

## CONCISE REPORTS

## Autoantibodies predicting the outcome of rheumatoid arthritis: evaluation in two subsets of patients according to severity of radiographic damage

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### Abstract

**Objective**—Autoantibodies such as rheumatoid factor (RF), antikeratin antibodies (AKA), antiperinuclear factor (APF), and anti-RA 33 antibodies are considered of value for the diagnosis of RA. The purpose of this study was to evaluate these autoantibodies as predictors of severe radiographic damage in rheumatoid arthritis (RA).

**Patients and methods**—Eighty six patients with RA (70 women, 16 men) fulfilling 1987 ACR criteria were selected from a cohort of 469 patients followed up since the first year of RA onset because they could be divided in two groups according to the severity of the radiographic damage. These 86 patients had a mean (SD) disease duration of eight (four) years: 43 patients had severe radiographic damage (Larsen score  $\geq 2$ ) and 43 had limited radiographic damage (Larsen score  $< 2$ ). The two groups were matched by disease duration and sex. The following autoantibodies were looked for: RF, ANA, AKA, APF, and anti-RA 33 antibodies. In addition, HLA class II DR  $\beta$  alleles and standard inflammatory parameters (erythrocyte sedimentation rate, C reactive protein) were determined.

**Results**—Patients with severe radiographic damage differed from those with limited radiographic damage in that they had higher RF ( $p=0.01$ ), APF ( $p<0.02$ ), and AKA ( $p=0.001$ ) titres. Stepwise regression analysis was done to calculate the odds ratios (OR) for each clinical and laboratory variable; only presence of cutaneous nodules (OR: 14.9; 95% CI: 7, 128), HLA DRB1\*04 or DRB1\*01 (OR: 7.53; 95% CI: 1.32, 42.9), AKA (OR: 3.11; 95% CI: 0.58, 16.8), a high erythrocyte sedimentation rate (OR: 2.66; 95% CI: 0.60, 11.9), and a high C reactive protein value (OR: 7.4; 95% CI: 1.43, 38.1) were predictive of severe radiographic damage.

**Conclusion**—These data suggest that the risk of severe radiographic damage in RA patients is higher when cutaneous nod-

ules, HLA DRB1\*04 or DRB1\*01, and/or AKA are present. The other autoantibodies of diagnostic significance are of little help for predicting joint destruction.

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Tests for circulating autoantibodies are used throughout the world for the diagnosis of rheumatoid arthritis (RA).<sup>1</sup> Autoantibodies considered to be early sensitive markers for RA include rheumatoid factors (RF), antibodies to the stratum corneum of rat oesophagus (called antikeratin antibodies (AKA)), and antiperinuclear factor (APF), which reacts with an antigen in the keratohyaline granules located in the cytoplasm of human buccal mucosa cells.<sup>2</sup> There is strong evidence that both AKA and APF react with epitopes of a filamentous protein called profilaggrin.<sup>3</sup> More recently, anti-RA 33 antibodies reacting with nuclear hnRNA-associated protein A2 were found in 30% of Austrian<sup>4</sup> or French RA patients,<sup>5</sup> irrespective of the presence of rheumatoid factors.

RF, AKA, APF, and anti-RA 33 antibodies are often detectable several years before the onset of clinical RA and are therefore considered helpful for the early diagnosis of RA.<sup>1,2</sup>

As RF may be predictive of the outcome of RA,<sup>6-8</sup> we investigated the value of the other RA associated autoantibodies for predicting articular damage in two subsets of patients differentiated on the basis of the severity of radiographic findings. Reference prognostic markers included clinical (extra-articular manifestations), biological (erythrocyte sedimentation rate (ESR) and C reactive protein (CRP) level) and immunogenetic (HLA DR4 and DR1 genotypes) parameters.<sup>3</sup>

### Patients and methods

All 558 adult patients who met ACR criteria for RA and had been regularly followed up at our institution since the first year of their disease (incipient RA) were included. Patients with RA of less than five years' duration were excluded. Follow up radiographs and an immunogenetic study were obtained in most of

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**Table 1** Sensitivity, specificity, and positive predictive value for severe destructive RA of five autoantibodies, inflammatory parameters (ESR, CRP), and high risk HLA alleles in two groups of RA patients

Variable	Sensitivity	Specificity	Positive predictive value	OR	95% CI
RF	0.69	0.51	0.58	2.41	0.99, 5.85
APF	0.46	0.74	0.64	2.52	1.01, 6.28
AKA	0.60	0.72	0.68	3.95	1.59, 9.75
RA 33	0.34	0.72	0.55	1.38	0.55, 3.45
ANA	0.20	0.72	0.42	0.68	0.25, 1.84
HLA DRB1*04 or DRB1*01	0.90	0.41	0.60	6.84	2.07, 22.60
ESR	1.0	0.27	0.55	—	—
CRP	0.91	0.44	0.56	8.53	1.68, 43.3

these RA patients (469 of 558 (84%)). Radiographs of the hands, wrists, feet, and joints with clinical symptoms were obtained in all patients after a mean disease duration of eight years. Two independent observers who were blinded to the status of the patients scored each radiograph using Larsen's method. The interobserver correlation coefficient determined after the observers received specific training in Larsen score determination was 0.94. Among the RA patients, two groups of white patients were differentiated based on severity of radiographic joint lesions, taking care to match the groups on sex distribution and disease duration (mean (SD) 8.95 (4.77) years in group I, 9.66 (7.20) years in group II). Group I patients (n = 43; 33 women and 10 men) had severe radiographic damage with a Larsen score  $\geq 2$  for each of both wrists, both hands, both feet, and at least one other joint. Group II patients (n = 43; 37 women and six men) had a Larsen score  $< 2$  for each joint studied. At symptom onset, mean age of the 86 patients in groups I and II combined was 43.29 years (range 18–69). Mean disease duration before the first visit to our clinic was 7.8 months (range 2–12).

In addition to a physical evaluation and to the usual laboratory tests, including ESR and CRP, the following immunological markers were looked for: RF by laser nephelometry (positive if  $\geq 40$  IU/ml), IgG AKA by indirect immunofluorescence on cryostat sections of rat oesophagus (positive if  $\geq 1:20$ ), IgGAM APF by indirect immunofluorescence on human buccal epithelial cells (positive if  $\geq 1:40$ ), and IgG anti-RA 33 antibodies by western blotting with HeLa cell nuclear extracts (serum dilution

**Table 2** Stepwise logistic regression to determine which variables best predicted severe articular damage in RA (adjusted for age, sex, and disease duration)

	OR	95% CI for OR
Cutaneous nodules	14.98	1.74, 128
RF	0.86	0.176, 4.19
ANA	0.36	0.07, 1.88
APF	0.66	0.14, 3.04
AKA	3.11	0.58, 16.8
RA-33ab	0.76	0.16, 3.70
HLA DRB1*04/DRB1*01	7.63	1.32, 42.9
ESR	2.66	0.60, 11.9
CRP	7.4	1.43, 38.1

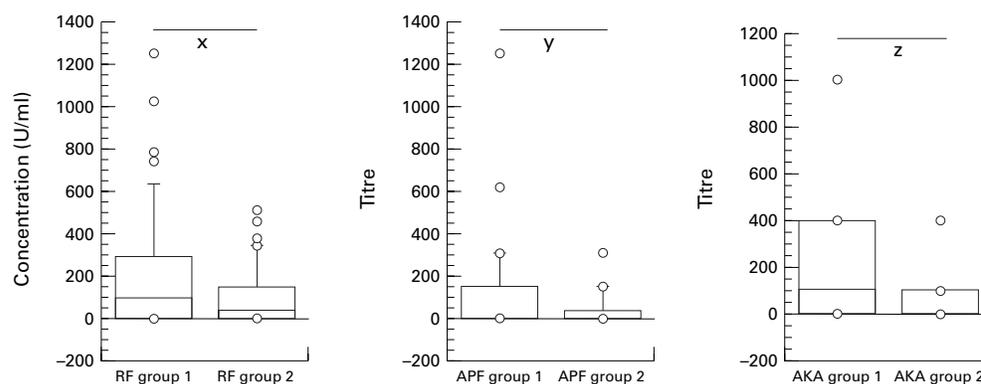
1:20) as described elsewhere.<sup>5</sup> Antinuclear antibody determination was performed routinely using HEP-2 cells as the substrate.

A frozen serum sample collected during the first year of the disease was used to determine the serological markers for RA. Non-routine tests (AKA, APF, anti-RA33ab) were performed in duplicate. HLA-DR genetic oligotyping was performed using a non-radioactive reverse dot blot technique as described elsewhere.<sup>8</sup>

Statistical analysis was performed using BMDP statistical software. Fisher's exact test or the  $\chi^2$  test and the non-parametric Kruskal-Wallis test were used for between group comparisons of qualitative and quantitative data, respectively. A stepwise multiple logistic regression model was constructed to look for relevant independent prognostic variables. The prognostic variables included in the multivariate model were selected based on the results of a univariate analysis. The significance level was 0.05.

## Results

Rates of occurrence (sensitivity) of RF, APF, AKA, anti-RA33 antibodies, and ANA in RA patients with (group I) and without (group II) severe articular damage are indicated in table 1, as well as the specificity and the positive predictive value of these five autoantibodies for severe radiographic damage. For purposes of comparison, the sensitivity, specificity, and positive predictive value of a high ESR ( $> 28$  mm 1st h) or a high CRP level ( $> 15$  mg/l) are also indicated. Of the five autoantibodies studied, AKA had the highest positive predictive



**Figure 1** Values of RF, APF, and AKA in patients with destructive (group 1) and non-destructive (group 2) RA. The horizontal line within boxes represents the median, boxes show the interquartile range ( $\pm 25\%$  of the median), vertical bars show the 95% confidence intervals (values above or below these levels were plotted separately). x:  $p < 0.01$ ; y:  $p < 0.02$ ; z:  $p < 0.001$ .

value (0.68) with the highest OR (3.95; 95% CI, 1.59, 9.76) for severe destructive RA.

Mean serum autoantibody titres were higher in group I than in group II, and the differences were significant for RF ( $p < 0.01$ ) APF ( $p < 0.02$ ), and AKA ( $p < 0.001$ ) (fig 1). Mean (SD) initial ESR and CRP levels were significantly higher in group I than in group II: 48 (31) versus 21 (17) ( $p < 0.0001$ ) for ESR and 51 (45) versus 12 (13) ( $p < 0.0001$ ) for CRP.

To determine which autoantibodies had the best predictive value for severe radiographic damage, all variables were entered into a dichotomous stepwise logistic regression model. Table 2 shows the results of this analysis.

Subcutaneous nodules, HLA DRB1\*04 or DRB1\*01 susceptibility alleles, AKA, and CRP were the four best variables for predicting severe articular damage.

A subsequent analysis excluding clinical parameters from the stepwise logistic regression model showed that AKA was the autoantibody with the best predictive value for severe articular damage (OR, 3.23; 95%CI of OR, 1.16, 8.96).

### Discussion

Recent research has concentrated on identifying clinical and laboratory abnormalities present in early RA, with the goal of predicting the long term outcome of the disease.<sup>6-8</sup> As radiological articular damage can occur within two years of the first manifestations of RA,<sup>6</sup> there is an urgent need for identifying independent predictive factors present at disease onset. Such factors would be of use for evaluating the risk-benefit ratio of potentially toxic second line drugs.

In addition to clinical, epidemiological, socioeconomic, and health assessment characteristics,<sup>10</sup> several studies have found a strong correlation between a persistently high ESR or CRP level and less favourable disease outcomes.<sup>7 8 11</sup>

DR4 and DR1 alleles, which share an epitope on the third hypervariable region of the  $\beta$  chain, have also been found to be associated with a poor prognosis.<sup>8 12 13</sup>

Among autoantibodies, IgM (or IgA) RF has been the most extensively studied. Presence of IgM RF was strongly predictive of an unfavourable outcome.<sup>11</sup> Very few published studies have evaluated the predictive value of the other autoantibodies, and most looked at only one antibody.

Westgeest *et al*<sup>4</sup> reported that APF indicated more severe disease in seronegative RA and also identified the most severe cases among RF positive patients. In a study by Paimela *et al*,<sup>15</sup> RA patients who were initially positive for AKA had more active disease as assessed based on clinical, laboratory tests, and radiological variables, as compared with AKA negative patients. However, none of these studies used multiple regression analysis to determine which autoantibodies were the best independent predictive variables, and which combinations of autoantibodies predicted the outcome in early RA. In an attempt to obtain more sharply contrasted findings regarding the prog-

nostic value of autoantibodies, we compared two groups of RA patients with and without severe radiographic damage. Although our study was not prospective, all patients had serum drawn within one year of RA onset and were followed up at our institution starting during the first year of their disease. All had initial radiographs with no articular damage, and in all a second series of radiographs was obtained after at least five years' follow up. In our model, presence of AKA (60.5% of group I patients with severe radiographic damage versus 27.9 % of group II patients with a benign articular outcome) was associated with a relative risk of 3.1. These data are very similar to those obtained with inflammatory markers such as ESR or CRP, which have been established as important prognostic indices for severe articular damage in retrospective and prospective studies.<sup>7 8 11</sup> Relative risks were 0.86 for RF, 0.66 for APF, 0.36 for ANA, and 0.76 for anti-RA 33 antibodies. Apparent discrepancies between our data and those from earlier studies may be ascribable to the fact that only patients with 'definite' RA after a mean follow up of eight years were included in our study; as a result, as many as 50% of patients in the favourable outcome group (group II) were positive for RF, contrasting with smaller proportions in most prospective studies in populations with short-term follow up periods.

The rate of occurrence of APF may seem low in our study compared with many others.<sup>1 16 17</sup> However, we used a cut off titre of 1:40 because in an earlier study<sup>5</sup> we found that a cut off of 1:10 had relatively poor specificity for RA. In several countries, the frequency of AKA<sup>18</sup> or anti-RA 33 antibodies<sup>1</sup> is considerably lower than in most of Europe.<sup>4 5</sup>

As the target of APF and AKA seems to be immunologically related to the same acidic form of profilaggrin,<sup>3</sup> determination of one or the other of these two antibodies is sufficient for diagnostic or prognostic purposes in early RA. These autoantibodies have been found in early RA,<sup>1 2</sup> and even before the onset of clinical symptoms.<sup>1</sup> Consequently, we recommend adding AKA to the list of clinical, physical function, laboratory (ESR, CRP), and immunogenetic parameters (HLA DRB1\*04/DRB1\*01 genotype) currently used for early prediction of the outcome of RA.

Even when they are statistically significant, however, differences between groups of patients are not necessarily helpful for making therapeutic decisions in individual patients. In the near future, development of a composite index including three or more independent variables may improve the ability of clinicians to predict the outcome of early RA.

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