

A retrospective study of the fluctuation in serum levels of anti-cyclic citrullinated peptide antibody in patients with rheumatoid arthritis

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Abstract

Objective

To investigate the fluctuation in serum levels of anti-cyclic citrullinated peptide antibody (anti-CCP) retrospectively in patients with rheumatoid arthritis (RA).

Methods

Serum levels of anti-CCP were measured retrospectively in 131 patients with RA and 90 patients with non-RA rheumatic diseases using a commercially available kit. All sera were collected from patients during the 22-year period, 1982-2004. To analyze the fluctuation in anti-CCP levels, 17 RA patients were selected on the basis of showing a significantly higher anti-CCP level in a serum sample taken at the first visit (> 80 U/ml), and availability of preserved serum samples that had been taken from each patient at 10 time points.

Results

The test gave a sensitivity of 88% (115/131) and a specificity of 81% (73/90). The longitudinal study of 17 RA patients showed that anti-CCP levels were elevated at the first visit in 12 (71%) patients and then decreased gradually, whereas those in the other five (29%) patients fluctuated substantially. In both cases, anti-CCP levels tended to fluctuate in parallel with the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) level, reflecting the spontaneous aggravation of arthritis and the efficacy of anti-rheumatic drugs. The courses of three representative RA patients are illustrated in detail along with their therapeutic regimens, and these further confirm the correlation of anti-CCP levels with laboratory parameters (ESR and CRP) as well as the activity of arthritis.

Conclusion

Measurement of serum anti-CCP levels was found to be useful for not only the diagnosis but also the management of RA.

Key words

Anti-cyclic citrullinated peptide antibody, rheumatoid arthritis, systemic sclerosis, Sjögren's syndrome, enzyme-linked immunosorbent assay.

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Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease of unknown etiology with a main characteristic of chronic inflammation of the joints leading to tissue degradation and destruction of bone and cartilage (1). Since organic joint damage is irreversible, early recognition and treatment of RA are important, with the goal of halting progression of the disease (2). RA is classified primarily according to the clinical features, and serologic markers are solely restricted to the determination of rheumatoid factor (RF) (3). Although RF can be detected in 60-80% of RA patients, its specificity is limited, since RF is also detected in non-RA rheumatic diseases and infectious diseases, as well as in healthy subjects, particularly the elderly.

Recently, Schellekens *et al.* discovered that antibodies specific for RA bind to antigenic determinants that contain the nonstandard amino acid, citrulline (4), formed by post-transcriptional modification of arginine residues by the enzyme peptidyl arginine deiminase (5). This observation was subsequently confirmed by Girbal-Neuhausser and coworkers (6), who showed that recombinant filaggrin fragments were recognized by RA autoantibodies only after enzymatic deimination *in vitro*. The antibodies directed against the citrullinated substrates were shown to be the same as those defined as anti-perinuclear factor (7) and anti-keratin antibody (8), and could be detected in over 80% of RA sera with a high disease specificity using several synthetic peptides containing citrulline (4). Schellekens *et al.* also described a modified peptide variant (cyclic citrullinated peptide [CCP]) that is recognized by RA autoantibodies with relatively high sensitivity in a reliable and convenient test format (9).

To date many reports have been published on the clinical significance of antibodies against CCP (anti-CCP), including the diagnostic accuracy of this antibody for RA (10-15), the timing of the appearance of anti-CCP antibodies in relation to rheumatic symptoms of RA (16), and the discriminatory power of these antibodies for erosive

arthritis (17,18).

In the present study, anti-CCP were measured in sera from patients with RA and patients with non-RA rheumatic diseases using a commercially available second-generation enzyme-linked immunosorbent assay (ELISA) kit. The fluctuation in antibody levels during the clinical course of RA was also determined in selected patients. The usefulness of measurement of anti-CCP is discussed, not only for the diagnosis but also the management of RA.

Materials and methods

Serum samples

Sera were obtained from the Department of Collagen Diseases, International Medical Center of Japan. Sera (n = 221) were collected from patients visiting the outpatient clinic or admitted to a hospital ward of the Department of Collagen Diseases between 1982-2004. One hundred and thirty-one patients had been diagnosed as having definite RA according to the revised criteria of the American College of Rheumatology (3). To further assess the specificity of the test, a group of serum samples taken from healthy volunteers (n = 200) and from patients with non-RA rheumatic diseases (n = 90) were tested as negative controls [20 with systemic lupus erythematosus (SLE, 19), 20 with systemic sclerosis (SSc, 20), 20 with primary Sjögren's syndrome (SS, 21), 10 with polymyositis/dermatomyositis (PM/DM, 22), 10 with mixed connective tissue disease (MCTD, 23) and 10 with adult-onset Still's Disease (AOSD, 24)]. All sera were stored at -80°C until use.

ELISA for anti-CCP

The second generation anti-CCP ELISA kit (DIASSTAT Anti-CCP; Axis-Shield, Dundee, UK) was purchased from Medical and Biological Laboratories (Nagoya, Japan) and serum anti-CCP levels were measured according to the manufacturer's instructions. Although a cut-off value of 5.0 U/ml was recommended by ROC analysis performed at the Axis-Shield Company using 575 RA patients and 1093 non-RA patients, we had originally determined a cut-off value of 5.6 U/ml by

ROC analysis of our data from RA and non-RA autoimmune patients. With this cut-off value, the sensitivity and specificity were slightly improved. Longitudinal measurement of serum anti-CCP levels was performed on 17 RA patients who were selected on the basis of the availability of preserved sera taken at 10 time points (mean ± SD of observation period: 6.1 ± 2.7 years, range: 2-11 years), and possessing significantly higher anti-CCP levels in a serum sample taken at the first visit (> 80 U/ml). As to the therapeutic regimen, all 17 patients were prescribed prednisolone (range of maximal dosage: 2.5 - 20 mg/day) and various non-steroidal anti-inflammatory drugs. The following disease-modifying anti-rheumatic drugs (DMARDs) were used: case A = none, case B = none, case C = gold sodium thiomalate (GST), case D = none, case E = GST, case F = GST, case G = GST, case H = none, case I = salazosulfapyridine (SASP), methotrexate (MTX), bucillamine (BUC), azathioprine (AZP), and cyclophosphamide (CPA), case J = GST, case K = BUC and CPA, case L = BUC and CPA, case M = GST, case N = GST and D-penicillamine (D-pc), case O = GST, case P = none, case Q = none.

Results

Serum levels of anti-CCP in RA and other rheumatic diseases

The titers of anti-CCP were compared in patients with RA and other rheumatic diseases. Using a cut-off value of 5.6 U/ml, the frequencies of anti-CCP were 115/131 (88%) in RA, 2/20 (10%) in SLE, 6/20 (30%) in SSc, 1/20 (5%) in SS, 3/10 (30%) in PM/DM, 4/10 (40%) in MCTD and 1/10 (10%) in AOSD (Fig.1). From these figures the diagnostic sensitivity and specificity of anti-CCP for RA were 88% and 81% (73/90), respectively. The clinical features of patients with non-RA rheumatic diseases and high serum levels of anti-CCP (>10 U/ml) are shown in Table I. The relatively high frequency of arthralgia, C-reactive protein (CRP) positivity and RF positivity suggests the presence of subclinical synovitis in some of these patients.

Table I. Clinical and laboratory features in non-RA rheumatic disease patients on the same day that high serum anti-CCP levels were detected (>10.0 U/ml).

| Pt. no. | Diagnosis | Sex | Anti-CCP* (U/ml) | Arthralgia/Arthritis | CRP# (mg/dl) | RF† (IU/ml) |
|---------|-----------|-----|------------------|----------------------|--------------|-------------|
| 1 | SSc | F | 1362.0 | - | 7.9 | 48 |
| 2 | PM/DM | F | 276.0 | + | 2.4 | 202 |
| 3 | SSc | F | 214.0 | + | 2.7 | 112 |
| 4 | SS | F | 194.0 | + | 0.3 | 45 |
| 5 | SSc | F | 84.0 | + | 0.8 | 173 |
| 6 | SSc | F | 72.0 | + | 1.2 | 58 |
| 7 | PM/DM | F | 35.1 | + | 1.2 | 79 |
| 8 | SSc | F | 29.0 | - | 0.7 | 67 |
| 9 | SSc | F | 15.3 | + | 0.5 | 260 |
| 10 | SLE | F | 11.0 | - | 0.3 | 10 |

CRP: C-reactive protein; RF: rheumatoid factor; SSc: systemic sclerosis; PM/DM: polymyositis/dermatomyositis; SS: Sjögren's syndrome; SLE: systemic lupus erythematosus; F: female; *Cut-off value = 5.6 U/ml; # Cut-off value = 0.4 mg/dl; †Cut-off value = 40 IU/ml.

Longitudinal measurement of serum levels of anti-CCP

Longitudinal measurement of serum anti-CCP levels was performed on 17 RA patients. For convenience, the results are shown in three figures according to the maximum value of anti-CCP levels detected during the course of RA in each patient (> 500 U/ml in Fig. 2a, 500-250 U/ml in Fig. 2b, and < 250 U/ml in Fig. 2c). The longitudinal study showed that anti-CCP levels were elevated in the initial sera and then decreased gradually in 11 (71%) patients, whereas anti-CCP levels fluctuated in

the other 5 (29%) patients. As to the therapeutic regimen, all 17 patients were prescribed prednisolone (range of maximal dosage: 2.5 - 20 mg/day) and various non-steroidal anti-inflammatory drugs. Disease-modifying anti-rheumatic drugs used in 12 patients with decreased anti-CCP were as follows: case A = none, case B = none, case C = GST, case D = none, case F = GST, case H = none, case I = SASP, MTX, BUC, AZP and CPA, case M = GST, case N = GST and D-pc, case O = GST, case P = none, case Q = none. Those used in the 5 patients with fluctuating

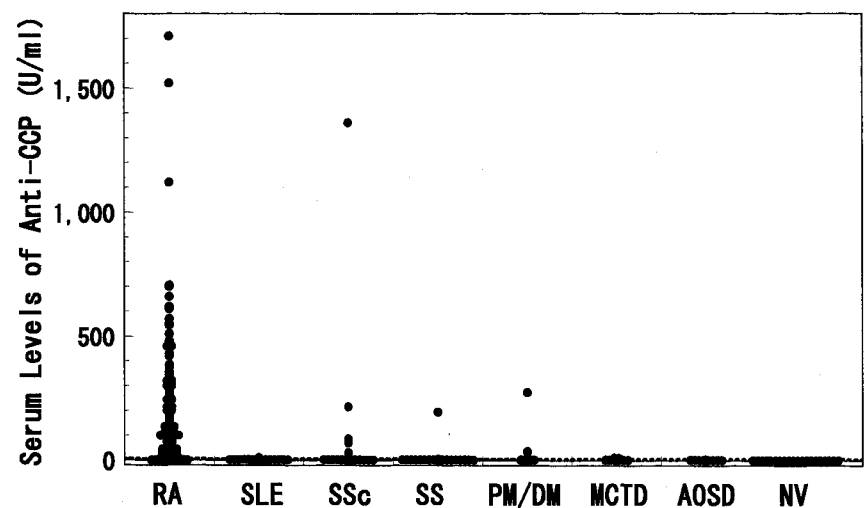


Fig. 1. Serum levels of anti-CCP in patients with RA and other rheumatic diseases, and normal volunteers. The horizontal line indicates the anti-CCP cut-off value (5.6 U/ml). RA: rheumatoid arthritis; SLE: systemic lupus erythematosus; SSc: systemic sclerosis; SS: Sjögren's syndrome; PM/DM: polymyositis/dermatomyositis; MCTD: mixed connective tissue disease; AOSD: adult-onset Still's disease; NV: normal volunteer.

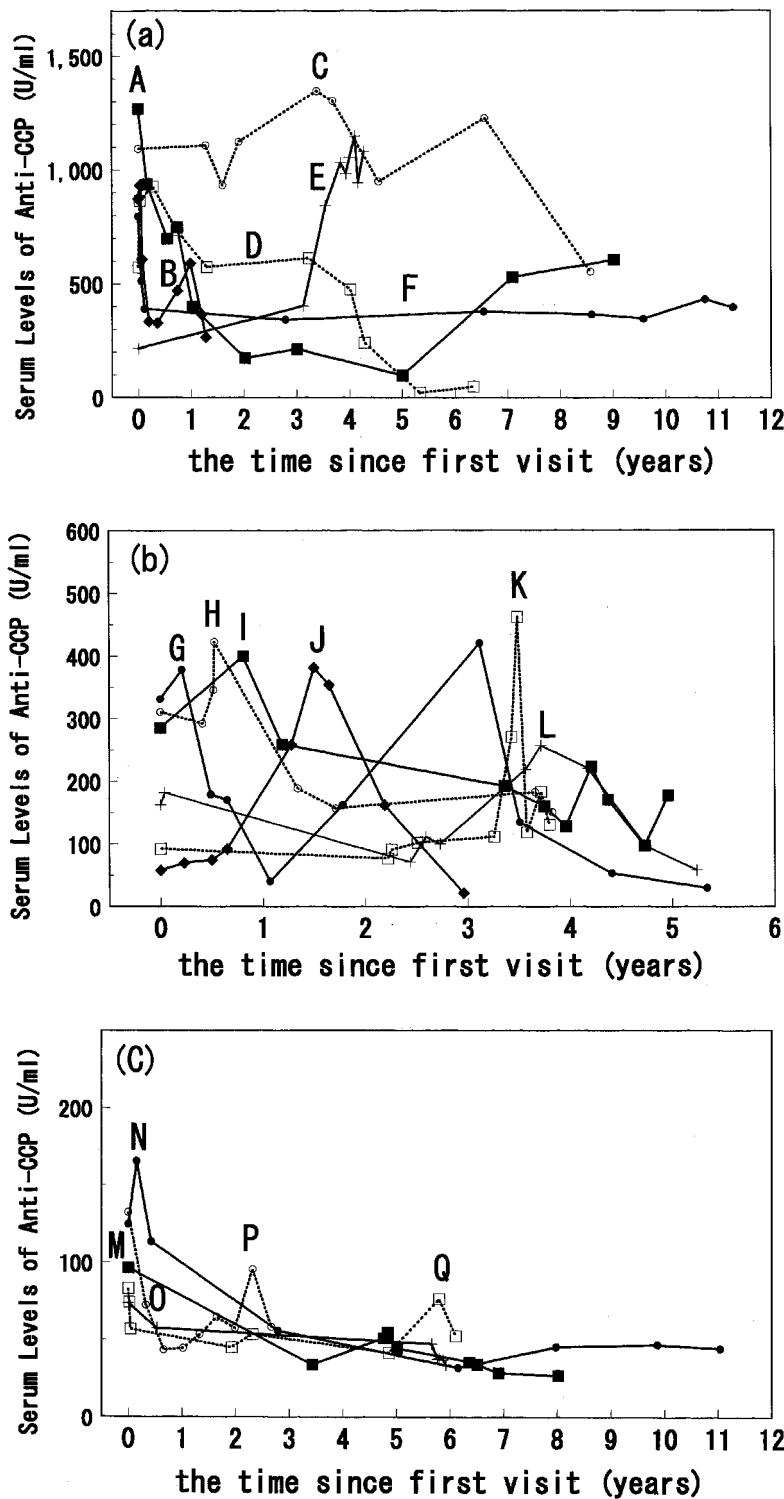


Fig. 2. Longitudinal fluctuation in serum levels of anti-CCP in (a) six RA patients (cases: A-F) with anti-CCP levels > 500 U/ml, (b) six RA patients (cases: G-L) with anti-CCP levels between 500 and 250 U/ml, and (c) five RA patients (cases: M-Q) with anti-CCP levels < 250 U/ml.

tuating anti-CCP were: case E = GST, case G = GST, case J = GST, case K = BUC and CPA, case L = BUC and CPA. There appeared to be no relationship between the change in pattern of

anti-CCP and DMARDs therapy. However, when the therapeutic regimens were effective in decreasing disease activity, anti-CCP levels also tended to decrease.

Representative cases demonstrating a relationship between serum anti-CCP levels and clinical or laboratory features

Case: A (53-year-old female, Fig. 3a). This patient experienced systemic polyarthrititis with low grade fever in 1971. A rheumatoid nodule appeared on the extensor surface of her right elbow joint in 1973 and ulnar deviations of both hands appeared in 1976. Carpal tunnel syndrome was diagnosed in 1981. A skin ulcer developed on her left leg in October, 1983. The presence of vasculitis was demonstrated by histologic examination of a biopsy from the leg lesion. After admission in January, 1984, the patient underwent plasmapheresis on three separate occasions using double-membrane filtration. Thereafter, her leg ulcer healed, her anemia improved and the erythrocyte sedimentation rate (ESR) decreased. Therapy was initiated with 15 mg/day prednisolone and 600 mg/day naproxen. The dose of prednisolone was tapered gradually. Prednisolone medication and the implementation of plasmapheresis appeared to improve her arthritis and laboratory parameters such as ESR and CRP. At the same time, anti-CCP levels were also reduced significantly. The coefficient of correlation (r_s) between anti-CCP levels and CRP values was 0.806 ($p < 0.01$).

Case: J (60-year-old male, Fig. 3b). This patient visited our outpatient clinic in 1991 complaining of xerophthalmia, xerostomia and mild arthralgia. Ophthalmologic examination revealed keratoconjunctivitis sicca on the basis of a positive Shirmer's test and positive Rose-Bengal test, and a diagnosis of Sjögren's syndrome was made. His chest X-ray indicated interstitial pneumonia in the bilateral lower lung fields. Arthritis of the bilateral elbows, bilateral wrists, bilateral knees and bilateral ankles, together with morning stiffness, continuing for at least 60 min, appeared in August, 1991, and led to a diagnosis of RA. Administration of 600 mg/day naproxen improved his arthritis. His polyarthrititis aggravated in January, 1993, and required the administration of 5 mg/day prednisolone, 75 mg/day diclofenac sodium and 10 mg sodium

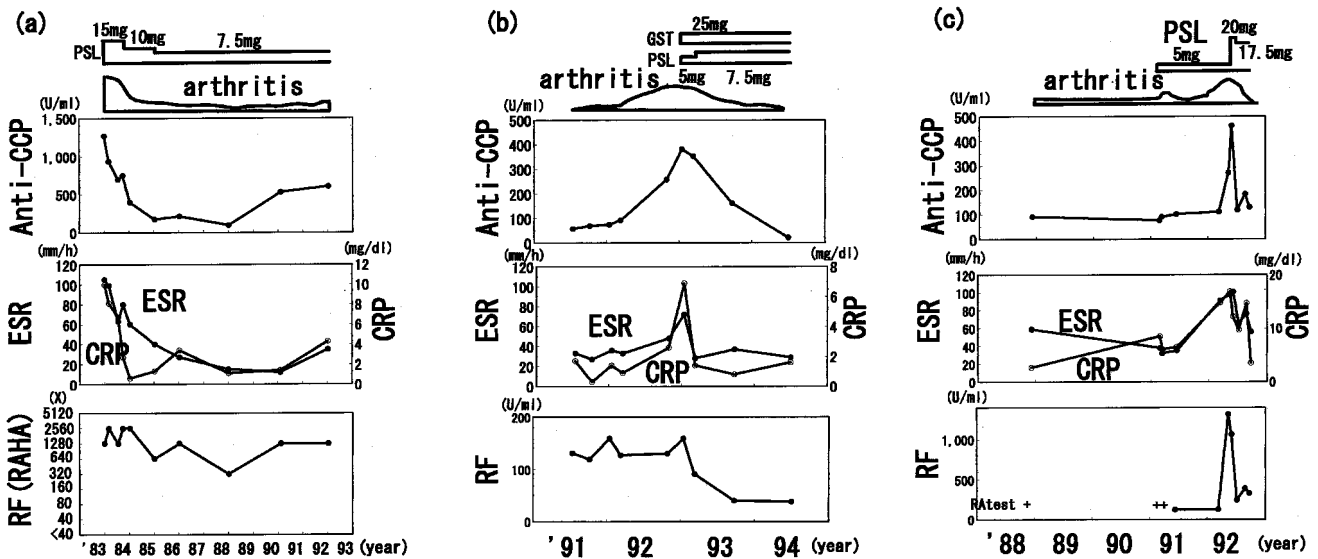


Fig. 3. Comparison of fluctuation in serum levels of anti-CCP with laboratory parameters such as erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) levels and rheumatoid factor (RF) titer during the clinical course of RA in three representative patients.

(a) Case A (53-year-old female). The coefficient of correlation (r_s) between anti-CCP levels and CRP values was 0.806 ($p < 0.01$).
 (b) Case J (60-year-old male). The coefficient of correlation (r_s) between anti-CCP levels and CRP values was 0.438 but did not reach statistical significance.
 (c) Case K (47-year-old male). The coefficient of correlation (r_s) between anti-CCP levels and CRP values was 0.564 but did not reach statistical significance.
 PSL: prednisolone; GST: gold sodium thiomalate; RAHA: rheumatoid arthritis hemagglutination.

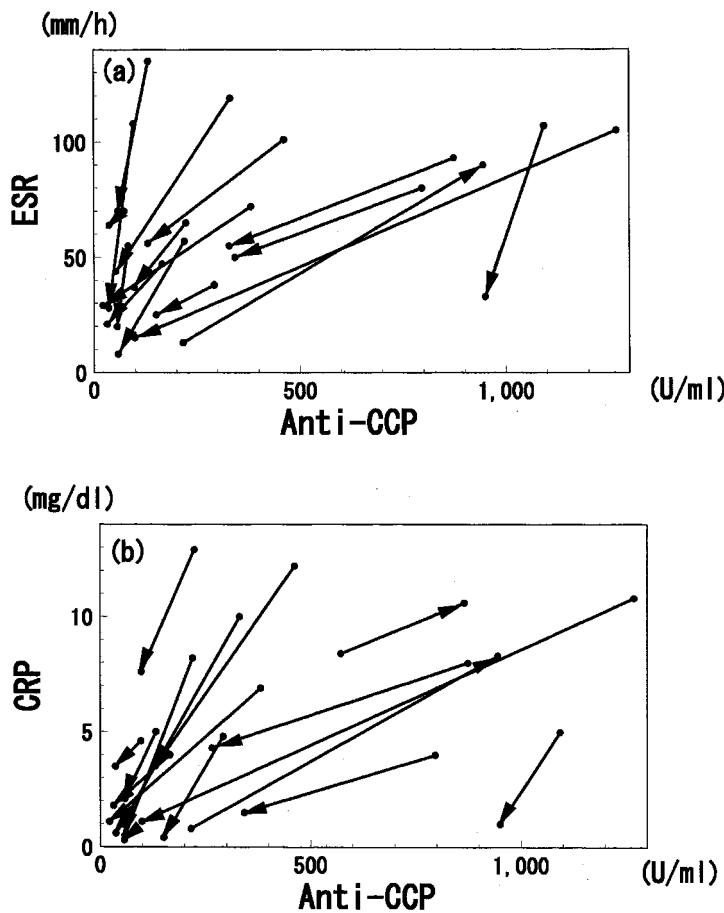


Fig. 4. Changes in serum anti-CCP levels compared with ESR or CRP levels in 17 RA patients. The maximum and minimum serum levels of anti-CCP (x-axis) in the 17 RA patients were plotted against the corresponding (a) ESR or (b) CRP value (y-axis), attaching lines and arrows to indicate the progression of time.

aurothiomalate by intramuscular injection weekly. Subsequently, 25 mg sodium aurothiomalate was injected intramuscularly biweekly. His arthritis then improved remarkably and marked improvement in laboratory findings such as ESR, CRP and RF was also observed. In this case, the elevation of ESR, CRP, RF and anti-CCP coincided with the deterioration of arthritis. The administration of prednisolone and sodium aurothiomalate improved not only his arthritis but also laboratory parameters such as ESR, CRP, RF and anti-CCP. The coefficient of correlation (r_s) between anti-CCP levels and CRP values was 0.438, but did not reach statistical significance.

Case: K (47-year-old male, Fig. 3c). This patient experienced polyarthritis of his right elbow joint, bilateral knee joints, and bilateral proximal interphalangeal joints in 1986. He visited our outpatient clinic as a new patient in 1988 and was prescribed 75 mg/day indometacin or 75 mg/day diclofenac sodium. His systemic polyarthritis grew more serious in 1990 and he was prescribed 2.5 mg/day prednisolone and 100 mg/day bucillamine. In spite of the prescription of 5 mg/day prednisolone, his polyarthritis deteriorated and he was admitted to our hospital. After ad-

mission, the prescription of 200 mg/day bucillamine improved his arthritis. In 1992, he was admitted to another hospital to receive medication for complicated diabetes and his prednisolone was discontinued. His polyarthritis then deteriorated strikingly and he was readmitted to our hospital. The administration of 20 mg/day prednisolone ameliorated his systemic polyarthritis remarkably. During the tapering of prednisolone, 50 mg/day cyclophosphamide was used simultaneously. Thereafter, his arthritis ameliorated remarkably and he was discharged from hospital. In this case, the elevation of ESR, CRP, RF and anti-CCP coincided with the deterioration of arthritis. The administration of prednisolone improved not only his arthritis but also laboratory parameters such as ESR, CRP, RF and anti-CCP. The coefficient of correlation (rs) between anti-CCP levels and CRP values was 0.564, but did not reach statistical significance.

Changes in serum anti-CCP levels and ESR or CRP in individual RA patients

The maximum and minimum serum levels of anti-CCP (x-axis) in the 17 RA patients were plotted against the corresponding ESR or CRP value (y-axis), and the attached lines and arrows indicate the progression of time in each individual RA patient (Figs. 4 and 5). These figures clearly indicate the corresponding changes in serum anti-CCP levels and ESR or CRP values in individual RA patients.

Discussion

This study has demonstrated that serum anti-CCP levels change substantially in RA patients; most patients showed an elevation in levels at the first visit followed by a decrease in the majority of cases (71%). In the remaining patients (29%), serum anti-CCP levels fluctuated considerably. Moreover, the change in anti-CCP levels in RA patients was shown to parallel disease activity (severity of arthritis and deterioration of laboratory parameters such as ESR and CRP). The reduction in anti-CCP levels seemed to follow an effective therapeutic

regimen, as demonstrated in the representative cases.

There have been many reports on the clinical significance of antibodies against CCP. The diagnostic accuracy of anti-CCP for RA has been described by several authors (10-15). In this study, a sensitivity of 88% and specificity of 81% were obtained using an originally determined cut-off value of 5.6 U/ml by receiver operating characteristic (ROC) curve analysis of our data for RA and non-RA autoimmune patients. The lower specificity of anti-CCP in the current study might be explained by the presence of subclinical synovitis in non-RA rheumatic disease patients with high serum anti-CCP levels, as indicated by clinical and laboratory features (high frequency of arthralgia/arthritis and high positivity of CRP and RF as shown in Table I).

In a previous study, anti-CCP was shown to be clinically significant and preceded the appearance of rheumatic symptoms of RA by several years (16). The discriminative power of anti-CCP for erosive arthritis has also been demonstrated by several authors (17, 18). All 17 patients who were longitudinally followed up in this study finally developed erosive arthritis. Although a precise analysis could not be performed in this study, partly because of loss of the medical records, RA patients who had high serum anti-CCP levels and a long disease duration tended to have erosive arthritis, whereas those who had high serum anti-CCP levels and a short disease duration often did not have erosive arthritis. It seems highly probable that elevated anti-CCP levels might be linked to erosive arthritis. In order to clarify this, further prospective analysis might be required.

The relationship between the citrullination of protein and the pathogenesis of RA was suggested in a recent report by genetic polymorphism of the citrullinating enzyme, peptidylarginine deiminase (PAD), in Japanese patients with RA (25). In the Japanese population, the distinct haplotype PADI4 was more frequent in RA patients than in healthy controls. Although the same haplotype has also been found in Caucasians, it is not significantly associated with RA in

this racial group (26).

There have been two previous reported studies about the anti-CCP test and infliximab therapy in patients with RA. Bobbio-Pallavicini *et al.* (27) reported that anti-CCP antibody levels were significantly decreased at 30 weeks but returned to the baseline thereafter. Rycke *et al.* (28) reported that rheumatoid factor, but not anti-CCP, was modulated by infliximab treatment in patients with RA.

In conclusion, the measurement of serum anti-CCP levels was found to be useful not only in the diagnosis but also the management of RA patients.

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