

Antibodies Against Cyclic Citrullinated Peptide and IgA Rheumatoid Factor Predict the Development of Rheumatoid Arthritis

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Objective. To evaluate the prevalence and predictive value of anti-cyclic citrullinated peptide (anti-CCP) antibodies in individuals who subsequently developed rheumatoid arthritis (RA) and to determine the relationship to rheumatoid factor (RF) of any isotype.

Methods. A case-control study was nested within the Northern Sweden Health and Disease Study and the Maternity cohorts of Northern Sweden. Patients with RA were identified among blood donors whose samples had been taken years before the onset of symptoms. Control subjects matched for age, sex, date of sampling, and residential area were selected randomly from the same cohorts. Anti-CCP antibody and RFs were determined using enzyme immunoassays.

Results. Eighty-three individuals with RA were identified as having donated blood before presenting with any symptoms of joint disease (median 2.5 years [interquartile range 1.1–4.7] before RA). In samples obtained before the onset of RA, the prevalence of autoantibodies was 33.7% for anti-CCP, 16.9% for IgG-

RF, 19.3% for IgM-RF, and 33.7% for IgA-RF (all highly significant compared with controls). The sensitivities for detecting these autoantibodies >1.5 years and ≤1.5 years before the appearance of any RA symptoms were 25% and 52% for anti-CCP, 15% and 30% for IgM-RF, 12% and 27% for IgG-RF, and 29% and 39% for IgA-RF. In conditional logistic regression models, anti-CCP antibody and IgA-RF were found to be significant predictors of RA.

Conclusion. Anti-CCP antibody and RFs of all isotypes predated the onset of RA by several years. The presence of anti-CCP and IgA-RF predicted the development of RA, with anti-CCP antibody having the highest predictive value. This indicates that citrullination and the production of anti-CCP and RF autoantibodies are early processes in RA.

Recent studies have indicated that posttranslational modification of proteins could be important in the initiation of autoimmune diseases such as rheumatoid arthritis (RA). Citrullination (i.e., conversion of peptidyl-arginine to peptidyl-citrulline) induces the appearance of a recently discovered autoantibody system, which can be measured efficiently by using cyclic citrullinated peptides (CCP) as antigens (1). The high specificity of anti-CCP antibodies for RA supports the importance of a modification process such as citrullination early in the development of the disease (1,2). Anti-CCP antibodies have a high specificity for RA in patients with recent-onset arthritis, and the detection of anti-CCP antibodies has been suggested as a new diagnostic test for use in early RA (3).

The specificity for RA can be further increased by combining the presence of anti-CCP antibody with the presence of rheumatoid factor (RF) (2). RFs and anti-CCP antibodies containing antigens such as filag-

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grin (i.e., the perinuclear factor) have been shown to precede the onset of clinical RA (4–6). The prevalence of RA is markedly increased in individuals with RF antibodies of more than one isotype, most frequently, a combination of IgM and IgA (7). Cigarette smoking has been suggested as an important environmental factor associated with an enhanced frequency of RF (8,9) and, in case-control and retrospective cohort studies, with an increased incidence of RA (10).

In this study, we sought to identify the prevalence and predictive value of anti-CCP antibodies for RA in population-based blood samples antedating the onset of RA symptoms. We also evaluated the occurrence of anti-CCP antibodies in relation to the presence of RF of IgG, IgM, and IgA isotypes as well as to smoking habits.

SUBJECTS AND METHODS

Subjects. A nested case-control study was performed in 2 cohorts: the Northern Sweden Health and Disease Study (NSHDS) cohort and the Maternity cohort of Northern Sweden. The NSHDS cohort contains 3 subcohorts: the Västerbotten Intervention Project, the Northern Sweden part of the World Health Organization (WHO) study for Monitoring of Trends and Determinants in Cardiovascular Disease (including the counties of Västerbotten and Norrbotten), and the Mammary Screening Project (for Västerbotten). In total, the cohort comprised 122,800 blood samples from 79,940 individuals (34,375 males and 45,565 females) as of January 2002. The Maternity cohort of Northern Sweden (78,700 women; 102,800 blood samples) comprises samples from pregnant women from the 4 northernmost counties of Sweden (population 900,000) who have undergone screening for rubella since November 1975. The cohorts are population-based, and no one was excluded. The study was approved by the Regional Ethics Committee of the University Hospital in Umeå.

At recruitment into the NSHDS cohort, subjects were asked to complete a self-administered questionnaire to collect demographic, medical, and lifestyle information, including smoking status and diet. A blood sample was collected into a heparinized tube and fractionated into plasma, buffy coat, and erythrocytes, and then stored at -80°C in a biorepository (the Northern Sweden Medical Research Biobank). The procedures for the collection and processing of blood samples were the same in all 3 subcohorts. In the Maternity cohort, blood samples were drawn from all pregnant women according to routine procedures. Sera were collected and stored at -20°C . The smoking habits of the women in the Maternity cohort were identified by means of a questionnaire.

The registry of patients who fulfilled the American College of Rheumatology (ACR; formerly, the American Rheumatism Association) classification criteria for RA (11) at the Department of Rheumatology, University Hospital, Umeå (the only rheumatology department in the county of Västerbotten), and whose date of presentation with symptoms or signs of joint disease were available was coanalyzed with the registries of the NSHDS and Maternity cohorts. The median

duration of disease after the diagnosis of RA was 3.0 years (interquartile range [IQR] 1.8–5.8 years). Eighty-six individuals were identified from the cohorts as having donated blood samples before the onset of signs or symptoms of joint disease. Figure 1 summarizes the identification of the RA patients and the RA pre-patients and the numbers of blood samples obtained.

For every sample from each cohort pre-patient identified, 4 control subjects, matched for sex, age at the time of blood sampling, the time point of blood sampling, and area of residence (rural or urban), were identified from the same cohort. Samples collected from 3 patients and 16 controls in the Maternity cohort during the autumn of 1975 were not available, and another 2 control tubes were empty. This resulted in only 2 controls in 2 sets of case-control pairs and 3 controls in 6 sets of pairs. In 15 of the 83 pre-patients, blood had been collected on 2 occasions within a period of 10 years antedating the onset of RA, yielding a total of 98 samples. Consequently, samples from 382 different control subjects (326 females and 56 males) and 83 pre-patients (69 females and 14 males) with 98 blood samples (84 females and 14 males) were identified for study.

The mean age of the pre-patients at the time of blood sampling was 54 years (range 31–67 years) among those in the NSHDS cohort (59 subjects with 72 samples) and 29 years (range 20–37 years) among those in the Maternity cohort (24 subjects with 26 samples). The mean age of the controls at the time of blood sampling was 55 years (range 30–69 years) among those in the NSHDS cohort (288 subjects) and 30 years (range 19–38 years) among those in the Maternity cohort (94 subjects). Additional samples were collected from 67 of the 83 pre-patients at their first visit to the early arthritis clinic, when RA was diagnosed. The antedating time for the samples was calculated as the time between blood collection and the onset of any symptoms of RA in all 83 pre-patients. On average, 7.2 ± 2.6 months ($\pm$ SD) should be added to the antedate time (i.e., the time from the onset of symptoms until the early arthritis was diagnosed as RA). The mean age at onset of RA was 53.5 years (range 27–68 years).

The mean storage time for the plasma from the NSHDS cohort (6.2 years [range 2.1–13.1] for the cases and 6.1 years [range 1.4–13.5] for the controls) was significantly shorter than the mean storage time for the sera from the Maternity cohort (14.4 years [range 3.3–26.2] for the cases and 14.7 years [range 2.6–26.3] for the controls). The samples from the early arthritis clinic were stored for a mean of 3.0 years (range 0.5–7.3) before being analyzed for the present study.

The frequencies of nonsmokers, ex-smokers, and current smokers were distributed fairly equally between the pre-patients (47.5%, 40.0%, and 12.5%, respectively) and the controls (53.6%, 28.7%, and 17.7%, respectively).

In addition to the case-control study, serum ($n = 16$) or plasma ($n = 5$) samples predating the diagnosis of RA by many years (long-term samples; median 10.9 years [IQR 8.7–18.4]) were also identified and evaluated independently. The inclusion of these 21 samples was intended to verify a pre-patient sample that was potentially positive for anti-CCP antibodies or RFs, but was collected closer to the time of disease onset. No control samples were identified for the long-term samples.

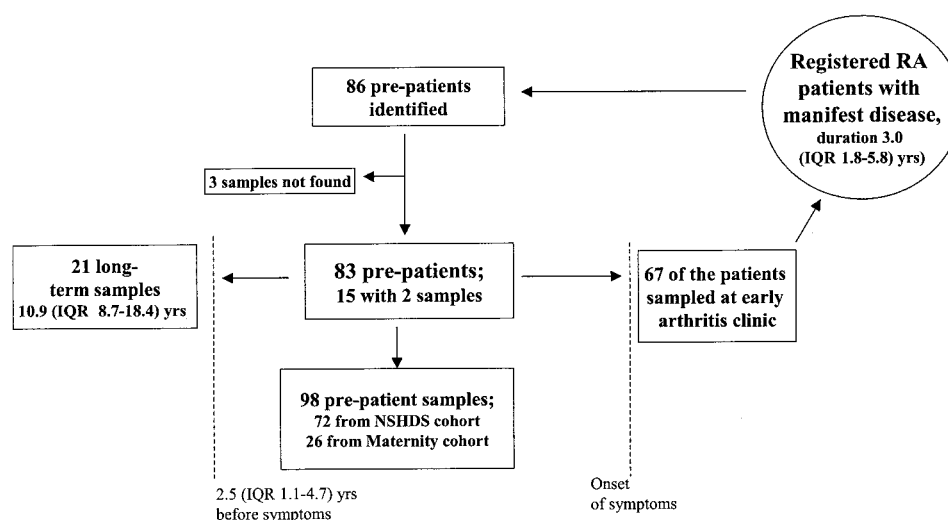


Figure 1. Overview of the patient and pre-patient blood samples. Comparison of the registry of patients who met the American College of Rheumatology criteria for rheumatoid arthritis (RA), including the date of symptom onset, with the registries of population-based blood sampling resulted in a total of 83 individuals who were blood donors prior to the onset of RA symptoms (pre-patients). Of these 83 pre-patients, 15 had donated blood on 2 occasions prior to diagnosis, resulting in 98 pre-patient blood samples. Blood had been collected from 67 of these individuals at the time of their first visit to the early arthritis clinic, when RA was diagnosed. Some long-term samples from these pre-patients (median 10.9 years before the first RA symptom) were also available for analysis. Durations are the median (interquartile range [IQR]) years. See Subjects and Methods for descriptions of the Northern Sweden Health and Disease Study (NSHDS) and the Maternity cohorts.

Enzyme immunoassay (EIA) for anti-CCP-2 antibody.

Anti-CCP antibody was measured in serum or plasma using the Immunoscan RA (Mark 2) EIA from Euro-Diagnostica (Arnhem, The Netherlands). This assay is also referred to as the anti-CCP-2 EIA, and it is different from the less sensitive CCP-1 assay previously described by Schellekens et al (2). The EIA was performed exactly as described by the manufacturer, including the use of a recommended cutoff value of 25 units/ml. Samples containing anti-CCP antibody at a concentration >25 units/ml (in duplicate measurements), which was the 98th percentile among controls, were considered positive. According to the manufacturer, the specificity of the CCP-2 assay is 97% compared with non-RA patients with other diseases and 99% compared with healthy individuals.

Enzyme-linked immunosorbent assays (ELISAs) for isotype-specific RFs. Microtiter plates (catalog number 3595; Costar, Cambridge, MA) were coated (100 μ l/well) with bovine serum albumin (BSA) (catalog number A7888; Sigma, St. Louis, MO), 10 μ g/ml in 50 mM carbonate/bicarbonate buffer, pH 9.6. The plates were initially washed 3 times with 0.15M NaCl containing 0.05% Tween 20 (Polysorbatum; Apoteksbolaget, Stockholm, Sweden) using an automatic plate washer. Rabbit IgG anti-BSA (Dako, Glostrup, Denmark) was diluted 1:500 in ELISA sample buffer (phosphate buffered saline, pH 7.4, containing 0.05% Tween 20 and 0.02% sodium azide) and added at 100 μ l/well. Unknown samples (serum or

plasma) and standard and control sera were diluted 1:500 in ELISA sample buffer supplemented with 1% BSA. Following overnight incubation and a washing step as described above, the diluted samples were added in duplicate (100 μ l/well). After 2 hours of incubation at room temperature with 4 washing cycles, the appropriate horseradish peroxidase-conjugated rabbit F(ab')₂ anti-human IgG, IgA, and IgM (Dako), diluted 1:1,000 in ELISA sample buffer plus BSA, was added (100 μ l/well) to the respective plates and incubated for 1 hour at room temperature.

Following another 4-cycle wash, tetramethylbenzidine (catalog number S1600; Dako) was added at 100 μ l/well to each well of the plate. When the optical density (OD; at 450 nm) of the standard reached a value of 0.6, the reactions were stopped by the addition of HCl (1.8M; 50 μ l/well) and the final readings were taken at 650 nm. Control sera with previously determined reactivity to IgG, IgA, and IgM RFs were used in all plates on each occasion for monitoring intra- and interassay variations. A previously analyzed serum with high RF reactivity for all 3 Ig classes (IgG, IgA, and IgM) was serially diluted and used as a standard. The activity of the undiluted standard was assigned a value of 100 units/ml for each of the 3 isotypes. The accepted variation in OD values for duplicates was set at <15%. If the variation exceeded this level, the samples were reassayed. The intra- and interassay coefficients of variation for the IgG-RF, IgA-RF, and IgM-RF ELISAs were 2.2% and 15.5%, 2.2% and 13.7%, and 4.4% and 16.8%, respectively.

The results for the control group served as a basis for setting the cutoff level for positive results. Since the distribution of the results was skewed toward higher values for all 3 RF isotypes measured, we decided to use the 95th percentile value of the controls as the cutoff point for all 3 RF classes. Calibration against the international reference preparation for RF (reference 64/1; WHO, Geneva, Switzerland) gave a cutoff value of ~19 IU/ml for the IgM-RF ELISA, which is consistent with the cutoff for the conventional nephelometric assay used in our laboratory. The isotype-specific RF ELISA methods were validated for both serum and various plasma types before starting the present study.

It is important to be aware of the possibility that the ELISA for IgG-RF may yield false-positive results if the samples also contain IgM-RF. Theoretically, normal IgG in the sample could be bound by IgM-RF and give rise to a false IgG signal in the ELISA. When examining our results, it became apparent that samples that were positive, and even strongly positive, for IgM-RF were often negative for IgG-RF; hence, we believe that there were no false-positive IgG-RF results. We believe that by preincubating the diluted samples overnight to allow the dissociation of loosely bound complexes, this problem was minimized, if not entirely eliminated.

All laboratory analyses were performed during the same time period and without any prior cycles of freezing and thawing of the samples. No significant differences have been found in any analyses comparing plasma and serum samples or of possible influences of long-term storage at -20°C or -80°C (Rantapää-Dahlqvist S, et al: unpublished data).

Statistical analysis. The chi-square test was used for testing categorical data between groups. The chi-square test for trends was used for testing several categorical data. Kruskal-Wallis one-way analysis of variance and Wilcoxon's rank sum test were used to compare continuous data. To assess the utility of the various antibodies in prospectively detecting RA patients, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value were calculated using a computer program (12). Comparisons were made between patients with the combination of anti-CCP antibodies plus each of the 3 RF isotypes versus those who had only 1 antibody and versus those who had neither antibody. The conditional logistic model was used to estimate the associations between the antibodies analyzed and the presence of RA. Odds ratios with 95% confidence intervals were also calculated. All *P* values are 2-sided, and *P* values less than or equal to 0.05 were considered statistically significant. When making multiple comparisons, the Bonferroni correction was applied. The calculations were performed using the SPSS software package for Windows (version 11.0; SPSS, Chicago, IL).

RESULTS

Ninety-eight samples had been obtained from 83 subjects before they presented with any symptoms of joint disease (median 2.5 years [IQR 1.1–4.7] before disease onset). The prevalence of all antibodies was significantly increased in pre-patient samples compared with controls. The prevalence of anti-CCP antibodies in the pre-patient samples was 33.7% ($\chi^2 = 103.5$, $P <$

Table 1. Presence of anti-CCP antibodies and IgG, IgM, and IgA RFs in 83 RA pre-patients, 67 patients with early RA, and 382 matched control subjects*

Antibody	No. (%) of control subjects (n = 382)	No. (%) of RA pre-patients (n = 83)	No. (%) of early RA patients (n = 67)
Anti-CCP	7 (1.8)	28 (33.7)	47 (70.1)
IgG-RF	21 (5.5)	14 (16.9)	31 (46.3)
IgM-RF	23 (6.0)	16 (19.3)	49 (73.1)
IgA-RF	21 (5.5)	28 (33.7)	47 (70.1)

* The rheumatoid arthritis (RA) pre-patients (those whose blood samples were obtained before the onset of any RA symptoms) were compared with their matched controls by McNemar's change test, and the early RA patient group was compared with the control group by chi-square test ($P < 0.0001$ for all comparisons). Controls were matched for sex, age at the time of blood sampling, the time point of blood sampling, and area of residence (rural or urban). Anti-CCP = anti-cyclic citrullinated peptide; RF = rheumatoid factor.

0.0001) (Table 1). IgG-RF was positive in 16.9% ($\chi^2 = 17.2$, $P < 0.0001$), IgM-RF in 19.3%, and IgA-RF in 33.7% ($\chi^2 = 26.4$, $P < 0.0001$, and $\chi^2 = 68.9$, $P < 0.0001$). When the pre-patients presented with early RA (mean $\pm$ SD symptom duration 7.2 ± 2.6 months; ≥ 4 ACR criteria fulfilled), 70.1% had anti-CCP antibodies and 46.3%, 73.1%, and 70.1% had IgG, IgM, and IgA RFs, respectively (Table 1).

The frequency of anti-CCP antibody and RFs of all isotypes in the 98 pre-patient samples increased significantly ($P < 0.001$ in anti-CCP antibody and all RF isotypes, by chi-square test for trend) over time approaching the onset of symptoms (Figure 2). The longest interval predating the onset of symptoms was 22 years for IgA-RF positivity and 19 years for both IgG-RF and IgM-RF positivity (Figure 2). Only 1 of the 21 long-term samples was positive for anti-CCP antibody (data not shown), with an interval of 9 years predating the onset of symptoms. Among the long-term samples, IgA-RF and IgM-RF were positive in 1 individual each, and both IgA-RF and IgG-RF were positive in another 2 individuals (data not shown). There were no significant differences between the sexes in the prevalence of anti-CCP antibody and RFs in any of the patient groups.

Once individuals began to produce anti-CCP antibody, all but 1 of them remained antibody positive, and the titer further increased significantly, until the disease was manifested ($P < 0.0001$, by Kruskal-Wallis one-way analysis of variance) (Figure 3). On an individual level, a comparison of anti-CCP antibodies between the pre-patient sample (or, in the 15 patients with 2 samples, the mean of the 2) and the sample collected at

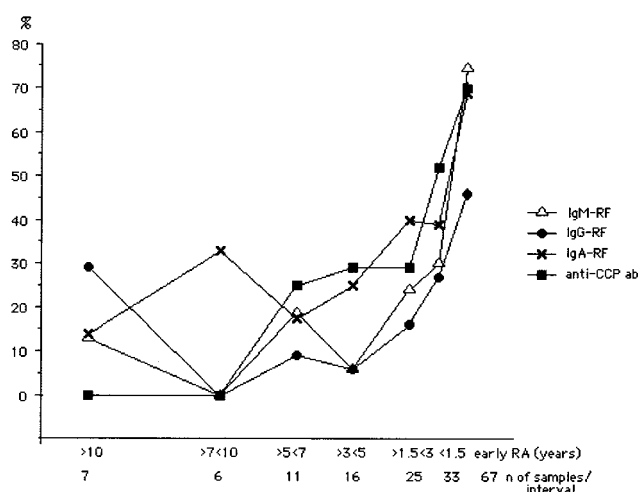


Figure 2. Frequency of anti-cyclic citrullinated peptide (anti-CCP) antibody (ab) and rheumatoid factor (RF) of IgM, IgG, and IgA isotypes in 98 blood samples collected from 83 subjects during the indicated time intervals (years) antedating the symptoms of rheumatoid arthritis (RA) and in 67 blood samples collected from 67 subjects at the time RA was diagnosed. $P < 0.001$ for all groups, by chi-square test for trend. Values are the percentages of samples positive (y-axis) at the time intervals shown (x-axis); numbers of samples evaluated at each interval are shown across the bottom.

the early arthritis clinic ($n = 67$) showed that the titers increased significantly over time ($P < 0.0001$, by Wilcoxon's rank sum test). The individual who became anti-CCP negative initially had a value of 56 units/ml; this had declined to 24 units/ml by the time of the second sampling (at the early arthritis clinic).

With regard to RF, the titers of all RF isotypes increased significantly ($P < 0.001$ for all isotypes, by Kruskal-Wallis one-way analysis of variance) prior to disease onset, although there was a decline in the titers in 10 individuals, 4 of whom had declines in all RF classes. However, in only 3 cases was the reduction significant. Two of these individuals had received corticosteroids before the second sampling (at the early arthritis clinic). For the other 7 samples, the RF levels were low and close to the cutoff value for positivity. On an individual level, a comparison of RF titers between the pre-patient sample (or, in the 15 patients with 2 samples, the mean of the 2) and the sample collected at the early arthritis clinic ($n = 67$) showed that the titers increased significantly over time ($P < 0.0001$, by Wilcoxon's rank sum test).

When the pre-patients were stratified according to the time before symptom onset, the highest sensitiv-

ities were 29% for IgA-RF in samples obtained >1.5 years before onset and 52% for anti-CCP antibodies in samples obtained ≤ 1.5 years before onset (Table 2). Specificities for the individual antibodies remained unchanged when the patients were stratified according to the time before symptom onset. Combining anti-CCP antibodies with any RF isotype increased the specificity, reaching 100% in some analyses (Table 2).

The positive predictive value was highest (82%) for anti-CCP antibodies when the estimated pretest probability for RA, as in this study with 4 controls per sample, was 20% before stratification of predisease samples (Table 2). When stratified for time to symptom onset, the PPVs for anti-CCP antibodies increased to 85% in samples obtained ≤ 1.5 years before onset and 91% in samples obtained at the time of early RA diagnosis (Table 2). The PPV was further increased when anti-CCP antibodies were combined with RF of any isotype. Furthermore, the PPV reached 100% when anti-CCP antibodies were analyzed in combination with either IgG-RF or IgM-RF in samples obtained ≤ 1.5 years before symptom onset.

When the probability of developing RA was set at the frequency of the disease in the general population (i.e., 1%), the PPV was highest for anti-CCP antibody

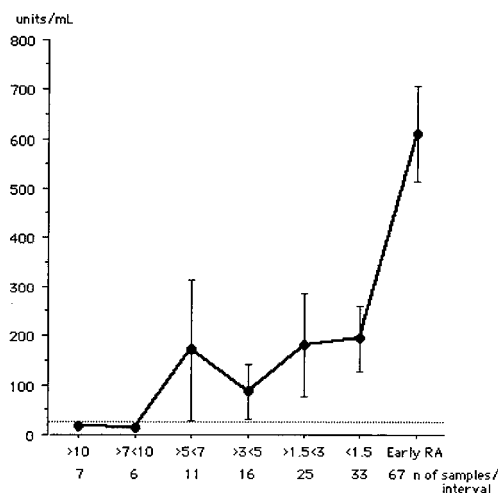


Figure 3. Titers of anti-cyclic citrullinated peptide antibody in 98 blood samples collected from 83 subjects during the indicated time intervals (years) antedating the symptoms of rheumatoid arthritis (RA) and in 67 blood samples collected from 67 subjects at the time RA was diagnosed. $P < 0.0001$, by Kruskal-Wallis one-way analysis of variance. Values are the mean $\pm$ SEM titers (y-axis) at the time intervals shown (x-axis); numbers of samples evaluated at each interval are shown across the bottom.

Table 2. Sensitivity, specificity, 95% CIs, PPV, and NPV data for the presence of anti-CCP antibodies and IgM, IgG, and IgA RFs in 98 blood samples from 83 RA pre-patients and from 67 of them with early RA*

	Sensitivity (95% CI)	Specificity (95% CI)	PPV	NPV
Samples from RA pre-patients (n = 98)				
Anti-CCP	34 (24.6–44.0)	98 (96.1–99.2)	82	86
IgM-RF	20 (13.2–30.0)	95 (92.5–97.1)	52	83
IgG-RF	17 (11.0–28.0)	95 (93.0–98.0)	47	82
IgA-RF	34 (24.6–44.0)	95 (92.5–97.1)	65	85
Anti-CCP + IgM-RF	15 (9.1–24.3)	99 (97.7–99.9)	83	82
Anti-CCP + IgG-RF	10 (5.3–18.4)	99 (97.7–99.9)	83	82
Anti-CCP + IgA-RF	21 (14.0–31.1)	99 (97.4–99.8)	87	83
Pre-patient samples taken >1.5 years before symptom onset (n = 65)				
Anti-CCP	25 (15.1–37.1)	98 (95.7–99.5)	80	84
IgM-RF	15 (8.0–27.0)	95 (91.6–97.4)	45	82
IgG-RF	12 (5.8–23.4)	94 (90.2–96.5)	34	81
IgA-RF	29 (18.9–42.0)	95 (91.2–97.1)	59	84
Anti-CCP + IgM-RF	11 (4.8–21.5)	99 (96.7–99.9)	77	82
Anti-CCP + IgG-RF	6 (2.0–15.8)	99 (96.6–99.9)	66	81
Anti-CCP + IgA-RF	17 (9.1–28.7)	99 (96.7–99.9)	84	83
Pre-patient samples taken ≤1.5 years before symptom onset (n = 33)				
Anti-CCP	52 (33.8–68.9)	98 (93.1–99.4)	85	89
IgM-RF	30 (16.2–48.9)	95 (89.1–97.7)	59	84
IgG-RF	27 (13.9–45.8)	98 (93.1–99.4)	75	84
IgA-RF	39 (23.4–57.8)	94 (88.1–97.2)	62	86
Anti-CCP + IgM-RF	24 (11.7–42.6)	100 (96.2–100)	100	84
Anti-CCP + IgG-RF	18 (7.6–36.1)	100 (96.3–100)	100	83
Anti-CCP + IgA-RF	30 (16.2–48.9)	99 (94.9–99.9)	91	85
Samples from patients with early RA (n = 67)				
Anti-CCP	70 (57.5–80.4)	98 (96.0–99.2)	91	93
IgM-RF	73 (60.6–82.9)	95 (92.5–97.1)	80	93
IgG-RF	46 (34.1–58.8)	95 (92.2–96.9)	70	88
IgA-RF	70 (57.5–80.4)	95 (92.5–97.1)	79	93
Anti-CCP + IgM-RF	58 (45.5–69.9)	99 (97.0–100)	97	91
Anti-CCP + IgG-RF	39 (27.4–51.5)	99 (97.0–100)	95	87
Anti-CCP + IgA-RF	60 (47.0–71.3)	99 (97.0–100)	95	91

* Rheumatoid arthritis (RA) pre-patients were those whose blood samples were obtained before the onset of any RA symptoms. The positive predictive value (PPV) and the negative predictive value (NPV) were calculated according to a 20% pretest probability of RA for this study. Values are percentages. 95% CIs = 95% confidence intervals; anti-CCP = anti-cyclic citrullinated peptide antibody; RF = rheumatoid factor.

(16%) (Table 3). When these calculations were made using an 8% frequency of RA (as an estimate of occurrence of RA in the patient population with pain and stiffness referred to our rheumatology clinic), the PPV of the presence of anti-CCP antibodies was 62%. At this 8% frequency, the PPVs for the RF isotypes (IgM, IgG, or IgA) in combination with anti-CCP antibody were further increased to 63%, 63%, and 70%, respectively (Table 3).

Anti-CCP antibodies in the pre-patient sera were significantly associated with all RF isotypes: $\chi^2 = 5.8$, $P < 0.05$ for IgG; $\chi^2 = 19.2$, $P < 0.0001$ for IgM; and $\chi^2 = 20.0$, $P < 0.0001$ for IgA. This was also the case in the controls: $\chi^2 = 8.4$, $P < 0.01$ for IgG; $\chi^2 = 9.0$, $P < 0.01$ for IgM; and $\chi^2 = 23.1$, $P < 0.0001$ for IgA. When

the pre-patient sera were stratified according to time before the onset of symptoms, only the anti-CCP antibodies were associated with IgM-RF ($\chi^2 = 13.1$, $P < 0.001$) and with IgA-RF ($\chi^2 = 16.0$, $P < 0.0001$) in sera obtained >1.5 years before the onset of symptoms. There were no associations in samples obtained ≤1.5 years before symptom onset.

In univariate conditional logistic regression analyses, all antibodies showed significant predictive values for RA, independently of time to symptom onset (Table 4). In multivariate conditional logistic regression models, only anti-CCP antibody and IgA-RF showed significant predictive values independently of time to symptom onset. Status as current and/or former smokers, whether analyzed separately or as a single group, did not

Table 3. PPV and NPV for anti-CCP antibodies and IgM, IgG, and IgA RFs in 98 blood samples from 83 RA pre-patients at 3 different probability estimates*

Antibody	Pretest probability of 20% for this study		Calculated hypothetical frequency of 8%		Population-based calculated frequency of 1%	
	PPV	NPV	PPV	NPV	PPV	NPV
Anti-CCP	82	86	62	94	16	99
IgM-RF	52	83	27	93	4	99
IgG-RF	47	82	23	93	3	99
IgA-RF	65	85	38	94	7	99
Anti-CCP + IgM-RF	83	82	63	93	16	99
Anti-CCP + IgG-RF	83	82	63	93	17	99
Anti-CCP + IgA-RF	87	83	70	94	22	99

* Rheumatoid arthritis (RA) pre-patients were those whose blood samples were obtained before the onset of any RA symptoms. The positive predictive value (PPV) and the negative predictive value (NPV) were calculated according to a 20% pretest probability of RA for this study. Values are percentages. Anti-CCP = anti-cyclic citrullinated peptide antibody; RF = rheumatoid factor.

significantly predict RA. In the pre-patients, current and former smokers were significantly more often positive for IgG-RF ($\chi^2 = 7.0$, 2 degrees of freedom, $P < 0.05$) but not for any other RF isotype or for anti-CCP antibodies. There was no increase in any of the RF isotypes in current or former smokers among the controls.

Table 4. Results of univariate and multivariate conditional logistic regression analyses for predictors of RA in pre-patients stratified according to time before the onset of symptoms and in controls*

	Univariate analysis, OR (95% CI)	Multivariate analysis, OR (95% CI)
≤1.5 years before symptom onset		
Anti-CCP antibody	31.4 (7.2–136.4)	28.9 (4.3–192.6)
IgG-RF	14.8 (3.1–69.6)	6.2 (0.6–61.0)
IgA-RF	10.9 (3.5–33.9)	11.4 (1.3–98.0)
IgM-RF	14.6 (3.1–67.8)	1.2 (0.1–22.1)
Current/former smoker	1.1 (0.7–2.3)	0.4 (0.1–1.3)
>1.5 years before symptom onset		
Anti-CCP antibody	29.6 (6.7–129.3)	16.1 (3.3–76.7)
IgG-RF	2.2 (0.8–5.5)	0.5 (0.1–2.4)
IgA-RF	8.4 (3.6–19.3)	5.1 (1.6–16.0)
IgM-RF	4.1 (1.5–10.5)	0.9 (0.1–4.5)
Current/former smoker	1.5 (0.8–2.6)	1.3 (0.6–2.4)

* Rheumatoid arthritis (RA) pre-patients were those whose blood samples were obtained before the onset of any RA symptoms. Controls were matched for sex, age at the time of blood sampling, the time point of blood sampling, and area of residence (rural or urban). OR = odds ratio; 95% CI = 95% confidence interval; anti-CCP = anti-cyclic citrullinated peptide antibody; RF = rheumatoid factor.

DISCUSSION

Anti-CCP antibodies were detected in pre-disease blood samples from 34% of individuals who subsequently developed RA and were identified as donors to a blood bank, the NSHDS, and the Maternity cohorts of Northern Sweden. The samples with positive antibodies predated the onset of RA symptoms by 4.5 months and up to 9 years, with the frequency of positive samples increasing the closer to the disease onset time that they had been collected. The diagnostic sensitivity, irrespective of time or when stratified for time antedating disease onset, was highest for anti-CCP antibodies as compared with the 3 RF isotypes, except in early RA, when IgM-RF had slightly higher sensitivity than anti-CCP (73% versus 70%). The prevalences of anti-CCP antibody and IgM and IgA RFs in the patients diagnosed as having early RA showed concordance with the frequencies reported in previous studies by other investigators (13,14).

Combining the presence of anti-CCP antibodies and the presence of any of the RF isotypes in samples antedating symptom onset resulted in high specificity, with increases to 99% and 100%. The odds ratio for anti-CCP antibody as a predictor of RA was high and was independent of time. Aside from anti-CCP antibody, only IgA-RF had a significant odds ratio in multivariate analyses. Of the 3 RF isotypes, IgA had the highest sensitivity and PPV, and it appeared earliest in the pre-patient sera. This is consistent with previous findings suggesting a primary role of IgA-RF in the pathogenesis of RA (15). The present study clearly shows that IgA-RF can be detected several years before any symptoms of RA, although IgA-RF does not reach as high a specificity for RA as anti-CCP antibody does.

In several case-control, retrospective, and prospective cohort studies, smoking has been postulated to contribute to the induction of RA (10). In the present study, we were unable to show a significant effect of smoking for the prediction of RA despite increased odds ratio in univariate conditional logistic regression analysis. However, we did not have detailed information concerning smoking histories (i.e., how long and how much they had smoked or, for former smokers, the time between stopping smoking and the blood sampling).

All 3 RF isotypes could be detected earlier than anti-CCP antibody in samples antedating symptoms of RA, but anti-CCP antibody was more prevalent in the pre-patient cohort when stratified according to the time before symptom onset, with titers increasing to the onset

of RA (Figure 3). This suggests that the underlying pathologic processes generating the production of these antibodies are different. Our results also suggest that the autoimmunologic process in RA precedes the clinical onset of the disease by several years and, in particular, that citrullination and the production of antibodies to citrullinated antigens are active and early processes in the majority of patients. With this study we have established that individuals who later develop RA have an increased capacity to produce autoantibodies against citrullinated proteins. The results of this study do not clarify whether the biochemical process of citrullination in RA is abnormal nor do they substantiate the pathogenic significance of these antibodies in individuals who develop RA.

During the last decade, there has been a growing body of evidence showing the importance of very early treatment of RA in order to reverse morbidity in these patients, as measured by disability and radiographic progression (16–18). Therefore, the question arises whether a screening of certain at-risk groups would be feasible or useful. The PPV based on a combination of anti-CCP antibody and RF isotypes was ~20% in the general population, in which the prevalence of RA has been estimated to be 1%; this is of the same magnitude as mammography screening for breast cancer, which has a PPV of 12–46% (19). However, in an at-risk group, for example, a group of patients attending an early arthritis clinic, this PPV would be much higher. Even for individuals with pain syndromes, in which the occurrence of RA could be estimated to be ~8%, the PPV of anti-CCP antibody for RA, either alone or in combination with any RF isotype, was found to be high (62% or 63–70%, respectively). Our data indicate that such a serologic screening is feasible and would be of considerable help in the clinical management of patients in whom RA is predicted to develop.

The study cohorts were population based, and all individuals in the county of Västerbotten were invited to participate. Since no exclusion criteria were applied, 3–4 individuals among the controls could already have RA or could have been developing the disease, assuming a disease prevalence of 0.8–1.0%. This might explain the frequency of positive individuals (1.8% with anti-CCP antibodies) among the controls. This was further supported by the fact that 2 of the 7 controls who had anti-CCP antibodies were also positive for RF of all isotypes and another control was positive for IgA and IgM RFs.

Another point to consider is that 26 pre-patient samples were obtained from the Maternity cohort, which

is a collection of sera from women who have been screened for rubella during pregnancy. Their controls came from the same cohort. In the pre-patient samples, but not in the control samples, there was a tendency toward a lower prevalence of antibodies in these samples obtained during pregnancy compared with samples collected in the other cohorts. The influence of hormonal changes during pregnancy (estrogen and prolactin) on the immune system is complex and is still not completely understood (20). There is a trend toward clinical improvement in RA patients during pregnancy, but usually there is a worsening of the disease after delivery (21). Glycosylation of IgG, a posttranslational modification, decreases during pregnancy and increases postpartum, both in normal pregnancies and in pregnancies in women with RA (22). However, the influence of pregnancy on antibody production, particularly production of anti-CCP antibody and RFs, remains speculative.

From the findings of this study we can conclude that anti-CCP antibodies and RFs, particularly IgA-RF, predate RA by up to several years and that the presence of anti-CCP antibody has a strong predictive value for later development of RA. This suggests that posttranslational modifications (e.g., citrullination of proteins) are early processes in the progression to disease.

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