Anti-citrullinated protein/peptide autoantibodies (ACPA) in rheumatoid arthritis

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Summary

Anti-citrullinated protein/peptide antibodies (ACPA) including anti-CCP and others have emerged as sensitive and specific serological markers of RA. First we examined the diagnostic performance of the newly developed anti-mutated citrullinated vimentin (MCV) antibody assay. In this study, anti-MCV levels together with those of anti-CCP2 and rheumatoid factors (RF) were determined in the sera of 237 individuals including 119 RA patients and 118 controls. Diagnostic properties were compared by receiving operating characteristic curve (ROC) analysis. When using manufacturer recommended cut-off values, sensitivity and specificity of anti-MCV antibodies was 75.6 % and 91.5% in RA, compared to 66.4 % and 98.3% for anti-CCP2. Introducing cut-off values to obtain the same 95% specificity resulted in decreased sensitivity of the anti-MCV test (69.7%), and increased sensitivity of the anti-CCP2 test (74.8%). At optimal cut-off levels, 41% of IgM RF negative cases, as well as 30% of anti-CCP2 negative cases in the RA group were anti-MCV positive. Double positivity for anti-MCV and anti-CCP2 provided 98.3% specificity with 97.5% positive predictive value in RA.

Next, the new generation CCP3 and CCP3.1 assays were also tested in comparison to the standard CCP2 assay. Again, samples from 119 RA patients and 118 controls were assessed. The sensitivity of the CCP3.1, CCP3 and CCP2 assays were 83%, 79% and 75%, respectively, while the specificity of the three assays were 98%, 97% and 96%, respectively. Among RA patients, 15 CCP2-negative subjects were tested positive using the third generation assays.

Finally, while the differential role of RF isotypes is more or less well-characterized, little information is available regarding anti-CCP isotypes. Therefore IgG, IgA and IgM anti-CCP2 and RF levels were measured in the sera of 119 RA patients and 118 controls. We assessed the diagnostic performance of IgA and IgM anti-CCP2 antibodies and their relationship with IgG anti-CCP2, RFs, disease duration and the presence of HLA-DRB1 shared epitope (SE) alleles. Patients with RA had significantly higher serum IgA and IgM anti-CCP2 antibody levels than healthy subjects and patients with other rheumatic diseases ($p<0.0001$). IgG, IgA and IgM anti-CCP2 antibodies were present in 74.8%, 52.9% and 44.5% of RA patients, and their diagnostic specificity was 95.8%, 95.8% and 91.6%, respectively. The presence of anti-CCP2 antibodies was significantly associated with SE alleles ($p=0.03$). The frequency of IgM anti-CCP2 positivity was lower in longstanding disease compared to early RA ($p=0.03$).

In conclusion, the performance of the novel anti-MCV ELISA for the diagnosis of RA is similar to that of the anti-CCP2 test, however, as the diagnostic spectrum of the anti-MCV assay is somewhat different from that of anti-CCP2, the combined application of the two assays can improve the laboratory diagnostics of RA. In our hands, the third generation CCP3 and CCP3.1 ELISAs performed better than the CCP2 assays. The CCP3.1 assay may represent a significant improvement over the other two assays. Furthermore, IgA and IgM anti-CCP2 antibodies are present in RA patients, and they are similarly specific for RA as IgG anti-CCP2. The higher frequency of IgM anti-CCP2 antibodies in early RA suggests that they are mostly generated during the first phase of immune response; nonetheless, their production seems to be sustained in some patients. The introduction of anti-MCV, anti-CCP3.1 assays and the determination of anti-CCP isotypes may enable a more refined characterization of RA patients.

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