

## CONCISE REPORT

# An eight year prospective study of outcome prediction by antiperinuclear factor and antikeratin antibodies at onset of rheumatoid arthritis

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**Objectives:** Antikeratin antibodies (AKA) and antiperinuclear factor (APF) are specific antibodies found very early in rheumatoid arthritis (RA). The objective of this eight year follow up study was to assess the value of early AKA and APF assays in predicting functional disability and cartilage damage.

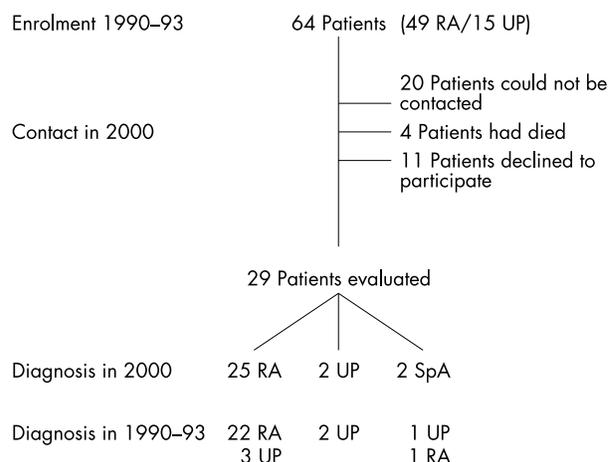
**Method:** In 2000, 64 patients tested for AKA and APF (antifilaggrin antibodies) between 1990 and 1993 during evaluation of early symmetric oligoarthritis or polyarthritis (suspected RA) were invited to participate in this non-concurrent cohort study. The Health Assessment Questionnaire (HAQ) score, disease activity score (DAS), and Larsen radiographic score were the primary evaluation criteria.

**Results:** Twenty nine patients were re-evaluated. Clinical and laboratory data obtained in 1993 were similar in this group and in the 35 other patients. Twenty five patients had received a diagnosis of RA. Nine (31%) were rheumatoid factor (RF) positive, nine (31%) were AKA positive, and six (21%) were APF positive during the first year of the disease. APF was correlated with the Larsen score ( $p=0.011$ ) and DAS ( $p=0.035$ ) evaluated after a mean disease duration of 8.55 years. All APF positive patients had erosive disease. AKA was correlated with the DAS ( $p=0.035$ ).

**Conclusion:** The presence of AKA or APF early in the course of RA was associated with the DAS or Larsen score eight years later. The number of patients was small, but the findings confirmed those of studies with shorter follow ups. Antifilaggrin antibodies should be included in the initial investigation of patients with RA and, when positive, should alert to a high risk of poor outcomes.

Several autoantibodies have been identified in rheumatoid arthritis (RA). The best known is rheumatoid factor (RF), which is the only autoantibody listed among diagnostic criteria. The value of RF for predicting severity of RA is controversial. Antiperinuclear factor (APF) is directed against a protein closely related to epidermal profilaggrin,<sup>1</sup> and antikeratin antibody (AKA) targets filaggrin epitopes.<sup>2</sup> APF and AKA show some overlap and belong to the group of antifilaggrin antibodies recognising citrullinated proteins.<sup>3</sup> They are both considered specific for RA but their predictive value over the course of the disease remains debatable.

This study was designed to determine whether serum APF/AKA in patients with early arthritis predicted severity of disease eight years later. Three aspects of disease severity were evaluated: disease activity, extent of joint destruction, and physical disability.



**Figure 1** Distribution and diagnosis of the 69 patients recruited between 1990 and 1993 as evaluated at the end of the recruitment period and at follow up in 2000. RA, rheumatoid arthritis; UP, undifferentiated polyarthritis; SpA, spondyloarthritis.

## PATIENTS AND METHODS

### Patients

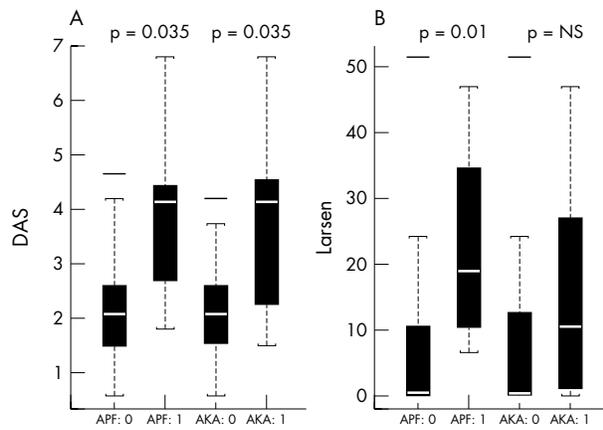
The study extended data from a cohort study on early polyarthritis conducted in the early 1990s. In 1993, two years after enrolment of 64 consecutive patients with early polyarthritis, 49 (77%) patients met the American College of Rheumatology (ACR) criteria for RA and 15 (23%) were considered to have undifferentiated polyarthritis. In 2000, extensive efforts were made to re-evaluate all 64 patients. Twenty nine patients agreed to undergo a full clinical, laboratory, and radiological evaluation (see fig 1 for reasons for drop out).

### Methods

The clinical diagnosis of RA was reassessed. Extra-articular features, criteria for remission according to the American Rheumatism Association (ARA)/ACR,<sup>4</sup> and the number of disease modifying drugs used were recorded. The disease activity score (DAS)<sup>5</sup> was calculated. Patients with a DAS score lower than 1.6 were classified as "in remission according to DAS".<sup>6</sup> The HLA generic type and the DRB1\*01 and DRB1\*04 subtypes were determined. Each patient completed a generic quality of life questionnaire (HAQ).

Radiographs of both hands and feet were read by two of us (SG and GH) and scored according to a modified version of the

**Abbreviations:** ACR, American College of Rheumatology; APF, antiperinuclear factor; AKA, antikeratin antibodies; DAS, disease activity score; HAQ, Health Assessment Questionnaire; RF, rheumatoid factor; SSOs, sequence specific oligonucleotides



**Figure 2** (A) Distribution of DAS values in APF negative (APF 0), APF positive (APF 1), AKA negative (AKA 0), and AKA positive (AKA 1) patients. Box plots represent the lower quartile, the median, and the upper quartile. Extremities of lines indicate the 10th and 90th centiles. The points are the extreme values, below the 10th centile or above the 90th centile. The p values are given for each statistical analysis. (B) Same representation as for Larsen score. NS, non-significant.

Larsen score,<sup>7</sup> the values of which can range from 0 to 160. Before the readings, a cut off was set at 5 to account for minor irregularities: patients whose score was lower than 5 were classified as having “non-erosive disease” and patients with a score of 5 or more as having “erosive disease”.

Primary outcome measures were the DAS, HAQ score, and Larsen score.

#### Laboratory test methods

Blood specimens for determination of APF, AKA, and RF were obtained at study entry (between 1990 and 1993). AKA IgGs were sought using indirect immunofluorescence as described by Young *et al*, with a few modifications<sup>8</sup>; serum samples were diluted 1/20. APF IgG were detected using indirect immunofluorescence on human buccal cells; serum samples were diluted to 1/40 to improve specificity and considered positive if more than 10% of cells were fluorescent. RF was assayed using the quantitative tube latex test (Pasteur production, positivity >40 IU/l) and the modified microplate Waaler-Rose test (Fumouze, positivity >40 IU/l).

Genomic DNA was extracted from peripheral blood cells using either exposure to proteinase K and phenol/chloroform extraction or a salting out procedure.<sup>9</sup> Generic HLA-DR oligotyping was performed using a non-radioactive reverse dot-blot technique, as described elsewhere. A set of 15 sequence specific oligonucleotides (SSOs) was used to determine generic DRB1 oligotyping<sup>9</sup>.

#### Statistical methods

Statistical analyses were conducted with SAS software (release 8.0; SAS Institute Inc, Nashville, TN, USA). Between observer agreement for Larsen score determination was evaluated using the within class correlation coefficient. Means were compared between groups by the non-parametric Mann-Whitney-Wilcoxon test and frequencies by Fisher's exact test. Associations between remission variables and presence of antibodies (APF, AKA, FR) were evaluated using univariable logistic regression.

#### RESULTS

The group of 29 patients evaluated in 2000 was not significantly different from the 35 other patients for sex, age at onset of disease, symptom duration, and serology (RF, APF, AKA); thus, our patient subgroup was representative of the entire cohort. Among the 29 patients, 25 (18 women, seven

**Table 1** Relationship between the three antibodies tested—namely, antiperinuclear factor (APF), stratum corneum (antikeratin) antibody (AKA), and rheumatoid factor (RF), and the three outcome criteria studied—namely, the disease activity score (DAS), the Larsen radiographic score, and the Health Assessment Questionnaire (HAQ) score

|     |          | DAS  |         | HAQ  |         | Larsen |         |
|-----|----------|------|---------|------|---------|--------|---------|
|     |          | Mean | p Value | Mean | p Value | Mean   | p Value |
| APF | Negative | 2.26 |         | 0.75 |         | 6.71   |         |
|     | Positive | 3.98 | 0.035   | 0.94 | NS      | 22.50  | 0.011   |
| AKA | Negative | 2.16 |         | 0.78 |         | 7.84   |         |
|     | Positive | 3.75 | 0.035   | 0.82 | NS      | 15.22  | NS      |
| RF  | Negative | 2.37 |         | 0.80 |         | 8.19   |         |
|     | Positive | 3.33 | NS      | 0.78 | NS      | 14.61  | NS      |

men) (86%) had a definite diagnosis of RA in 2000 and were entered in the analyses reported below. Their mean age at onset of RA was 50.6 (SD 15.5) years (range, 18.9–72) and mean follow up was 8.5 (SD 0.64) years (range, 7.2–9.9).

All APF positive patients were AKA positive, and four of the six APF and AKA positive patients were RF positive. Seven of the nine AKA positive patients were also positive for RF. The shared epitope was assessed in 24 patients, of whom six were negative and 18 had one or two alleles. Of the 24 patients whose DAS was available, five had less than 1.6 units, indicating disease remission. Six patients fulfilled ARA criteria for remission. The DAS ranged from 0.55 to 6.8 (mean 2.69 (SD 1.46)) and the HAQ score ranged from 0.0 to 2.625 (mean 0.79 (SD, 0.8)).

Because between observer agreement for determination of the Larsen score was high ( $r=0.956$ ,  $p<0.001$ ), we used the mean of the two determinations. The scores ranged from 0 to 51 (mean, 10.5 (SD 14.95)). Twelve patients had a Larsen score greater than 5, defining erosive disease; of the 13 other patients, four had scores between 1 and 4 and nine had a score of 0.

Patients who were APF positive early on had more severe disease eight years later, as shown by the higher DAS score (3.98 (95% confidence interval (95%CI) 2.18 to 5.78) v 2.26 (95%CI 1.71 to 2.810;  $p=0.009$ , fig 2) and Larsen score (22.5 (95%CI 5.86 to 39.14) v 6.71 (95%CI 0.52 to 12.90);  $p=0.02$ ). Patients who were AKA positive at study entry had a higher DAS (3.75 (95%CI 2.30 to 5.20) v 2.16 (95%CI 1.63 to 2.68);  $p=0.009$ ) and a non-significantly higher Larsen score (15.22 v 7.84;  $p=0.24$ ) (table 1). All APF positive patients were in the erosive disease group; half the patients with erosive disease were APF positive. A strong correlation was found between APF and “erosive disease” ( $p=0.005$ ). Conversely, AKA was not correlated with “erosive disease”. The positive predictive value of APF for “erosive disease” was 100% and the negative predictive value was 68.4%.

Although HAQ scores were higher in patients who were positive for either APF or AKA early in the disease, the difference was significant.

None of the three scores (DAS, Larsen, HAQ) were statistically correlated with presence of RF early in the disease, although a trend was seen for DAS ( $p=0.08$ ). Female sex predicted absence of remission ( $p=0.027$  by univariable analysis), with women having an odds ratio of 10.67 (1.31–86.93) compared with men. Nevertheless, sex did not affect the associations between serological markers and primary evaluation criteria (data not shown).

#### DISCUSSION

This eight year prospective cohort study found a correlation linking baseline APF positive patients with severity of joint

destruction and clinical RA activity. Moreover, all APF positive patients were classified in the erosive disease group. Patients who were AKA positive at baseline had more active disease, confirming the close association between APF and AKA antibodies.

Few longitudinal studies on associations linking APF or AKA to RA severity have been published. Some short term studies found that AKA was correlated with clinical indices but not with Larsen score,<sup>10,11</sup> whereas others found the opposite.<sup>12</sup> In a long term study of APF and AKA, Kurki *et al* found no association linking these antibodies to severity of erosions.<sup>13</sup> Nevertheless, all AKA positive and 98% of APF positive patients presenting with polyarthritis had erosive disease after eight years. A more recently published three year study of APF<sup>14</sup> found results very similar to ours. Interestingly, the same correlation was found for anticitrullinated cyclic peptide (anti-CCP) antibodies, which have been shown to recognise both perinuclear factor and (pro)-filaggrin.<sup>3,15</sup>

Surprisingly, RF was not associated with any of the study indices. Possible explanations include the very early time of serological marker determination and the limited statistical power of the study related to the few patients. However, this finding suggests that, in patients with very early RA, determination of APF/AKA may supply additional information on the risk of unfavourable outcomes.

In conclusion, although limited by the few patients, this study is the first to investigate associations between APF/AKA in patients with early RA and several aspects of RA severity evaluated eight years later. The associations with disease activity and with the Larsen score accord with most published longitudinal studies. Our data indicate that these autoantibodies provide additional information to clinicians and, consequently, should be added to the list of initial tests in patients with early RA.

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