

EXPERT
REVIEWSThe case for measuring
antibodies to specific
citrullinated antigens*Expert Rev. Clin. Immunol.* 9(12), 1185–1192 (2013)**Anna B Montgomery¹,
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Anti-citrullinated protein/peptide antibodies (ACPA) are the principal autoantibody system associated with rheumatoid arthritis (RA), with diagnostic sensitivity of 70% and specificity of 95%. Current testing for ACPA uses the anti-cyclic citrullinated peptide assay (anti-CCP) which measures a generalized reactivity with citrulline-containing peptides, thus giving no insight into reactivity to specific RA antigens. Of these, the best characterized are, α -enolase, fibrinogen/fibrin, vimentin, Type 2 collagen and filaggrin, antibodies to each of which are found in approximately 30–60% of RA cases. Given reports of cross-reactivity between citrullinated antigens, we discuss whether or not measuring these specific antibodies could aid: clinical diagnosis, identification of clinical subsets and drug responses, or provide insight into pathogenic mechanisms or etiology of RA.

KEYWORDS: ACPA, anti-CCP, auto-antibody, citrullination, rheumatoid arthritis

Antibodies to citrullinated peptides were first described only 15 years ago when it was discovered that the previously described antiperinuclear factor assay, known to be diagnostically specific for rheumatoid arthritis (RA), was detecting antibodies to filaggrin that had undergone the post-translational modification of citrullination (or deimination) [1,2]; the conversion of an arginine residue to a citrulline residue by peptidylarginine deiminase (PAD) enzymes. Although not a true auto-antigen due to lack of presence in the joint, filaggrin was found to be an effective capture antigen for ACPA due to high numbers of arginine residues and thus citrulline residues after citrullination. However, cross-reactivity between citrullinated filaggrin and fibrinogen peptides indicates filaggrin could still be of pathological significance in RA through epitope spreading, as well as giving an early indication of the potential for cross-reactivity between ACPA. Using peptides spanning the filaggrin molecule, reactivity of serum antibodies occurred only with citrullinated variants of the peptides and not with their arginine-containing controls. So citrulline-specific was this response, many commentators have used the term ‘anti-citrulline autoimmunity’ to describe the auto-antibody response in RA. However, it is clear

that a citrulline residue alone is insufficient to determine antibody binding to a peptide, because not all citrulline-containing sequences act as epitopes for autoantibodies. As can be seen in TABLE 1, the immunodominant epitope in citrullinated filaggrin (cit-filaggrin) has citrulline residues immediately flanked by glycine, serine or threonine. Interestingly, on the immunodominant epitope of citrullinated enolase (CEP-1), the citrulline at position 15 is flanked by glycine and serine [3], however, other epitopes on enolase, and the major epitopes on citrullinated fibrinogen (cit-fibrinogen), vimentin (cit-vimentin) and collagen (cit-collagen) are seemingly flanked by an almost random assortment of amino acids, though there does seem to be a preference for small and relatively neutral amino acids, with glycine or serine in particular, being common neighbors of the citrulline residues. Therefore, it can be concluded that the mere presence of citrulline is not enough to determine an ACPA response: there is also some dependence on the flanking amino acids.

The question then arises: in the context of the whole protein, can these epitopes be described as truly antigen-specific? In favor of such specificity are the numerous studies which show that ACPA react with only a tiny

Table 1. Well-characterized citrullinated autoantigens in rheumatoid arthritis.

Antigen	Present in joint	Sensitivity	Reactive epitope(s)	Ref.
α -enolase	Yes	50%	'CEP-1' 5^{KIHAXEIFDSXGNPTVE}21 324 ^{NPXKXIAKAVNEKSCNCLL} 341	[3]
Fibrinogen/fibrin	Yes	66%	α-chain 36^{GPXVVEHXQSACKDS}50 171 ^{VDIDIKIXSCXGSCS} 185 501 ^{SGIGTLDGFHXHPD} 515 621 ^{XGHAKSXPVXGIHTS} 635 β-chain 60^{XPAPPPISGGGYXAX}74	[7]
Vimentin	Yes	40%	59^{VYATXSSAVXLXSSVP}74 1^{STXSVSSSSYXXMFGG}16	[69]
Collagen type 2	Yes	40%	59^{AXGLTGXPDA}369	[70]
Filaggrin	No	28%	306^{SHQESTXGXSRGRSGRSG}324	[1]

Peptide sequences in bold: immunodominant; peptide sequences not in bold: reactive; X: Citrulline residue.

proportion of the citrullinated proteins found in the synovial membrane or in citrullinated cell extracts [4,5]. Other studies have pointed out how there is a distinct pattern of autoantibody specificity for each serum examined (reviewed in [6]).

However, against the concept of distinct antigen specificity are the numerous demonstrations of cross-reactivity. As well as the cross-reactivity initially observed between cit-filaggrin and cit-fibrinogen [7,8], Amara *et al.* showed that a number of monoclonal ACPA from the joints of RA patients had cross-reactivity between CEP-1 and cit-vimentin peptides, and a smaller number between CEP-1 and cit-fibrinogen peptides [9]. van de Stadt *et al.* also demonstrated cross-reactivity between CEP-1 and citrullinated fibrinogen using two monoclonal anti-citrullinated fibrinogen antibodies isolated from RA sera [10]. Snir *et al.* however found a low level of cross-reactivity between CEP-1 and cit-fibrinogen in a limited number of sera using absorption experiments, indicating some patients had non-cross-reactive antibodies [11]. In addition, other antigens, including the cit-collagen peptide and CEP-1 showed no cross-reactivity. Other studies with ACPA have also demonstrated heterogeneous and non-overlapping reactivities [12,13].

Although cross-reactive ACPA have been shown to bind these secondary substrates with lower avidity than their primary substrates, the lower avidity of ACPA binding seen in general when compared with recall antigens does question if primary reactivities exist or if indeed there is an as yet unknown progenitor antigen [14]. There is also potential for cross-reactivity between ACPA and a newly characterized group of autoantibodies in RA: anti-carbamylated protein antibodies (anti-CarP) in light of similarity between homocitrulline residues found in carbamylated proteins and citrulline [15].

All of these studies of cross-reaction can be criticized on a number of grounds, including the use of peptides, which may

not express conformational epitopes on naturally occurring proteins, and the fact that monoclonal antibodies may not be representative of serum antibodies. Nevertheless, there does appear to be an emerging consensus that antibodies to specific citrullinated antigens do indeed exist in RA, though they appear to be much more cross-reactive than the autoantibodies found in other autoimmune diseases.

Clinical diagnosis

Prior to the discovery of the relevance of ACPA to RA diagnostics, antibody testing was restricted to the presence of rheumatoid factor (RF): auto-antibodies reactive for the Fc region of native IgG class antibodies. Although useful in diagnostics with sensitivity of 60–80%, the presence of RF in other autoimmune diseases, systemic lupus erythematosus (SLE)

and Sjögren's syndrome reduces diagnostic specificity. Testing for ACPA (ACPA⁺/ACPA⁻) is useful in both classification of RA and in predicting progression of undifferentiated arthritis or arthralgia. It has been shown that ACPA⁺ arthralgia patients are more likely to develop RA than ACPA⁻ patients and van de Stadt *et al.* included ACPA status in a 9-point prediction rule for development of RA [16]. Studies have also shown a large range of ACPA specificities increases this risk of disease progression [17–19], with one study comparing ACPA⁺ RA patients with ACPA⁺ non-RA controls finding 82% of RA patients had more than 2 ACPA, compared with only 4.8% of controls [20].

Defining ACPA positivity typically uses a generic test such as the second- or third-generation anti-CCP assays (anti-CCP2/CCP3). However, there are others which efficiently capture the majority of ACPA, such as the anti-mutated citrullinated vimentin (anti-MCV) ELISA [21]. Although the anti-MCV test uses recombinant vimentin, the amino acid mutations introduced to capture more ACPA reactivity than citrullinated vimentin alone make the anti-MCV a generic test for ACPA rather than a specific antigen assay. A number of studies have compared anti-MCV and anti-CCP, in general finding they are similar in sensitivity and specificity both in diagnosis and prediction of RA [22]. Moreover, the two assays overlap considerably, and using the two assays in combination confers only a marginal increase of sensitivity and predictive value [23].

Given the considerable overlap between different generic tests for ACPA, it is questionable whether testing for specific antigens would improve diagnostic sensitivity or specificity. An analysis of 291 RA sera tested for antibodies to whole citrullinated fibrinogen, cit-vimentin, cit-collagen and CEP-1 showed almost all of the antibodies to the specific peptides fell within the anti-CCP positive population and that each test on its own

had a much lower diagnostic sensitivity than anti-CCP and thus would be of limited use in diagnosing established RA [11]. Antibodies to the same specific peptides in patients with very early and undifferentiated arthritis showed no increase in predictive value over anti-CCP testing alone [24]. Studies which use larger numbers of peptides pick up a proportionally larger number of patients who are anti-CCP negative [25], but the small increase in overall diagnostic sensitivity does not really justify their use in routine diagnosis.

Clinical subsets & response to treatment

Of those diagnosed with RA, ACPA status effectively divides patients into two large subsets which differ in severity and clinical outcome [26]. ACPA⁺ RA is associated with a more severe phenotype measured by 28-joint Disease Activity Score (DAS28), Visual Analog Scale (VAS) and radiographic scores of joint damage [27–29] as well as an increased risk of secondary disorders of the cardiovascular system [30,31]. However, it could be argued that, at over 70% of RA patients, the ACPA⁺ population is hardly a ‘subset’ and therefore the subgroups within this population, as defined by antibodies to specific peptides, are likely to be of more interest. One study of 661 RA patients examined antibodies to citrullinated whole myelin basic protein, and a number of citrullinated peptides including CEP-1, cit-vimentin and cit-fibrinogen [26]. None of the antibody assays showed any association with standard clinical characteristics, such as swollen joint count, length of morning stiffness, erythrocyte sedimentation rate (ESR) or C-reactive protein CRP, suggesting that measurements of fine specificity were not identifying clinical subsets. However, the study over time was limited to the examination of subsets defined by joint disease severity. Therefore, if fine specificities are linked to disease subsets it is likely to be extra-articular, such as lung disease or eye involvement, and much more work and more complete documentation of the clinical phenotype is required to identify such links. In a recent study by Harlow *et al.*, citrullinated proteins were immunoprecipitated from cell extracts using sera from RA patients with interstitial lung disease (ILD) [32]. This identified two citrullinated autoantigens potentially associated with RA-ILD, heat shock protein 90 α and β (Hsp90 α Hsp90 β). ELISA assays using these proteins and non-citrullinated controls, found antibodies against citrullinated Hsp90 α and Hsp90 β were largely restricted to RA-ILD patients compared with RA non-ILD patients. While this suggests that clinical subsets associated with specific ACPA may indeed exist, these findings need to be confirmed in other cohorts.

Some studies have suggested that the whole ACPA⁺ RA population may have a poorer response to anti-TNF- α therapy when compared with ACPA⁻ [33–35]. However, we were unable to further define this association by subdividing the ACPA⁺ group according to the presence of antibodies to CEP-1 and immunodominant peptides from cit-fibrinogen and cit-vimentin in a cohort of 450 anti-TNF- α -treated RA patients [36]. Conversely, ACPA⁺ RA patients have been found to respond better to treatment with methotrexate than ACPA⁻

patients, although these beneficial therapeutic effects may be reduced in patients with a high yield of ACPA [37,38]. However, this has not been studied in relation to ACPA fine specificity.

An important limiting factor of studies which fail to identify worse clinical outcomes associated with specific ACPA subsets is that these are usually not corrected for number and dose of disease-modifying anti-rheumatic drugs (DMARDs), both of which may be varied according to disease severity [27].

Pathogenic mechanisms

ACPA have been found in RA patient sera years before disease onset, and studies have shown that ACPA specificities, isotypes and titer increase during the pre-clinical phase before stabilizing after disease onset [17,39,40]. This broadening of ACPA profile pre-onset is hypothesized to occur through a process of epitope spreading. In the context of ACPA⁺ RA, this could suggest an explanation for the variation in ACPA⁺ patients in early RA (50%) and developed RA (70%), indicating a number of early RA patients have a titer or range of ACPA not yet caught by conventional testing (FIGURE 1). While this observation may also reflect a bias in cohorts of established RA toward more severe disease, it is of interest that the largest study of pre-RA ACPA fine specificity to date observed different clusters of peptide specificities [41]. One antibody pattern (anti-Fib α 591 and Vim60-75) was observed early with fluorescence intensity only increasing modestly prior to disease onset, suggesting a role in the earliest phases of ACPA autoimmunity. Two other patterns increased markedly before symptom onset, and may be involved in the initiation of clinical disease. It is also relevant to note that avidity maturation of ACPA occurs before disease onset, again confirming ACPA development pre-disease [42]. All these data indicate the presence of ACPA is integral to disease development, wherein the antibody range and titer increase until pathology can no longer be contained by immunoregulatory systems and disease ensues. ACPA contribute to the pathogenesis of RA in numerous ways. Immune complexes containing citrullinated fibrinogen and vimentin have been identified in the peripheral blood and synovial fluid of patients with RA [43,44], with experimental evidence showing these can stimulate cells of the monocyte/macrophage lineage which play a central role in the pathogenesis of RA [45]. It has also been shown that citrullination of fibrinogen increases its ability to bind to Toll-like receptor 4 (TLR4), allowing ACPA containing immune complexes to enhance macrophage TNF- α production through co-ligation of Fc γ -receptor and TLR4 [44]. ACPA can also form immune complexes with RF, with the preferential binding of RF to hypoglycosylated peptides making hypoglycosylated ACPA an ideal substrate [46], and can activate complement via both classical and alternative complement pathways [47].

Recent data suggest that antibodies to citrullinated vimentin may have a part to play in the bone loss associated with RA. Noting that high levels of ACPA were associated with raised markers of bone turnover, Harre *et al.* affinity-purified ACPA

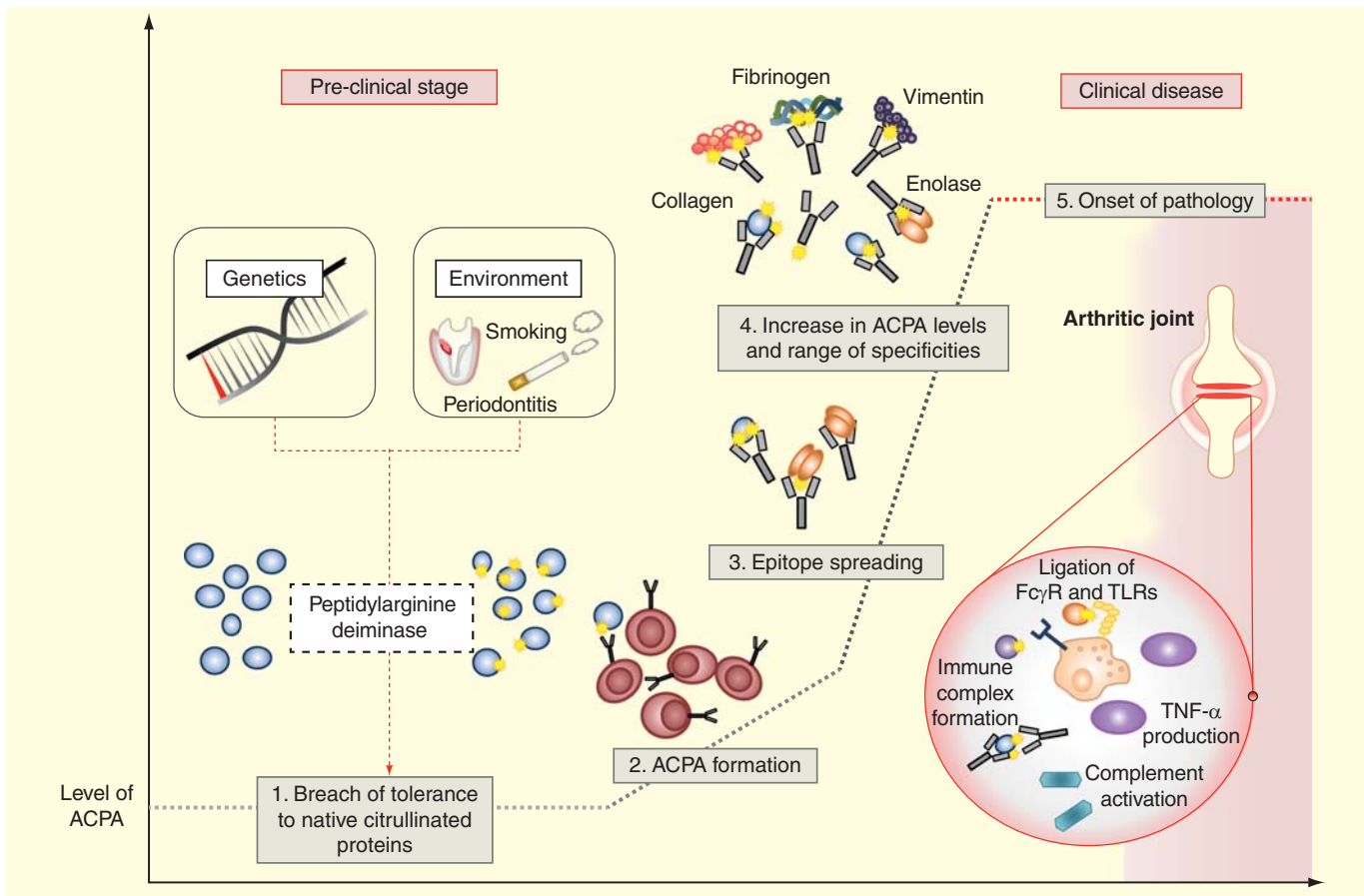


Figure 1. The formation and development of anti-citrullinated protein antibodies prior to rheumatoid arthritis onset. Breach of tolerance occurs to constitutively expressed citrullinated proteins. Integral to this are genetic rheumatoid arthritis susceptibility alleles with probable involvement of environmental factors such as periodontitis infection and smoking. 2) An antibody response occurs to native citrullinated proteins, forming ACPA. 3) Epitope spreading of citrullinated protein epitopes leads to an 4) increase in ACPA titer and range of specificities pre-rheumatoid arthritis. 5) Clinical disease occurs as ACPA begin to mediate inflammation and damage in the joint. ACPA: Anti-citrullinated protein antibodies.

using anti-MCV [48]. They observed that osteoclasts expressed increasing levels of cell-surface citrullinated vimentin during differentiation, and that addition of anti-MCV antibodies enhanced osteoclastogenesis and bone resorption both *in vitro* and *in vivo* using lymphocyte-deficient mice. The mechanism appeared to depend on production of TNF- α by osteoclast precursors. While this encourages further study of specific ACPA in relation to pathogenesis, it should be noted that the possibility of cross-reactivity was not fully excluded and would seem likely given the MCV-based purification. Thus, the possibility remains that stimulation of other citrullinated cell-surface molecules may have enhanced TNF- α production [49]. While these observations may challenge the conclusions from the more comprehensive studies of ACPA-fine specificity and clinical outcomes referred to above, these have significant limitations as observed and have not investigated systemic osteoporosis. Clearly this is an area that warrants further research and, importantly, the development of specific ACPA reagents including monoclonal antibodies, will significantly aid investigation into pathogenic mechanisms.

Etiology

Smoking has been an established risk factor for RA since 1987, but more recently has been shown to have a stronger, dose-dependent association with ACPA⁺ RA [50] proposed to be due to increased levels of citrullination in the lungs of smokers [51]. Although our group has recently shown no significant difference in the levels of citrullination in the lungs of smokers and non-smokers [UNPUBLISHED DATA], this study and others have shown the potential for the lung to act as a priming site for ACPA⁺ RA due to the presence of RA-associated citrullinated proteins and the citrullinating enzyme peptidylarginine deiminase 2 (PAD2) [51], which is also found in the RA synovium. Smoking is also linked to another risk factor for RA: periodontitis (PD). As well as the characteristic bone loss and inflammation in both conditions [52], RA patients have an increased incidence of PD [53] and an overlapping antibody response, with ACPA found in PD sera and gingival crevicular fluid [54]. Interestingly, the presence of IgA ACPA in saliva would be compatible with a mucosal trigger [55].

Porphyromonas gingivalis, an important causative agent of PD, also carries the only known prokaryotic PAD enzyme (PPAD) and is therefore capable of creating novel citrullinated antigens [56]. We and others have also demonstrated that PPAD is capable of autocitrullination [57]. We also found a more frequent antibody response to *P. gingivalis* PPAD protein in RA and PD patients (25 and 11%, respectively) compared with non-RA non-PD controls (5%) [57]. However, a citrulline-dependent antibody response to PPAD was confined to the RA group, being found in 38% compared with 2% of PD. This suggests another potential role for *P. gingivalis* in RA development, whereby an antibody response to auto-citrullinated PPAD could induce an antibody response to native citrullinated peptides through epitope spreading.

de Pablo *et al.* investigated associations with specific ACPA reactivities and periodontitis in a study of 194 patients (96 PD and 98 non-PD) [58]. PD patients had a significantly higher frequency of CEP-1 antibody positivity than non-PD patients (12 and 3%, respectively) while there was no significant difference in levels of antibodies to cit-vimentin or cit-fibrinogen. This study also noted a significantly increased antibody response to the non-citrullinated variant of the CEP-1 peptide in PD patients, which might indicate a role for non-citrullinated peptides in tolerance breakdown. While these data suggest that periodontitis may confer a higher risk toward anti-CEP-1-positive RA, potentially due to the cross-reactivity between human and *P. gingivalis* α -enolase [3], this would need to be confirmed in longitudinal studies.

Potential mechanisms for tolerance breakdown have also been investigated *in vivo* using transgenic mice expressing the RA-associated risk allele HLA-DRB1*0401 (DR4-IE-Tg). In one study, Kinloch *et al.* found that both citrullinated and non-citrullinated forms of recombinant *P. gingivalis* and human α -enolase induced a rapid onset of arthritis in DR4-IE-Tg mice but not in wild-type controls [59]. Notably, immunization with the uncitrullinated protein resulted in an antibody response to both uncitrullinated and citrullinated α -enolase, again suggesting a role for uncitrullinated proteins in tolerance breakdown. Immunization with citrullinated human fibrinogen also leads to arthritis in DR4-IE-Tg mice, although with a lower incidence (35%) [60]. Interestingly in this model, the transgenic mice were not distinguishable from controls by the presence of antibodies to citrullinated fibrinogen, but rather by antibodies recognizing citrullinated vimentin, suggesting a role for epitope spreading, influenced by HLA risk alleles, in the onset of arthritis [61]. However, these results are yet to be reproduced.

Genetic factors are integral to the development of RA (reviewed in [62], the most prominent of which are the HLA-DRB1 'shared epitope' (SE) allele and the PTPN22 R620W gene variant. The SE includes RA-associated HLA-DRB1 alleles with a similar amino acid sequence at the P4 pocket of the peptide-binding region. It is thought that SE alleles allow preferential binding and presentation of citrullinated peptides [63], whereas PTPN22 mutations may affect B- or T-cell tolerance [64]. To date, the HLA-DRB1 SE alleles have shown to be significantly

associated with cit-vimentin [65], CEP-1 [25] and cit-collagen II [11] antibodies in RA, whereas contradictory results have been seen for cit-fibrinogen peptides and whole fibrinogen [11,65].

In an extensive study of 1985 RA patients (63% ACPA⁺) and 2252 age- and sex-matched controls, Lundberg *et al.* [25] investigated the association of antibodies to CEP-1, cit-vimentin, cit-fibrinogen and cit-collagen II with the SE, PTPN22 variant and smoking. The combination of SE, PTPN22 and smoking was strongly associated with CEP-1 and cit-vimentin antibody positive subsets, and most strongly with the subset positive for both antibodies. Fisher *et al.* [66] carried out a similar study in a Korean cohort, finding the strongest association of the SE and smoking combination to be with cit-vimentin, however the PTPN22 gene variant was not investigated. Fisher *et al.* also found a gene-dosing effect of SE alleles on the number and levels of specific ACPA. Similarly in a study of 521 RA patients, Montes *et al.* demonstrated that 71% of anti-CCP⁺ patients with antibodies to cit-vimentin had HLA-DRB1 SE alleles compared with 58% of the CCP⁺ cit-vimentin-negative group, with a particularly strong association of cit-vimentin antibodies and the presence of two SE alleles being noted [67]. From what is known so far about genetic associations with ACPA specificity, the strongest correlations are seen between the SE and antibodies to CEP-1 and cit-vimentin antibodies. These associations become stronger in the presence of two SE alleles, and the addition of the PTPN22 R620W gene variant and smoking. These data indicate that genetic factors confer a greater risk for developing an antibody response to certain citrullinated autoantigens.

In summary, the data presented encourage further study of specific ACPA as this is likely to shed further light on the etiology and also the pathogenesis of RA.

Expert commentary & five-year view

There is little evidence to date that measurement of individual ACPA fine specificities adds much to the diagnosis of RA, or the prediction of disease severity and clinical outcomes. The further development of multiplex assays such as that described by Hansson *et al.* [68] may help the prediction of disease onset in those at risk of RA, as well as reducing false positive results by simultaneously testing control peptides. However, it is unknown whether the incremental gain may be enough to justify the additional cost. In the next 5 years, we may begin to see specific ACPA linked to different extra-articular manifestations of RA. The greatest advances though are likely to be seen in the further elucidation of etiology and pathogenesis in RA, with the potential for developing novel treatment strategies based on these specific pathways.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending or royalties.

No writing assistance was utilized in the production of this manuscript.

Key issues

- Anti-citrullinated protein/peptide antibodies (ACPA⁺) rheumatoid arthritis (RA) patients make up 70% of RA cases, with ACPA positivity being defined by generic tests such as anti-cyclic citrullinated peptide assay (anti-CCP).
- A number of specific, well-defined citrullinated autoantigens have been described.
- Cross-reactivity is observed to some degree between specific ACPA, however distinct patterns of reactivity and non-cross-reactive antibodies are also seen.
- Measurement of specific ACPA adds little benefit over generic ACPA tests in the diagnosis of RA or prediction of disease severity, but higher numbers of specific ACPA may predict RA in those at risk.
- No conclusive evidence yet links individual specific ACPA with any particular extra-articular manifestation.
- ACPA may be involved in unique pathogenic mechanisms; citrullination of fibrinogen induces increased TNF- α production from macrophage/monocytes and antibodies to citrullinated vimentin increase osteoclast differentiation.
- Immunodominant citrullinate enolase peptide and immunodominant citrullinated vimentin peptide ACPA are most strongly associated with the combination of HLA-DRB1 SE allele, PTPN22 gene and smoking.
- Non-citrullinated variants of citrullinated autoantigens may be involved in breach of tolerance mechanisms.

References

- Schellekens GA, de Jong BA, van den Hoogen FH, van de Putte LB, van Venrooij WJ. Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. *J. Clin. Invest.* 101(1), 273–281 (1998).
- Vincent C, Simon M, Sebbag M *et al.* Immunoblotting detection of autoantibodies to human epidermis filaggrin: a new diagnostic test for rheumatoid arthritis. *J. Rheumatol.* 25(5), 838–846 (1998).
- Lundberg K, Kinloch A, Fisher BA *et al.* Antibodies to citrullinated alpha-enolase peptide 1 are specific for rheumatoid arthritis and cross-react with bacterial enolase. *Arthritis Rheum.* 58(10), 3009–3019 (2008).
- van Beers JJ, Schwarte CM, Stammen-Vogelzangs J, Oosterink E, Božić B, Pruijn GJ. The rheumatoid arthritis synovial fluid citrullinome reveals novel citrullinated epitopes in apolipoprotein E, myeloid nuclear differentiation antigen, and β -actin. *Arthritis Rheum.* 65(1), 69–80 (2013).
- Trouw LA, Mahler M. Closing the serological gap: promising novel biomarkers for the early diagnosis of rheumatoid arthritis. *Autoimmun. Rev.* 12(2), 318–322 (2012).
- Wegner N, Lundberg K, Kinloch A *et al.* Autoimmunity to specific citrullinated proteins gives the first clues to the etiology of rheumatoid arthritis. *Immunol. Rev.* 233(1), 34–54 (2010).
- Sebbag M, Moinard N, Auger I *et al.* Epitopes of human fibrin recognized by the rheumatoid arthritis-specific autoantibodies to citrullinated proteins. *Eur. J. Immunol.* 36(8), 2250–2263 (2006).
- Trier NH, Leth ML, Hansen PR, Houen G. Cross-reactivity of a human IgG₁ anticitrullinated fibrinogen monoclonal antibody to a citrullinated profilaggrin peptide. *Protein Sci.* 21(12), 1929–1941 (2012).
- Amara K, Steen J, Murray F *et al.* Monoclonal IgG antibodies generated from joint-derived B cells of RA patients have a strong bias toward citrullinated autoantigen recognition. *J. Exp. Med.* 210(3), 445–455 (2013).
- van de Stadt LA, van Schouwenburg PA, Bryde S *et al.* Monoclonal anti-citrullinated protein antibodies selected on citrullinated fibrinogen have distinct targets with different cross-reactivity patterns. *Rheumatology (Oxford)*, 52(4), 631–635 (2012).
- Snir O, Widhe M, von Spee C *et al.* Multiple antibody reactivities to citrullinated antigens in sera from patients with rheumatoid arthritis: association with HLA-DRB1 alleles. *Ann. Rheum. Dis.* 68(5), 736–743 (2009).
- Goules JD, Goules AV, Tzioufas AG. Fine specificity of anti-citrullinated peptide antibodies discloses a heterogeneous antibody population in rheumatoid arthritis. *Clin. Exp. Immunol.* 174(1), 10–17 (2013).
- Ioan-Facsinay A, el-Bannoudi H, Scherer HU *et al.* Anti-cyclic citrullinated peptide antibodies are a collection of anti-citrullinated protein antibodies and contain overlapping and non-overlapping reactivities. *Ann. Rheum. Dis.* 70(1), 188–193 (2011).
- Suwannalai P, Scherer HU, van der Woude D *et al.* Anti-citrullinated protein antibodies have a low avidity compared with antibodies against recall antigens. *Ann. Rheum. Dis.* 70(2), 373–379 (2011).
- Shi J, Willemze A, Janssen GM *et al.* Recognition of citrullinated and carbamylated proteins by human antibodies: specificity, cross-reactivity and the ‘AMC-Senshu’ method. *Ann. Rheum. Dis.* 72(1), 148–150 (2013).
- van de Stadt LA, Witte BI, Bos WH, van Schaardenburg D. A prediction rule for the development of arthritis in seropositive arthralgia patients. *Ann. Rheum. Dis.* doi:10.1136/annrheumdis-2012-202127 (2012) (Epub ahead of print).
- van der Woude D, Rantapää-Dahlqvist S, Ioan-Facsinay A *et al.* Epitope spreading of the anti-citrullinated protein antibody response occurs before disease onset and is associated with the disease course of early arthritis. *Ann. Rheum. Dis.* 69(8), 1554–1561 (2010).
- van de Stadt LA, de Koning MH, van de Stadt RJ *et al.* Development of the anti-citrullinated protein antibody repertoire prior to the onset of rheumatoid arthritis. *Arthritis Rheum.* 63(11), 3226–3233 (2011).
- Sokolove J, Bromberg R, Deane KD *et al.* Autoantibody epitope spreading in the pre-clinical phase predicts progression to rheumatoid arthritis. *PLoS ONE* 7(5), e35296 (2012).
- Barra L, Scinocca M, Saunders S *et al.* Anti-citrullinated protein antibodies (ACPA) in unaffected first degree relatives of rheumatoid arthritis patients. *Arthritis Rheum.* 65(6), 1439–1447 (2013).

- 21 Nicaise Roland P, Grootenboer Mignot S, Bruns A *et al.* Antibodies to mutated citrullinated vimentin for diagnosing rheumatoid arthritis in anti-CCP-negative patients and for monitoring infliximab therapy. *Arthritis Res. Ther.* 10(6), R142 (2008).
- 22 Bartoloni E, Alunno A, Bistoni O *et al.* Diagnostic value of anti-mutated citrullinated vimentin in comparison to anti-cyclic citrullinated peptide and anti-viral citrullinated peptide 2 antibodies in rheumatoid arthritis: an Italian multicentric study and review of the literature. *Autoimmun. Rev.* 11(11), 815–820 (2012).
- 23 Nicaise-Roland P, Nogueira L, Demattei C *et al.* Autoantibodies to citrullinated fibrinogen compared with anti-MCV and anti-CCP2 antibodies in diagnosing rheumatoid arthritis at an early stage: data from the French ESPOIR cohort. *Ann. Rheum. Dis.* 72(3), 357–362 (2013).
- 24 Pratt AG, Charles PJ, Chowdhury M, Wilson G, Venables PJ, Isaacs JD. Serotyping for an extended anti-citrullinated peptide autoantibody panel does not add value to CCP2 testing for diagnosing RA in an early undifferentiated arthritis cohort. *Ann. Rheum. Dis.* 70(11), 2056–2058 (2011).
- 25 Lundberg K, Bengtsson C, Kharlamova N *et al.* Genetic and environmental determinants for disease risk in subsets of rheumatoid arthritis defined by the anticitrullinated protein/peptide antibody fine specificity profile. *Ann. Rheum. Dis.* 72(5), 652–8 (2012).
- 26 Willemze A, Böhringer S, Knevel R *et al.* The ACPA recognition profile and subgrouping of ACPA-positive RA patients. *Ann. Rheum. Dis.* 71(2), 268–274 (2012).
- 27 Fisher BA, Plant D, Brode M *et al.* Antibodies to citrullinated alpha-enolase peptide 1 and clinical and radiological outcomes in rheumatoid arthritis. *Ann. Rheum. Dis.* 70(6), 1095–1098 (2011).
- 28 van Oosterhout M, Bajema I, Levarht EW, Toes RE, Huizinga TW, van Laar JM. Differences in synovial tissue infiltrates between anti-cyclic citrullinated peptide-positive rheumatoid arthritis and anti-cyclic citrullinated peptide-negative rheumatoid arthritis. *Arthritis Rheum.* 58(1), 53–60 (2008).
- 29 van der Woude D, Syversen SW, van der Voort EI *et al.* The ACPA isotype profile reflects long-term radiographic progression in rheumatoid arthritis. *Ann. Rheum. Dis.* 69(6), 1110–1116 (2010).
- 30 Aubart F, Crestani B, Nicaise-Roland P *et al.* High levels of anti-cyclic citrullinated peptide autoantibodies are associated with co-occurrence of pulmonary diseases with rheumatoid arthritis. *J. Rheumatol.* 38(6), 979–982 (2011).
- 31 Giles JT, Danoff SK, Sokolove J *et al.* Association of fine specificity and repertoire expansion of anticitrullinated peptide antibodies with rheumatoid arthritis associated interstitial lung disease. *Ann. Rheum. Dis.* doi:10.1136/annrheumdis-2012-203160 (2013) (Epub ahead of print).
- 32 Harlow L, Rosas IO, Gochuico BR *et al.* Identification of citrullinated hsp90 isoforms as novel autoantigens in rheumatoid arthritis-associated interstitial lung disease. *Arthritis Rheum.* 65(4), 869–879 (2013).
- 33 De Rycke L, Baeten D, Kruithof E, Van den Bosch F, Veys EM, De Keyser F. The effect of TNFalpha blockade on the antinuclear antibody profile in patients with chronic arthritis: biological and clinical implications. *Lupus* 14(12), 931–937 (2005).
- 34 Caramaschi P, Biasi D, Tonolli E *et al.* Antibodies against cyclic citrullinated peptides in patients affected by rheumatoid arthritis before and after infliximab treatment. *Rheumatol. Int.* 26(1), 58–62 (2005).
- 35 van Dongen H, van Aken J, Lard LR *et al.* Efficacy of methotrexate treatment in patients with probable rheumatoid arthritis: a double-blind, randomized, placebo-controlled trial. *Arthritis Rheum.* 56(5), 1424–1432 (2007).
- 36 Fisher BA, Plant D, Lundberg K *et al.* Heterogeneity of anticitrullinated peptide antibodies and response to anti-tumor necrosis factor agents in rheumatoid arthritis. *J. Rheumatol.* 39(5), 929–932 (2012).
- 37 van Aken J, Heimans L, Gillet-van Dongen H *et al.* Five-year outcomes of probable rheumatoid arthritis treated with methotrexate or placebo during the first year (the PROMPT study). *Ann. Rheum. Dis.* doi:10.1136/annrheumdis-2012-202967 (2013) (Epub ahead of print).
- 38 Visser K, Verpoort KN, van Dongen H *et al.* Pretreatment serum levels of anti-cyclic citrullinated peptide antibodies are associated with the response to methotrexate in recent-onset arthritis. *Ann. Rheum. Dis.* 67(8), 1194–1195 (2008).
- 39 Verpoort KN, Jol-van der Zijde CM, Papendrecht-van der Voort EA *et al.* Isotype distribution of anti-cyclic citrullinated peptide antibodies in undifferentiated arthritis and rheumatoid arthritis reflects an ongoing immune response. *Arthritis Rheum.* 54(12), 3799–3808 (2006).
- 40 Chibnik LB, Mandl LA, Costenbader KH, Schur PH, Karlson EW. Comparison of threshold cutpoints and continuous measures of anti-cyclic citrullinated peptide antibodies in predicting future rheumatoid arthritis. *J. Rheumatol.* 36(4), 706–711 (2009).
- 41 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.* 65(4), 899–910 (2013).
- 42 Suwannalai P, van de Stadt LA, Radner H *et al.* Avidity maturation of anti-citrullinated protein antibodies in rheumatoid arthritis. *Arthritis Rheum.* 64(5), 1323–1328 (2012).
- 43 Zhao X, Okeke NL, Sharpe O *et al.* Circulating immune complexes contain citrullinated fibrinogen in rheumatoid arthritis. *Arthritis Res. Ther.* 10(4), R94 (2008).
- 44 Sokolove J, Zhao X, Chandra PE, Robinson WH. Immune complexes containing citrullinated fibrinogen costimulate macrophages via Toll-like receptor 4 and Fc gamma receptor. *Arthritis Rheum.* 63(1), 53–62 (2011).
- 45 Laurent L, Clavel C, Lemaire O *et al.* Fcγ receptor profile of monocytes and macrophages from rheumatoid arthritis patients and their response to immune complexes formed with autoantibodies to citrullinated proteins. *Ann. Rheum. Dis.* 70(6), 1052–1059 (2011).
- 46 Scherer HU, van der Woude D, Ioan-Facsinay A *et al.* Glycan profiling of anti-citrullinated protein antibodies isolated from human serum and synovial fluid. *Arthritis Rheum.* 62(6), 1620–1629 (2010).
- 47 Trouw LA, Haisma EM, Levarht EW *et al.* Anti-cyclic citrullinated peptide antibodies from rheumatoid arthritis patients activate complement via both the classical and alternative pathways. *Arthritis Rheum.* 60(7), 1923–1931 (2009).
- 48 Harre U, Georgess D, Bang H *et al.* Induction of osteoclastogenesis and bone loss by human autoantibodies against citrullinated vimentin. *J. Clin. Invest.* 122(5), 1791–1802 (2012).
- 49 Lu MC, Lai NS, Yu HC, Huang HB, Hsieh SC, Yu CL. Anti-citrullinated protein antibodies bind surface-expressed citrullinated Grp78 on monocyte/macrophages and stimulate tumor necrosis

- factor alpha production. *Arthritis Rheum.* 62(5), 1213–1223 (2010).
- 50 Klareskog L, Malmström V, Lundberg K, Padyukov L, Alfredsson L. Smoking, citrullination and genetic variability in the immunopathogenesis of rheumatoid arthritis. *Semin. Immunol.* 23(2), 92–98 (2011).
- 51 Makrygiannakis D, Hermansson M, Ulfgrén AK *et al.* Smoking increases peptidylarginine deiminase 2 enzyme expression in human lungs and increases citrullination in BAL cells. *Ann. Rheum. Dis.* 67(10), 1488–1492 (2008).
- 52 Mercado FB, Marshall RI, Bartold PM. Inter-relationships between rheumatoid arthritis and periodontal disease. A review. *J. Clin. Periodontol.* 30(9), 761–772 (2003).
- 53 Scher JU, Ubeda C, Equinda M *et al.* Periodontal disease and the oral microbiota in new-onset rheumatoid arthritis. *Arthritis Rheum.* 64(10), 3083–3094 (2012).
- 54 Lappin DF, Apatzidou D, Quirke AM *et al.* Influence of periodontal disease, *Porphyromonas gingivalis* and cigarette smoking on systemic anti-citrullinated peptide antibody titres. *J. Clin. Periodontol.* 40(10), 907–915 (2013).
- 55 Svärd A., Kastbom A, Sommarin Y, Skogh T. Salivary IgA antibodies to cyclic citrullinated peptides (CCP) in rheumatoid arthritis. *Immunobiology* 218(2), 232–237 (2013).
- 56 Wegner N, Wait R, Sroka A *et al.* Peptidylarginine deiminase from *Porphyromonas gingivalis* citrullinates human fibrinogen and α -enolase: implications for autoimmunity in rheumatoid arthritis. *Arthritis Rheum.* 62(9), 2662–2672 (2010).
- 57 Quirke AM, Lugli EB, Wegner N *et al.* Heightened immune response to autocitrullinated *Porphyromonas gingivalis* peptidylarginine deiminase: a potential mechanism for breaching immunologic tolerance in rheumatoid arthritis. *Ann. Rheum. Dis.* (2013) (Epub ahead of print).
- 58 de Pablo P, Dietrich T, Chapple IL *et al.* The autoantibody repertoire in periodontitis: a role in the induction of autoimmunity to citrullinated proteins in rheumatoid arthritis? *Ann. Rheum. Dis.* (2013).
- 59 Kinloch AJ, Alzabin S, Brintnell W *et al.* Immunization with *Porphyromonas gingivalis* enolase induces autoimmunity to mammalian α -enolase and arthritis in DR4-IE-transgenic mice. *Arthritis Rheum.* 63(12), 3818–3823 (2011).
- 60 Hill JA, Bell DA, Brintnell W *et al.* Arthritis induced by posttranslationally modified (citrullinated) fibrinogen in DR4-IE transgenic mice. *J. Exp. Med.* 205(4), 967–979 (2008).
- 61 Bell DA, Hill JA, Cairns E. An intriguing relationship between the immune response to citrullinated vimentin and the HLA shared epitope: comment on the article by Verpoort *et al.* *Arthritis Rheum.* 58(10), 3277, author reply 3277–3278 (2008).
- 62 Perricone C, Ceccarelli F, Valesini G. An overview on the genetic of rheumatoid arthritis: a never-ending story. *Autoimmun. Rev.* 10(10), 599–608 (2011).
- 63 Hill JA, Southwood S, Sette A, Jevnikar AM, Bell DA, Cairns E. Cutting edge: the conversion of arginine to citrulline allows for a high-affinity peptide interaction with the rheumatoid arthritis-associated HLA-DRB1*0401 MHC class II molecule. *J. Immunol.* 171(2), 538–541 (2003).
- 64 Menard L, Saadoun D, Isnardi I *et al.* The PTPN22 allele encoding an R620W variant interferes with the removal of developing autoreactive B cells in humans. *J. Clin. Invest.* 121(9), 3635–3644 (2011).
- 65 Verpoort KN, Cheung K, Ioan-Facsinay A *et al.* Fine specificity of the anti-citrullinated protein antibody response is influenced by the shared epitope alleles. *Arthritis Rheum.* 56(12), 3949–3952 (2007).
- 66 Fisher BA, Bang SY, Chowdhury M *et al.* Smoking, the HLA-DRB1 shared epitope and ACPA fine-specificity in Koreans with rheumatoid arthritis: evidence for more than one pathogenic pathway linking smoking to disease. *Ann. Rheum. Dis.* (2013) (Epub ahead of print).
- 67 Montes A, Perez-Pampin E, Calaza M, Gomez-Reino JJ, Gonzalez A. Association of anti-citrullinated vimentin and anti-citrullinated α -enolase antibodies with subsets of rheumatoid arthritis. *Arthritis Rheum.* 64(10), 3102–3110 (2012).
- 68 Hansson M, Mathsson L, Schleder T *et al.* Validation of a multiplex chip-based assay for the detection of autoantibodies against citrullinated peptides. *Arthritis Res. Ther.* 14(5), R201 (2012).
- 69 Snir O, Widhe M, Hermansson M *et al.* Antibodies to several citrullinated antigens are enriched in the joints of rheumatoid arthritis patients. *Arthritis Rheum.* 62(1), 44–52 (2010).
- 70 Schulte S, Unger C, Mo JA *et al.* Arthritis-related B cell epitopes in collagen II are conformation-dependent and sterically privileged in accessible sites of cartilage collagen fibrils. *J. Biol. Chem.* 273(3), 1551–1561 (1998).

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