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Anti-cyclic citrullinated peptide antibodies, IgM and IgA rheumatoid factors in the diagnosis and prognosis of rheumatoid arthritis

S. Bas, S. Genevay, O. Meyer[1] and C. Gabay

Objectives. **To evaluate the association of anti-cyclic citrullinated peptide (anti-CCP) antibodies with immunoglobulin (Ig) M and IgA rheumatoid factors (RF) in discriminating between rheumatoid arthritis (RA) and other rheumatic diseases, and to determine, in a longitudinal study, whether clinical signs of disease severity are associated with the presence of these autoantibodies at the time of patient inclusion.**

Methods. **The presence of these three markers was determined in 196 patients with established RA, 239 non-RA controls with various rheumatic diseases (cross-sectional study) and in 27 patients with arthritis of less than 1 yr disease duration who were subsequently followed during about 8 yr (longitudinal study). At the time of follow-up, 21 of these 27 patients had RA. They were further evaluated and several clinical variables were recorded.**

Results. **The specificity was significantly increased (from 82–90% to 98% in the cross-sectional study) when the combination of anti-CCP antibodies with IgA RF or the combination of the three serological markers was used. An association was observed between the presence of the three autoantibodies and clinical signs of disease severity (functional disability, presence of erosions and absence of clinical remission).**

Conclusions. **The presence either of anti-CCP antibodies and IgA RF or of the three markers appears to be useful in the diagnosis of RA. Their association with clinical signs of disease severity at the time of patient inclusion suggests their potential usefulness as markers for prognosis.**

KEY WORDS: Rheumatoid arthritis, Anti-cyclic citrullinated peptide antibodies, IgM rheumatoid factors, IgA rheumatoid factors.

Rheumatoid factors (RF) are currently used in the diagnosis of rheumatoid arthritis (RA) and constitute one of the classification criteria proposed by the American College of Rheumatology (ACR) [1]. However, RF positivity shows low diagnostic specificity because RF are present in patients with other autoimmune and infectious diseases, and even in a noticeable proportion of normal healthy subjects, particularly in ageing individuals [2–4]. Another test of interest in the diagnosis of RA is the assay for anti-cyclic citrullinated peptide (CCP) antibody, which has high specificity (91–98%) but wide variability in diagnostic sensitivity (41–68%) [5–10].

In a recent study, we compared the performances of 11 different assays to detect RFs [11] and found substantial differences in sensitivity and specificity, with questionable reliability for some assays. However, the anti-CCP antibody test was not studied comparatively, no follow-up study was performed and healthy subjects were not tested. We therefore compared the sensitivities and specificities of the anti-CCP antibodies with those of immunoglobulin (Ig) M and IgA RF, which have been

Division of Rheumatology, Department of Internal Medicine, University Hospital, Geneva, Switzerland and [1]Service de Rhumatologie, Hôpital Bichat, 46, rue Huchard, 75018 Paris, France.

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Correspondence to: S. Bas, Research Laboratory, Division of Rheumatology, University Hospital, 1211 Geneva 14, Switzerland. E-mail: sylvette.bas@hcuge.ch

found previously to be the most accurate [11]. Two different studies were performed, including a cross-sectional study with samples from 196 patients with established RA, 239 non-RA controls with various rheumatic diseases and two groups of healthy blood donors, and an 8-yr follow-up study on 27 patients with arthritis of less than 1 yr disease duration.

Patients and methods

Patients

In the cross-sectional study, different groups were compared: (i) 196 patients with RA according to the 1987 ACR revised criteria [1] (clinical characteristics of these patients have been described previously [10]); median age 64 yr (range 22–87 yr); 75% were women; (ii) 239 non-RA control patients, including 160 patients recruited over the same period as the RA patients in the out-patient clinic of the University Hospital of Geneva, with other rheumatological diagnoses [connective tissue diseases ($n=45$), seronegative spondylarthropathies ($n=45$), crystal-induced arthritis ($n=25$), other inflammatory diseases, including Crohn's disease, polymyalgia rheumatica and sarcoidosis ($n=22$), osteoarthritis ($n=18$), transient hip synovitis ($n=3$), undifferentiated polyarthralgias ($n=2$)], and 79 patients with seronegative spondylarthropathies recruited in a EULAR (European League against Rheumatism)-sponsored trial of long-term azithromycin treatment in reactive arthritis (Gaston JSH, Kvien TK, Bardin T, Dijkmans BAC, Altwegg M, Plan PA, Vischer TL, in preparation); median age 42 yr (range 4–101 yr); 49% were women. Two groups of healthy blood donors were also used as negative controls: (i) 99 individuals matched with the RA patients of the cross-sectional study for age, sex and geographical distributions [median age 60 yr (range 50–75 yr), 71% were women]; and (ii) 57 individuals $\geqslant 60$ yr of age (because RF positivity is known to occur particularly in healthy ageing individuals [2–4]) [median age 66 yr (range 60–71 yr); 21% were women].

The longitudinal study, conducted with 64 consecutive patients, has been described previously [12]. After 8 yr (± 8 months) of follow-up, 29 patients were re-evaluated but two of them were subsequently excluded because initial serum samples were not available. Clinical evaluation of the 27 remaining patients confirmed the diagnosis of RA in 21 of them [median age at time of follow-up 54 yr (range 27–77 yr); 76% were women]. The non-RA patients comprised four with seronegative spondylarthropathies and two with undifferentiated polyarthritis. Patients with RA were further evaluated. Radiographs were scored according to a modified version of the Larsen score [13]. We recorded the presence of the HLA-DRB1 70–74 shared epitope, extra-articular manifestations (nodules, pleuritis, pericarditis, Felty's syndrome, Sjögren's syndrome, vasculitis), criteria for remission according to ARA [14], daily life function assessed with the Health Assessment Questionnaire (HAQ), the Disease Activity Score (DAS) [15], and the number of treatments with disease-modifying anti-rheumatic drugs (DMARDs).

A serum sample was drawn from each patient (at the time of inclusion for patients of the longitudinal study), was aliquoted and stored at -80°C until use.

Anti-CCP antibody determination by enzyme immunoassay

The enzyme-linked immunosorbent assay (ELISA) kits detecting the IgG anti-CCP antibodies were purchased from Euro-Diagnostica (Arnhem, The Netherlands). Excepting the standard curve, which was a plot of the logarithm of optical density *vs* the logarithm of units of anti-CCP antibodies, the assay was performed according to the manufacturer protocol.

IgM and IgA RF determinations by enzyme immunoassays

One ELISA using Fc fragments of human IgG as antigen, developed in our laboratory, was used to detect IgM RF [11]. One commercially available kit, purchased from Inova Diagnostics (San Diego, CA, USA), was used to detect IgA RF.

Immunogenetic analysis

HLA DR generic oligotyping was performed using a non-radioactive reverse dot-blot technique as described elsewhere [12, 16].

Statistical analysis

Statistical analysis was performed using SPSS statistical software (SPSS for Macintosh, version 10; SPSS, Chicago, IL, USA).

The sensitivity and specificity were computed for each of three tests, along with 95% confidence intervals (CI). Differences were tested with the McNemar test.

In the longitudinal study, subgroups of RA patients who were positive and negative for anti-CCP antibodies, IgM and IgA RF were compared using Student's *t*-test (for continuous variables) and the χ^2 test (for discrete variables).

Results

Sensitivity and specificity of anti-CCP antibodies and IgM and IgA RF for the diagnosis of RA in the cross-sectional study

When individual tests were considered, sensitivity for RA was highest for IgM RF (73%), followed by IgA RF (63%) and anti-CCP antibodies (56%). The difference between IgA RF and anti-CCP antibodies was not significant. Specificity was significantly greater for anti-CCP antibodies and IgA RF (90%) than for IgM RF (82%). The combination of anti-CCP antibodies and IgA RF and the combination of the three tests gave the lowest sensitivities (44 and 41% respectively) but the highest specificity (98%) (Table 1).

Presence of autoantibodies in samples from healthy blood donors

When individual tests were considered, 13% of matched donors and 9% of elderly donors ($\geqslant 60$ yr of age) had IgM RF and 5% had IgA RF in both groups. Anti-CCP antibodies were detected in 2% of matched donors but not in elderly donors. With the combinations of two or three tests, no healthy blood donor was found to be positive (data not shown).

Anti-CCP antibody and IgM and IgA RF determinations at the time of patient inclusion in the longitudinal study

When individual tests were considered, sensitivity for the diagnosis of RA was highest for IgA RF (48%), followed by IgM RF (38%) and anti-CCP antibodies (33%). The combination of anti-CCP antibodies and IgA RF was

TABLE 1. Comparison of the three tests, alone or combined, for the diagnosis of RA: sensitivity and specificity in patients with well-characterized diseases

Test	Sensitivity (95% CI)	Specificity (95% CI)
Anti-CCP antibodies (ACCP)	0.56 (0.49–0.63)	0.90 (0.86–0.93)
IgM RF	0.73 (0.67–0.79)	0.82 (0.77–0.87)
IgA RF	0.63 (0.56–0.70)	0.90 (0.86–0.94)
ACCP + IgM RF	0.48 (0.41–0.55)	0.96 (0.93–0.98)
ACCP + IgA RF	0.44 (0.37–0.51)	0.98 (0.96–1.00)
IgM RF + IgA RF	0.59 (0.52–0.66)	0.94 (0.91–0.97)
ACCP + IgM RF + IgA RF	0.41 (0.34–0.48)	0.98 (0.97–1.00)

	Intertest difference in	
	Sensitivity (*P**)	Specificity (*P**)
ACCP *vs* IgM RF	<0.0001	0.013
ACCP *vs* IgA RF	0.072	1
IgM RF *vs* IgA RF	0.002	0.003
(ACCP + IgM RF) *vs* (ACCP + IgA RF)	0.12	0.13
(ACCP + IgM RF) *vs* (IgM RF + IgA RF)	0.003	0.45
(ACCP + IgA RF) *vs* (IgM RF + IgA RF)	<0.0001	0.012
ACCP *vs* (ACCP + IgM RF + IgA RF)	<0.0001	<0.0001
IgM RF *vs* (ACCP + IgM RF + IgA RF)	<0.0001	<0.0001
IgA RF *vs* (ACCP + IgM RF + IgA RF)	<0.0001	<0.0001
(ACCP + IgM RF) *vs* (ACCP + IgM RF + IgA RF)	<0.0001	0.031
(ACCP + IgA RF) *vs* (ACCP + IgM RF + IgA RF)	0.031	1
(IgM RF + IgA RF) *vs* (ACCP + IgM RF + IgA RF)	<0.0001	0.002

Positive patients were 196 patients with established rheumatoid arthritis and negative patients were 239 patients with various other rheumatological diseases (cross-sectional study).

CI, confidence interval.

**P* values are based on the McNemar test.

TABLE 2. Follow-up characteristics of patients with RA according to anti-CCP antibody and RF positivities at the time of patient inclusion

	Anti-CCP antibodies		IgM RF		IgA RF	
	+ ($n=7$)	− ($n=14$)	+ ($n=8$)	− ($n=13$)	+ ($n=10$)	− ($n=11$)
Women (%)	100	64	88	69	90	64
Age at time of patient inclusion (yr; mean ± S.D.)	58 ± 10	55 ± 16	50 ± 16	49 ± 16	51 ± 14	48 ± 17
HLA-DRB1 70–74 shared epitope (%)	100	69	88	75	90	73
Presence of radiological signs of joint damage (%)	86	29*	75	31*	70	27
Presence of extra-articular manifestations† (%)	14	29	25	23	30	18
Clinical remission (ARA) (%)	0	43*	0	46*	0	55**
HAQ at time of follow-up (mean ± S.D.)	1.1 ± 1.0	0.7 ± 0.8	1.1 ± 0.9	0.6 ± 0.8	1.0 ± 0.9	0.6 ± 0.9
DAS at time of follow-up (mean ± S.D.)	3.6 ± 1.7	2.2 ± 1.3	3.9 ± 1.6	1.9 ± 0.9***	3.8 ± 1.5	1.9 ± 0.9***
Number of DMARDs at time of follow-up (mean ± S.D.)	2.4 ± 1.0	2.1 ± 1.1	2.5 ± 0.9	2.0 ± 1.2	2.6 ± 1.0	1.8 ± 1.1

†Nodules, pleuritis, pericarditis, Felty's syndrome, Sjögren's syndrome, vasculitis.

*$P < 0.05$; **$P < 0.01$ (χ^2 test); ***$P < 0.005$ (Student's *t*-test).

found in 33% of the patients and the three autoantibodies in 29%. The intertest differences in sensitivities were not significant (McNemar test) (data not shown). The prevalence of anti-CCP antibodies and IgM RF was significantly higher in patients with radiological signs of joint damage (86 *vs* 29% and 75 *vs* 31% respectively). At the time of patient inclusion, the presence of autoantibodies (43–55%) was detected only in RA patients who had a chronic course with clinical signs of active disease after 8 yr. In contrast, these markers were absent in six patients with a favourable outcome (remission). Patients without RF had significantly lower DAS (1.9 ± 0.9) than patients with IgM (3.9 ± 1.6) or IgA RF (3.8 ± 1.5) (Table 2).

Discussion

The diagnostic and prognostic values of anti-CCP antibody determination, alone or associated with IgM RF, have already been evaluated carefully [5–10], but the value of its association with IgA RF has not been reported hitherto. The present study shows that the specificity was slightly but significantly increased (from 82–90% to 98% in the cross-sectional study) when the combination of anti-CCP antibodies and IgA RF or the combination of the three serological markers was used. This conclusion was validated in the longitudinal study, as none of the six non-RA patients had, at the time of patient inclusion, one of these combinations of

autoantibodies. The usefulness of the combination of anti-CCP antibodies and IgA RF is reinforced by their low prevalence (0–5%) in the healthy adult population, whereas IgM RF has a prevalence of 9–13%. However, the problem is the relative low sensitivity, as this combination of autoantibodies is detected in only 44% of patients with well-characterized RA and 33% of patients with RA duration of <1 yr, underlining the need for improvement in the serological diagnosis of RA.

As already reported [5, 7, 8, 17–20], the determination of these serological markers appeared to be important for prognosis. The presence of anti-CCP antibodies and IgM RF was associated with a higher probability of radiological signs of joint damage and that of RFs with higher functional disability. The presence of the three markers predicted the absence of clinical remission after 8 yr. Thus, although this study was performed in a small group of patients with early inflammatory arthritis, it shows that these markers deserve to be evaluated further in a larger population of patients in order to better determine their usefulness as diagnosis and prognosis markers in early arthritis.

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