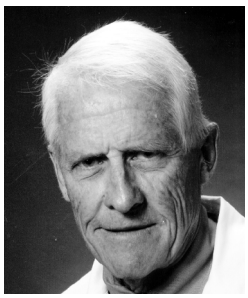
Editorial

Can We Rely on Anti-Citrulline Antibody Determination for the Diagnosis of Early Rheumatoid Arthritis?



While intuitively the early diagnosis of any disease is important, it has now become dogma that the early diagnosis and treatment of rheumatoid arthritis (RA) is critical to prevent crippling. Accordingly, if one accepts this view, there is an increasing need to have readily available tools to identify early RA. The challenge in very early RA is to distinguish this disease from other disorders that mimic it or are transient (e.g., viral arthritis) or are not anticipated to lead to destructive arthritis [e.g., systemic lupus erythematosus (SLE)]. Perhaps a more important objective is to also identify which patients with RA are likely to have an aggressive course of their disease. The serologic measurement of anti-citrulline antibodies has been proposed as a means to achieve these objectives, since the test has an apparent high specificity for established RA and is at least as sensitive for the diagnosis as rheumatoid factor (RF). While anti-citrulline antibodies have been known to be associated with RA for over 40 years[1], it is only recently that their measurement has been facilitated by a relatively simple ELISA employing a proprietary cyclic polypeptide, CCP2 [2].

In this issue of *The Journal*, Matsui and coworkers[3] examine the performance of a series of RA-associated markers, including anti-CCP2 antibodies, in a cohort of patients with early RA (very early or < 6 months and relatively early or < 2 years) compared to patients with other disorders that could mimic RA. These included SLE, mixed connective tissue disease, and Sjögren's syndrome (SS). The authors found that anti-CCP2 antibodies had a sensitivity greater than 67% for the diagnosis of very early RA, and 82% for the overall diagnosis of RA, among a group of Japanese patients seen in one academic medical center. Other diagnostic markers were also prevalent in this population group, including RF (84%) and anti-galactosyl IgG antibodies (approximately 90%). However, anti-CCP2 antibodies were more specific for RA than any combination of other markers (anti-agalactosyl IgG, RF, matrix metalloproteinase-3). Further, anti-CCP2 antibodies had a positive predictive value of approximately 91% for RA. Importantly, RF was positive in 61% of anti-CCP2-negative patients with very early RA, while anti-CCP2 was positive in 22% of RF-negative subjects. The authors concluded that anti-CCP2 antibodies are the single most useful test for the overall diagnosis of RA, but that a combination of anti-CCP2 and RF is more useful than either test alone for the diagnosis of very early RA.

How do these results compare to the reports from other population groups with RA? The sensitivity of anti-CCP2 antibodies for the diagnosis of RA in most other reported series, particularly in Caucasians, is generally lower (60%–70%) and the specificity for RA is generally higher (95%–98%)[4]. In the series of patients reported here by Matsui, *et al*, a relatively high frequency of anti-CCP2 antibodies was also seen in patients with non-RA connective tissue disorders such as SLE (15%), SS (14%), polymyositis/dermatomyositis (23%), and scleroderma (16%). The median level of anti-CCP2 antibodies was, however, generally lower in these non-RA patients (7–35 units per ml) compared to RA (100 units per ml or more).

These are surprising results, and they urge some caution in generalizing from the conclusions of the studies in this somewhat selected group of Japanese patients with RA, since some of the patients with anti-CCP2 antibodies may have arthritis that can clinically mimic early RA but that is not generally recognized to lead to joint destruction. This may account for the fact that among non-Asian population groups previously studied, the specificity of anti-CCP2 antibodies for RA has been reported to be higher than in the studies by Matsui, *et al*. These authors also suggested the interesting possibility that the common denominator predicting the likelihood of positive anti-CCP2 antibodies was the occurrence of inflammatory arthritis, regardless of its association with disease. While there is no evidence yet supporting this hypothesis, there are a growing number of reports of anti-citrulline antibody in non-RA subjects.

Symmons, *et al*[5] have recently stated that to date, "no set of predictive criteria has been able to discriminate between

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individuals destined to develop ultimate RA (those with persistent destructive disease) and those not developing RA." These authors assert that the majority of patients with early inflammatory polyarthritis have undifferentiated disease, and that the only justification for using American College of Rheumatology (ACR) criteria or any criteria to define RA in patients with early inflammatory polyarthritis is to satisfy the insistence of manuscript reviewers! They have argued that the label "rheumatoid arthritis" should be reserved for those with "established disease" and that "its use in the first few months of early disease should be dropped."

There are a few reports of anti-citrulline antibodies (measured by CCP2) in psoriatic arthritis[6,7] and even in psoriasis without arthritis[8]. We have identified patients who appear to have primary SS with an inflammatory polyarthritis who have anti-citrulline antibodies (by CCP2 assay)[9]. Many of these patients had clinical evidence of erosive or destructive arthritis similar to RA. The occurrence of anti-citrulline antibody in human RA is closely linked to the expression of the RA shared epitope (SE). HLA typing among these patients with anti-Ro/SSA-positive SS with anti-citrulline antibody revealed that many expressed one copy of the SE but only on one parental allele and DR3 (often linked to anti-Ro/SSA antibody expression) on the other allele. It has been pointed out that citrullination (due to deimination by the enzyme peptidyl arginine deiminase of arginine residues in certain polypeptides) may be a nonspecific event linked to inflammation in general[10]. The ability to produce anti-citrulline antibodies experimentally, however, is linked to the expression of the SE, as we have recently shown in DR4 transgenic mice[11]. Therefore, the presence of synovial inflammation in individuals with the appropriate genetic background (the SE) may be necessary to generate an immune response to citrullinated polypeptides. We did not observe anti-citrulline antibodies among a carefully selected group of patients with psoriatic arthritis who lacked the SE[12].

Consistent with these latter experimental observations[11] are numerous reports linking the presence of anti-citrulline antibodies in human RA to the expression of MHC class II molecules with the SE[13-15]. While this combination of circumstances is more likely to occur in what we understand as RA, it is entirely conceivable that patients with other disorders not classically recognized as fulfilling ACR criteria for this diagnosis may have anti-citrulline antibodies. Van der Helm-van Mil and coworkers[16] recently provided evidence that SE alleles did not independently contribute to RA among a group of patients with undifferentiated arthritis, but rather, influenced the development of anti-citrulline antibodies. The level of anti-citrulline antibodies was higher among patients with the SE than in those without this genetic marker. Similarly, Inigoyen and coworkers[15] recently reported that the SE is strongly associated with the production of anti-CCP antibodies, but not RF, and that the odds ratio of having anti-citrulline antibodies is doubled when 2 copies of the SE are expressed. These observations, taken together, are consistent with the hypothesis that SE expression is linked directly to the immune response to citrulline, confirming the experimental observations[11]. Given these findings, the expression of anti-citrulline antibody may be more closely linked to the SE than to RA itself, and could account for the growing number of observations that anti-citrulline antibodies may be present in patients with other diseases who express the SE. It appears, therefore, that the dust has not yet settled on the issue of whether anti-citrulline antibodies, usually tested by reactivity to CCP2, perform as an ideal marker for the diagnosis of early RA.

A second issue is whether or not anti-citrulline antibodies are useful in predicting severity of RA. This had been alluded to earlier. There are a number of studies among Caucasians that suggest this association[17] and with the SE. It is possible, as stated, that the linkage here is with anti-citrulline antibodies. That is, given the studies described above, it is possible that the association of the SE with more severe disease is a reflection of its influence on the production of anti-citrulline antibodies. Moreover, direct experimental evidence exists that anti-citrulline antibodies are pathogenic. First, the studies by Kuhn and collaborators, showing that in mice, citrullinated collagen II enhances its arthritogenicity compared to uncitrullinated collagen II, support this possibility[18]. Second, we have shown that mice transgenic for the SE (but not non-SE transgenic mice) can be induced to develop inflammatory arthritis following immunization with citrullinated but not non-citrullinated fibrinogen[19]. However, the thesis that anti-citrulline antibodies are pathogenic in RA, and predict severity, is difficult to reconcile with their observed appearance years before the onset of obvious RA[20]. This suggests that additional factors influence the expression of synovitis and its severity.

The rediscovery of anti-citrulline antibodies and their relationship to RA has opened a new chapter in our understanding of this disease. Like the earlier discovery of RF, the strong association of anti-citrulline antibodies in RA challenges us to examine how the immune response to citrullined polypeptides is involved in the pathogenesis of the disease, and how reliable anti-citrulline antibodies are in its early diagnosis and in predicting its progression. More work is needed.

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