies exist for measuring ACPAs, including the widely available tests for anticyclic citrullinated peptide antibodies and for antibodies to mutated/modified citrullinated vimentin. These methodologies overall have similar diagnostic accuracies for RA, although there is some variability. The discovery of ACPAs and the biology of citrullination have also led to important advances in the understanding of the pathophysiology and development of RA, especially regarding the relationship between potential genetic and environmental risk factors for RA. Going forward, research into autoimmunity to citrullinated proteins may help identify the specific etiology of RA and provide approaches for the prediction of future risk of disease, and ultimately prevention of RA.

Keywords

Anticyclic citrullinated peptide antibody; Anti-CCP antibody; Antibodies to citrullinated peptide antigens; ACPAs; Rheumatoid arthritis; Diagnosis; Pathogenesis; Autoimmunity

Introduction

Rheumatoid arthritis (RA) is a systemic inflammatory autoimmune disease that affects approximately 1% of the population, leading to significant morbidity and even increased mortality [1]. However, there is increasing awareness that an early diagnosis of RA followed by aggressive, disease activity-directed therapies can lead to improved long-term outcomes [2]. A key aspect of this approach to the management of RA is an accurate, early diagnosis of disease, with the diagnosis depending heavily on serologic testing, in particular testing for rheumatoid factor (RF) and autoantibodies to citrullinated proteins. These latter

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autoantibodies are collectively termed *antibodies to citrullinated protein antigens* (ACPAs). ACPAs are highly specific for RA, and as such, they have become one of the prime biomarkers for the diagnosis of RA [3•]. Additionally, the biology of autoimmunity to citrullination is driving investigations into the pathogenesis of RA [4]. Herein we review the role of ACPA testing in the management of RA. Additionally, we review the potential role that citrullinated proteins potentially play in the pathophysiology of RA development.

Discovery of Antibodies to Citrullinated Protein Antigens

Antibodies to citrullinated proteins were first reported in association with RA in the 1960s and were initially termed *antiperinuclear factors* (APFs) [5]. Over time, other highly RA-specific autoantibodies to keratin were discovered, although it later became apparent that both APFs and antikeratin antibodies were likely binding to the same antigen: filaggrin [6]. Ultimately, the binding of APFs and antikeratin antibodies was realized to be directed to sites within the protein containing the post-translationally modified amino acid citrulline [6–10]. Multiple citrullinated protein targets have since been identified that serve as targets in RA-related autoimmunity, including vimentin, fibrin, fibrinogen, fibronectin, filaggrin, and enolase, with each of these proteins likely having multiple citrullinated sites that may serve as antigens [4].

Antibodies to Citrullinated Protein Antigens Testing in Clinical Practice

Many assays are available to test for ACPAs to specific antigens; however, most of these are used primarily for research purposes. For the clinical management of RA, most ACPA testing is performed using widely available commercial assays that utilize a synthetic cyclic citrullinated protein (CCP) as the antigen to detect ACPAs, although for proprietary reasons, the specific antigens present within these kits are not widely known. (The cyclic nature of the antigen in these assays is thought to improve antigen stability and diagnostic accuracy [10].) Several generations of anti-CCP tests are now available, each with different features and diagnostic accuracy for RA. Additionally, there is an ACPA test that identifies autoantibodies to modified/mutated citrullinated vimentin (anti-MCV).

CCP1

The earliest version of anti-CCP testing was designated anti-CCP1 and contained antigen constructed of a cyclic version of citrullinated filaggrin (CCP1 is the only one of the CCP assays in which the structure is published in widely available literature) [9–12]. However, this first-generation synthetic CCP antigen has been altered to improve diagnostic accuracy; this early version subsequently has largely been replaced in clinical use by later generations of tests.

CCP2

The second generation anti-CCP test (CCP2) has been available for clinical use since the early-2000s and is currently the most commonly used assay in clinical and research applications. Multiple versions of anti-CCP2 diagnostic kits are commercially available; however, as these kits all use a similar antigen plate and have similar overall diagnostic accuracy for RA, herein we focus on two commonly used versions: the INOVA Quanta Lite

CCP2 IgG kit (I-CCP2) (INOVA Diagnostics, San Diego, CA) and the Axis-Shield Diastat CCP2 IgG kit (AS-CCP2) (Axis-Shield, Dundee, Scotland, United Kingdom). Both are enzyme-linked immunosorbent assay (ELISA) kits that detect IgG antibodies to a second-generation synthetic CCP antigen (the specific citrullinated proteins are proprietary and therefore not widely known). In studies reported by the manufacturer, when tested in 949 individuals (252 with established RA, 216 random blood donors, 336 individuals with other rheumatic diseases, and 145 with infectious diseases), the sensitivity and specificity of I-CCP2 were found to be 76.6% and 94.8%, respectively [13]. Studies conducted by the manufacturer of AS-CCP2 demonstrated a sensitivity for RA of 62% when tested in 412 individuals with RA, and a specificity for RA ranging from 67% to 100% when tested against healthy individuals and those with other rheumatic diseases [14].

Several studies have compared the diagnostic accuracy of CCP2 with that of other CCP tests. Overall, the diagnostic accuracy for RA seems to be modestly greater with CCP2 compared with other ACPA kits; in particular, in a large meta-analysis published in 2010, CCP2 was equally sensitive but overall more specific than RF, CCP1, CCP3, or anti-MCV [3•]. Additionally, anti-CCP2 appears to be a better prognostic indicator for joint damage than anti-CCP1 [11]. Going forward, CCP2 as an antigen to detect ACPAs likely will be in clinical use for some time, as it is being utilized in multiple large clinical laboratories as the antigen for newer high-throughput, bead-based methodologies for autoimmune rheumatic disease using platforms such as the BioPlex 2200 (Bio-Rad Laboratories, Hercules, CA). However, the diagnostic accuracy of testing using the CCP2 antigen on such platforms will need to be carefully evaluated in adequately powered studies in comparison to standard ELISA-based testing.

CCP3

The INOVA Quanta Lite CCP3 IgG kit (CCP3) (INOVA Diagnostics, San Diego, CA) is an ELISA that detects IgG antibodies to a new third-generation, synthetic CCP antigen designed to increase the sensitivity while maintaining a high specificity for RA. Despite the relatively high sensitivity of the anti-CCP2 test, some patients with RA who are negative for CCP2 are positive for using the CCP3 assay [15, 16]. For example, in testing of 156 patients with established RA, 166 random blood donors, 113 individuals with other rheumatic diseases, and 86 with infectious diseases, the manufacturers reported a slightly higher sensitivity of 74% with CCP3 (compared with 69% with CCP2), although the specificity of the anti-CCP3 kits was slightly lower at 96% (compared with 98% for the CCP2 assay) [17]. However, in a 2010 meta-analysis, testing with CCP3 demonstrated a similar overall sensitivity for RA when compared with CCP2 (range, 48%–81% vs 57%–78%) but lower specificity (range, 85%–95% vs 94%–96%) [3•].

CCP3.1

All previously described assays for anti-CCP detect only IgG antibodies to CCP, but the INOVA Quanta Lite CCP3.1 IgG/IgA kit (CCP3.1) detects both IgA and IgG antibodies against the third-generation CCP. The manufacturer (INOVA Diagnostics, San Diego, CA) reports that this CCP3.1 kit is slightly more sensitive, with equivalent specificity as compared with CCP2 [18]. Testing of 942 individuals (495 with RA, 275 random blood

donors, 74 individuals with other rheumatic diseases, 98 with infectious diseases) found a sensitivity and specificity for CCP3.1 of 70% and 98%, respectively, whereas the sensitivity and specificity for CCP2 were 65% and 98%, respectively. In early RA (diagnosis within the past 2 years), CCP3.1 was more sensitive than CCP2 (64% vs 55%) [18].

Anti–Modified/Mutated Citrullinated Vimentin

The clinically available anti-MCV tests evolved out of the original anti-Sa antibodies (so named because they were identified in a patient whose name began with “Sa”) [19]. These autoantibodies were later discovered to be directed to vimentin, with the anti-MCV test now utilizing a genetically altered form of vimentin (arginines replaced with glycines) [20]. There are several manufacturers of the anti-MCV test, including Exagen Diagnostics (Albuquerque, NM) and Orgentec Diagnostika (Mainz, Germany). For clinical purposes, the anti-MCV test is not performed in local laboratories (as are anti-CCP tests); rather, blood is sent to centralized laboratories for testing, with results reported back to the clinician. A recent meta-analysis of anti-MCV testing for the diagnosis of RA suggested that it has a sensitivity ranging from 64% to 84% and a specificity of 79% to 96%. Although anti-CCP2 testing has been demonstrated to be more specific than anti-MCV testing [21], several reports have suggested that anti-MCV testing may be positive in patients with RF and anti-CCP–negative RA, allowing for improved classification of such patients as having RA, especially in early disease [22].

Data are limited regarding which patient-specific factors may influence the diagnostic accuracy of ACPA testing for RA. However, the anti-CCP kits appear to have similar diagnostic accuracy across age, gender, and racial/ethnic groups, although some groups of Native American patients with RA may have higher rates of positivity when compared with other racial/ethnic groups [23]. Smokers with RA have been shown to have higher levels of anti-CCP2 than nonsmokers [24]; however, the implications of this finding related to the diagnostic accuracy of ACPA testing for RA are unclear.

In summary, anti-CCP and anti-MCV tests have overall fairly similar sensitivity and specificity for RA (summarized in Table 1), although anti-CCP2 kits may have modestly increased overall diagnostic accuracy. However, a caveat to the reported diagnostic accuracies of ACPA testing for RA is that they have been calculated largely using the American College of Rheumatology (ACR) 1987 revised RA classification criteria as the “gold standard” for disease—a gold standard that did not include ACPA testing [25]. With the introduction of the new ACR/European League Against Rheumatism (EULAR) classification criteria for RA that incorporate ACPA testing [26•], these diagnostic accuracies will need to be revised; importantly, these calculations will need to account for the inclusion of ACPA testing within the criteria.

Antibodies to Citrullinated Protein Antigens Testing in Early Versus Late Rheumatoid Arthritis

In addition to being highly specific for established RA, ACPA testing may improve the sensitivity of early classification of patients as having RA. In particular, if the 1987 ACR

criteria are used as the gold standard for a diagnosis of RA, and ACPA positivity is allowed as a criterion for diagnosis, then several studies suggest that ACPA testing by anti-CCP methodologies may improve the overall sensitivity for RA compared with RF testing alone, with greater increases in sensitivity in the classification of patients with a shorter duration of disease (<6 months) [27]. However, these results are not conclusive, as a 2010 meta-analysis suggested that ACPA testing overall may be less sensitive (48%–73%) in early RA (patients with disease of <2 years' duration) compared with established RA (sensitivities of 79%–81%), perhaps limiting the usefulness of ACPA testing in patients with a short duration of symptoms of idiopathic arthritis (IA) [3•]. These reported differences in assay sensitivity in early versus late RA do have their limitations because they have been analyzed largely using separate cohorts and not in the same individuals over time [3•]. Also, early disease cohorts may have contained patients with IA from causes other than RA, thereby falsely reducing the sensitivity of ACPA testing for early RA-related disease. To inform this latter point, to date, limited published data are available regarding the evolution of ACPA positivity over time in patients who present with early, undifferentiated IA. However, in a recent meta-analysis, the development of new anti-CCP positivity occurred in less than 10% of individuals originally presenting with anti-CCP-negative IA, suggesting that in most, anti-CCP presents at least concurrently with the onset of symptomatic IA (and, as discussed below, perhaps prior to the onset of clinically apparent IA).

There are methodologies currently in development that will allow for testing of autoantibodies to a wide variety of specific citrullinated proteins, although it remains to be seen how these broad-spectrum ACPA testing platforms may increase our ability to classify early IA as RA, as well as guide management and influence outcomes. Additionally, the new ACR/EULAR classification criteria for RA were established in part to utilize ACPA testing to establish a methodology by which patients with IA that would prove to be persistent and destructive could be classified as having RA earlier in their disease course [26•]. In limited studies to date, these new criteria appear to identify patients with RA earlier in the time course of disease than the 1987 ACR RA criteria, although the specific contribution that ACPA testing has made to these new RA criteria allowing for an earlier diagnosis of RA has yet to be elucidated fully [25, 28].

Antibodies to Citrullinated Protein Antigens Testing in Predicting (and Improving) Outcomes in Rheumatoid Arthritis

Multiple studies suggest that ACPA testing can determine which patients with IA who may or may not meet full classification criteria for RA at initial presentation will have persistent, aggressive disease [29, 30]. Furthermore, in both early and established RA, elevated anti-CCP levels are associated with other markers of RA severity, including the presence or development of erosive disease, extra-articular disease, elevated C-reactive protein, and worse patient- and physician-reported measures of disease [31–33]. Also, there has been an association of anti-CCP positivity with increased risk of cardiovascular disease and total mortality [1, 34].

However, it is not clear if ACPA testing is always a significantly better predictor of more severe disease (including extra-articular disease) than other biomarkers, including RF. This

is a difficult issue to sort out, as most patients with seropositive RA are both RF and ACPA positive, although studies that have included patients with isolated RF or ACPA positivity suggest that ACPAs are a better predictor of worse prognosis than RF [32]. Therefore, additional studies are needed to determine the exact role that ACPA testing will have in predicting outcomes in RA, especially in relationship to other biomarkers.

While ACPAs may predict persistent, more severe disease, even in patients with undifferentiated IA, a major question is whether or not ACPA testing leads to improvements in the overall outcomes of disease. If an earlier diagnosis of RA based on ACPA testing leads to earlier use of appropriate therapies that in turn improves outcomes of disease, then ACPA testing may well lead to improved disease outcomes. Also, if ACPA testing leads to identification of patients at a high risk of adverse long-term outcomes, and this knowledge is used to guide therapy, then outcomes may be improved. However, it is not clear if other clinical parameters, such as tender and swollen joint counts, functional status, or inflammatory markers, may be better measures than ACPA testing to guide therapy. Further studies are needed to evaluate these issues.

Antibodies to Citrullinated Protein Antigens Elevations and Responses to Disease-Modifying Antirheumatic Drugs and Biologic Therapies

ACPA elevations may predict responses to certain antirheumatic therapies. In the Probable RA Methotrexate Versus Placebo Therapy (PROMPT) trial, in which patients with early, undifferentiated IA were randomly assigned to receive methotrexate or placebo, patients with anti-CCP positivity who received methotrexate appeared to have the highest rates of remission and reduction in disease progression [35]. Also, ACPA testing in conjunction with cytokine testing has been shown to predict improved response to the anti-tumor necrosis factor (TNF) agent etanercept [36]. However, there are also data that suggest that ACPA positivity is associated with decreased response to therapy, although differences in duration of RA and prior treatments for disease may affect these findings. For example, Potter et al. [37] found in a study of 642 patients in the United Kingdom with longstanding RA that ACPA (and RF) positivity was associated with a decreased response to anti-TNF therapy at 6 months, as measured by Disease Activity Score 28. It is also unclear whether ACPA elevations may predict response to B-cell depletion with rituximab. In a study of 208 patients with RA who had failed prior therapies, elevations of anti-CCP or RF predicted an improved response to rituximab [38]. However, in a study of 110 patients with RA in Italy, elevations of RF, but not anti-CCP, were associated with improved responses to rituximab [39].

Several other features of ACPA elevations suggest that they may be related to severity of RA, and that ACPAs may be used as a marker to predict response to therapies. In particular, higher ACPA levels may predict worse disease activity, and ACPA levels may decrease to some extent in RA after treatment with a variety of agents, although ACPA levels rarely become negative. In particular, Roland et al. noted that anti-MCV antibodies fell faster than other autoantibodies, including anti-CCP, in response to treatment with the anti-TNF agent infliximab, although the significance of this finding in the management of patients with RA is unclear [40–43]. Additionally, a 2011 Swedish study demonstrated that current smoking is

associated with a decreased response to disease-modifying antirheumatic drugs and biologic therapies in established RA [44]. Because smoking has been associated with increased ACPA levels [24], it may be that there is a mechanistic link among smoking, elevations in ACPAs, and decreased disease response to therapy [44]. Overall, however, more studies are needed before we can determine the specific mechanisms by which ACPAs may be associated with responses to therapy with disease-modifying antirheumatic drugs and biologic therapies.

Antibodies to Citrullinated Protein Antigens Testing and Juvenile Idiopathic Arthritis

The classification of juvenile idiopathic arthritis (JIA) encompasses a variety of types of arthritis, including disease that resembles adult-type RA (persistent polyarticular, erosive disease), as well as other entities, including Still's disease and seronegative spondyloarthropathies. Most forms of JIA are ACPA negative, especially monoarticular or oligoarticular disease in children with disease onset at younger than 16 years of age; however, if ACPA testing is positive in JIA, it is often in patients with age at onset of disease greater than 16 years, in RF-positive patients, and in patients with more diffuse (ie, polyarticular) and persistent inflammatory arthritis that resembles adult-onset RA [45]. Therefore, ACPA testing in children with IA may be a predictor of more persistent, destructive disease.

Antibodies to Citrullinated Protein Antigens Positivity in Other Diseases

Despite the high specificity of ACPAs for RA, individuals with other rheumatic diseases, including psoriatic arthritis, systemic lupus erythematosus (SLE), systemic sclerosis, and primary biliary cirrhosis, may be ACPA positive, although the types of ACPA testing used in these studies have varied widely [46–50]. The implications of ACPA positivity in these supposedly non-RA rheumatic diseases are unclear; however, most studies have identified that ACPA elevations are associated with more severe joint disease regardless of the other features of autoimmune disease, suggesting that ACPAs are part of inflammatory joint disease even if RA is not clearly classifiable as the primary disease. For example, two studies including more than 200 patients with psoriatic skin disease (with or without arthritis) found that about 7% to 8% of those with psoriasis were positive for anti-CCP2; however, anti-CCP2 positivity was rare (<1%) among patients with psoriasis in the absence of joint disease [51, 52]. Also, a high proportion of patients with psoriasis, arthritis, and anti-CCP2 positivity also fulfilled 1987 ACR classification criteria for RA, suggesting that these patients may have actually had RA, or at least that anti-CCP2 positivity in the setting of psoriatic arthritis was associated with a more RA-like arthritis [51]. Kakumanu et al. [46] also found that about 17% of SLE patients had elevated anti-CCP1 antibodies, and that anti-CCP1 elevations in patients with SLE correlated with the presence of erosive/deforming arthritis, perhaps representing an overlap syndrome with RA. There are conflicting data regarding anti-CCP positivity in patients with familial Mediterranean fever (FMF). Ceri et al. [53] reported a prevalence of anti-CCP positivity (specific kit not described) of 14.5% in 83 patients with FMF, and in these patients, anti-CCP was associated with arthritis [53].

However, in another study of 49 patients with FMF (23 with arthritis during attacks), Karatay et al. [54] found no elevations of anti-CCP (CCP generation not specified).

Importantly, ACPA positivity in non-RA rheumatic diseases may also represent nonspecific antibody reactivity and not true autoimmunity to citrullinated proteins. For example, Kakumanu et al. [46] noted that a subset of patients with SLE who were anti-CCP1 positive also had high rates of reactivity to a noncitrullinated cyclic arginine antigen, suggesting that some anti-CCP reactivity in SLE might actually not be specific for citrulline. Also, the anti-CCP3 test has been reported to have increased positivity in patients with scleroderma, especially limited disease. Wu et al. [55] found that 29% of 52 patients with the CREST syndrome were anti-CCP3 positive (although these patients were not anti-CCP2 positive). The authors postulated that anti-CCP3 reactivity in this study may have been due to cross-reactivity of the CCP3 antigen with other antigens found in scleroderma, as there was a high correlation between anti-CCP3 antibodies and anticentromere antibodies [55, 56]. However, another study of patients with scleroderma found similar rates of positivity (~14%) between these two kits (CCP2 and CCP3), although in this latter study, the patients may not have had limited scleroderma; thus, it may not have evaluated the same types of patients as did Wu et al. [50, 55]. It has been reported that higher ACPA elevations are typically more specific for RA than for non-RA [3•]; however, this has not yet been demonstrated conclusively, as Ingegnoli et al. [57] reported high-titer anti-CCP in scleroderma. As such, further investigations are needed to determine how titers of ACPA elevations may be used to distinguish RA from non-RA conditions.

Finally, ACPA positivity has been noted in approximately 30% of patients with active pulmonary tuberculosis and no evidence of articular RA, with the citrullinated antigens in the two major studies with these findings being CCP2 (Elkayam et al. [58]) and CCP1 (filaggrin) (Kakumanu et al. [12]). However, it was later demonstrated that in these patients with tuberculosis, ACPA reactivity may have been due to non-citrulline-specific antibody reactivity, as these patients had similar antibody reactivity to noncitrullinated arginine residues [12, 59]. As such, ACPA positivity in non-RA conditions needs to be interpreted cautiously, with consideration of use of control antigens to determine the true ACPA reactivity.

Antibodies to Citrullinated Protein Antigens and the Pathophysiology of Rheumatoid Arthritis

As discussed previously, detecting the presence of ACPAs is of diagnostic and prognostic value in individuals with RA; however, autoimmunity to citrullinated proteins also has implications in the pathogenesis of RA, and ACPAs have formed the core of a great deal of recent research into the mechanisms by which RA may develop.

Biology of Citrullination

Citrulline contained within proteins is created post-translationally by the action of the enzyme peptidyl arginine deiminase (PAD) on the amino acid arginine [4]. Multiple forms of PAD are found in humans (types 1–4 and 6), and PAD is present in a variety of cell types

[4]. The two forms of PAD that appear to be most important for citrullination related to RA are PAD2 (expressed in multiple tissues, including macrophages) and PAD4 (expressed primarily in bone marrow and granulocytes, especially neutrophils) [4]. Citrullination takes place in several normal cellular processes, including inflammation, apoptosis, and cellular differentiation. Additionally, tissues involved in inflammation from a multitude of diseases have increased levels of citrullinated proteins; these tissues and diseases include neural plaques in multiple sclerosis, hippocampal tissue in patients with Alzheimer's disease, and glomerular tissue in patients with obstructive nephropathy [4, 60].

Role of Antibodies to Citrullinated Protein Antigens in the Pathogenesis of Rheumatoid Arthritis

Because citrullination is a common biologic occurrence, the reasons for the high specificity of ACPAs for RA are not known; however, it may be that the underlying genetic and environmental factors related to RA act in concert to generate citrullinated proteins and then present in a specific manner these citrullinated proteins to the immune system so as to generate autoimmunity [61]. For example, smoking (one of the strongest known risk factors for RA) or other environmental factors may lead to increased formation of citrullinated proteins [62]. Then, in the presence of genetic risk factors for RA, including the shared epitope, citrullinated proteins may be presented to the immune system, breaking tolerance to these antigens and leading to the subsequent development of ACPAs [62]. The strong association of ACPA elevations with specific *HLA* alleles containing the shared epitope supports the idea that genetic factors may be an important aspect in the development of autoimmunity to citrullinated proteins [4]. Additionally, the work by Hill and colleagues [61] that demonstrated that citrullination of proteins increased their affinity for *HLA DR4* molecules provides a mechanistic basis by which genetic factors may lead to ACPA generation [61].

However, despite their strong association with RA, it is unclear (even if they are present) what precise role(s) ACPAs play in the pathogenesis of RA, although many studies suggest that autoimmunity to citrullinated proteins is likely a key aspect of the development of RA. For example, as discussed previously, the association of ACPAs with more severe RA (or arthritis in non-RA conditions such as SLE) suggests that autoimmunity to citrullinated proteins is directly pathogenic. Also, immunization with citrullinated peptides can lead to inflammatory arthritis in animal models of disease, and infusion of antibodies to citrullinated proteins leads to more severe arthritis [63, 64]. Additionally, in several studies of human RA, increased levels of citrullinated proteins have been found within the diseased joints and in circulating immune complexes [4]. T cells from humans with RA also have been noted to react to citrullinated vimentin, which induced arthritis in an experimental murine model [65]. Also, recent work demonstrating that circulating immune complexes containing citrullinated fibrinogen activate inflammation through interactions with Toll-like receptor 4 provides a potential mechanism by which circulating ACPAs may induce joint-specific inflammation [66]. The association of specific polymorphisms in the PAD enzyme and increased RA risk in certain Asian populations also suggests a pathogenic role for citrullination in RA, although a similar association between PAD polymorphisms and RA has not been seen in patients of European descent [67].

Perhaps most importantly in determining the role of ACPAs in the earliest pathogenic processes in RA are the findings in multiple human studies of elevated ACPAs as well as RF a substantial period of time prior to the onset of symptomatic synovitis, which suggest that autoimmunity to citrullinated proteins is an important factor in the development of RA [68–71]. In particular, the “preclinical” elevations of ACPAs have led to the creation of several specific hypotheses regarding the role of autoimmunity to citrullinated proteins in the pathogenesis of RA. For example, the strong association of exposure to tobacco smoke with ACPA positivity in RA in the setting of the shared epitope has led researchers to hypothesize a potential mechanism for RA development that is highly dependent on ACPA generation [62]. In this model, smoking leads to increased citrullination, which in the appropriate genetic background then leads to autoimmunity to citrullinated proteins and, subsequently, the development of clinically apparent RA [62]. This model of RA development is compelling, at least in terms of ACPA-positive RA that occurs in smokers, although the specific anatomic sites and mechanisms by which smoking leads to ACPA generation and RA in in vitro biologic systems have yet to be elucidated. Additionally, citrullinated proteins as a possible link among periodontal disease, infection with the organism *Porphyromonas gingivalis*, and the initiation and development of RA are being explored [72•].

Antibodies to Citrullinated Protein Antigens Testing in Prediction of Future Rheumatoid Arthritis: A Pathway Toward Prevention?—

The discovery of preclinical elevations of ACPAs has led many researchers to hope that testing for these autoantibodies in currently asymptomatic individuals may identify individuals at high risk of future RA, and that such high-risk individuals, once identified, may be targeted with preventive interventions [73]. Certainly, the high specificity of ACPAs for RA leads to it having a high positive predictive value (PPV) for future disease; in several studies, the PPV of ACPA for future RA has been reported to be greater than 90% [73]. However, there are limitations to these findings. Rantapaa-Dahlqvist et al. [70] demonstrated this by showing that if the prevalence of RA is estimated at 1% in widespread population screening, the PPV for future RA of ACPAs is less than 50%.

Additional issues need to be considered to understand the predictive capability of ACPA testing for future RA in currently asymptomatic individuals. Most studies suggest that ACPA positivity and levels are relatively stable throughout the course of RA, with most patients presenting with inflammatory arthritis and ACPA positivity simultaneously, although it still may take some time for the patient to develop full criteria for classification as RA. However, some studies have noted that some patients who initially presented with IA and negative ACPA testing (anti-CCP) later developed ACPA positivity. A meta-analysis published in 2009 found that 1.3% to 8.9% of patients with IA were initially ACPA negative but developed positivity over time [74]. The apparent conversion of patients with IA from ACPA negative to positive status over time may be a result of our current lack of the correct ACPA assays to detect early autoimmunity, or it may be that certain individuals truly do not have pre-IA, circulating, asymptomatic, RA-related autoimmunity. Also, because there may be differential ability of the currently available ACPA tests (in particular the various versions of anti-CCP tests) to detect early ACPAs, the diagnostic accuracy of these tests for

future RA needs to be examined in prospective studies of currently asymptomatic individuals. These issues will need to be further explored before ACPA testing can be utilized widely for the evaluation of preclinical RA, although such testing holds great promise for our future ability to predict accurately the future onset of RA, and perhaps prevent disease.

Conclusions

ACPAs are a class of autoantibodies that are highly specific for RA. Importantly, ACPAs are predictive of persistent, severe arthritis and may allow for earlier identification of patients who require more aggressive therapy. Additionally, ACPAs have provided valuable clues to the underlying pathophysiology of RA. Going forward, further studies are needed to determine the exact role of clinical ACPA testing in the improvement of outcomes of RA. Further studies are also needed to determine the specific role that ACPAs play in the development of RA, as well as in the prediction and prevention of future disease in currently asymptomatic individuals.

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Table 1
Summary of the diagnostic accuracy for rheumatoid arthritis of the clinically-available
ACPA diagnostic kits^a

Kit	Sensitivity, %	Specificity, %
CCP2	51–80	92–98
CCP3	48–82	81–96
CCP3.1	64–81	98
Anti-MCV	66–82	72–91

^aThese represent the sensitivities and specificities for rheumatoid arthritis as determined by the 1987 American College of Rheumatology classification criteria ACPA—antibody to citrullinated protein antigen; CCP—cyclic citrullinated protein; MCV—modified/mutated citrullinated vimentin

(Data from Whiting et al. [3•], Axis-Shield [14], INOVA Diagnostics [17], and INOVA Diagnostics [18].)