Value of Antibodies to Citrulline-Containing Peptides for Diagnosing Early Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** To compare the diagnostic values of antiperinuclear factor (APF), antikeratin antibody (AKA), and anti-cyclic citrullinated peptides (anti-CCP) to discriminate between patients with and without rheumatoid arthritis (RA) and to determine the diagnostic value of anti-CCP used alone or with other tests.

Methods. Two hundred and seventy patients with early arthritis underwent standardized investigations in 1995–1997. The clinical utility of APF, AKA, and anti-CCP in first-visit sera was evaluated using receiver-operating characteristic curves. Combinations of anti-CCP with other laboratory tests were assessed by multiple logistic regression.

Results. Anti-CCP, APF, and AKA were not perfectly correlated with one another. Anti-CCP with 53 UI as the cutoff was 47% sensitive and 93% specific, versus 52% and 79%, and 47% and 94%, for APF and AKA, respectively. Multiple logistic regression selected anti-CCP, AKA, IgM-rheumatoid factor (RF) ELISA, and the latex test.

Conclusion. Rheumatologists can routinely use 2 or 3 tests for diagnosing RA (latex and/or IgM RF ELISA, and either AKA or anti-CCP ELISA) and can add a third or fourth test when the diagnosis remains in doubt. (J Rheumatol 2003;30:2535–9)

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While the early diagnosis of rheumatoid arthritis (RA) is difficult, it has become a key priority because there is now convincing evidence that early treatment with disease modifying antirheumatic drugs improves the outcome of RA[1]. Although the only laboratory test included in the classification criteria for RA is serum rheumatoid factor (RF) determination[2], strong data indicate that the diagnosis can benefit from assays for antibodies to citrulline-containing peptides [anti-cyclic citrullinated peptides antibodies (anti-CCP), antifilaggrin antibodies (AFA), antiperinuclear factors (APF), or antikeratin antibodies (AKA), in reference to the various methods used for their detection][3-5].

We previously found wide variations in opinions among rheumatologists about which laboratory and imaging studies are useful for identifying the cause of recent-onset polyarthritis[6]. AKA was among the main laboratory tests used for the diagnosis of RA before anti-CCP kits were introduced commercially. However, the diagnostic usefulness of anti-CCP detected using various methods has not been studied comparatively, and the diagnostic value of anti-CCP in combination with other tests has not been fully evaluated.

In a study conducted before the identification of anti-CCP, we found that IgG-AKA, IgM-RF by ELISA, and the latex test was the best combination for diagnosing RA in a cohort of 270 patients with early arthritis[7]. We also found that IgG and IgA-RF, IgA and IgM-AKA, antinuclear antibodies, anti-RA33 antibodies, and HLA-DR4 had little value in distinguishing RA from non-RA. We have now evaluated the same cohort with 2 objectives: (1) to compare the diagnostic value of IgG-APF, AKA, and anti-CCP and (2) to determine the diagnostic value of anti-CCP used alone or in combination with other tests.

MATERIALS AND METHODS

Study design. The study design has been reported[7,8]. Briefly, the study cohort was composed of patients first evaluated for early arthritis between 1995 and 1997 in Brittany, France. Inclusion criteria were as follows: age

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16 years or older, swelling of at least one joint, absence of a previous diagnosis of joint disease, and disease duration of no more than one year. At baseline, all patients had a standardized interview; a general physical examination; and a rheumatological examination. Radiographs of the chest, pelvis, hands, and feet were obtained. The following laboratory tests were performed: blood counts; serum creatinine; proteinuria; serum C-reactive protein; a latex test and ELISA for IgM, IgG and IgA-RF; tests for APF, AKA, antinuclear antibody (ANA), and anti-DNA antibody; and HLA AB DR type.

Each patient was followed by an office-based rheumatologist at 6-month intervals up to a clinical diagnosis of a specific joint disease and fulfillment of published criteria for that joint disease. The patients were asked to attend a last visit between June and November 1999, after which a panel of 5 rheumatologists determined whether RA was present, based on all available data for each patient[8]. This final diagnosis was used as the gold standard against which the diagnostic value of laboratory tests at the first visit was evaluated.

Laboratory methods. All sera were centralized and tested blindly in the Brest laboratory of Immunology (PY). RF was measured by the latex fixation test (LFT, expressed in IU/ml) where human IgG serves as a target antigen, and by 3 class-specific ELISA (with results expressed as optical density) where the capture antigen was rabbit IgG. The IgM-specific ELISA is thus equivalent to the sensitized sheep cell agglutination test (SCAT)[9]. The SCAT has been shown to be the most specific in the diagnosis of RA, and the LFT the most sensitive but the least specific. Regardless, occasionally, RA patients are LFT-negative and SCAT-positive.

We used anti-human IgG antibody as the revealing agent for detecting APF, AKA, and anti-CCP.

APF was assayed as described[10] and expressed as the reciprocal of the titer. In brief, a wooden tongue depressor was used to scrape buccal mucosal cells from the inside of both cheeks of healthy volunteers. The cells were washed 3 times in PBS, pH 7.4, resuspended in PBS containing colimycin and sodium azide, and transferred dropwise on multispot slides, with approximately 5,000 cells per well.

AKA was detected by indirect immunofluorescence using a section of the middle third of a rat esophagus as the substrate. Antibodies were detected using goat anti-human antibodies coupled to alkaline phosphatase, and expressed as the reciprocal of the titer.

Anti-CCP were detected by ELISA, using a commercial kit (Eurodiagnostica, Arnhem, The Netherlands) according to the manufacturer's instructions, expressed in optical density (OD) in the scattergram (Figure 1) and UI in the text.

Statistical analysis. Distributions of laboratory test results at the first visit were described as the percentage per category for qualitative items and as the median (range) for quantitative items. The distributions of laboratory test results were compared between patients with and without RA at the final visit using the chi-square test (or the Fisher exact test, where necessary) for qualitative items and the Mann-Whitney test[11] for quantitative items. For each test, diagnostic value was described by plotting sensitivity against 1-specificity to obtain the receiver operating characteristic (ROC) curve[12]. ROC curves were plotted for each continuous laboratory test by varying the cutoffs (values lower than the cutoff were considered negative and other values positive). Diagnostic value was described by determining the sensitivity and specificity obtained with all cutoffs. The optimal cutoff for a continuous laboratory test was defined as the value nearest to the northwest point of the ROC curve. To evaluate how well laboratory test combinations discriminated between patients with and without RA, a multiple logistic regression procedure with backward selection using the likelihood ratio test[13] was applied. Data were analyzed using the Statistical Package for the Social Sciences (SPSS 9.0, 1999).

RESULTS

Study population. Two hundred and seventy patients with arthritis of less than one year's duration constituted the study cohort. Demographic and clinical data have been described[7,8]. For 27 patients, sera were not available at the end of the study. This left 243 patients for whom all laboratory test results were known. Demographic and clinical data are presented in Table 1. At the last visit, 86 patients (35%) were given a diagnosis of RA by the panel of 5 rheumatologists.

Correlations among APF, AKA, and anti-CCP. These variables were not perfectly correlated with one another (Figure 1). The correlation coefficients for anti-CCP and AKA, for anti-CCP and APF, and for AKA and APF were 0.575, 0.599, and 0.523, respectively.

Diagnostic value of laboratory tests in isolation. For each of these tests, diagnostic value was described by the ROC curve (Figures 2a, b, and c). For each test (APF, AKA, and anti-CCP), the value in discriminating between patients with and without RA was described by determining the sensitivity and specificity obtained using all cutoff points.

The optimal cutoff titers for APF, AKA, and anti-CCP, defined as the value nearest to the northwest point of the ROC curve, were ≥ 1/80, ≥ 1/10, and 53 IU, respectively. Zero, one, 2, or 3 tests were positive in 30, 14, 16, and 26 patients with RA and in 116, 31, 8, and 2 patients without RA (Tables 2 and 3). The diagnostic values for RA are shown in Table 2. AKA and anti-CCP had similar values for the diagnosis of RA, and for both tests, optimal titers gave a good trade-off between sensitivity (47%) and specificity (93 or 94%). Zero, one, 2, or 3 tests were positive in 33 (42%), 15 (19%), 12 (15%), and 19 (24%) patients fulfilling 1987 American College of Rheumatology (ACR) criteria for RA at the first visit and in 113 (69%), 30 (18%), 12 (7%), and 9 (5%) in the remainder. APF, AKA, and anti-CCP were found more often in RF positive patients than in RF negative patients (data not shown, $p < 0.0001$). For example, anti-CCP were positive in 38 of 65 latex positive patients versus 15 of 184 latex negative patients.

These tests were found in the subgroup of patients who would develop erosive disease (defined using item 7 of the 1987 ACR criteria) more often than in the remainder (25/46, 20/46, and 20/46 patients had APF, AKA, and anti-CCP at the first visit in the erosive subgroup versus 51/197, 30/197, and 31/197 in the remainder; $p < 0.001$, < 0.0001 and < 0.0001 respectively).

We also studied the sensitivity of these tests in diagnosing RA for a 95% specificity level. Sensitivity of ELISA IgM RF, latex test, APF, AKA, and anti-CCP at the first visit were 41%, 45%, 28%, 39%, and 35%, respectively. Anti-CCP tests were not better than RF alone.

Diagnostic value of laboratory tests in combination. The diagnostic value of the latex test, IgM-RF ELISA, and AKA combination was not changed substantially when anti-CCP was used instead of AKA (Table 3).

Multiple logistic regression analysis, which included all laboratory data, with backward selection using the likeli-

Table 1. Demographic, clinical, and biological data for patients presenting with arthritis at the first visit and diagnosis at last visit. Distributions at the first visit are described as n (%) per category and median (range) for qualitative and quantitative items, respectively.

Age, yrs	49.4 (19–86)
Sex, F/M	162/81
Synovitis, n	2 (0–34)
Diagnosis of RA by the panel of 5 rheumatologists, n (%)	86/243 (35.4)
Diagnoses in non-RA patients, n (%)	157/243 (64.6)
Gouty arthritis-chondrocalcinosis-hydroxyapatitis	12
Spondyloarthropathy	53
Connective tissue disease (SS, scleroderma, polymyositis, SLE)	17
Giant cell arteritis-polymyalgia rheumatica	3
Other defined	24
Undifferentiated	48
Latex test	0 (0–1600)
IgM RF ELISA	0.063 (0–0.851)
Antinuclear antibodies	0 (0–1000)

SS: Sjögren's syndrome; SLE: systemic lupus erythematosus.

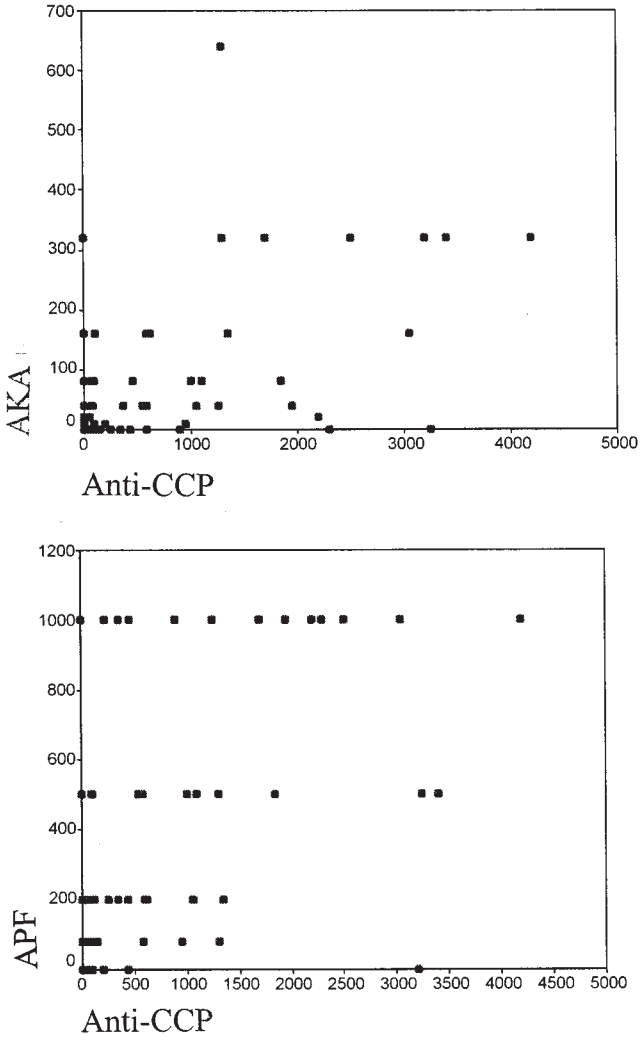

Figure 1. Scattergram of antiperinuclear factor (APF), antikeratin antibody (AKA), and antibody to citrullinated peptides (anti-CCP). 1a: Scattergram of, in the Y-axis, the reciprocal of the titers of AKA (Y axis) and optical density of antibody to CCP (X axis). 1b: Scattergram of the reciprocal of the titers of APF (Y axis) and of optical density of antibody to CCP (X axis).

hood ratio test selected IgM-RF ELISA, the latex test, AKA, and anti-CCP. The performance of the multiple logistic regression function is shown in Figures 2D and 2E. Compared with our previous combination of laboratory tests, anti-CCP combined with AKA, IgM-RF, and the latex test slightly improved the diagnostic value of testing.

DISCUSSION

RF and anti-CCP are the best validated tests for diagnosing RA. Three earlier studies found that combining anti-CCP with another test for RA was useful for the diagnosis[8,14,15]. Nevertheless, whether anti-CCP is superior to older tests remains a matter of debate[16].

Our study was designed to determine the respective diagnostic values of anti-CCP assayed using 3 different methods. We found that AKA and anti-CCP were similarly useful for diagnosing RA and were better than APF, with optimal cutoff yielding a good trade-off between sensitivity (47%) and specificity (93 or 94%). All these tests are related to patients who are RF positive at the first visit and are more frequently observed in the group of patients who will develop erosive and/or definite RA 2 years later but remain frequent in seronegative and/or nonerosive RA. These results suggest that rheumatologists can choose either AKA or anti-CCP for the diagnosis of RA.

However, in some of our patients, only one or 2 of the 3 tests used to detect anti-CCP were positive, suggesting differences in the auto-antibody spectra detected by anti-CCP ELISA, APF, and AKA. This led us to investigate which combination of these laboratory tests best discriminated between early RA and other forms of arthritis at the first visit. Multiple logistic regression showed that it was useful to combine IgM-RF ELISA, the latex test, AKA, and anti-CCP. Using anti-CCP slightly improved the diagnostic value compared to that observed in our earlier study of laboratory test combinations without anti-CCP. These results suggest that rheumatologists can routinely use 2 or 3 tests

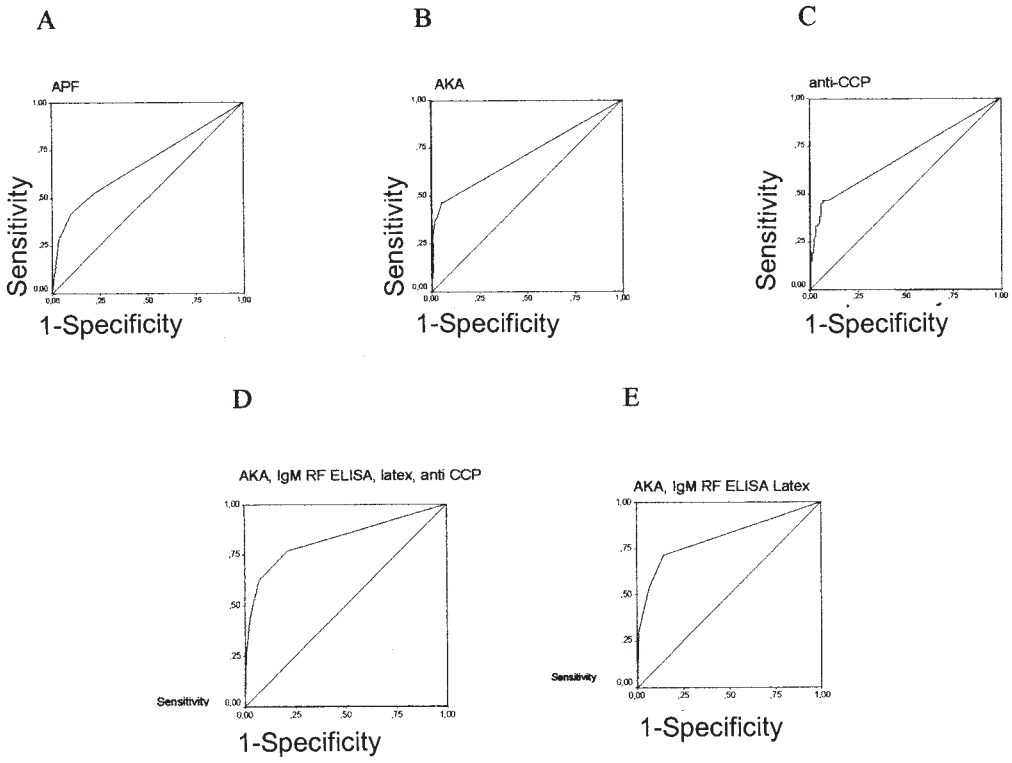

Figure 2. Value of laboratory tests (tests for APF, AKA, and anti-CCP used alone or in combination, evaluated by logistic regression) in discriminating between patients with and without RA. For each test or combination of tests, value was described by determining the sensitivity and specificity obtained using all cutoff points. ROC curves for A: APF; B: AKA; C: anti-CCP; D: the combination of AKA, IgM RF ELISA, latex test, and anti-CCP; and E: the combination of AKA, IgM RF ELISA, and latex test.

Table 2. Proportions of patients with and without RA who had positive tests for APF, AKA, and anti-CCP.

	Patients with RA n = 86 (%)	Patients without RA n = 157 (%)
APF–/AKA–/anti-CCP–	30 (35)	116 (74)
APF+/AKA–/anti-CCP–	6 (7)	23 (15)
APF–/AKA+/anti-CCP–	5 (6)	6 (4)
APF–/AKA–/anti-CCP+	3 (3)	2 (1)
APF+/AKA+/anti-CCP–	5 (6)	1 (1)
APF–/AKA+/anti-CCP+	5 (6)	0 (0)
APF+/AKA–/anti-CCP+	6 (7)	7 (4)
APF+/AKA+/anti-CCP+	26 (30)	2 (1)

RA: rheumatoid arthritis; APF: antiperinuclear factor; AKA: antikeratin antibody; anti-CCP: antibody to citrullinated peptides.

Table 3. Sensitivity and specificity of APF, AKA, anti-CCP, and rheumatoid factor (latex test and RF IgM ELISA). Values are percentages.

Laboratory Test	Sensitivity, %, n = 86	Specificity, %, n = 157
APF	52	79
AKA	47	94
Anti-CCP	47	93
At least 1/3 of AKA-RF IgM ELISA-latex	75	82
At least 2/3 of AKA-RF IgM ELISA-latex	56	94
3/3 of AKA-RF IgM ELISA-latex	33	99
At least 1/3 of CCP-RF IgM ELISA-latex	75	86
At least 2/3 of CCP-RF IgM ELISA-latex	57	95
3/3 of CCP-RF IgM ELISA-latex	32	97
At least 1/4 of AKA-RF IgM ELISA-latex-CCP	77	79
At least 2/4 of AKA-RF IgM ELISA-latex-CCP	63	93
At least 3/4 of AKA-RF IgM ELISA-latex-CCP	43	97
4/4 of AKA-RF IgM ELISA-latex-CCP	27	100

RF: rheumatoid factor; APF: antiperinuclear factor; AKA: antikeratin antibody; anti-CCP: antibody to citrullinated peptides.

for diagnosing RA (latex and/or RF ELISA, and either AKA or anti-CCP ELISA) and can add the third or the fourth test when the diagnosis remains in doubt.

We hope that ELISA-based tests that employ second generation cyclic citrullinated peptides will once again improve the diagnostic value of anti-CCP.

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