Clinical and Experimental Rheumatology 2008; 26: 461-463.

Evaluation of third generation anti-CCP antibodies in the diagnosis of rheumatoid arthritis from undifferentiated polyarthritis after 4 years of follow-up

J.L. Caro-Oleas[1], A. Fernández-Suárez[2], S. Reneses Cesteros[3], C. Porrino[4], A. Núñez-Roldán[1], I. Wichmann Schlipf[1]

Departments of [1]Immunology and [3]Rheumatology, University Hospital Virgen del Rocío, Sevilla, Spain; Department of [2]Biotechnology, Hospital Alto Guadalquivir, Andújar, Spain; [4]Biotechnology Area, Empresa Pública Hospital de Poniente de Almería, El Ejido, Spain.

J.L. Caro-Oleas, A. Fernández-Suárez, S. Reneses Cesteros, C. Porrino, A. Núñez-Roldán, I. Wichmann Schlipf.

This work was supported by grants from Red Temática de Investigación en Trasplante C03/03 and the Research Proyect FIS 05/0374 of the Spanish Ministry of Health.

Please address correspondence to: Dr. J.L. Caro-Oleas, Avenida Manuel Siurot s/n, Edificio de Laboratorios. Servicio de Inmunología 3ª planta, Hospital Universitario Virgen del Rocío, 41013, Sevilla, Spain.
E-mail: joseluiscaro@hotmail.com

Received on April 14, 2007; accepted in revised form on December 13, 2007.

Key words: RA diagnosis, undifferentiated polyarthritis, anti-cyclic citrullinated peptide antibodies, third generation CCP ELISA.

Competing interests: none declared.

ABSTRACT

Objectives. *Rheumatoid arthritis (RA) is a complex pathology to identify at an early stage. A large number of patients with recent onset polyarthritis (ROP) do not usually fulfil the ACR criteria for diagnosis of the disease and are classified as having undifferentiated polyarthritis. The aim of this study is to verify with certainty the diagnosis of patients whose illness has not been classified after four years of follow-up, and correlate their actual status with the levels of anti-cyclic citrullinated peptide (anti-CCP) antibodies and rheumatoid factor (RF) found.*

Methods. *After one year of follow-up, 56 patients from a total of 322, included from January 2002 in the ROP Unit, did not meet ACR criteria for any rheumatic disease. The anti-CCP antibodies and RF levels were determined in the initial clinical assessment.*

Results. *After four years of follow-up, 12 new diagnoses were made in the 56 patients with undifferentiated polyarthritis: 3 seronegative RA, 8 seropositive RA and 1 psoriatic arthritis. The anti-CCP antibodies levels were positive for 5 of these 12 patients (median anti-CCP 228.6 U/mL), and all were RF positive. Six of the 7 anti-CCP antibodies negative patients were diagnosed of RA (3 seropositive and 3 seronegative for RF) and 1 was diagnosed of psoriatic arthritis (anti-CCP antibodies negative and RF positive). Forty-four patients still displayed undifferentiated polyarthritis and were RF negative.*

Conclusion. *A positive result for RF and anti-CCP antibodies in patients who do not meet the ACR diagnostic criteria could be a useful indicator of the presence of future RA.*

Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease which is characterised by the destruction of joints due to chronic inflammation. The diagnosis and classification of RA is based on clinical data, radiological findings and laboratory results. Rheumatoid factor (RF) forms part of the American College of Rheumatology (ACR) diagnostic criteria (1) and is the main serological marker used routinely in clinical practice (2-3). Nevertheless, the detection of RA is difficult in the early stages of the illness due to the fact that many of its clinical characteristics, such as inflammation of the joints, are also present in other rheumatic diseases.

Numerous efforts have been made in order to find new serological markers which are useful for the diagnosis and follow-up of RA, since RF shows acceptable sensitivity and low specificity (4). The study of cyclic citrullinated peptides (5) and the research into the techniques used to detect anti-cyclic citrullinated peptide (anti-CCP) antibodies have already displayed results (6). There are many published studies which show that anti-CCP antibodies are a good indicator of the presence of rheumatoid arthritis (8-10) and that the combination of positive anti-CCP antibodies and/or positive RF increases sensitivity levels (11). The presence of these autoantibodies seems to predict a more aggressive disease, with greater activity and more severe radiological damage (12).

The problem of definitively diagnosing RA or any other type of rheumatic disease has been analysed in other studies we have previously carried out (13). In patients with recent onset polyarthritis (ROP) after one year of follow-up from the initial consultation at our hospital, 17.4% of patients did not meet ACR diagnostic criteria for any rheumatic disease and were classified as having undifferentiated polyarthritis. The aim of this study is to present the results of a new revision, after a four years follow-up, of all the patients with undifferentiated polyarthritis included in our hospital register, who could not be diagnosed with certainty, and to correlate the new diagnosis obtained with the initial levels of anti-CCP antibodies and RF.

Patients and methods

Patients

We analysed patients referred from primary healthcare centres, emergency services and outpatient rheumatology clinics (Public Health Area comprising 774,619 patients according to the 2002 census) to the Recent-onset Polyarthritis Unit of the Hospital Virgen del Rocío

in Seville (Spain). All the patients were included in a prospective computerised register from January 2002 through today. (14).

The patients were referred to our unit with a form that included the referral criteria of each patient: age ≥16 years, inflammation of two or more joints for a minimum period of 4 weeks, and arthritis with a period of evolution ≤1 year, together with personal patient information and the physician recommending referral. During the first visit, these criteria were revised and used to include the patients in the Recent-Onset Polyarthritis Unit register. We excluded from the study: patients with arthrosis, crystal-induced arthritis, infectious or paraneoplastic arthritis; patients with non-inflammatory joint effusion; patients previously treated with anti-inflammatory drugs and/or steroids; and those who did not give informed consent.

Several studies have been carried out on the 322 patients included in the ROP register from 2002 in order to determine the diagnostic usefulness of anti-CCP antibodies (13, 14), using serum from these patients' initial clinical assessment. Having revised the ACR diagnostic criteria, 56 patients continued to present undifferentiated polyarthritis after one year of follow-up. The clinical histories of all of these patients were revised (after four years of follow-up in each case) in order to establish a diagnosis which meets the ACR criteria.

Laboratory tests

All of the analysis were carried out during the patients' initial consultation.

Anti-CCP antibodies

An ELISA kit, QUANTA Lite™ CCP IgG ELISA (INOVA Diagnostic, San Diego, CA, USA) called third generation (anti-CCP3) test, was used to detect the presence of anti-CCP antibodies. Antigenic differences between second and third generation tests are not available. The procedures were conducted manually and according to the manufacturers' recommendations. We analysed the results obtained using the cut-offs determined by the manufacturers (20 U/mL).

Table I. Patients with undifferentiated polyarthritis with the diagnosis confirmed after four years of follow-up. The levels of anti-CCP antibodies and RF were determined at the beginning of follow-up.

Patient number	Sex	Age	Diagnosis after 4 years of follow-up	Anti-CCP ab. (U/mL)	RF (U/mL)
1	W	38	RA seronegative	1.8	30.0
2	W	67	RA seronegative	0.0	10.0
3	W	40	RA seronegative	4.9	9.3
4	M	65	Psoriatic arthropathy	5.0	53.0
5	W	48	RA seropositive	4.9	94.0
6	M	23	RA seropositive	4.8	55.0
7	W	48	RA seropositive	4.9	95.0
8	W	31	RA seropositive	270.0	598.0
9	W	44	RA seropositive	195.0	75.0
10	W	37	RA seropositive	140.0	66.0
11	W	36	RA seropositive	305.0	316.0
12	W	31	RA seropositive	233.0	598.0

RF: rheumatoid factor; anti-CCP ab.: levels of anti-cyclic citrullinated peptide antibodies; W: woman; M: man.

Rheumatoid factor

RF (anti-total) was determined by means of nephelometry in a BN® II (Dade Behring, Marburg, Germany) using the N latex RF method (Dade Behring) according to the manufacturer's instructions. Results over 50 U/mL were considered positive, using the optimum cut-off reported by other authors (10).

Results

From a total of 56 patients with undifferentiated polyarthritis included in this study, 47 were seronegative and 9 were seropositive for RF. The average age of the patients was 45.34±2.03 (SEM) years (age range from 16 to 77 years). Twenty men (35.7%; average age 37.3±3.19 years) and 36 women (64.3%; average age 49.81±2.33 years) were included in this study.

Only 12 patients met diagnostic criteria for a rheumatic disease. Three cases of seronegative rheumatoid arthritis, 8 cases of seropositive rheumatoid arthritis and one case of psoriatic arthritis were diagnosed (Table I). The anti-CCP antibody levels were positive (and high) for 5 of these 12 patients diagnosed with RA (median anti-CCP 228.6 U/mL); all patients also presented positive RF. Of the 7 anti-CCP antibodies negative patients, 6 were diagnosed of RA (3 were seropositive and 3 were seronegative for RF); one patient had psoriatic arthritis with very low levels of anti-CCP antibodies (5 U/mL) and was RF positive, although with low levels (53 U/mL). In 5 out of 6 of the patients diagnosed from RA we could obtain recently a new serum and mesure the present anti-CCP antibody levels, that were all negative (median anti-CCP 7.9±2.0 SEM).

After four years of follow-up and according to this last revision, there are still 44 patients with undifferentiated polyarthritis. All of these patients were RF negative [median rheumatoid factor, 9.85±0.47 (SEM)] and do not have detectable levels of anti-cyclic citrullinated peptide antibodies [median anti-CCP, 4.40±0.27 (SEM)].

Discussion

At times, it can be difficult to diagnose rheumatoid arthritis, since over a period of 6 weeks or more, a minimum of 4 of the 7 ACR criteria may not be fulfilled. The parameters used in the criteria reflect the chronic phase of the disease (1), and it takes a long time to reach this phase. Therefore, there are problems in finding an exact diagnosis of RA in the early stages of the disease. Patients who visit for an initial consultation their rheumatologist or general practitioner do not often meet the criteria of this classification and usually present atypical symptoms. The disease is thus frequently classified as undifferentiated polyarthritis. This may

explain why 44 patients included in our study after 4 years from the initial diagnosis remained in the undifferentiated polyarthritis group.

The presence of serological markers such as RF and anti-CCP antibodies help to diagnose the disease but it is noteworthy that the majority of patients with no definite diagnosis are RF and anti-CCP antibodies negative. This negative result may indicate that patients will not develop RA, or that development of the disease will occur at a later date, although this can only be verified over time.

The anti-CCP antibodies positive patients in this study to date display a specificity of 100%. In other words, all anti-CCP antibodies positive patients subsequently developed RA. Nevertheless, the presence of 6 patients with anti-CCP antibodies negative results who were diagnosed as RA (false negatives) reduces the predictive value of this test. Recently we tested anti-CCP antibody levels in 5 out of these 6 patients and they are still negative. Further follow-up and more patients are necessary to confirm that individuals negative for anti-CCP at the moment of diagnosis, will not develop later these antibodies.

In addition, the only psoriatic arthropathy found was RF positive (however, this was well within the lower limit, 53 U/mL, with a cut-off value of 50 U/mL). The fact that the patient in our study did not display typical clinical symptoms of psoriatic arthropathy and showed positive values for RF, could explain the delay in diagnosis. The anti-CCP antibody test was negative, which may show the usefulness of this marker in the differential diagnosis, although its presence has been described in approximately 12.5% of patients with psoriatic arthritis (15).

In light of the results obtained in this study, it can be concluded that the concurrence of a positive result for RF and anti-CCP antibodies in a patient, who does not meet the ACR criteria for diagnosing RA, could be a useful indicator of future RA.

Acknowledgements

The authors thank the manufacturers for providing the ELISA kits analysed, which made the present study possible.

References

1. 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.
2. WOLFE F, CATHEY MA, ROBERTS FK: The latex test revisited. Rheumatoid factor testing in 8,287 rheumatic disease patients. *Arthritis Rheum* 1991; 34: 951-60.
3. VISSER H, GELINCK LB, KAMPFRAATH AH, BREEDVELD FC, HAZES JM: Diagnostic and prognostic characteristic of the enzyme linked immunosorbent rheumatoid factor assays in rheumatoid arthritis. *Ann Rheum Dis* 1996; 55: 157-61.
4. VAN BOEKEL MA, VOSSENAAR ER, VAN DEN HOOGEN FH, VAN VENROOIJ WJ: Autoantibody systems in rheumatoid arthritis: specificity, sensitivity and diagnostic value. *Arthritis Res* 2002; 4: 87-93.
5. 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.
6. COENEN D, VERSCHUEREN P, WESTHOVENS R, BOSSUYT X: Technical and diagnostic performance of 6 assays for the measurement of citrullinated protein/peptide antibodies in the diagnosis of rheumatoid arthritis. *Clin Chem* 2007; 53: 498-504.
7. VAN GAALEN FA, LINN-RASKER SP, VAN VENROOIJ WJ *et al*: Autoantibodies to cyclic citrullinated peptides predict progression to rheumatoid arthritis in patients with undifferentiated arthritis: a prospective cohort study. *Arthritis Rheum* 2004; 50: 709-15.
8. SODERLIN MK, KASTBOM A, KAUTIAINEN H, LEIRISALO-REPO M, STRANDBERG G, SKOGH T: Antibodies against cyclic citrullinated peptide (CCP) and levels of cartilage oligomeric matrix protein (COMP) in very early arthritis: relation to diagnosis and disease activity. *Scand J Rheumatol* 2004; 33: 185-8.
9. RIEDMAN JP, MUÑOZ S, KAVANAUGH A: The use of second generation anti-CCP antibody (anti-CCP2) testing in rheumatoid arthritis – A systematic review. *Clin Exp Rheumatol* 2005; 23 (Suppl. 39): S69-76.
10. 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.
11. RAZA K, BREESE M, NIGHTINGALE P *et al*.: Predictive value of antibodies to cyclic citrullinated peptide in patients with very early inflammatory arthritis. *J Rheumatol* 2005; 32: 231-8.
12. DEL VAL DEL AMO N, IBANEZ BOSCH R, FITO MANTECA C, GUTIERREZ POLO R, LOZA CORTINA E: Anti-cyclic citrullinated peptide antibody in rheumatoid arthritis: relation with disease aggressiveness. *Clin Exp Rheumatol* 2006; 24: 281-6.
13. FERNANDEZ-SUAREZ A, RENESES S, WICHMANN I, CRIADO R, NUNEZ A: Efficacy of three ELISA measurements of anti-cyclic citrullinated peptide antibodies in the early diagnosis of rheumatoid arthritis. *Clin Chem Lab Med* 2005; 43: 1234-9.
14. RENESES S, PESTANA L, FERNANDEZ-SUAREZ A *et al*: Predictors of remission in recent onset polyarthritis. *Scand J Rheumatol* 2007; 37: 378-85.
15. INANC N, DALKILIC F, KAMALI S *et al*.: Anti-CCP antibodies in rheumatoid arthritis and psoriatic arthritis. *Clin Rheumatol* 2007; 26: 17-23.