Diagnostic Utility of Anti-Cyclic Citrullinated Peptide Antibodies for Very Early Rheumatoid Arthritis

TOSHIHIRO MATSUI, KOTA SHIMADA, NAOKO OZAWA, HIROMI HAYAKAWA, FUTOSHI HAGIWARA, HISANORI NAKAYAMA, SHOJI SUGII, YOSHINORI OZAWA, and SHIGETO TOHMA

ABSTRACT. ***Objective***. To compare the diagnostic utility of anti-cyclic citrullinated peptide (anti-CCP) antibodies with other serological markers including rheumatoid factor (RF), anti-agalactosyl immunoglobulin G (IgG) antibody, and matrix metalloproteinase (MMP)-3 in very early rheumatoid arthritis (RA).

Methods. Serum concentrations of anti-CCP antibodies, RF, anti-agalactosyl IgG antibody, and MMP-3 were measured in 262 patients with RA ("total RA") including 55 patients with disease duration of less than 6 months who had not been treated before entry ("very early RA") and 116 patients with rheumatic diseases other than RA.

Results. The diagnostic sensitivity of anti-CCP antibodies was 82.4% in total RA and 67.3% in very early RA and was lower than that of RF (84.0% total RA, 83.6% very early RA) and anti-agalactosyl IgG antibody (90.5%, 90.9%), whereas specificity, positive predictive value, and diagnostic accuracy were the best among markers tested both in total RA and in very early RA. The presence of either anti-CCP antibodies or RF increased the sensitivity, but any combination of serological markers was not significantly better in diagnostic accuracy than anti-CCP antibodies alone. The rates of RF-positive subjects in anti-CCP antibody-negative patients both in total RA (43.5%) and in very early RA (61.1%) were higher than those of anti-CCP antibody-positive subjects in RF-negative patients (38.1% and 22.2%, for total RA and early RA, respectively).

Conclusion. Measurement of anti-CCP antibodies, by itself, is useful for the diagnosis of RA; however, combined use of anti-CCP antibodies with RF may be more useful than either method alone for the diagnosis of very early RA. (First Release Aug 15 2006; J Rheumatol 2006;33:2390–7)

Key Indexing Terms:
ANTI-CYCLIC CITRULLINATED PEPTIDE ANTIBODY
ANTI-AGALACTOSYL IgG ANTIBODY
RHEUMATOID ARTHRITIS
RHEUMATOID FACTOR

Rheumatoid arthritis (RA) is a systemic autoimmune disease of unknown etiology, characterized by chronic joint inflammation that often leads to joint destruction. Diagnosis of RA is currently based on the revised classification criteria of the American College of Rheumatology (ACR)[1]; however, it remains imprecise, especially early in the course of disease. Rheumatoid factor (RF) has been widely used in clinical practice as a useful serological marker for diagnosis of RA. Although RF is the only serological test in the criteria of the ACR and can be detected in up to 70-80% of patients with RA[2], its specificity is limited, since RF can also be detected in other rheumatic diseases, infectious diseases, and in healthy individuals, especially the elderly. Because the current therapeutic strategies in RA employ increasingly aggressive regimens from an early stage of the disease, more specific serological markers than RF are desirable.

Recently, anti-cyclic citrullinated peptide (anti-CCP) antibodies have attracted attention as a useful marker for the diagnosis of RA with high specificity. Several studies have shown that anti-CCP antibodies are moderately sensitive but highly specific for RA and that their specificity is higher than that of RF[3-7]. Anti-CCP antibodies can be detected very early in the disease and may be used as an indicator for the progression and prognosis of RA[8-13]. The clinical utility of anti-CCP antibodies has been compared mainly with that of RF, but not often with that of other serological markers.

Parekh, *et al* reported that RA was associated with an altered glycosylation pattern of serum immunoglobulin G (IgG), resulting in increased levels of oligosaccharides that lack terminal galactose residues[14]. Interestingly, RF in patients with RA could bind better to galactose-free IgG

From the Department of Rheumatology; Division of Rheumatology, Clinical Research Center for Allergy and Rheumatology; and the Department of Rehabilitation, Sagamihara National Hospital, National Hospital Organization, Kanagawa, Japan.

Supported in part by grants from the Ministry of Health, Labour and Welfare, Japan; the National Hospital Organization; and the Manabe Foundation.

T. Matsui, MD, PhD, Staff Doctor; H. Nakayama, MD, Staff Doctor; Y. Ozawa, MD, Staff Doctor, Department of Rheumatology; K. Shimada, MD, PhD, Research Resident; N. Ozawa, Research Assistant; H. Hayakawa, Research Assistant; F. Hagiwara, MD, Research Associate; S. Tohma, MD, Department Head, Division of Rheumatology, Clinical Research Center for Allergy and Rheumatology; S. Sugii, MD, Staff Doctor, Department of Rehabilitation, Sagamihara National Hospital.

Address reprint requests to Dr. T. Matsui, Department of Rheumatology, Sagamihara National Hospital, National Hospital Organization, 18-1, Sakuradai Sagamihara-City, Kanagawa, 228-8522, Japan.
E-mail: t-matsui@sagamihara-hosp.gr.jp

Accepted for publication May 6, 2006.

(agalactosyl IgG) than to galactosylated IgG[15]. In addition, anti-agalactosyl IgG antibodies have been detected in patients with RA and the titers positively correlated with disease activity[16,17]. There are no reports comparing the clinical utility of anti-CCP antibodies and anti-agalactosyl IgG antibodies, except one study in a small number of Japanese patients[18].

We compared the diagnostic utility of anti-CCP antibodies (measured by a second generation kit) for RA with other serological markers including RF, anti-agalactosyl IgG antibodies, and matrix metalloproteinase (MMP)-3, especially in patients with an early stage of RA.

MATERIALS AND METHODS

Patients. All patients were selected consecutively from outpatients being treated at the Department of Rheumatology, Sagamihara National Hospital. Serum samples were obtained from 262 patients with RA (214 women and 48 men, mean age: 56.7 years, range: 24–83 years, mean disease duration ± SD: 8.2 ± 10.5 years) diagnosed according to the revised criteria of the ACR (defined as "total RA" group) (Table 1). There were a total of 128 patients with early RA with disease duration of less than 2 years (defined as "early RA" group), including 55 patients with disease duration of less than 6 months who had never been treated with any drugs except nonsteroidal antiinflammatory drugs (NSAID) before blood sampling (defined as "very early RA" group). A total of 116 patients with rheumatic diseases other than RA were used as controls. No patient in the control group had comorbid RA. The control group included 26 patients with systemic lupus erythematosus (SLE; all women, mean age: 48.0 yrs, range: 24–83 yrs), 13 patients with mixed connective tissue disease (MCTD) (all women, mean age: 48.5 yrs, range: 25–69 yrs), 24 patients with systemic sclerosis (SSc; all women, mean age: 58.2 yrs, range: 32–87 yrs), 21 patients with primary Sjögren's syndrome (SS; all women, mean age: 55.0 yrs, range: 27–80 yrs), 21 patients with polymyositis (PM)/dermatomyositis (DM) (7 men, mean age: 58.4 years, range: 28–85 years), and 11 patients with vasculitis (3 men, mean age: 62.6 yrs, range: 43–86 yrs). The diseases were diagnosed according to the standard criteria of each disease[19-23]. All serum samples were stored at –20°C until assayed.

Measurements of serological markers. Anti-CCP antibodies were measured using anti-CCP second generation ELISA kit (Diastat™ Anti-CCP, Axis-Shield, Dundee, UK), according to the manufacturer's instructions. The cutoff value of 4.5 U/ml for anti-CCP antibodies used in this study had been determined in the previous study that determined the cutoff value of this ELISA kit for Japanese subjects[24]. RF was measured by latex-enhanced immunonephelometric assay (Dede Behring, Marburg, Germany). The cutoff value for RF was 15 IU/ml. Anti-agalactosyl IgG antibodies were measured using an electro-chemiluminescence immunoassay (ECLIA) kit (Picolumi® CARF, Sanko Jyunyaku, Tokyo, Japan), henceforth referred to as "CARF". This detects all isotypes of immunoglobulins that bind to agalactosyl IgG and has been reported to have a higher sensitivity for RA than that of RF[25]. The cutoff value of CARF was 6.0 AU/ml[26]. MMP-3 was measured by ELISA (The Binding Site Limited, Birmingham, UK). Cutoff values for MMP-3 were 45.3 ng/ml for men and 21.0 ng/ml for women. C-reactive protein (CRP) measured by latex turbidimetric immunoassay and erythrocyte sedimentation rate (ESR) were used as inflammatory markers for RA. To evaluate the overall diagnostic utilities of serological markers for RA, the diagnostic accuracy was calculated as follows: diagnostic accuracy = ([number of test-positive subjects in RA] + [number of test-negative subjects in control group]) / (total number of subjects in RA and control group).

Statistical analyses. The Statistical Package for Bioscience (Comworks, Saitama, Japan) was used for statistical analysis. Comparison of the titer distributions between RA and other rheumatic diseases was made using the Mann-Whitney U-test. Comparisons of sensitivity and specificity were made using chi-square test. For the construction of receiver-operating characteristic (ROC) curves, the relationship between sensitivity and 1–specificity for various cutoff points was plotted. The area under the ROC curve (AUC) provides an index of the overall discriminative ability of the test[24]. Differences in AUC were analyzed using the z-test. Spearman's rank correlation coefficient was used to assess the importance of the different variables. Differences were considered significant where $p < 0.05$.

RESULTS

Prevalence of anti-CCP antibodies in RA and other rheumatic diseases. Anti-CCP antibodies were detected in the sera of 216 of 262 patients with RA (82.4%) and 20 of 116 patients in the control group (17.2%) (Figure 1). Prevalence of anti-CCP antibodies in controls was 4 of 26 patients with SLE (15.4%), 2 of 13 patients with MCTD (15.4%), 4 of 24 patients with SSc (16.7%), 3 of 21 patients with SS (14.3%), 5 of 21 patients with PM/DM (23.8%), and 2 of 11 patients with vas-

Table 1. Demographic and clinical characteristics of patients and controls.

	Total, n	Men, n	Men, %	Mean Age, yrs	Range, yrs
RA					
< 6 mos*	83	16	19.3	53.6	24–83
< 6 mos (no therapy)	55	10	18.2	53.4	28–83
6–12 mos	20	6	30.0	58.0	24–73
1–2 yrs	25	9	36.0	56.0	29–75
> 2 yrs	134	17	12.7	58.6	24–83
RA total	262	48	18.3	56.7	24–83
Controls					
SLE	26	0	0	48.0	29–79
MCTD	13	0	0	48.5	25–69
SSc	24	0	0	58.2	32–87
Primary SS	21	0	0	55.0	27–80
PM/DM	21	7	33.3	58.4	28–85
Vasculitis	11	3	37.5	62.6	43–86
Control total	116	10	8.6	54.6	25–87

RA: rheumatoid arthritis; SLE: systemic lupus erythematosus; MCTD: mixed connective tissue disease; SSc: systemic sclerosis; SS: Sjögren's syndrome; PM/DM: polymyositis/dermatomyositis. * Disease duration.

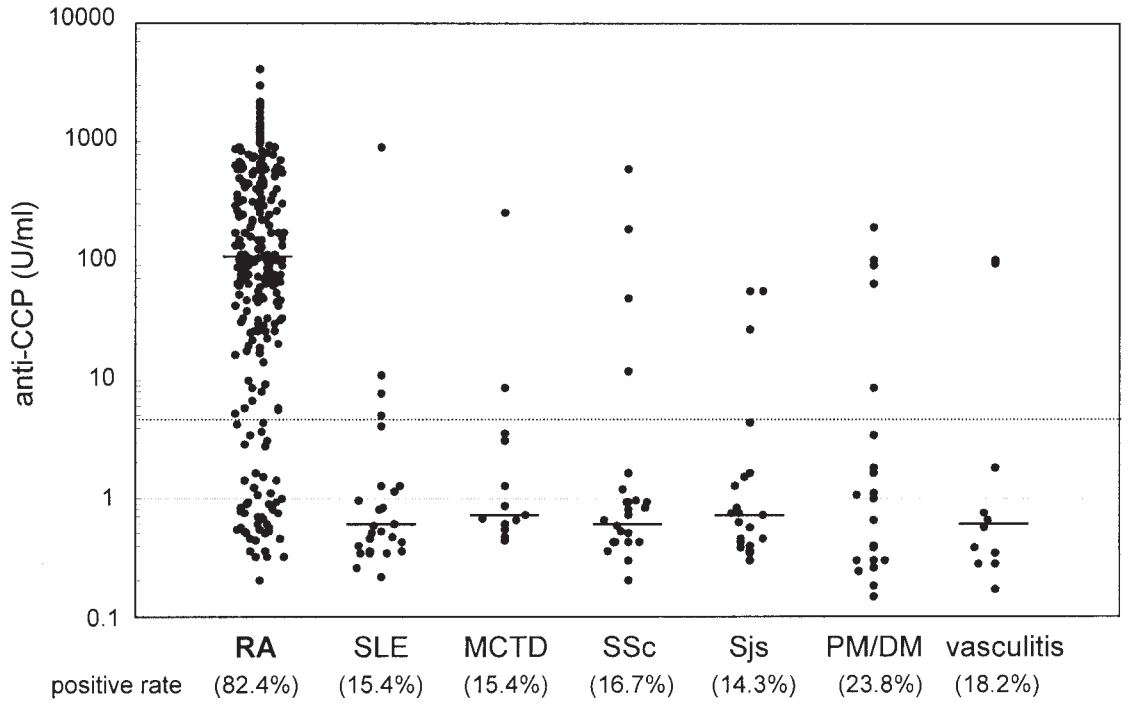

Figure 1. Prevalence of anti-CCP antibodies in RA and controls. Positive rate and median value of anti-CCP antibodies in patients with RA were significantly higher than those in all control diseases ($p < 0.001$ by chi-square test and $p < 0.001$ by Mann-Whitney U-test, respectively). Broken line is the cutoff value of anti-CCP antibodies (4.5 U/ml). Black bar indicates a median value of anti-CCP antibodies in each disease.

culitis (18.2%). The median and the mean values of anti-CCP antibodies in total RA were 100 U/ml and 311 U/ml, respectively. For the control groups, the median and the mean, respectively, were: total of controls: 0.7 U/ml, 25 U/ml; SLE: 0.2 U/ml, 37 U/ml; MCTD: 0.5 U/ml, 21 U/ml; SSc: 0.2 U/ml, 35 U/ml; SS: 0.3 U/ml, 7 U/ml; PM/DM: 0.2 U/ml, 22 U/ml; vasculitis: 0.2 U/ml, 18 U/ml. The median and the mean values of anti-CCP antibodies in total RA were significantly higher than the median and mean of the overall control group of rheumatic diseases ($p < 0.001$) and each disease subset composing the control group ($p < 0.001$, each disease subset).

Relationship between RA disease progression and the occurrence of anti-CCP antibodies. To address the relationship between the disease progression in RA and the occurrence of anti-CCP antibodies, we examined the occurrence of anti-CCP antibodies according to the disease duration or to the disease stage (Figure 2). Anti-CCP antibodies were detected in 60 of 83 (72.3%) patients with RA whose disease duration was less than 6 months. Prevalence of anti-CCP antibodies increased with prolongation of disease duration and it reached almost a plateau by one year. However, mean titers of anti-CCP antibodies were not affected by the disease duration. When the prevalence of anti-CCP antibodies was correlated with the degree of joint damage evaluated by Steinbrocker's staging method (Figure 2B)[27], 85 of 113 patients (75.2%) in stage I (patients with no erosions) were positive for anti-CCP antibodies. Prevalence of anti-CCP antibodies increased with the progression of joint destruction, reached a peak at stage III (94.7%), and then decreased at stage IV (88.2%). Mean titers of anti-CCP antibodies reached a peak at stage II and then decreased.

Comparison of the correlation of serological and inflammatory marker measurements. Next, we examined the correlation in the values for serological and inflammatory markers. Anti-CCP antibodies were significantly correlated with RF ($r = 0.399$, $p < 0.001$), CARF ($r = 0.398$, $p < 0.001$), and MMP-3 in women ($r = 0.165$, $p = 0.016$) (Table 2). CARF was strongly correlated with RF ($r = 0.952$, $p < 0.001$), but not with MMP-3. Serological markers tested here (anti-CCP antibodies, RF, CARF, and MMP-3) were all significantly correlated with inflammatory markers (CRP and ESR). In particular, MMP-3 strongly correlated with inflammatory markers (MMP-3 vs ESR in women: $r = 0.543$, $p < 0.001$; MMP-3 vs CRP in women: $r = 0.620$, $p < 0.001$).

Comparison of the diagnostic utility of anti-CCP antibodies with other serological markers. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy of anti-CCP antibodies were compared with those of other serological markers (RF, CARF, and MMP-3; Table 3). In total RA, the sensitivity of anti-CCP antibodies (82.4%) was lower than those of CARF (90.5%, $p < 0.05$) and RF (84.0%, not significant). By comparison, the specificity of anti-CCP antibodies (82.8%) was significantly higher than that of any other markers (RF: 70.7%, $p < 0.01$;

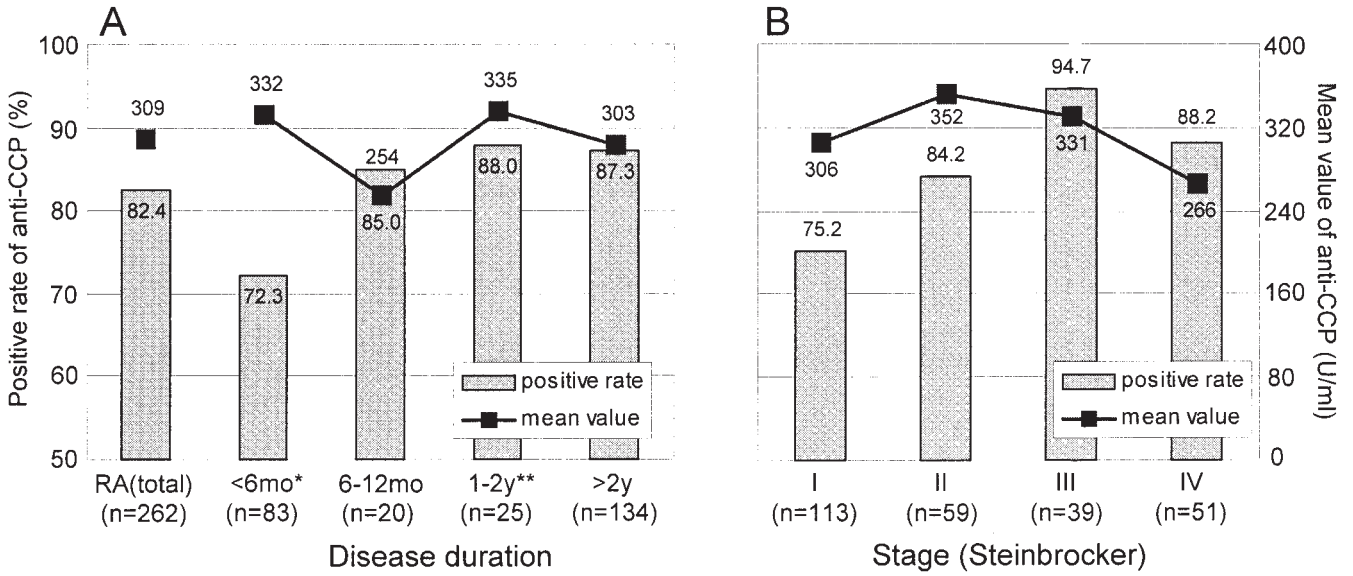

Figure 2. Relationship between the positive rate of anti-CCP antibodies and disease progression. A. Comparison of the positive rate and mean value of anti-CCP antibodies and disease duration at blood sampling. B. Comparison of the positive rate and mean value of anti-CCP antibodies and stage of RA. Disease stage was evaluated by Steinbrocker's staging method (see Methods).

Table 2. Spearman's rank correlation coefficients between serological markers (inflammatory markers).

	RF (p)	CARF (p)	MMP-3 (women) (p)	MMP-3 (men) (p)	ESR (p)	CRP (p)
Anti-CCP	r = 0.399 (< 0.001)	0.398 (< 0.001)	0.165 (0.016)	0.115 (0.440)	0.258 (< 0.001)	0.192 (0.002)
RF	—	0.952 (< 0.001)	0.129 (0.059)	0.066 (0.661)	0.210 (< 0.001)	0.216 (< 0.001)
CARF	—	—	0.091 (0.182)	0.026 (0.864)	0.163 (0.008)	0.170 (0.006)
MMP-3 (women)	—	—	—	—	0.543 (< 0.001)	0.620 (< 0.001)
MMP-3 (men)	—	—	—	—	0.300 (< 0.040)	0.491 (< 0.001)
ESR	—	—	—	—	—	0.714 (< 0.001)

Anti-CCP: anti-cyclic citrullinated peptide antibodies; RF: rheumatoid factor; CARF: anti-agalactosyl IgG; MMP-3: matrix metalloproteinase-3; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein.

CARF: 50.0%, $p < 0.001$; and MMP-3: 46.6%, $p < 0.001$). PPV of anti-CCP antibodies (91.5%) was also best among the markers tested. To compare the overall diagnostic utilities of serological markers for RA, we calculated the diagnostic accuracy (see Methods). The diagnostic accuracy of anti-CCP antibodies (82.5%) was higher than that of any other markers (RF: 79.9%, CARF: 78.0%, MMP-3: 62.4%), but differences were statistically significant only in MMP-3 ($p < 0.001$). These characteristics of anti-CCP antibodies in diagnostic value were also observed in early RA (patients with disease duration < 2 yrs) and in very early RA (patients with disease duration < 6 mos and with no medicinal treatments before blood sampling). In early RA and very early RA, the diagnostic accuracies of anti-CCP antibodies (79.9% and 77.8%, respectively) were significantly higher than those of CARF (71.3%, $p < 0.05$; 63.2%, $p < 0.01$, respectively) and MMP-3 (54.1%, $p < 0.001$; 47.4%, $p < 0.001$, respectively).

ROC analyses. For the comparison of the diagnostic utility of each marker, we additionally undertook an ROC analysis and calculated the AUC (Figure 3 and Table 4). The AUC of anti-CCP antibodies (0.873) was higher than that of any other serological markers (RF: 0.805, $p < 0.05$; CARF: 0.811, $p = 0.050$; MMP-3: 0.663, $p < 0.001$) in total RA. However, in very early RA, the AUC of anti-CCP antibodies (0.805) was comparable with that of RF (0.788, $p = 0.754$) and CARF (0.689, $p = 0.689$).

Combination of serological markers for a diagnosis of RA. We examined whether combined use of serological markers would increase the diagnostic value for RA compared with use of anti-CCP antibodies alone (Table 3). With anti-CCP antibodies in combination with either RF, CARF, or MMP-3, the diagnostic sensitivity was significantly increased to over 90% (90.1%, 93.1%, or 90.1%, for combination with either RF, CARF, or MMP-3, respectively), whereas the specificity significantly decreased to under 66% (65.5%, 45.7%, or 40.5%, respectively) compared with that of anti-CCP antibodies alone. In total RA, no combination of serological markers was better than use of anti-CCP antibodies alone in the diagnostic

Table 3. Diagnostic values of serological markers for RA.

	Sensitivity			Specificity	PPV			NPV			Diagnostic Accuracy		
	< 6 mo†	< 2 yrs	Total		< 6 mo	< 2 yrs	Total	< 6 mo	< 2 yrs	Total	< 6 mo	< 2 yrs	Total
Anti-CCP	67.3	77.3	82.4	82.8	64.9	83.2	91.5	84.2	76.8	67.6	77.8	79.9	82.5
RF	83.6	84.4	84.0	70.7***	57.5	76.1	86.6	90.1	80.4	66.1	74.9	77.9	79.9
CARF	90.9**	90.6**	90.5***	50.0*	46.3***	66.7**	80.3*	92.1	82.9	69.9	63.2**	71.3***	78.0
MMP-3	49.1	60.9**	69.5*	46.6*	30.3*	55.7*	74.6*	65.9***	51.9*	40.3*	47.4*	54.1*	62.4*
Anti-CCP or RF	87.3***	87.5***	90.1***	65.5**	54.5	73.7	85.5***	91.6	82.6	74.5	72.5	77.0	82.5
Anti-CCP or CARF	92.7**	93.0*	93.1*	45.7*	44.7***	65.4*	79.5*	93.0	85.5	74.6	60.8*	70.5***	78.6
Anti-CCP or MMP-3	76.4	85.2	90.1***	40.5*	37.8**	61.2*	77.4*	78.3	71.2	64.4	52.0*	63.9*	74.9***
RF or CARF	92.7**	91.4**	91.2**	50.0*	46.8***	66.9**	80.5*	93.5	84.1	71.6	63.7**	71.7***	78.6
RF or MMP-3	94.5*	93.8*	94.3*	28.4*	38.5**	59.1*	74.8*	91.7	80.5	68.8	49.7*	62.7*	74.1**
CARF or MMP-3	94.5*	95.3*	95.8*	19.8*	35.9*	56.7*	73.0*	88.5	79.3	67.6	43.9*	59.4*	72.5*
Anti-CCP and RF	63.6	74.2	76.3	88.8	72.9	88.0	93.9	83.7	75.7	62.4	80.7	81.1	80.2
Anti-CCP and CARF	65.5	75.0	79.8	87.1	70.6	86.5	93.3	84.2	75.9	65.6	80.1	80.7	82.0
Anti-CCP and MMP-3	40.0**	53.1*	61.8*	88.8	62.9	84.0	92.6	75.7	63.2***	50.7**	73.1	70.1***	70.1*
RF and CARF	81.8	83.6	83.2	71.6	57.7	76.4	86.9	89.2	79.8	65.4	74.9	77.9	79.6
RF and MMP-3	38.2**	51.6*	59.2*	89.7	63.6	84.6	92.8	75.4	62.7***	49.3*	73.1	69.7***	68.5*
CARF and MMP-3	45.5***	56.3*	64.1*	76.7	48.1	72.7	86.2	74.8	61.4**	48.6*	66.7***	66.0*	68.0*

* $p < 0.001$, p values vs anti-CCP; ** $p < 0.01$; *** $p < 0.05$; † disease duration < 6 months, with no treatments before blood sampling; PPV: positive predictive value; NPV: negative predictive value. For other definitions see Table 2.

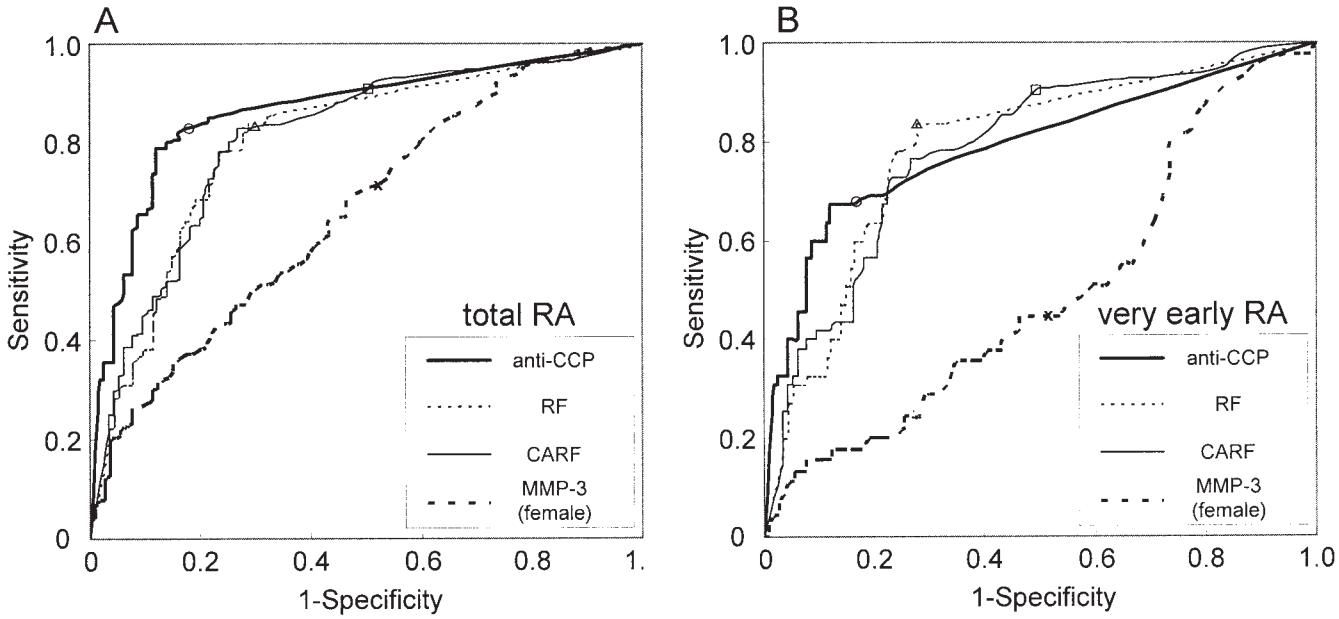

Figure 3. ROC curves of anti-CCP antibodies, RF, CARF, and MMP-3 (women). A. ROC curves in total RA. ROC curves of anti-CCP, RF, CARF, and MMP-3 (women) were based on data from patients with RA (n = 262) and other rheumatic diseases as a control (n = 116). Since the distribution of MMP-3 in normal individuals differs between women and men, ROC curves of MMP-3 were constructed separately. ROC curve of MMP-3 based on data from women with RA (n = 214) and other rheumatic diseases (n = 106). B. ROC curves in very early RA. ROC curves of anti-CCP, RF, and CARF were based on data from patients with very early RA (n = 55) and ROC curve of MMP-3 was based on data from women with very early RA (n = 45). Controls were the same as in A. Cutoff value of each variable used in this study was plotted on the ROC curve.

accuracy; however, in very early RA, the combination of anti-CCP antibodies with RF or with CARF was superior to anti-CCP antibodies alone (80.7% and 80.1%, respectively, compared with 77.8%).

Occurrence of anti-CCP antibodies in seronegative RA. Finally, we evaluated the utility of anti-CCP antibodies in seronegative RA. Anti-CCP antibodies were detected in 22.2% of RF-negative very early RA and the incidence was lower than that of total RA (38.1%) (Table 5). Conversely, RF was detected in 61.1% of anti-CCP antibody-negative very early RA and the incidence was higher than that of total RA (43.5%).

Table 4. Comparison of the area under the curves (AUC) of anti-CCP antibodies, RF, CARF, and MMP-3.

	AUC			p* (RA < 6 mo with No Treatment)			p (< 2 yrs)			p (RA total)		
	RA (< 6 mo with No Treatment)	RA (< 2 yrs)	RA (total)	RF	CARF	MMP-3	RF	CARF	MMP-3	RF	CARF	MMP-3
Anti-CCP	0.805	0.855	0.873	0.754	0.688	< 0.001	0.215	0.195	< 0.001	0.035	0.050	< 0.001
RF	0.788	0.808	0.805	—	0.689	< 0.001	—	0.968	0.002	—	0.871	0.002
CARF	0.783	0.806	0.811	—	—	< 0.001	—	—	< 0.001	—	—	< 0.001
MMP-3	0.492	0.621	0.663	—	—	—	—	—	—	—	—	—

* Differences in AUC were analyzed using the z-test. For definitions, see Table 2.

Table 5. Comparison of occurrence of anti-CCP antibodies and RF in RA.

Anti-CCP	RF	< 6 mo* with No Treatment (%)	< 2 yrs* (%)	Total (%)
Positive	Positive	35 (63.6)	95 (74.2)	200 (76.3)
Positive	Negative	2 (3.6)	4 (3.1)	16 (6.1)
Negative	Positive	11 (20.0)	13 (10.2)	20 (7.6)
Negative	Negative	7 (12.7)	16 (12.5)	26 (9.9)
Anti-CCP-positive in RF-negative		2/9 (22.2)	4/20 (20.0)	16/42 (38.1)
RF-positive in anti-CCP-negative		11/18 (61.1)	13/29 (44.8)	20/46 (43.5)

* Disease duration. For definitions see Table 2.

DISCUSSION

Currently, RA is diagnosed according to the revised classification criteria of the ACR in which RF is the only serological marker adopted; however, RF is not fully specific for RA. For prevention of joint destruction, RA has to be diagnosed early and treatment started as soon as possible. Therefore, diagnostic markers of RA with higher sensitivity and specificity than RF are desirable. We compared the diagnostic efficiency of anti-CCP antibodies and other serological markers for RA, especially in very early RA.

In total RA, anti-CCP antibodies were superior to MMP-3, comparable with RF, and inferior to CARF in sensitivity, but the best in specificity among the serological markers tested. Although the previous reports using an anti-CCP first generation kit (anti-CCP1) suggested a lower sensitivity for anti-CCP antibodies compared with RF[28], the anti-CCP second generation kit (anti-CCP2) improved the diagnostic sensitivity to a level comparable to that of RF[29]. Our results using anti-CCP2 also supported the data. However, in very early RA, anti-CCP antibodies were inferior to RF in sensitivity, although the difference was not statistically significant. In contrast, RF and CARF were positive in approximately 80% and 90% of very early RA sera, respectively. By comparison, the occurrence of anti-CCP antibodies was not as high as RF and CARF in the very early stage of RA but tended to increase as the disease duration increased, as previously reported[30]. For this reason, use of anti-CCP antibodies alone is not thought to be the best diagnostic test for very early RA.

The best known property of anti-CCP antibodies is the high specificity for RA. It had been shown that the specificity of anti-CCP antibodies for RA is over 95%[29], but recent reports using anti-CCP2 showed that it is around 90%[7,18,24]. In our study, the specificity (83%) of anti-CCP antibodies was highest among serological markers tested; however, it was lower than that reported in the earlier studies. Recently, a number of studies reported that anti-CCP antibodies can be detected before the onset of RA[31,32]. However, no subject in our control group developed RA after 2 years of observation from baseline. For the production of anti-CCP antibodies, citrullination or existence of citrullinated proteins in the synovium is essential. Vossenaar, *et al* suggested that the presence of citrullinated proteins in the inflamed synovium is not specific for RA, but rather may be an inflammation-associated phenomenon[33]. We recently reported that anti-CCP antibody-positive patients showed significantly higher incidence of joint symptoms than anti-CCP antibody-negative patients in rheumatic diseases other than RA[34]. Our results, together with other reports, suggest that anti-CCP antibodies may be useful as a joint inflammation marker not only in RA but also in non-RA rheumatic diseases. Further studies regarding anti-CCP antibodies or citrullination of proteins in non-RA diseases should clarify this issue.

Despite the use of the anti-CCP2 kit, the results of sensitivity and specificity were diverse. It seems that in some studies higher specificity (> 95%) tended to be associated with a lower sensitivity (< 70%)[35,36], and conversely, others

with higher sensitivity (> 80%) had lower specificity (< 92%)[7,18,24]. However, because of several different factors, such as the background of the study group (e.g., early RA or not) or of the control group (e.g., healthy donors or patients with rheumatic disease), or the racial distribution of the subjects studied, it was difficult to compare the results of the diagnostic utility of anti-CCP antibodies equally with that obtained in the previous studies. To compare the overall diagnostic utility, we used the "diagnostic accuracy"[9] as an overall index in this study. As a result, anti-CCP antibodies showed the highest diagnostic accuracy (82.5%) among serological markers tested here. This value was found to be comparable with that which we calculated using the data of the previous studies (80–88%)[7,18,24,36].

The combination of anti-CCP antibodies with RF was reported to increase the diagnostic utility for RA[5,37]. In our study, any combinations of serological markers (presence of marker A or marker B) significantly increased the sensitivity compared with the use of anti-CCP antibodies alone, both in total and in very early RA (except a combination of "anti-CCP antibodies and MMP-3" in very early RA). For diagnostic accuracy, the combinations of anti-CCP antibodies with RF or with CARF could be superior to anti-CCP antibodies alone in very early RA, but the difference between them was not statistically significant. Although anti-CCP antibodies were detected in 38% of total RA and 22% of very early RA in RF-negative patients, the frequency of RF-positive subjects in anti-CCP antibody-negative patients was higher both in total RA (44%) and in very early RA (61%). This finding was similar to the previous reports[7,30] and was also observed with CARF (data not shown). Therefore, we suggest that both anti-CCP antibodies and RF (or CARF) should be measured for the diagnosis of very early RA.

We examined anti-agalactosyl IgG antibodies (CARF) in detail. CARF showed very strong correlation with RF (r = 0.952) and higher sensitivity for RA (90%) than RF (84%). But its specificity for RA (50%) was much lower than that of anti-CCP antibodies (83%) and RF (71%). Also, its diagnostic accuracy, PPV, and AUC were all inferior to those of RF. Agalactosyl IgG is specific for RA, and RF binds more strongly to agalactosyl IgG than to normal IgG[15]. In addition, CARF detection methods have the advantage that all subclasses of anti-agalactosyl IgG antibodies can be detected. Because of these characteristics of CARF, earlier studies paid attention to its high sensitivity but not to its low specificity for RA. Recently, Nishijima, *et al* showed that anti-agalactosyl IgG antibodies are frequently detected in patients with systemic sclerosis (74%)[38], and Maeno, *et al* showed that anti-agalactosyl IgG antibodies can be detected in juvenile onset SS (93%), both at higher rates than in juvenile idiopathic arthritis (37%)[39]. Thus, despite the low specificity, CARF is superior in sensitivity and may be useful in combination with anti-CCP antibodies to enhance the diagnostic accuracy for very early RA. Further studies of CARF are expected.

Our study indicated that detection of anti-CCP antibodies is useful, by itself, for the diagnosis of RA; however, combined use of anti-CCP antibodies with RF may be more useful than use of anti-CCP antibodies alone for the diagnosis of RA and for the diagnosis of very early RA. When new diagnostic criteria for RA are considered, it is desirable that anti-CCP antibodies be included unless RF is excluded.

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. Smolen JS. Autoantibodies in rheumatoid arthritis. In: van Venrooij WJ, Maini RN, editors. Manual of biological markers of disease. Dordrecht: Kluwer; 1996: C1.1/1-18.
3. 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.
4. 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.
5. Bizzaro N, Mazzanti G, Tonutti E, Villalta D, Tozzoli R. Diagnostic accuracy of the anti-citrulline antibody assay for rheumatoid arthritis. Clin Chem 2001;47:1089-93.
6. Visser H, le Cessie S, Vos K, Breedveld FC, Hazes JM. How to diagnose rheumatoid arthritis early: a prediction model for persistent (erosive) arthritis. Arthritis Rheum 2002;46:357-65.
7. Lee DM, Schur PH. Clinical utility of the anti-CCP assay in patients with rheumatic diseases. Ann Rheum Dis 2003;62:870-4.
8. Kroot EJ, de Jong BA, van Leeuwen MA, et al. The prognostic value of anti-cyclic citrullinated peptide antibody in patients with recent-onset rheumatoid arthritis. Arthritis Rheum 2000;43:1831-5.
9. Jansen LM, van Schaardenburg D, van der Horst-Bruinsma I, van der Stadt RJ, de Koning MH, Dijkmans BA. The predictive value of anti-cyclic citrullinated peptide antibodies in early arthritis. J Rheumatol 2003;30:1691-5.
10. De Rycke L, Peene I, Hoffman IE, et al. Rheumatoid factor and anticitrullinated protein antibodies in rheumatoid arthritis: diagnostic value, associations with radiological progression rate, and extra-articular manifestations. Ann Rheum Dis 2004;63:1587-93.
11. Forslind K, Ahlmen M, Eberhardt K, Hafstrom I, Svensson B. Prediction of radiological outcome in early rheumatoid arthritis in clinical practice: role of antibodies to citrullinated peptides (anti-CCP). Ann Rheum Dis 2004;63:1090-5.
12. Kastbom A, Strandberg G, Lindroos A, Skogh T. Anti-CCP antibody test predicts the disease course during 3 years in early rheumatoid arthritis (the Swedish TIRA project). Ann Rheum Dis 2004;63:1085-9.
13. Lindqvist E, Eberhardt K, Bendtzen K, Heinegard D, Saxne T. Prognostic laboratory markers of joint damage in rheumatoid arthritis. Ann Rheum Dis 2005;64:196-201.
14. Parekh RB, Dwek RA, Sutton BJ, et al. Association of rheumatoid arthritis and primary osteoarthritis with changes in the glycosylation pattern of total serum IgG. Nature 1985;316:452-7.
15. Watson M, Rudd PM, Bland M, Dwek RA, Axford JS. Sugar printing rheumatic diseases: a potential method for disease differentiation using immunoglobulin G oligosaccharides. Arthritis Rheum 1999;42:1682-90.
16. Kondo S, Mashima T, Kai M, Yamada Y, Yoshizawa M, Hosoda T. The study of serum antiagalactosyl IgG antibody in early rheumatoid arthritis [in Japanese]. Rinshou to Kenkyu 1997;72:190-4.

17. Das H, Atsumi T, Fukushima Y, et al. Diagnostic value of antiagalactosyl IgG antibodies in rheumatoid arthritis. Clin Rheumatol 2004;23:218-22.
18. Araki C, Hayashi N, Moriyama M, et al. Usefulness of anti-cyclic citrullinated peptide antibodies (anti-CCP) for the diagnosis of rheumatoid arthritis [in Japanese]. Rinsho Byori 2004;52:966-72.
19. 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.
20. Alarcon-Segovia D, Cardiel MH. Comparison between 3 diagnostic criteria for mixed connective tissue disease. Study of 593 patients. J Rheumatol 1989;16:328-34.
21. Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Arthritis Rheum 1980;23:581-90.
22. Vitali C, Bombardieri S, Moutsopoulos HM, et al. Preliminary criteria for the classification of Sjogren's syndrome. Results of a prospective concerted action supported by the European Community. Arthritis Rheum 1993;36:340-7.
23. Bohan A, Peter JB. Polymyositis and dermatomyositis (first of two parts). N Engl J Med 1975;292:344-7.
24. Suzuki K, Sawada T, Murakami A, et al. High diagnostic performance of ELISA detection of antibodies to citrullinated antigens in rheumatoid arthritis. Scand J Rheumatol 2003;32:197-204.
25. Ichikawa Y, Yamada C, Horiki T, et al. Anti-agalactosyl IgG antibodies and isotype profiles of rheumatoid factors in Sjogren's syndrome and rheumatoid arthritis. Clin Exp Rheumatol 1998;16:709-15.
26. Ebizuka T, Kobayashi S, Akimoto T, et al. Standard range, setting of cutoff levels, and clinical usefulness of a kit for measuring oligosaccharide chains in anti-agalactosyl IgG antibodies [in Japanese]. Risho to Kenkyu 1997;74:477-82.
27. Steinbrocker O, Traeger CH, Batterman RC. Therapeutic criteria in rheumatoid arthritis. JAMA 1949;140:659-62.
28. 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.
29. Vossenaar ER, Van Venrooij WJ, Pruijin GJM. Anti-CCP antibodies in (early) rheumatoid arthritis. In: Conrad K, Fritzler M, Meurer M, Sack U, Shoenfeld Y, editors. Proteomics to molecular epidemiology: relevance of autoantibodies. Lengerich: Pabst Science Publishers; 2002:454-62.
30. Dubucquoi S, Solau-Gervais E, Lefranc D, et al. Evaluation of anti-citrullinated filaggrin antibodies as hallmarks for the diagnosis of rheumatic diseases. Ann Rheum Dis 2004;63:415-9.
31. Rantapaa-Dahlqvist S, de Jong BA, Berglin E, et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. Arthritis Rheum 2003;48:2741-9.
32. Nielen MM, van Schaardenburg D, Reesink HW, et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. Arthritis Rheum 2004;50:380-6.
33. Vossenaar ER, Smeets TJ, Kraan MC, Raats JM, van Venrooij WJ, Tak PP. The presence of citrullinated proteins is not specific for rheumatoid synovial tissue. Arthritis Rheum 2004;50:3485-94.
34. Matsui T, Shimada K, Tohma S. Anti-cyclic citrullinated peptide antibody in rheumatic diseases other than rheumatoid arthritis. Clin Rheumatol 2005 Nov;10:1-2 [Epub ahead of print]
35. 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.
36. Vallbracht I, Rieber J, Oppermann M, Forger F, Siebert U, Helmke K. Diagnostic and clinical value of anti-cyclic citrullinated peptide antibodies compared with rheumatoid factor isotypes in rheumatoid arthritis. Ann Rheum Dis 2004;63:1079-84.
37. Jansen AL, van der Horst-Bruinsma I, van Schaardenburg D, van de Stadt RJ, de Koning MH, Dijkmans BA. Rheumatoid factor and antibodies to cyclic citrullinated peptide differentiate rheumatoid arthritis from undifferentiated polyarthritis in patients with early arthritis. J Rheumatol 2002;29:2074-6.
38. Nishijima C, Sato S, Takehara K. Anti-agalactosyl IgG antibodies in sera from patients with systemic sclerosis. J Rheumatol 2001;28:1847-51.
39. Maeno N, Takei S, Fujikawa S, et al. Antiagalactosyl IgG antibodies in juvenile idiopathic arthritis, juvenile onset Sjogren's syndrome, and healthy children. J Rheumatol 2004;31:1211-7.