

# Antikeratin antibodies: diagnostic and prognostic markers for early rheumatoid arthritis

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## Abstract

**Antibodies to the stratum corneum of rat oesophagus (antikeratin antibodies) were assayed by indirect immunofluorescence in a prospective study of patients with early rheumatoid arthritis (RA). At the beginning of the study, antikeratin antibodies of IgG class were detected in serum samples from 27/71 (38%) patients compared with 1/20 (5%) control patients with reactive arthritis, and 1/38 (3%) healthy blood donors. At the end of the two year follow up, 27/67 (40%) patients with RA were positive for antikeratin antibodies. The patients with RA who were initially positive for antikeratin antibodies had a more active disease course than the patients negative for antikeratin antibodies as measured by clinical, laboratory, and radiological variables. The prevalence of positivity for antikeratin antibodies fluctuated during the follow up, the variation paralleling the disease activity. The occurrence of HLA-DR4 was similar in patients with RA who were positive and negative for antikeratin antibodies. Antikeratin antibodies were also found in seronegative patients with RA, confirming that antikeratin antibodies do not have rheumatoid factor activity. These results show that antikeratin antibodies are detectable at the time of the initial diagnosis of RA and that the positivity for antikeratin antibodies may have prognostic significance in early RA.**

Circulating autoantibodies are found in most patients with rheumatoid arthritis (RA). Only rheumatoid factors are generally used in the diagnosis of RA, however. In the early phase of RA, rheumatoid factor is also an important prognostic marker.<sup>1,2</sup> A number of patients with RA nevertheless remain negative for rheumatoid factor and other diagnostic and prognostic markers would be of clinical importance. During the last ten years several studies have suggested that antibodies to the stratum corneum of rat oesophagus (antikeratin antibodies) fulfil many requirements for an additional serological marker of RA.

The increased occurrence of antikeratin antibodies in patients with RA was first described by Young *et al* in 1979.<sup>3</sup> The presence of positivity for antikeratin antibodies in RA has varied in previous studies, ranging from 36 to 69%.<sup>4-14</sup> Antikeratin antibodies have occasionally been found in other rheumatic diseases such as systemic lupus erythematosus,<sup>4,9,12</sup> systemic sclerosis,<sup>8,9,12</sup> and ankylosing spondylitis.<sup>8,12</sup>

Several workers have reported an association between disease activity assessed by various clinical and laboratory parameters and positivity for antikeratin antibodies in RA.<sup>9,10,13,14</sup> It has also been suggested that antikeratin antibodies are present in a fraction of seronegative patients with RA.<sup>13,14</sup> The exact clinical significance of antikeratin antibodies in RA, however, has remained controversial.

This prospective study of early RA was undertaken to find out whether antikeratin antibodies are present in early disease and whether an early appearance of antikeratin antibodies can predict disease progression.

## Patients and methods

### PATIENTS AND CONTROLS

Seventy one consecutive patients (57 women, 14 men) with newly diagnosed definite or classical RA according to the 'old' criteria of the American Rheumatism Association<sup>15,16</sup> were entered into a prospective study. The mean age at entry was 44.4 years (range 19-65). All patients had early RA with a disease duration of less than one year (mean 7.8 months, range 3-12). At the time of diagnosis, 48 patients (68%) were seropositive by the Rose-Waaler test (titre  $\geq 1/64$ ), 41 patients (58%) were HLA-DR4 positive, and joint erosions were present in 23 (32%) of patients. None of the patients had previously received any second line disease modifying drugs. After the diagnosis was made, treatment with second line drugs (either parenteral gold, sulphasalazine, or an antimalarial drug) was initiated. Two patients were treated with low dose corticosteroids (prednisone 5-7.5 mg daily) and gold. Most patients were also receiving non-steroidal anti-inflammatory drugs.

The clinical rheumatoid activity was determined by LP every three months during the first year and every four months during the second year of the follow up. Clinical activity was determined by the Ritchie articular index score,<sup>17</sup> duration of morning stiffness, grip strength, and a visual analogue scale for pain.<sup>18</sup> The erythrocyte sedimentation rate and C reactive protein were measured at the same time. Rheumatoid factor was assayed by the Rose-Waaler test at 0 and 24 months. Human leucocyte antigen (HLA) typing was performed on all patients with RA using a standard lymphocytotoxicity technique.

Radiographs of the hands and feet were taken at entry to the study and at 12 and 24 months' follow up. The radiographs were interpreted by hospital radiologists who were unaware of the patients' clinical and laboratory data. New

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erosions were recorded in the hands and feet of each patient at one and two years follow up.

Blood samples of the 71 patients with RA were collected at the start of the prospective study, at six months (70 patients) and at 24 months (67 patients). Serum samples were stored at  $-20^{\circ}\text{C}$  until analysed. Control samples for the determination of antikeratin antibodies included 20 serum samples from patients with reactive arthritis and 38 serum samples from adult blood donors. Control serum samples were treated in a similar way to those from patients with RA. Serum samples were tested without knowing the clinical details of the patients.

#### ANTIKERATIN ANTIBODIES

Unfixed cryostat sections of rat oesophagus were used as targets in the indirect immunofluorescence assay with fluorescein (FITC) labelled antihuman IgG (SBL, Stockholm, Sweden) and FITC labelled antihuman IgA and IgM (Behring, Marburg, Germany).

An antigen preparation containing epithelial keratins was made from human epidermis. The minced epidermal material was extracted with 0.5 ml Triton X-100 in 50 mM TRIS-HCl buffer. The insoluble material was then dissolved in Laemmli sample buffer.<sup>19</sup> Sodium dodecyl sulphate polyacrylamide gel electrophoresis was performed with 8% slab gels. The separated polypeptides were transferred onto nitrocellulose sheets.<sup>20</sup> For immunoblotting the serum samples were diluted 1:100. The bound antibodies were detected by peroxidase labelled antihuman IgG (Dakopatts, Glostrup, Denmark). In this work only antibodies giving the laminar staining of stratum corneum were designated as antikeratin antibodies. Nine serum samples were analysed by immunoblotting using human epidermis as the antigen source. None of the serum samples reacted with keratin polypeptides.

#### STATISTICAL ANALYSIS

The differences between the mean values of unpaired parametric observations were tested with Student's *t* test.  $\chi^2$  test with Yates's correction was used to compare percentages.

### Results

#### OCCURRENCE OF ANTIKERATIN ANTIBODIES

Antikeratin antibodies of IgG class were found in 38% of patients with RA with early disease at month 0 compared with 3% of the 58 control subjects ( $p < 0.001$ ) (table 1). Antikeratin anti-

*Table 1 Occurrence of antikeratin antibodies of different immunoglobulin classes in patients with early rheumatoid arthritis (RA) at month 0 and in controls*

Subject group	No (%) positive for antikeratin antibodies		
	IgG	IgM	IgA
Patients with RA (n=71)	27 (38)	10 (14)	4 (6)
Control patients (n=20)*	1 (5)	1 (5)	0 (0)
Healthy control subjects (n=38)	1 (3)	0 (0)	0 (0)

\*Patients with reactive arthritis.

bodies of IgM or IgA class were present in low amounts in patients with RA, usually simultaneously with IgG class antibodies. The occurrence of antikeratin antibodies of IgG class fluctuated in parallel with the clinical activity of RA with 26% positivity at six months and 40% two years after the start of the study. As antikeratin antibodies of IgM and IgA classes were so rare, the results were further analysed for the presence of antikeratin antibodies of IgG class only.

At the beginning of the study there were no major differences between patients with RA who were positive or negative for antikeratin antibodies in clinical and laboratory characteristics or in the treatment given (table 2).

#### ASSOCIATION OF ANTIKERATIN ANTIBODIES WITH RHEUMATOID FACTOR

Most of the patients who were positive for antikeratin antibodies were seropositive at the beginning of the study and two years later (table 3).

#### ASSOCIATION OF ANTIKERATIN ANTIBODIES WITH CLINICAL ACTIVITY

Several clinical and laboratory features of the patients positive for antikeratin antibodies were compared with those of patients negative for antikeratin antibodies (fig). At the start of the study the clinical activity did not differ between patients with RA who were positive or negative for antikeratin antibodies. At the end of the follow up, patients initially positive for antikeratin antibodies had developed more active disease than patients negative for antikeratin antibodies, in spite of similar treatment with second line disease modifying drugs (fig).

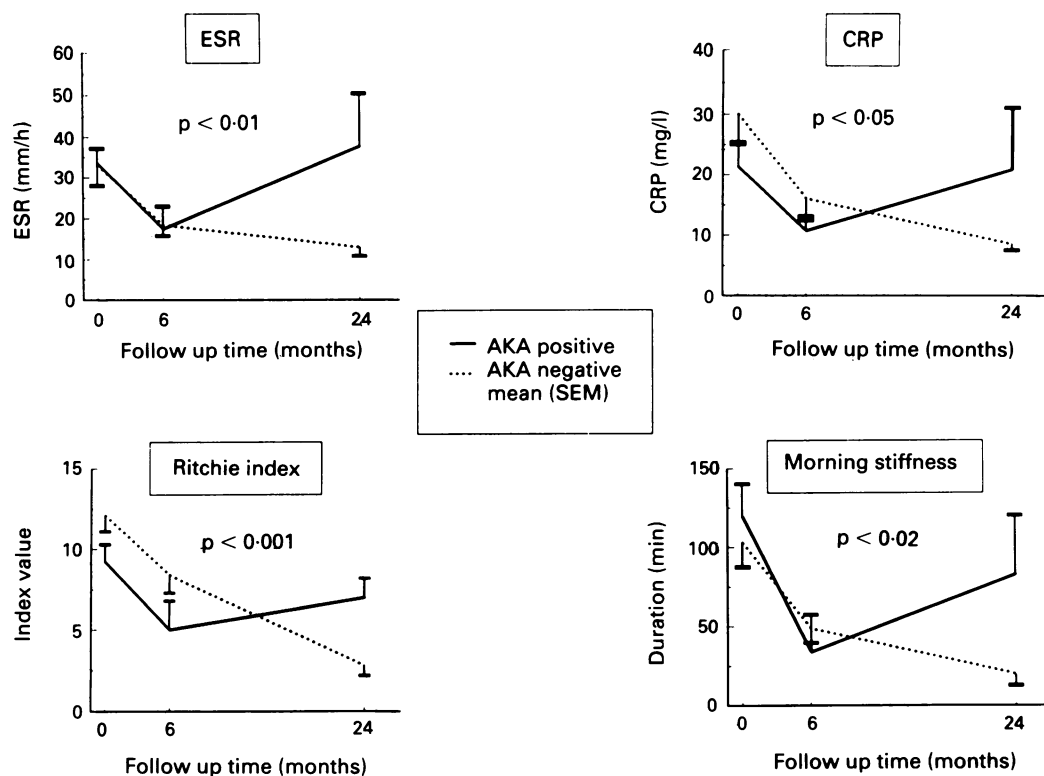
*Table 2 Comparison of patients with rheumatoid arthritis (RA) who were positive (AKA+) and negative (AKA-) for antikeratin antibodies at the beginning of the study. p Values not significant in any instance*

Variable	AKA+ (n=27)	AKA- (n=44)
Sex (M/F)	24/3	33/11
Mean (SEM) age (years)	45.7 (2.0)	42.9 (1.9)
Mean (SEM) disease duration (months)	7.5 (0.6)	7.8 (0.5)
Number (%) positive for rheumatoid factor	22/27 (81)	26/44 (59)
Number (%) positive for HLA-DR4	17/27 (63)	24/44 (55)
Number (%) of erosions	8/27 (30)	15/44 (34)
Number (%) starting treatment with:		
Parenteral gold	21/27 (78)	34/44 (77)
Sulphasalazine	5/27 (19)	11/44 (25)
Antimalarial drugs	1/27 (4)	0

*Table 3 Occurrence (%) of IgG antikeratin antibodies (AKA) and rheumatoid factor (RF) in patients with early rheumatoid arthritis (RA) over 24 months of follow up. Results are given as number (percentage) of patients*

Rheumatoid factor positivity	0 months (n=71)		24 months (n=67)	
	AKA+ (n=27)	AKA- (n=44)	AKA+ (n=27)	AKA- (n=40)
RF+*	22 (31)	26 (37)	19 (28)	14 (21)
RF-	5 (7)	18 (25)	8 (12)	26 (39)

\*Rose-Waaler titre  $\geq 1/64$ .



Comparison between patients initially antikeratin antibody (AKA) positive or negative during the follow up. ESR=erythrocyte sedimentation rate; CRP=C reactive protein.

#### ASSOCIATION OF ANTIKERATIN ANTIBODIES WITH RADIOLOGICAL PROGRESSION

At entry erosions were present in 30% of patients positive for antikeratin antibodies and in 34% of patients negative for antikeratin antibodies. During the following two years the two patient groups showed radiological progression in spite of treatment with disease modifying drugs. The patients who were initially positive for antikeratin antibodies had a trend to a more rapid radiological progression; 51% showed progression with new erosions compared with 36% of patients negative for antikeratin antibodies.

#### Discussion

Previous studies have shown the specificity of IgG type antikeratin antibodies for RA. These results confirm the diagnostic significance of antikeratin antibodies in RA by showing their presence in early disease. The occurrence of antikeratin antibodies in our patients was within the range reported in other studies with advanced RA.<sup>5 12</sup> Furthermore the follow up of the patients also showed that antikeratin antibodies are markers for active and progressive disease.

The results obtained with the immunofluorescence test for antikeratin antibodies show remarkable specificity for RA. This is in striking contrast to the results obtained with assays using purified or electrophoretically separated keratins as antigens.<sup>21 22</sup> These tests have shown a high incidence of keratin antibodies in patients with various diseases and in healthy subjects. We were unable to show antibodies to human epidermal keratin by immunoblotting in

early RA. Therefore, the antikeratin antibodies shown by the indirect immunofluorescence test probably represent a different antigen-antibody system from that defined by specific keratin antibody tests. It should be noted that until now there has been no direct evidence for the antikeratin specificity of antikeratin antibodies in RA.

No correlation between disease duration and the incidence of antikeratin antibodies has been observed previously.<sup>9 10 14 23 24</sup> On the other hand, few patients with early RA have been included in earlier studies. Our results show that antikeratin antibodies are present at the beginning of the disease.

The ability to predict the clinical course in the early stages of RA is important to establish a rational treatment. At the onset of this follow up the clinical activity and the rate of erosions were similar in patients with RA who were positive and negative for antikeratin antibodies. After two years follow up, however, patients initially positive for antikeratin antibodies had more active disease and a trend towards a more rapid radiological progression of joint damage than patients with RA who were negative for antikeratin antibodies. HLA-DR4 positivity has been related to a more aggressive RA.<sup>25</sup> In line with previous studies, HLA-DR4 positivity was not associated with antikeratin antibodies.<sup>8</sup> Thus our results suggest that antikeratin antibodies are an independent, diagnostic, and prognostic marker in patients with RA.

The immunofluorescence test for antikeratin antibodies is only semiquantitative and the lowest serum dilution is set to 1:10 to maintain the specificity of the test. Therefore the variation

of the incidence of antikeratin antibodies during the follow up is probably due to the variation of serum levels of antikeratin antibodies. Interestingly, this variation paralleled the disease activity; at six months follow up the occurrence of antikeratin antibodies was at its lowest and at that point the degree of clinical improvement, after treatment with disease modifying drugs, was at its highest (fig).

The diagnostic value of antikeratin antibodies has been assessed by several workers.<sup>9 13 14</sup> IgG class antikeratin antibodies have been considered to be more specific for RA than rheumatoid factor.<sup>4 6</sup> In our patients with early RA, the percentage of patients who were both rheumatoid factor negative and positive for antikeratin antibodies was low. These findings confirm, however, that antikeratin antibodies and rheumatoid factor are different antibody specificities.

In conclusion, our data suggest that in a fraction of patients with RA antikeratin antibodies are present in the early stages of disease and that in these patients antikeratin antibodies may be a marker for a more severe and active disease.

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