EXTENDED REPORT

Autoantibodies can be prognostic markers of an erosive disease in early rheumatoid arthritis

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Objective: To evaluate a contribution of selected laboratory parameters for a prediction of progressive and erosive development in patients with early rheumatoid arthritis (RA).

Methods: In a prospective study baseline levels of antibodies to cyclic citrullinated peptide (anti-CCP), IgM, IgA, and IgG rheumatoid factors (RFs) were measured by enzyme linked immunosorbent assay (ELISA) in 104 patients with RA with disease duration <2 years. Antikeratin antibodies (AKA) and anti-perinuclear factor (APF) were detected by indirect immunofluorescence. Patients were divided into two groups based either on the presence or absence of erosions or according to progression of Larsen score at the end of the 24 months' follow up.

Results: Sixty seven (64%) patients developed radiographic erosions, 49 (47%) had progression in Larsen score, and 36 (35%) progressed by more than 10 Larsen units. Significant differences in erosions and progression between the two groups were detected for anti-CCP, AKA, APF, IgM RF, IgA RF, and IgG RF. Baseline Larsen score correlated significantly with anti-CCP, IgM RF, and IgA RF levels, and all measured antibodies correlated with the progression >10 units. The combination of anti-CCP and IgM RF increased the ability to predict erosive and progressive disease.

Conclusion: The data confirmed that measurement of anti-CCP, AKA, APF, and individual isotypes of RFs was useful for prediction of structural damage early in the disease course. Combined analysis of anti-CCP and IgM RF provides the most accurate prediction.

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The course of rheumatoid arthritis (RA) is heterogeneous, spanning from mild forms tending to remission and reacting well to treatment, to aggressive forms resistant to classical therapeutic measures. Fairly common is an early onset of severe structural damage to joints and extra-articular manifestations of the disease, which may shorten the life of patients.[1] The advance of structural damage at the beginning of the disease to a certain extent predicts further disease development and the progression of radiographic damage.[2] Disease modifying drugs and particularly new biological agents that have come into use have been shown to control both clinical and laboratory signs of the disease and, as observed recently, even arrest development of joint damage.[3] These substances should therefore be given early and in sufficient doses. However, in view of the risks and the high costs of these drugs, there is a clear need to identify effective prognostic indicators of more aggressive disease development.

Contributions of several laboratory parameters have been explored in attempts to find good prognostic markers and diagnostic tools. In addition to rheumatoid factors (RFs), other autoantibodies have been associated with RA, such as antikeratin antibodies (AKA),[4 5] antiperinuclear factor (APF),[6] anti-RA33 antibodies,[7] anti-Sa,[8] or anti-calpastatin antibodies.[9] In some reports the presence of AKA or APF has been associated with more rapid progress of RA[10] or more severe disease,[11] although the association with disease severity has not yet been confirmed.[12] AKA or APF have been detected in sera of individuals before the manifestation of disease symptoms, which may suggest their role in the pathogenesis of the disease.[13 14]

Recent results have shown that antibodies to a cyclic citrullinated peptide (anti-CCP) seem be highly specific for RA, including the early form, and that they may possibly be of prognostic value, either individually or in combination with RF, as a marker of a more serious disease.[15–17] Recently anti-CCP antibodies were incorporated into newly proposed diagnostic criteria for RA, and their positivity showed the strongest association with erosive arthritis.[18]

In this study we evaluated the predictive importance of anti-CCP, AKA, APF, and RF isotypes on the development of radiological progression in a group of patients with early RA.

PATIENTS AND METHODS

Patients

One hundred and four patients with RA of <24 months' duration were assessed in a prospective study. Only patients who fulfilled the diagnostic criteria for RA either at the beginning of the disease or during the follow up period were finally evaluated.[19] Patients were followed up over a period of two years. Hands and feet radiographs were taken at the onset of the study and repeated after 24 months. Patients were split into erosive and non-erosive groups based on the presence of erosions at the end of the study. The finding of at least one definite erosion on any of the hands or feet radiographs was sufficient for inclusion in the erosive group. Radiological progression was estimated by the Larsen score.[20]

Laboratory methods

Serum antibodies directed to cyclic citrullinated peptide (anti-CCP) were assessed with a commercial enzyme linked immunosorbent assay (ELISA; Immunoscan RA, Euro-Diagnostics, Arnhem, The Netherlands).[15–17] Serum samples were diluted 1:50, or more for cases in which the antibody level was very high with optical densities not falling on a standard curve at the original dilution. Values greater than the mean value plus 5SD obtained from 62 middle aged healthy

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Abbreviations: AKA, antikeratin antibodies; APF, antiperinuclear factor; CCP, cyclic citrullinated peptide; ELISA, enzyme linked immunosorbent assay; PBS, phosphate buffered saline; RA, rheumatoid arthritis; RF, rheumatoid factor

Table 1 AKA, APF, anti-CCP antibodies, and RF isotypes determined at the presenting visit of patients with early RA with erosive and non-erosive disease. Results are shown as No (%) unless otherwise stated

	All early RA (n=104)	Erosive RA (n=67)	Non-erosive RA (n=37)	Difference erosive v non-erosive (p value)
AKA+	39 (38)	33 (49)	6 (16)	0.001
APF+	38 (37)	30 (45)	8 (22)	0.021
Anti-CCP+	44 (42)	36 (54)	8 (22)	0.002
IgM RF+	50 (48)	39 (58)	11 (30)	0.008
IgA RF+	47 (45)	36 (54)	11 (30)	0.024
IgG RF+	38 (37)	30 (45)	8 (22)	0.021
Anti-CCP (units), mean (SD)	140.8 (211)	159.1 (224)	85.8 (165)	0.007
IgM RF (index), mean (SD)	2.9 (2.7)	3.3 (2.8)	2.0 (2.2)	0.003
IgA RF (index), mean (SD)	2.6 (2.7)	2.9 (2.6)	1.9 (2.8)	<0.001
IgG RF (index), mean (SD)	2.7 (2.8)	3.0 (2.8)	2.1 (2.8)	0.013
Anti-CCP+ and/or IgM RF+	64 (62)	50 (75)	14 (38)	<0.001

controls of the same ethnic origin (17.0±0.84 units) raised by five standard deviations—that is, 21.4 units, were regarded as positive.

AKA were examined by indirect immunofluorescence using frozen sections of a rat oesophagus.[21] Serum samples were diluted 1:10 in phosphate buffered saline (PBS) and results were read by two independent observers. An intensive laminar fluorescence of the stratum corneum was regarded as positive.

APF was determined on buccal mucosa cells from a "positive" donor by indirect immunofluorescence.[21]

RFs of IgG, IgA, and IgM isotypes were assessed by ELISA,[22] with minor modifications.[23] Briefly, ELISA plates were coated with rabbit IgG (Sigma, St Louis, USA) at a concentration of 30 μg/ml. Samples were diluted 1:75 for IgA RF and 1:150 for IgM RF detection. Sera for the detection of IgG RF were first digested with 0.06% pepsin (Sigma) in acetate buffer (pH 4.4) at 37°C overnight and then used in dilution 1:100 in PBS. IgG RF was detected using porcine antibodies against the Fab fragment of human IgG (Sevac, Praha, Czech Republic) followed by peroxidase conjugated rabbit antibodies against porcine immunoglobulin (Sevac). IgA RF and IgM RF were detected using peroxidase conjugated antibodies against human α or μ chains (Sevac). Results were expressed as an index determined by the ratio of the optical density value from the serum under consideration and the mean value calculated from the optical densities of five normal control sera. These five identical normal sera were used on every plate to ensure interplate reproducibility, which was previously found not to exceed 15%.[23] Index values raised by two standard deviations above the mean index values obtained from 50 healthy middle aged controls were regarded as positive (IgG RF=2.1, IgA RF=2.0, and IgM RF=2.2). A good correlation of levels of individual RF isotypes with latex and haemagglutination assays has previously been shown.[23]

Statistics

An evaluation of contingency tables by Fisher's exact test or the χ^2 test was used for qualitative signs. The non-parametric Mann-Whitney test was used to compare non-paired sets. Correlation between quantitative variables was assessed by Spearman's correlation coefficient. The level of significance was recorded. Statistical analysis was performed using GraphPad Prism 3.0 (GraphPadSoftware, San Diego, California, USA).

RESULTS

Antibody status in erosive and non-erosive disease

Erosions were present in 67 (64%) and absent in 37 (36%) patients. AKA, APF, anti-CCP, IgM RF, IgA RF, and IgG RF were found significantly more often in patients with erosions than in those without (table 1). To obtain a definite meaning for the

assessed values, only levels of anti-CCP antibodies greater than a mean level of controls + 5SD were regarded as positive. This threshold value was never exceeded by anti-CCP antibodies in any of the healthy control sera. In addition, in patients with RA with raised anti-CCP antibodies, the levels were markedly different from those of normal controls. The lowest level of a positive patient with RA was 48.3 units, which was more than twice the mean level recorded for controls. These findings confirmed that the test differentiated excellently between positive and negative values.

The differences in the mean levels of anti-CCP and all rheumatoid factor isotypes were also significant between the two groups (table 1).

Positivity in anti-CCP or IgM RF, or both was detected in 64 (62%) patients, which showed the additional sensitivity obtained by using both tests. Although there was a good correlation between anti-CCP and IgM RF levels (r_s=0.4; p<0.001), the two tests differed in their positivity and negativity (p<0.001) as only 30 of the 64 patients were both anti-CCP+ and IgM RF+, 14 were anti-CCP+/IgM RF−, and 20 were anti-CCP−/IgM RF+. Fifty (75%) of the 67 patients with erosive disease were positive for both or one of these tests, and this was significantly more often than the positivity in patients without erosive changes, which was found in 14/37 (38%) patients (p<0.001). Positivity in anti-CCP and IgM RF had good predictive ability for erosive development (p=0.019; χ^2 9.93). Twenty five of 30 patients (83%) who were positive in both tests belonged to the group with erosive changes, whereas only five (17%) were found among patients with non-erosive disease. Furthermore, another 11/14 patients (79%) who were anti-CCP+/IgM RF− and 14/20 (70%) anti-CCP−/IgM RF+ were found in the erosive group.

Autoantibodies in relation to radiographic progression assessed by Larsen method

Radiographic impairment was assessed by Larsen score at baseline and again after two years. The mean (SD) Larsen score in the whole group at presentation was 13.9 (17.5) and the mean (SD) progression was 9.2 (15.0). There was a weak, but significant, correlation between baseline Larsen score in individual patients and the levels of anti-CCP antibodies (r_s=0.227; p=0.02), IgM RF (r_s=0.23; p=0.019), and IgA RF (r_s=0.28; p=0.004) measured at the onset of the study. Furthermore, progression of the Larsen score correlated well with the initial levels of anti-CCP (r_s=0.45; p<0.001), IgM RF (r_s=0.3; p=0.002), IgA RF (r_s=0.31; p=0.001), and IgG RF (r_s=0.34; p<0.001).

An analysis of the results, carried out separately for the two groups according to whether any radiological progression in Larsen score occurred (radiographic progressors) or not (radiographic non-progressors) during the two years of follow

Table 2 Serum levels and status of anti-CCP, IgM RF, IgA RF, IgG RF, APF, and AKA in patients who showed any radiographic progression in Larsen score (radiographic progressors) and in those who did not have any increase in Larsen score after two years' follow up (radiographic non-progressors). Results are shown as No (%) unless otherwise stated

	Radiographic progressors (n=49)	Radiographic non-progressors (n=55)	Significance (p value)	OR (95% CI)
Anti-CCP+	32 (65)	12 (22)	<0.001	6.7 (2.8 to 16.1)
IgM RF+	29 (59)	21 (38)	0.05	2.4 (1.1 to 5.7)
IgA RF+	30 (61)	17 (30)	0.003	3.5 (1.6 to 7.9)
IgG RF+	27 (55)	11 (20)	<0.001	4.9 (2.1 to 11.7)
APF+	25 (51)	13 (24)	0.005	3.4 (1.5 to 7.8)
AKA+	29 (59)	10 (18)	<0.001	6.5 (2.7 to 15.9)
Anti-CCP+ and IgM RF+	23 (47)	7 (13)	0.002	4.8 (1.8 to 12.8)
Anti-CCP+ and/or IgM RF+	39 (80)	25 (45)	<0.001	4.7 (2.0 to 11.2)
Anti-CCP (units), mean (SD)	217.2 (248)	72.7 (141)	<0.001	–
IgM RF (index), mean (SD)	3.7 (3.1)	2.2 (2.1)	0.002	–
IgA RF (index), mean (SD)	3.2 (2.7)	2.0 (2.5)	<0.001	–
IgG RF (index), mean (SD)	3.5 (3.0)	1.9 (2.5)	<0.001	–

Table 3 Anti-CCP, IgM RF, IgA RF, IgG RF, APF and AKA levels and status measured at the beginning of the study in patients designated as fast progressors (Larsen score progression >10) and slow progressors (Larsen score <10) after two years of follow up. Results are shown as No (%) unless otherwise stated

	Fast progressors (n=36)	Slow progressors (n=68)	Significance	OR (95% CI)
Anti-CCP+	24 (67)	20 (29)	<0.001	4.8 (2.0 to 11.4)
IgM RF+	23 (64)	27 (40)	0.024	2.7 (1.2 to 6.2)
IgA RF+	19 (53)	20 (29)	0.032	2.7 (1.2 to 6.2)
IgG RF+	19 (53)	19 (28)	0.018	2.9 (1.2 to 6.7)
APF+	18 (5)	20 (29)	0.054	2.4 (1.0 to 5.5)
AKA+	23 (64)	16 (24)	<0.001	5.2 (2.2 to 12.5)
Anti-CCP+ and IgM RF+	18 (50)	12 (18)	0.001	4.7 (1.9 to 11.5)
Anti-CCP+ and/or IgM RF+	29 (81)	35 (51)	0.006	3.9 (1.5 to 10.1)
Anti-CCP (units), mean (SD)	240.0 (258)	88.3 (159)	<0.001	–
IgM RF (index), mean (SD)	3.3 (2.6)	2.6 (2.7)	0.035	–
IgA RF (index), mean (SD)	2.8 (2.4)	2.4 (2.8)	0.032	–
IgG RF (index), mean (SD)	3.2 (2.9)	2.4 (2.8)	0.034	–

up, showed significant differences in anti-CCP, IgM RF, IgA RF, IgG RF levels and in anti-CCP, IgM RF, IgA RF, IgG RF, AKA, and APF status (table 2). Similarly, increased levels of anti-CCP and RF isotypes or an increased frequency of anti-CCP, RF isotypes, AKA, and APF positivity were found in a group with significant progression defined as an increase in the Larsen score of 10 or more (fast progressors) (table 3).

Positivity in anti-CCP and IgM RF is well associated with progression in the Larsen score (p<0.001; χ^2 24.0). Twenty three of 30 patients (77%) who were positive for both tests belonged to the group with progression in Larsen score, whereas only 7/30 (23%) were found among patients with non-erosive disease. Furthermore, another 10/14 patients (71%) who were anti-CCP+/IgM RF– were found in the erosive group.

Fast progression was also associated well with anti-CCP and IgM RF status (p=0.02; χ^2 15.0). Eighteen of 30 patients (60%) who were positive for both tests were found in the group with fast progression.

There were significantly more patients with anti-CCP+ and/or IgM RF+ in the group with any or fast radiological progression (tables 2 and 3).

DISCUSSION
Reliable predictive parameters of the disease course in RA are needed, particularly to help in therapeutic decisions at early

disease stages. Such prognostic factors for a development of RA have been reviewed recently.[24] It has generally been accepted that high serum levels of RFs or a combination of indicators, such as IgM RF, number of swollen joints, and number of erosions, have a predictive ability. A disadvantage of several of these studies is that they used parameters more typical of a relatively advanced stage of the disease and therefore of limited use for the disease in its very early stages.

Laboratory investigations performed at the presentation of patients enrolled into this study showed differences between early destructive and non-destructive disease. Anti-CCP, AKA, APF, and RF isotypes were all different between the two groups. Measurement of anti-CCP antibodies was slightly more sensitive than detection of AKA and APF, which are assumed to recognise the same antibodies. The simplicity of the ELISA for the anti-CCP antibodies offers another advantage. Of particular interest was the wide difference between anti-CCP levels in the control group and the raised levels in patients with RA, providing a clear distinction for positive samples.

Similar differences were found when radiographic impairment was assessed by a more precise method, and correlation of laboratory parameters with the Larsen score was found at the beginning of the study and also with the further radiological advancement. This documents an important predictive role for early detection of antibodies, which may alert

the doctor to take a more aggressive approach to treatment. Although all the measured parameters are useful in the differentiation of progressive disease, the highest odds ratios are found for anti-CCP or AKA antibodies. Given that an excellent specificity for anti-CCP and RA was found in several studies, it seems that it would be advisable to measure anti-CCP in all patients at their presentation with arthritis.

The possibility of predicting development of RA by detecting anti-CCP antibodies has recently been described.[15–17] Anti-CCP antibodies became an important part of a newly proposed model for diagnostic criteria of early arthritis and strongly predicted persistent and erosive disease.[18] The association of anti-CCP antibodies with disease severity measures was not, however, seen in one study of 106 patients with early RA, where anti-Sa positive patients had a higher incidence of erosions.[25] The results of our study, however, confirmed earlier reports of an association with anti-CCP. However, not all the patients who developed early erosive disease were positive for autoantibodies—the combined sensitivity for destructive disease of anti-CCP and/or IgM RF was only around 75%. On the other hand, the presence of both anti-CCP antibodies and IgM RF is the best predictor of erosive or progressive disease. Eighty three per cent of patients who were positive for both antibodies showed early erosions, 76% showed progressive disease, and 60% progressed by more than 10 in the Larsen score. Therefore, when both tests are positive, they have a very good predictive ability for early destructive RA.

In conclusion, early development of erosive disease in RA is associated with the presence of several autoantibodies. The most sensitive are RFs and anti-CCP antibodies, and their combined occurrence, particularly, is highly predictive for early erosions and more progressive disease.

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