Anti-Cyclic Citrullinated Peptide versus Anti-Sa Antibodies in Diagnosis of Rheumatoid Arthritis in an Outpatient Clinic for Connective Tissue Disease and Spondyloarthritis

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ABSTRACT. ***Objective***. To compare the diagnostic value of anti-cyclic citrullinated peptide (anti-CCP) and anti-Sa antibodies in serum for prediction of rheumatoid arthritis (RA) in an outpatient clinic for connective tissue diseases and spondyloarthritides.

Methods. A cross-sectional study was carried out to analyze the presence or absence of anti-CCP and anti-Sa antibodies in the sera of 250 randomly selected patients. The disease distribution in the study was as follows: 87 patients had RA (34.8%); 90 (36%) had other connective tissue diseases (CTD); 50 (20%) spondyloarthritis; 19 (7.6%) polymyalgia rheumatica; and 4 (1.6%) juvenile idiopathic arthritis.

Results. Anti-CCP antibodies were detected in 63 patients with RA and in 9 patients with other illnesses [sensitivity 72.4%, specificity 94.4%, positive predictive value (PPV) 87.5%]. Anti-Sa antibodies were detected in 38 patients with RA and in 6 patients with other illnesses (sensitivity 43.6%, specificity 96.3%, PPV 86.3%). Anti-CCP and anti-Sa results were discordant in up to 47 of 87 RA patients. No relation between the presence of anti-Sa and higher or lower titers of anti-CCP antibodies was observed.

Conclusion. The diagnostic value in RA is similar for both antibodies. However, the sensitivity of anti-CCP detection is higher than that of anti-Sa. Our results suggest that presence of anti-Sa antibodies in serum may be useful as a complementary assay when anti-CCP antibodies are negative and RA is suspected. (J Rheumatol 2006;33:1476–81)

Key Indexing Terms:
ANTI-CYCLIC CITRULLINATED PEPTIDE ANTIBODIES — ANTI-Sa ANTIBODIES
DIAGNOSTIC FACTORS — RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease of unknown etiology that primarily affects the joints, afflicting about 1% of the population. Autoantibodies in sera of patients with RA have been described; these can be divided, according to van Boekel, *et al*[1] into the antibodies that are also present in other diseases and antibodies that are more specific to RA, and thus more relevant to clinical practice. Several studies have shown that antibodies targeting the citrullinated antigens anti-Sa and anti-cyclic citrullinated peptide (anti-CCP) are very specific for RA diagnosis[2-5], occurring at very early stages of the disease.

Detection of anti-CCP is currently performed by ELISA. Antiperinuclear factors (APF) and antikeratin antibodies (AKA) usually appear simultaneously in patients with RA and target filaggrin modified by posttranslational deimination of peptidylarginine into peptidylcitrulline. Antibodies from sera containing APF and AKA recognize citrullinated peptides derived from filaggrin in a first-generation ELISA (anti-CCP1). Further modification of the citrullinated peptides, including their cyclization to ensure that the citrulline residue will be exposed after binding of the peptide to ELISA plates, led to a better performing second generation anti-CCP assay, anti-CCP2[3,6-8].

Other antibodies specific for RA are anti-Sa antibodies, which react by immunoblotting with a protein of molecular weight of about 48–50 kDa[9-11]. Studies have demonstrated that these autoantibodies occur in patients with recent onset RA, and seem to be related to more rapid and severe joint damage[12,13]. Recently, some authors have shown that the Sa antigen is a citrullinated vimentin[14,15]. Therefore, there is a

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need to compare anti-CCP and anti-Sa antibodies for purposes of diagnosis of RA. We investigated the usefulness of both autoantibodies in a specialized outpatient clinic for connective tissue diseases (CTD) and spondyloarthritides.

MATERIALS AND METHODS

Study population. Two-hundred fifty patients were randomly recruited from a cohort of 2072 patients diagnosed with either RA or another CTD or spondyloarthritis by the Rheumatology Service at the Gregorio Marañón University Hospital, Madrid, between 1988 and 2004. The serum samples collected showed the following disease distribution: 87 patients who fulfilled the 1987 American College of Rheumatology criteria for RA[16]; 27 patients with systemic lupus erythematosus[17]; 19 with polymyalgia rheumatica (PMR)[18]; 18 with primary Sjögren's syndrome[19]; 18 with undifferentiated connective tissue disease (CTD); 18 with ankylosing spondylitis[20]; 17 with psoriatic arthritis[20]; 10 with undefined spondyloarthritis[21]; 8 with polymyositis[22]; 7 with progressive systemic sclerosis[23]; 5 with mixed connective tissue disease[24]; 5 with reactive arthritis (arthritis, conjunctivitis, and urethritis); 4 with juvenile idiopathic arthritis (JIA)[25] (2 polyarticular, 1 pauciarticular, 1 systemic); 4 with palindromic rheumatism[26]; and 3 patients with Behçet's disease[27]. The study fulfilled the requirements from the Gregorio Marañón University Hospital Ethics Committee.

Methods. The anti-CCP assay was carried out using the RA-96RT second-generation kit (Euro-Diagnostica Inmunoscan RA, Medeon, Sweden), according to the manufacturer's instructions. Briefly, serum samples (dilution 1:50) were incubated with a citrullinated synthetic peptide in an ELISA (incubation for 1 h at room temperature). A secondary anti-IgG antibody was then added and incubated for 1 h at room temperature. After washing, the substrate was added to each well in the microplate and the reaction was photometrically visualized (450 nm). The optical density (OD) results are given in U/ml units. The kit also provided calibrator samples and control samples to evaluate the quality of the results. The standard curve was found to be close to linearity within the range 25–1600 U/ml. A cutoff value of 25 U/ml was determined by receiver-operating characteristic (ROC) curves to be the best cutoff level for our population, in perfect agreement with that recommended by the manufacturer.

The anti-Sa assay was performed as reported[28]. Briefly, human placenta extracts were prepared using the method described by Clark, *et al*[29] with some modifications[9,30]. The sample was electrophoresed (12% sodium dodecyl sulfate-polyacrylamide gel) and semidry electrotransferred onto nitrocellulose membrane, as described[28,31-33]. Membranes were blocked (3% bovine serum albumin in Tris-saline buffer solution) and incubated with patient sera (dilution 1:5; incubation for 1 h at room temperature). After extensive washing, membranes were incubated with an anti-IgG/peroxidase conjugated antibody (dilution 1:1000; incubation for 2 h at room temperature; Nordic). Antibodies bound to the membrane were visualized using a substrate solution containing 4-Cl-1-naphtol (0.05%) and H_2O_2 (0.04%). The presence of a triplet band in membranes at about 48/50 kDa was indicative of anti-Sa antibodies in patient serum.

Statistical methods. Data were analyzed using the nonparametric Mann-Whitney U test ($p < 0.05$). Contingency tables were used to calculate the sensitivity, specificity, and positive and negative predictive values (PPV, NPV) and the likelihood ratios of both assays for the diagnosis of RA[34].

RESULTS

Each of the assays described above was performed by blind operators. The patients with RA represented 34.8% of the study group (87 of 250). The frequency of antibodies for each diagnosis is shown in Table 1.

Table 2 shows the sensitivity, specificity, PPV, NPV, and positive and negative likelihood ratios of the assays. As shown in Table 1, the specificity and predictive values were similar in both assays. However, the sensitivity of anti-Sa antibodies was lower than that of anti-CCP antibodies. The likelihood ratio for positive findings was 12.92 and 11.78 for the anti-CCP and anti-Sa antibodies, respectively (Table 2). The sensitivity of prediction of RA was higher if both assays were considered together.

The results of the anti-CCP assay showed that 72 out of 250 sera had positive anti-CCP antibodies, from which 87.5% were diagnosed with RA and 12.5% with other diseases. The OD (mean ± standard error) values were 403 ± 53 and 21 ± 11 U/ml (OD range 0–1600 U/ml) in patients with RA and with other illnesses, respectively. When we considered only the OD values of positive anti-CCP (OD range 25–1600 U/ml), the OD values were 557 ± 63 and 379 ± 175 U/ml in patients with RA and with other illnesses, respectively. The results of the anti-Sa assay showed that 44 out of 250 sera had anti-Sa anti-

Table 1. Frequency of anti-cyclic citrullinated peptide (anti-CCP) and anti-Sa antibodies in systemic connective tissue diseases.

	N	Anti-CPP Antibodies, no. (%)	Anti-Sa Antibodies, no. (%)
Rheumatoid arthritis	87	63 (72.4)	38 (43.6)
Systemic lupus erythematosus	27	1 (3.7)	0 (0)
Polymyalgia rheumatica	19	3 (15.7)	0 (0)
Primary Sjögren's syndrome	18	0 (0)	1 (5.5)
Undifferentiated connective tissue disease	18	1 (5.5)	2 (11.1)
Ankylosing spondylitis	18	1 (5.5)	1 (5.5)
Psoriatic arthritis	17	0 (0)	0 (0)
Undefined spondyloarthritides	10	1 (10)	0 (0)
Polymyositis	8	0 (0)	0 (0)
Progressive systemic sclerosis	7	0 (0)	0 (0)
Mixed connective tissue disease	5	0 (0)	0 (0)
Reactive arthritis	5	0 (0)	0 (0)
Palindromic rheumatism	4	2 (50.0)	1 (25.0)
Juvenile idiopathic arthritis	4	0 (0)	1 (25.0)
Behçet's disease	3	0 (0)	0 (0)

Table 2. Sensitivity, specificity, predictive values, and likelihood ratios of anti-cyclic citrullinated peptide (anti-CCP) and anti-Sa antibodies for the diagnosis of rheumatoid arthritis.

	Anti-CCP Antibodies	Anti-Sa Antibodies	Anti-CCP or Anti-Sa Antibodies
Sensitivity, %	72.4	43.6	85.0
Specificity, %	94.4	96.3	90.8
Positive predictive value, %	87.5	86.3	83.1
Negative predictive value, %	86.5	76.2	91.9
Positive likelihood ratio	12.9	11.7	9.4
Negative likelihood ratio	0.29	0.58	0.1

bodies, from which 86.4% were diagnosed with RA and 13.6% with other diseases.

The dissociation between these 2 tests is illustrated in Table 3. Thirty-one percent of patients with RA presented simultaneously with positive anti-CCP and anti-Sa antibodies (27 out of 87). By contrast, 15% of patients with RA showed neither anti-CCP nor anti-Sa antibodies (13 out of 87). Agreement of the 2 different assays was thus 46%. Anti-CCP antibodies were exclusively detected in 41.3% of patients, whereas 12.6% of sera samples showed only positive anti-Sa antibodies. Differences between anti-CCP and anti-Sa results were found in up to 47 out of 87 patients with RA.

Only one non-RA patient, diagnosed with undifferentiated connective tissue disease (disease duration 6 yrs), presented simultaneously with anti-Sa and anti-CCP (OD value 185.25 U/ml) antibodies. On the other hand, anti-CCP antibodies were detected in the sera of 8 non-RA patients (OD mean value 403.25 U/ml, range 26.9–1600); none of these patients had positive anti-Sa antibodies. These 8 patients had been diagnosed as follows: 3 PMR (mean disease duration 1.6 yrs); 2 palindromic rheumatism (mean disease duration 7 yrs); 1 systemic lupus erythematosus (disease duration 10 yrs); 1 with ankylosing spondylitis (disease duration 6 yrs); and 1 with undifferentiated spondyloarthritis (disease duration 3 yrs).

Anti-Sa antibodies were detected in the sera of 5 non-RA patients, none of whom were positive for anti-CCP antibodies. These 5 patients had the following diagnoses: 1 polyarticular JIA (disease duration 4 yrs); 1 primary Sjögren's syndrome (disease duration 1 yr); 1 undifferentiated connective tissue disease (disease duration 12 yrs); 1 palindromic rheumatism (disease duration 2 yrs); and 1 with ankylosing spondylitis (disease duration 20 yrs). Interestingly, 4 of the non-RA patients who were positive for either anti-CCP or anti-Sa antibodies presented with persistently positive rheumatoid factor and chronic oligoarthritis (2 patients with palindromic rheumatism and anti-CCP; 1 patient with PMR and anti-CCP; and 1 patient with undifferentiated connective tissue disease and anti-Sa antibodies).

The Mann-Whitney U test disclosed no statistical differences ($p = 0.39$) between the titer of anti-CCP antibodies in sera with anti-CCP positive/anti-Sa positive antibodies (OD 453 ± 90 U/ml, range 38.25–1600) and the titer of anti-CCP in sera with anti-CCP positive/anti-Sa negative antibodies (OD 536 ± 79 U/ml, range 26.9–1600). Similarly, the titer of anti-CCP antibodies in anti-CCP positive/anti-Sa positive sera (OD 463 ± 92 U/ml, 38.25–1600) and in anti-CCP positive/anti-Sa negative sera (OD 517 ± 86 U/ml, range 36.53–1600) showed no differences in patients diagnosed with RA ($p = 0.21$). In contrast, the titers of anti-CCP antibodies (OD range 0–1600) in sera with anti-Sa positive (OD 288 ± 65 U/ml) and with anti-Sa negative antibodies (OD 125 ± 24 U/ml) were statistically different ($p < 0.0001$). However, these differences were not observed when analysis was restricted to patients with RA (Figure 1).

DISCUSSION

Previous studies have shown that the assay based on detection of serum anti-CCP antibodies by ELISA is useful in the diagnosis of RA. The reported sensitivity and specificity of anti-CCP antibodies varies between 42% and 80% and 85% and 99%, respectively, depending on the progression of the disease and on the use of first (anti-CCP-1) or second (anti-CCP-2) generation antibody tests[1,4-6,35,36]. On the other hand, several authors have proposed the usefulness of anti-Sa antibody detection by immunoblotting in the diagnosis of RA. The sensitivity and specificity of this assay varied between 31% and 43% and 94% and 99%, respectively[9-12].

We conclude that detection of anti-CCP antibodies is much

Table 3. Differences between anti-cyclic citrullinated peptide-2 (anti-CCP) and anti-Sa antibodies in patients with rheumatoid arthritis.

	Positive Anti-CCP	Negative Anti-CCP	Total No. (%)
Positive anti-Sa, no. (%)	27 (31.0)	11 (12.6)	38 (43.6)
Negative anti-Sa, no. (%)	36 (41.3)	13 (14.9)	49 (56.3)
Total	63 (72.4)	24 (27.5)	87

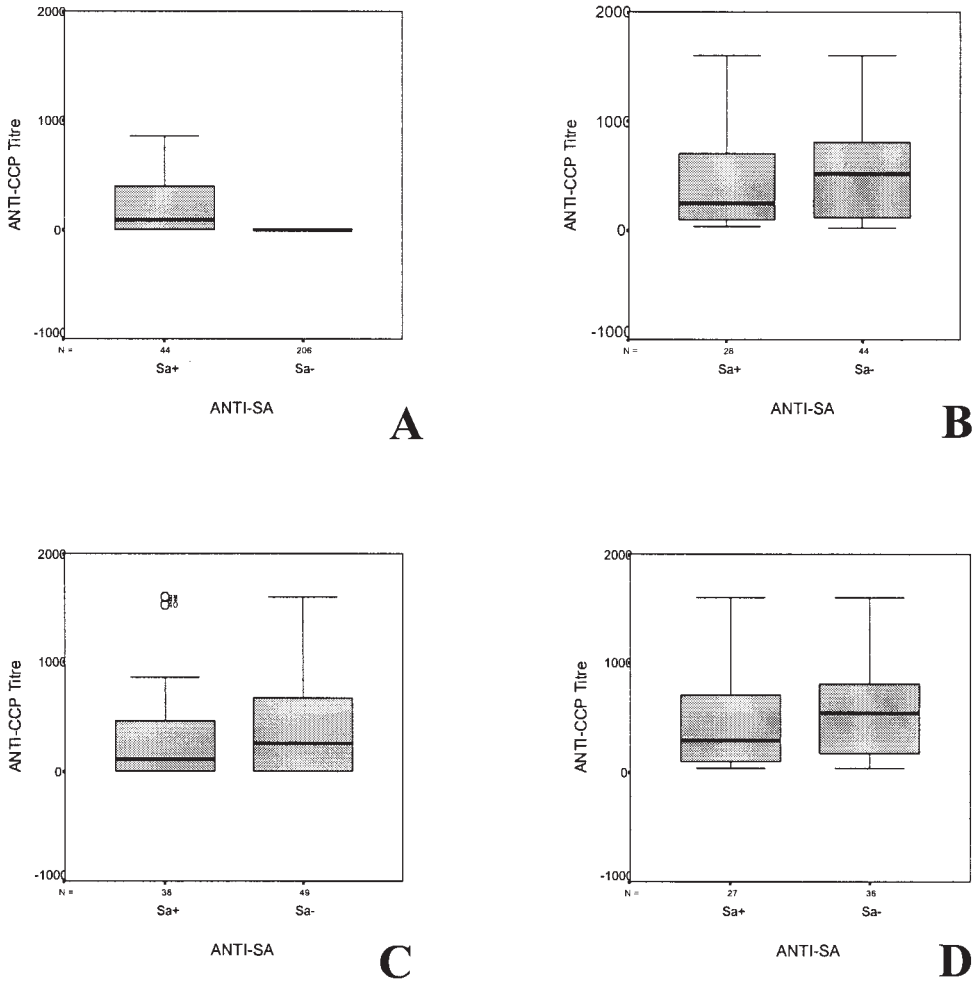

Figure 1. Comparison of anti-CCP titers in Sa positive and Sa negative sera from various groups of patients. A. Anti-CCP positive and negative groups. B. Anti-CCP positive group. C. Anti-CCP positive and negative in patients with RA. D. Anti-CCP positive in patients with RA.

more sensitive than detection of anti-Sa antibodies for the diagnosis of RA. The agreement between the anti-CCP and anti-Sa antibodies in patients with RA was only about 46%. Moreover, 12% of RA patients presented anti-Sa antibodies without anti-CCP antibodies.

There may be several possible explanations for the discordances between anti-Sa and anti-CCP in our study: (1) The source of the antigen in the anti-Sa assay is unfortunately not yet perfectly optimized. Moreover, the CCP test uses a single citrullinated epitope, while the Sa antigen may contain many different citrulline residues and thus many different citrullinated epitopes. (2) Although anti-CCP and anti-Sa are both specifically reactive with citrullinated epitopes[14], they are detected by different assays that may have a different sensitivity for RA. Our data suggest that the ELISA technique is more sensitive than the present immunoblotting technique for anti-Sa detection. (3) The antigen in the ELISA technique is a synthetic peptide, which could result in a lower antibody/antigen affinity compared to the true antigen.

The statistical values obtained in this study are similar to those previously described in the literature. However, Vossenaar, *et al*[14,37] obtained a much higher sensitivity for anti-Sa (about 76%) than that obtained in our study (43.6%). The discrepancy may be due to a random selection of patients in our study. It could be also ascribed to a higher sensitivity in the reaction developed in the immunoblotting membrane, since Vossenaar, *et al*[14] used chemiluminescence methodology, whereas we have employed a chromogenic reagent. On the other hand, we did not find a higher titer of anti-CCP antibodies in anti-CCP positive/anti-Sa positive sera versus in anti-CCP positive/anti-Sa negative sera; however, as previously reported, both anti-CCP and anti-Sa antibodies are very specific to RA. Both assays had a likelihood ratio above 10, which indicates a strong effect on pre- and post-test likelihood[32]. From these results it can be concluded that patients with anti-CCP antibodies have a 12.9-fold higher probability for presenting RA than patients with negative anti-CCP antibodies. The likelihood of having RA when anti-Sa antibodies

are detected was 11.7. Considering some recent reports that have pointed to the presence of anti-CCP antibodies in some patients many years before clinical diagnosis of RA[38], some patients not currently fulfilling RA criteria but with anti-CCP or anti-Sa antibodies may develop RA features later on. This could be the case in patients with palindromic rheumatism, seropositive polyarticular JIA, PMR, or other atypical-onset forms of RA. Indeed, anti-CCP antibodies have been detected in patients with palindromic rheumatism[39]. Nevertheless, possible false-positive results in both autoantibodies cannot be completely ruled out.

It is currently accepted that the detection of anti-CCP antibodies is the best diagnostic test for RA and that the ELISA technique is simple and reproducible. However, up to 12% of patients with RA have anti-Sa antibodies, while they are negative for anti-CCP antibodies. We suggest that in cases with negative anti-CCP antibodies by ELISA, anti-Sa by immunoblotting could be of great use.

REFERENCES

1. Van Boekel MAM, Vossenaar ER, van den Hoogen FHJ, van Venrooij WJ. Autoantibody systems in rheumatoid arthritis: specificity, sensitivity and diagnostic value. Arthritis Res 2002;4:87-93.
2. Schellekens GA, Visser H, de Jong BA, et al. The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. Arthritis Rheum 2000;43:155-63.
3. Van Venrooij WJ, Pruijn GJM. Citrullination: a small change for a protein with great consequences for rheumatoid arthritis. Arthritis Res 2000;2:249-51.
4. Vencovsky J, Machacek S, Sedova L, et al. Autoantibodies can be prognostic markers of an erosive disease in early rheumatoid arthritis. Ann Rheum Dis 2003;62:427-30.
5. Meyer O, Labarre C, Dougados M, et al. Anticitrullinated protein/peptide antibody assays in early rheumatoid arthritis for predicting five year radiographic damage. Ann Rheum Dis 2003;62:120-6.
6. Schellekens GA, de Jong BA, van den Hoogen FH, van de Putte LBA, van Venrooij WJ, Visser H. Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. J Clin Invest 1998;101:273-81.
7. Girbal-Neuhauser E, Durieux JJ, Arnaud M, Dalbon P, Sebbag M, Vincent C. The epitopes targeted by the rheumatoid arthritis-associated antifilaggrin autoantibodies are posttranslationally generated on various sites of (pro)filaggrin by deimination of arginine residues. J Immunol 1999;162:585-94.
8. Van Boekel MA, van Venrooij WJ. Modifications of arginines and their role in autoimmunity. Autoimmun Rev 2003;2:57-62.
9. Despres N, Boire G, Lopez-Longo FJ, Menard HA. The Sa system: a novel antigen-antibody system specific for rheumatoid arthritis. J Rheumatol 1994;21:1027-33.
10. Hayem G, Chazerain P, Combe B, et al. Anti-Sa antibody is an accurate diagnostic and pronostic marker in adult rheumatoid arthritis. J Rheumatol 1999;26:7-13.
11. Menard HA, Lapointe E, Rochdi MD, Zhou ZJ. Insights into rheumatoid arthritis derived from the Sa immune system. Arthritis Res 2000;2:429-32.
12. Hueber W, Hassfeld W, Smolen JS, Steiner G. Sensitivity and specificity of anti-Sa autoantibodies for rheumatoid arthritis. Rheumatology Oxford 1999;38:155-9.
13. Boire G, Cossette P, de Brum-Fernandes AJ, et al. Anti-Sa antibodies and antibodies against cyclic citrullinated peptide are not equivalent as predictors of severe outcomes in patients with recent-onset polyarthritis. Arthritis Res Ther 2005;7:R592-603.
14. Vossenaar ER, Despres N, Lapointe E, et al. Rheumatoid arthritis specific anti-Sa antibodies target citrullinated vimentin. Arthritis Res Ther 2004;6:R142-50.
15. Goldbach-Mansky R, Lee J, McCoy A, et al. Rheumatoid arthritis associated autoantibodies in patients with synovitis of recent onset. Arthritis Res 2000;2:236-43.
16. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
17. Tan EM, Cohen AS, Fries JF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271-7.
18. Jones JG, Hazleman BL. The prognosis and management of polymyalgia rheumatica. Ann Rheum Dis 1981;40:1-5.
19. Vitali C, Moutsopoulos HM, Bombardieri S. The European Study Group on diagnostic criteria for Sjögren's syndrome. Sensitivity and specificity of tests for ocular and oral involvement in Sjögren's syndrome. Ann Rheum Dis 1994;53:637-47.
20. Bennett PH, Burch TA. New York symposium on population studies: new diagnostic criteria. Bull Rheum Dis 1968;17:453-8.
21. Gladman DD, Shuckett R, Russell ML, Thorne JC, Schachter RK. Psoriatic arthritis: an analysis of 220 patients. QJ Med 1987;62:127-41.
22. Dougados M, van der Linden S, Juhlin R, Huitfeldt B, Amor B, Calin A. The European Spondylarthropathy Study Group preliminary criteria for the classification of spondylarthropathy. Arthritis Rheum 1991;34:1218-27.
23. Tanimoto K, Nakano K, Kano S, Mori S, Veki H, Nishitani H. Classification criteria for polymyositis and dermatomyositis. J Rheumatol 1995;22:668-74.
24. Masi AT, Rodnan GP, Medsger TA, Altman RD, D'Angelo WA, Fries JF. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Arthritis Rheum 1980;23:581-90.
25. Kasukawa R, Tojo T, Miyawaki S, Yoshida H, Tanimoto K, Nobunaga M. Preliminary diagnotic criteria for classification of mixed connective tissue disease. In: Kasukawa R, Sharp GC, editors. Mixed connective tissue disease and antinuclear antibodies. Amsterdam: Elsevier; 1987:41-7.
26. Gonzalez-Lopez L, Gámez-Nava JI, Jhangri GS, Ramos-Remus C, Russell AS, Suárez-Almazor ME. Prognostic factors for the development of rheumatoid arthritis and other connective tissue diseases in patients with palindromic rheumatism. J Rheumatol 1999;26:540-5.
27. Criteria for diagnosis of Behcet's disease. International Study Group for Behcet's Disease. Lancet 1990;335:1078-80.
28. Escalona M, Lopez-Longo FJ, Gonzalez CM, et al. Anti-Sa sera from patients with rheumatoid arthritis contain at least 2 different subpopulations of anti-Sa antibodies. J Rheumatol 2002;29:2053-60.
29. Clark G, Reichlin M, Tomasi TB. Characterization of a soluble cytoplasmic antigen reactive with sera from patients with systemic lupus erythematosus. J Immunol 1969;102:117-22.
30. Boire G, Lopez-Longo FJ, Lapointe S, Menard HA. Sera from patients with autoimmune disease recognize conformational determinants on the 60-kd Ro/SS-A protein. Arthritis Rheum 1991;34:722-30.
31. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 1979;76:4350-4.
32. Rodriguez-Mahou M, López-Longo FJ, Lapointe N, Carreño L, Grau R, Gurbindo D. Autoimmune phenomena in children with

human immunodeficiency virus infection and acquired immunodeficiency syndrome. Acta Pediatr Scand 1994;Suppl 400:31- 4.
33. Lopez-Longo FJ, Rodriguez-Mahou M, Escalona M, et al. Heterogeneity of the anti-Ro(SSA) response in rheumatic diseases. J Rheumatol 1994;21:1450-6.
34. Sturgess A, Edmonds J. Improving the effectiveness of autoantibody testing in the clinic. Autoimmun Rev 2002;1:273-8.
35. van Venrooij WJ, van de Putte LB. Early diagnosis of rheumatoid arthritis with a test based upon a specific antigen: cyclic citrullinated peptide [Dutch]. Ned Tijdschr Geneeskd 2003;147:191-4.
36. van Venrooij WJ, Vossenaar ER, Zendman AJ. Anti-CCP antibodies: the new rheumatoid factor in the serology of rheumatoid arthritis. Autoimmun Rev 2004;3 Suppl 1:S17-9.
37. Vossenaar ER, van Venrooij WJ. Citrullinated proteins: sparks that may ignite the fire in rheumatoid arthritis. Arthritis Res Ther 2004;6:107-11.
38. Nell V, Machold KP, Stamm TA, et al. Autoantibody profiling as early diagnostic and prognostic tool for rheumatoid arthritis. Ann Rheum Dis 2005;64:1731-6.
39. Salvador G, Gomez A, Vinas O, et al. Prevalence and clinical significance of anti-cyclic citrullinated peptide and antikeratin antibodies in palindromic rheumatism. An abortive form of rheumatoid arthritis? Rheumatology Oxford 2003;42:972-5.