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Increased Prevalence of Anti-Third Generation Cyclic Citrullinated Peptide Antibodies in Patients With Rheumatoid Arthritis and CREST Syndrome

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Abstract

To investigate the prevalence of anti-third generation cyclic citrullinated peptide antibodies (anti-CCP3) in patients with systemic connective tissue diseases, we assembled a training set consisting of 115 patients with rheumatoid arthritis (RA), 52 with Calcinosis, Raynaud's phenomenon, oesophageal dysmotility, sclerodactyly, telangiectasia (CREST) syndrome, 21 with scleroderma, 20 with ankylosing spondylitis, 18 with reactive arthritis, 25 with juvenile rheumatoid arthritis (RA), 51 with osteoarthritis, 26 with mixed connective tissue disease, 23 with primary Sjogren's syndrome, 74 with systemic lupus erythematosus, 49 with Polymyalgia rheumatica, and 39 with polymyositis/dermatomyositis. The commercial enzyme-linked immunosorbent assay (ELISA) was used to detect anti-CCP antibodies, including anti-CCP2 (regular, second generation of CCP antigen) and anti-CCP3 (third generation of CCP antigen) in disease-related specimens and normal controls. These serum samples were also evaluated for anti-centomere antibodies by anti-centromere ELISA kit. The higher frequencies of anti-CCP3 and anti-CCP2 were detected in 75.6 and 70.4% patients with RA, respectively. At the same time, anti-CCP3 (not anti-CCP2) was significantly increased in samples isolated from patients with CREST syndrome. The clinical sensitivity of IgG anti-CCP3 for the patients with CREST syndrome was 29% (15 of 52) and the specificity was 96% (384 of 397), with the exception of the RA group. The anti-centromere antibodies were significantly higher in patients with CREST only. The results of our study suggest that compared to anti-CCP2 assay, the new anti-CCP3 assay can enhance the clinical sensitivity for diagnosis of RA and, as an associate marker combined with anti-centromere, can distinguish CREST syndrome from other systemic connective tissue diseases, especially RA. The clinical specificity of anti-CCP3 was lower than anti-CCP2 assay in diagnosis of RA because of the crossreaction to the patients with CREST syndrome.

Index Entries

Anti-CCP; rheumatoid arthritis; CREST syndrome; antibodiies.

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Introduction

A major characteristic of most connective tissue diseases is an autogenous profile of autoantibodies directed to distinct intracellular antigens in vivo *(1)*. Citrulline is generated by the deimination of arginine in humans *(2)*. Citrullination can be catalyzed to generate cyclic citrullinated peptide (CCP) by a family of calcium-dependent enzymes known as peptidyl arginine deiminase. These enzymes are presented in several different cell and tissue types, including inflammatory cells. The generated CCP is immunogenic and can induce a series of humoral responses in vivo *(3)*.

Rheumatoid arthritis (RA) is a systemic auto-immune disease of unknown etiology that is distinguished by chronic inflammation of joints, resulting in tissue degradation and joint deformation. The course of RA varies, ranging from a mild to an aggressive form. Early diagnosis and treatment reduce joint destruction, preserve function, and improve survival *(4)*. Because a current therapeutic strategy for RA recommends increasingly aggressive treatment early in the course of the disease, diagnostic tests with high specificity are desirable for choosing the optimal treatment strategy.

RA is associated with elevated titers of antibodies, including rheumatoid factor (RF), anti-CCP, antibodies (anti-CCP) directed against RA-33, calpastatin, keratin, and antifilaggrin; most of these have failed to demonstrate adequate diagnostic and prognostic value *(5)*. RF, an auto-antibody directed against the constant region of IgG, is elevated in 75% of the patients with RA and is widely used in clinical practice. However, RFs are not very specific for this disease and can also be detected in people with infections, other auto-immune diseases, and some healthy individuals. In addition to RF, the diagnostic properties of RA antibodies that recognize a CCP have been identified *(6,7)*, and anti-CCPs are frequently observed in patients with RA—especially in early and even in preclinical disease *(8,9)*. The elevated titers of anti-CCP are more specific for RA than RF, with a disease specificity approaching 100% *(7)*. The comparison of diagnostic utility of anti-CCPs and RFs in patients with RA and other autoimmune diseases suggests that anti-CCPs proved to be superior to RFs and MMP-3 *(10)*. Anti-CCPs are strongly associated with disease severity *(11)* and represent an independent predictor for erosive RA *(12)*. The presence of anti-CCP in early disease is highly predictive for more rapid radiographical disease progression, a clinical hallmark of aggressive RA *(13–17)*. Citrullinated proteins are found in the joints of patients with RA but not in joints from people with other forms of joint disease *(18)*. These results lend a theoretical basis for a use of anti-CCPs as predictive, diagnostics and prognostic markers in RA and suggest a possible pathogenic role for these antibodies in the disease development.

Anti-CCPs can easily be detected in sera using commercially available enzyme-linked immunosorbent assays (ELISA) based on highly purified synthetic peptides containing modified arginine residues (citrulline) serving as antigen. The first anti-CCP test revealed a high diagnostic specificity of about 98% and sensitivity around 70% *(19)*. The sensitivity of anti-CCP ELISA has increased during recent years with the second-generation anti-CCP assay, which uses cyclic synthetic citrullinated peptide antigen *(20)*. The anti-CCP2 test has shown a comparable sensitivity (41–88%) but a much higher specificity than IgM-RF in diagnosing RA, and therefore, anti-CCP testing has been increasingly accepted in laboratory medicine *(7,10,21,22)*.

Recently, several studies have suggested *(23–25)* that antibodies from some patients with RA who are negative for anti-CCP2 are reactive to other citrullinated proteins, demonstrating that there are additional epitopes that are not present in CCP2 antigen sequence. Inova Diagnostics (San Diego, CA) provided an anti-CCP3 (CCP3) assay kit for the detection

of anti-CCP IgG antibodies in patient sera. In the current study, the prevalence of anti-CCP3 antibodies was evaluated in patients with clinical diagnosis of RA and other systemic autoimmune diseases.

Materials and Methods

The specificities and sensitivities of the Quanta Lite™ CCP3 IgG EIA kit (cat. no. 704535), CCP IgG EIA kit (cat. no. 708790), and Centromere (CENP-A and CENP-B) IgG EIA kit (cat. no. 708770; INOVA Diagnostics) were evaluated by testing serum samples from healthy subjects and patients with different connective tissue diseases.

The study group included 116 patients with RA, 52 patients with Calcinosis, Raynaud's phenomenon, oesophageal dysmotility, sclerodactyly, telangiectasia (CREST) syndrome, 21 patients with scleroderma (SSc), 20 patients with ankylosing spondylitis, 18 patients with reactive arthritis, 25 patients with juvenile RA, 51 patients with osteoarthritis, 26 patients with mixed connective tissue disease (MCTD), 23 patients with primary Sjogren's syndrome (pSS), 74 systemic lupus erythematosus (SLE), 49 patients with Polymyalgia rheumatica, and 39 patients with polymyositis/dermatomyositis; 115 patients were on the registry of patients who fulfilled the American College of Rheumatology (ACR; formerly, the American Rheumatism Association) classification criteria for RA *(26)*. All disease-related samples were randomly selected from the practices of the Morris and Metzger rheumatologist group in Los Angeles, CA. The identification of patients was based on a physical examination and laboratory testing and a review of all previously available medical records. The sera of 51 healthy individuals between ages 21 and 65 yr were collected from the blood donors of blood banks in Southern California as control group. All sera were separated after the blood was drawn and were stored at room temperature for 30 min; the serum was then stored at –70°C until testing.

Auto-Antibody Determination

The levels of anti-CCP2, anti-CCP3, and anti-centromere were determined using the commercially Quanta Lite CCP (CCP2) IgG EIA kit, Quanta Lite CCP3 IgG EIA kit, and Quanta Lite Centromere (CENP-A and CENP-B) from INOVA Diagnostics.

Competition assays were performed to investigate whether there was any immunological crossreactivity between the anti-CCP3 and anti-CCP2. Sera at a dilution ratio of 1:200 that yielded 50% of maximal binding to the CCP3-coated plate were pre-incubated with CCP3, CCP2, PPD, and without any antigen coated plates, respectively. The plates were covered with sealing tape and were incubated for 60 min at room temperature on a micro-titer plate mixing apparatus at 500 rpm. After the incubation, the sera solution were collected, and inhibition of binding to CCP3 was tested. The percentage of inhibition was calculated as follows:

$$\text{Percent inhibition} = (\text{units control} - \text{units with competitor})/\text{units control} \times 100.$$

Statistical Analysis

Conventional methods were used for calculation of means and standard deviations. For skewed variables, nonparametric tests were used for comparisons between among groups (Mann-Whitney *U* test), whereas Student's *t*-test was used for normal distributed variables and Spearman rank correlation coefficients were calculated to estimate interrelations between antibody levels. Paired Student's *t*-test was used to compare inhibition of binding to CCP3 with control and between compounds tested.

Results

Table 1 summarizes the results for anti-CCP3 screening of disease-related samples and sera from normal subjects. The higher frequency of anti-CCP3 was found in patients

Table 1
Frequency of Positive Results of Anti-CCP3 in Patients With Connective Tissue Disease and Normal Subjects

Diagnosis	Anti-CCP3 Positive/numbers	Anti-CCP2
CREST syndrome	15/52	2/52
Rheumatoid arthritis	87/115	81/115
Scleroderma	0/21	0/21
Ankylosing spondilitis	0/20	0/20
Reactive arthritis	0/18	0/18
Juvenile arthritis	2/25	1/25
Osteoarthritis	0/51	1/51
Mixed connective tissue disease	3/26	3/26
Sjogren's syndrome	3/23	1/23
Lupus erythematosus	3/74	2/74
Polymyalgia rheumatica	0/49	0/49
Dermatomyositis	1/35	0/35
Polymyositis	0/4	0/4
Healthy individuals	0/51	0/51

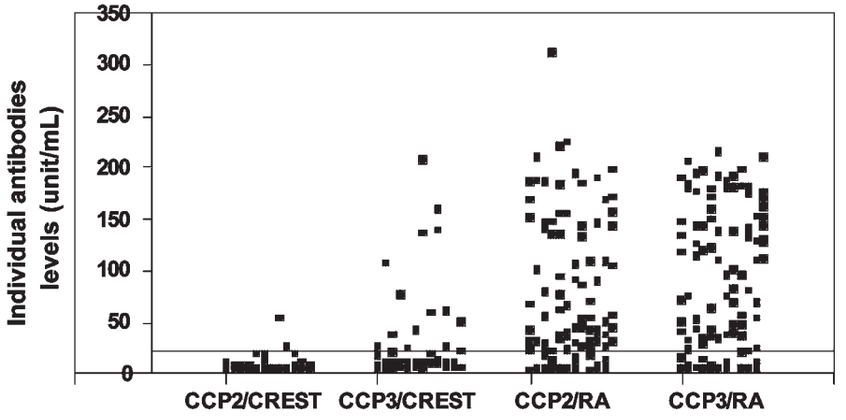

Fig. 1. Levels of anti-CCP3 and anti-CCP2 IgG antibodies in patients with CREST syndrome and RA.

with RA and CREST syndrome at 69 and 29%, respectively. No anti-CCP3 antibodies were detected in normal subjects.

Additionally, anti-CCP3 antibodies were found in three patients with SLE, three patients with MCTD, three patients with pSS, two patients with juvenile RA, and one patient with dermatomyositis.

Increased levels of both anti-CCP2 and anti-CCP3 were detected in samples from patients with RA. The clinical sensitivities were 70.4% for IgG anti-CCP2 antibodies and 75.6% for IgG anti-CCP3 antibodies. Additionally, there was a significant correlation between the anti-CCP2 antibody concentrations and the anti-CCP3 antibody concentrations ($p < 0.0001$; Fig. 1).

Meanwhile, anti-CCP3 (not anti-CCP2) antibodies (Fig. 1) were significantly increased in samples from patients with CREST syndrome. There was no correlation between anti-CCP2

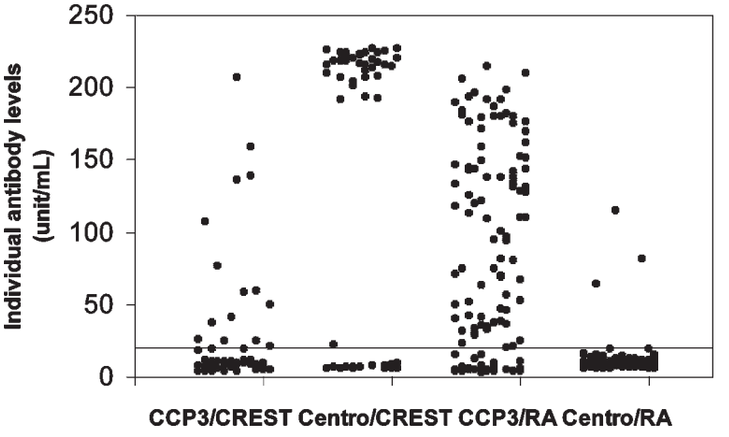

Fig. 2. Levels of anti-CCP3 and anti-centromere IgG antibodies in patients with CREST syndrome and RA.

antibody concentrations and anti-CCP3 antibody concentrations. The clinical sensitivity of IgG anti-CCP3 antibodies for the CREST was 29% (15 of 52) and the specificity was 96% (384 of 397), with the exception of in the RA group. The anti-centromere antibodies were significantly higher only in patients with CREST (positive patient 76.9% [40 of 52]; $p < 0.0001$; Fig. 2). There was a significant correlation between levels of anti-CCP3 and anti-centromere ($p < 0.05$; in all 15 anti-CCP3-positive patients with CREST, their anti-centromere is also positive) in the CREST patient group.

To study possible crossreactivity between the anti-CCP3 and anti-CCP2 antibodies, we performed competition experiments at a dilution ratio of 1:200 that yielded 50% of maximal binding to CCP3. An irrelevant antigen PPD was used as a control. Table 2 shows that the serum solutions pre-incubated with CCP3 had about 50% capacity to inhibit serum binding to CCP3-coated plates ($p < 0.01$ compared to CCP2 and PPD), whereas CCP2 and PPD showed no inhibitory effect in this respect.

Table 2
Percentage Inhibition of Antibody Binding to CCP3 by CCP2, CCP3, and an Irrelevant Antigen PPD in Four High-Titer Sera Compared With Control Values Without the Antigen

	Competitor compounds		
	CCP2	CCP3	PPD
Sample No.	Inhibition %		
1	10.2	57.3	0
2	14. 6	44.1	10.3
3	2.6	47.6	–3.8
4	–0.7	52.9	–1.3
Average	6.7	50.5	1. 3

Discussion

The study demonstrates that anti-CCP3 antibodies (and not anti-CCP2 antibodies) are present not only in the patients with RA but also in the sera as patients with the CREST syndrome. The measurement of anti-CCP3 associating with anti-centromere antibodies may be used to distinguish CREST syndrome from RA and other systemic connective tissue diseases.

Studying the crossreactive serum antibodies binding to CCP3 could compete when pre-incubated at the same concentration with CCP3-coated plate. However, CCP2 could not inhibit the antibodies binding to CCP3, indicating different antigenicities to CCP3 and CCP2. Our data suggest that both anti-CCP2 and anti-CCP3 were significantly elevated and correlated in patients with RA. The anti-CCP3 titers were higher than anti-CCP3, and there was no correlation between the anti-CCP2 and anti-CCP3 in the group of patients with CREST syn-

drome. To analyze the difference of antibody responses to CCP3 and CCP2 in patients with RA and patients with CREST syndrome, a probable explanation is that antibodies from patients with RA can recognize the different epitopes of both CCP3 and CCP2 antigens. The antibodies from CREST syndrome recognize only epitopes of antigen on CCP3. Another explanation is that the antibodies have different reactivity that results from the development of the immune response in the different conditions that induce against antigenic site on CCP3 or CCP2, respectively. It has been reported that the antibodies recognize an epitope that contains the deimidated form of arginine, as an L-citrulline residue *(20)*.

In recent years, interesting data have accumulated regarding the diagnostic utility of anti-CCP antibodies in patients with RA and their role in the pathogenesis.

In this study, we evaluated the diagnostic utility of a new anti-CCP3 EIA test in patients with RA and other connective tissue diseases. We found that the levels and the frequency of elevated titers of anti-CCP3 antibodies were higher in patients with RA and CREST syndrome compared with other systemic connective tissue diseases conditions. Compared with the anti-CCP2 test, the anti-CCP3 test indicates a higher clinical sensitivity and a lower specificity for patients with RA. At the same time, levels of anti-CCP3 antibodies are significantly higher than levels of anti-CCP2 antibodies in patients with CREST syndrome.

Sensitivity and specificity of anti-CCP2 antibodies in patients with RA have been investigated in previous studies using other systemic connective tissue diseases as a control group, including SLE, progressive systemic sclerosis, pSS, and vasculitis *(7,22,25,27)*.

Mediwake et al. *(28)* evaluated the sensitivity and specificity of anti-CCP antibodies in patients with SLE with erosive and non-erosive arthritis. They found that this test is specific for RA only, and the presence of anti-CCP2 antibodies distinguish RA from erosive SLE. Similarly, anti-CCP2 antibodies may be a useful tool in the differential diagnosis of elderly onset RA and polymyalgia rheumatica *(29)*.

The reported frequency of anti-CCP positivity in pSS was 2 to 7% in different studies *(7,25,27)*. Recently, Gottenberg et al. *(30)* investigated the prevalence of these antibodies in a cohort of patients with pSS. They found that 7.5% of affected patients (not fulfilling ACR criteria for rheumatoid arthritis) were positive for anti-CCP2. Intestinally, the researchers identified another group of anti-CCP2-positive patients with pSS that fulfilled ACR classification criteria for RA but who had with non-erosive arthritis and response to DMARDs. In another study by Kamali et al. *(31)*, the frequency of anti-CCP antibodies was investigated in patients with RF-positive early arthritis, including patients with Wegener's vasculitis, pSS, and RA. The low anti-CCP positivity in patients with Wegener's vasculitis and pSS suggested that this test appeared to be helpful for the differential diagnosis in patients with RF-positive early arthritis.

Simultaneously, Alenius et al. *(32)* assessed the incidence of anti-CCP2 antibodies in psoriatic patients with and without arthritis compared with patients with early RA and controls. They established that the anti-CCP2 positivity is more prevalent in patients with psoriatic arthritis than with psoriasis alone but is less frequent than in patients with early RA. The anti-CCP2-positive patients with psoriatic arthritis had polyarticular disease, whereas there was no recognized correlation between the presence of anti-CCP2 antibodies and radiological changes and/or deformity and functional instability.

In this study, anti-CCP3 auto-antibodies were found in three patients with SLE, three patients with MCTD, three patients with pSS, two patients with juvenile RA, and one patient with dermatomyositis. The similar prevalence of anti-CCP3 positivity in these diseases has been reported in the prescription information of an ELISA kit for anti-CCP3. The most promi-

nent unexpected finding in our study was the observation that an increased level of anti-CCP3 antibodies was identified in 15 (29%) patients with CREST syndrome.

Another study also revealed that the presence of citrullinated protein is not specific for RA; it may be an inflammation-associated phenomenon. The high specificity of the anti-CCP antibodies is most likely the result of an abnormal humoral response to the proteins *(33)*.

CREST syndrome has been described as a form of progressive systemic sclerosis in which there is relatively limited involvement of the skin, prominence of CREST. Early studies emphasized that antinuclear antibodies recognizing chromosomal centromere proteins (ACAs) had a close association with CREST syndrome *(34,35)*. Centromere antibodies have been recognized as a serological marker of a form of systemic sclerosis or scleroderma that is commonly referred to as CREST syndrome *(36)*. However, later studies did not confirm a close relationship between ACAs and CREST syndrome because of their occurrence in other rheumatic and in nonrheumatic diseases *(37,38)*. The clinical and serological heterogeneity in patients with ACA was recently evaluated *(39)*. It has been demonstrated that ACAs were positive primarily in patients with SSc with CREST features and partly in other rheumatic disorders. The high levels of ACAs may be necessary for the development of CREST features. Because not all patients develop the features of CREST at the same time and ACAs are present in more than 50%—but not all—cases, elevated levels of anti-CCP antibodies detected by the third generation of ELISA may be useful for the associate diagnosis of CREST syndrome.

Conclusion

The results of our study suggest that a new CCP3 assay offers a good diagnostic performance for detection of RA. The presence of an elevated level of anti-CCP3 antibodies in a subset of patients with CREST syndrome has raised the possibility that anti-CCP antibodies could serve as an immune marker in this disease. Further studies on larger patient populations are needed to assess the value of anti-CCP3 in clinical practice, especially for patients with CREST syndrome.

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References

1. Von Muhien and Tan, E. M. (1995), Autoantibodies in the diagnosis of systemic rheumatic diseases. *Semin Arthritis Rheum.* **24,** 323–358.
2. Senshu, T., Kan, S., Ogawa, H., Manabe, M., and Asaga, H (1996), Preferential deimination of keratin K1 and filaggrin during the terminal differentiation of human epidermis. *Biochem Biophys Res Commun* **225,** 712–719.
3. Vossenaar, E. R., Zendman, A. J., van Venrooij, W. J., Pruijn, G. J., (2003), PAD, a growing family of citrullinating enzymes: genes, features and involvement in disease. *Bioassays* **25,** 1106–1118.
4. ACR Subcommittee on RA Guidelines (2002), Guidelines for the management of rheumatoid arthritis. *Arthritis Rheum* **46,** 328–346.
5. Goldbach-Mansky, R., Lee, J., McCoy, A., et al. (2000), Rheumatoid arthritis associated autoantibodies in patients with synovitis of recent onset. *Arthritis Res* **2,** 236–243.
6. Schellekens, G. A., de Jong, B. A., van den Hoogen, F. H., van de Putte, L. B., and van Venrooij, W. J. (1998), Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. *J Clin Invest* **101,** 273–281.
7. Schellekens, G. A., Visser, H., de Jong, B. A., et al. (2000), The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinate peptide. *Arthritis Rheum* **43,** 155–163.
8. Nielen, M. M., van Schaardenburg, D., Reesink, H. W., et al. (2004), Specific autoantibodies precede

the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis Rheum* **50,** 380–386.
9. Rantapaa-Dahlqvist, S., de Jong, BA., Berglin, E., et al. (2003), Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum* **48,** 2741–2749.
10. Suzuki, K., Sawada, T., Murakami, A., et al. (2003), High diagnostic performance of ELISA detection of antibodies to citrullinated antigens in rheumatoid arthritis. *Scand J Rheumatol* **32,** 197–204.
11. Agrawal, S., Misra, R., and Aggarwal, A. (2007), Autoantibodies in rheumatoid arthritis: association with severity of disease in established RA. *Clin Rheumatol* **26,** 201–204.
12. Shovman, O., Gilburd, B., Zandman-Goddard, G., et al. (2005), The diagnostic utility of anti-cyclic citrullinated peptide antibodies, matrix metalloproteinase-3, rheumatoid factor, erythrocyte sedimentation rate, and C-reactive protein in patients with erosive and non-erosive rheumatoid arthritis. *Clin Dev Immunol* **12,** 197–202.
13. Vencovsky, J., Machacek, S., Sedova, L., et al. (2003), Autoantibodies can be prognostic markers of an erosive disease in early rheumatoid arthritis. *Ann Rheum Dis* **62,** 427–430.
14. Meyer, O., Labarre, C., Dougados, M., et al. (2003), Anticitrullinated protein/peptide antibody assays in early rheumatoid arthritis for predicting five year radiographic damage. *Ann Rheum Dis* **62,** 120–126.
15. Forslind, K., Ahlmen, M., Eberhardt, K., et al. (2004), Prediction of radiological outcome in early rheumatoid arthritis in clinical practice: role of antibodies to citrullinated peptides (anti-CCP). *Ann Rheum Dis* **63,** 1090–1095.
16. Visser, H., le Cessie, S., Vos, K., Breedveld, F. C., and Hazes, J. M. (2002), How to diagnose rheumatoid arthritis early: a prediction model for persistent (erosive) arthritis. *Arthritis Rheum* **46,** 357–365.
17. Orbach, H., Gilburd, B., Brickman, C., Gerli, R., and Shoenfeld, Y. (2002), Anti-cyclic peptide antibodies as a diagnostic test for rheumatoid arthritis and predictor of an erosive disease. *IMAJ* **4,** 892–893.
18. Baeten, D., Peene, I., Union, A., et al. (2001), Specific presence of intracellular citrullinated proteins in rheumatoid arthritis synovium: relevance to antifilaggrin autoantibodies. *Arthritis Rheum* **44,** 2255–2262.
19. van Boekel, M. A., Vossenaar, E. R., van den Hoogen, F. H., van Venrooij, W. J. (2002), Autoantibody systems in rheumatoid arthritis: specificity, sensitivity and diagnostic value. *Arthritis Res* **4,** 87–93.
20. Vossenaar, E. R. and van Venrooij, W. J. (2004) Anti-CCP antibodies, a specific marker for (early) rheumatoid arthritis. *Clin Appl Immunol Rev* **4,** 239–262.
21. Vallbracht, I., Rieber, J., Oppermann, M., Forger, F., Siebert, U., and Helmke, K. (2004), Diagnostic and clinical value of anti-cyclic citrullinated peptide antibodies compared with rheumatoid factor isotypes in rheumatoid arthritis. *Ann Rheum Dis* **63,** 1079–1084.
22. Bas, S., Genevay, S., Meyer, O., and Gabay, C. (2003), Anti-cyclic citrullinated peptide antibodies, IgM and IgA rheumatoid factors in the diagnosis and prognosis of rheumatoid arthritis. *Rheumatology (Oxford)* **42,** 677–680.
23. van Jaarsveld, C-. H., ter Borg, E. J., Jacobs, J. W., et al. (1999), The prognostic value of the antiperinuclear factor, anti-citrullinated peptide antibodies and rheumatoid factor in early rheumatoid arthritis. *Clin Exp Rheumatol.* **17,** 689–697.
24. Saraux, A., Berthelot, J. M., Devauchelle, V., et al. (2003), Value of antibodies to citrulline-containing peptides for diagnosing early rheumatoid arthritis. *J Rheumatoln* **30,** 2535–2539.
25. Vittecoq, O., Incaurgarat, B., Jouen-Beades, F., et al. (2004), Autoantibodies recognizing citrullinated rat filaggrin in an ELISA using citrullinated and non-citrullinated recombinant proteins as antigens are highly diagnostic for rheumatoid arthritis. *Clin Exp Immunol* **135,** 173–180.
26. Arnett, F. C., Edworthy, S. M., Bloch, D. A., et al. (1988), The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* **31,** 315–324.
27. Zeng, X., Ai, M., Tian, X., et al. (2003), Diagnostic value of anti-cyclic citrullinated Peptide antibody in patients with rheumatoid arthritis. *J Rheumatol* **30,** 1451–1455.
28. Mediwake, R., Isenberg, D. A., Schellekens, G. A., and van Venrooij, W. J. (2001), Use of anti-citrullinated peptide and anti-RA33 antibodies in distinguishing erosive arthritis in patients with systemic lupus erythematosus and rheumatoid arthritis. *Ann Rheum Dis* **60,** 67–68.
29. Lopez-Hoyos, M., Ruiz de Alegria, C., Blanco, R., et al. (2004), Clinical utility of anti-CCP antibodies in the differential diagnosis of elderly-onset rheumatoid arthritis and polymyalgia rheumatica. *Rheumatology (Oxford)* **43,** 655–657.
30. Gottenberg, J. E., Mignot, S., Nicaise-Rolland, P., et al. (2005), Prevalence of anti-cyclic citrullinated peptide and anti-keratin antibodies in patients with primary Sjogren's syndrome. *Ann Rheum Dis* **64,** 114–147.
31. Kamali, S., Polat, N. G., Kasapoglu, E., et al. (2005), Anti-CCP and antikeratin antibodies in rheumatoid arthritis, primary Sjogren's syndrome, and Wegener's granulomatosis. *Clin Rheumatol* **24,** 673–676.
32. Alenius, G. M., Berglin, E., Rantapaa Dahlqvist, S. (2006), Antibodies against cyclic citrullinated peptide (CCP) in psoriatic patients with or without joint inflammation. *Ann Rheum Dis* **65,** 398–400.
33. Vossenaar, E. R., Smeets, T. J., Kraan, M. C., Raats, J. M., van Venrooij, W. J., and Tak, P. P. (2004), The

presence of citrullinated proteins is not specific for rheumatoid synovial tissue. *Arthritis Rheum* **50,** 3485–3494.
34. Fritzler, M. J. and Kinsella, T. D. (1980), The CREST syndrome: a distinct serologic entity with anticentromere antibodies. *Am J Med* **69,** 520–526.
35. Weiner, E. S., Earnshaw, W. C., Senecal, J. L., Bordwell, B., Johnson, P., and Rothfield, N. F. (1988), Clinical associations of anticentromere antibodies and antibodies to topoisomerase I. A study of 355 patients. *Arthritis Rheum* **31,** 378–385.
36. Tan, E. M., Rodnan, G. P., Garcia, I., Moroi, Y., Fritzler, M. J., and Peebles, C. (1980), Diversity of antinuclear antibodies in progressive systemic sclerosis. Anti-centromere antibody and its relationship to CREST syndrome. *Arthritis Rheum* **23,** 617–625.
37. Caramaschi, P., Biasi, D., Manzo, T., Carletto, A., Poli, F., and Bambara, L. M. (1995), Anticentromere antibody—clinical associations. A study of 44 patients. *Rheumatol Int* **14,** 253–255.
38. Zuber, M., Gotzen, R., Filler, I. (1994), Clinical correlation of anticentromere antibodies. *Clin Rheumatol* **13,** 427–432.
39. Miyawaki, S., Asanuma, H., Nishiyama, S., and Yoshinaga, Y. (2005), Clinical and serological heterogeneity in patients with anticentromere antibodies. *J Rheumatol* **32,** 1488–1494.