Antikeratin Antibodies in Rheumatoid Arthritis

Methods and Clinical Significance

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A method to determine antikeratin antibodies (AKA) is described. AKA were detected by indirect immunofluorescence technique on rat esophagus as antigen in sera of patients with definite rheumatoid arthritis (RA). The frequency of AKA in rheumatoid factor (SCAT/Waaler-Rose) positive RA was 64% and in SCAT-negative RA, 28%. Of 61 control patients with non-RA rheumatic diseases, none was AKA-positive. Of healthy controls, 2.5% were AKA-positive. In serum from 88 definite RA patients, AKA were compared with precoded clinical features. A highly significant correlation to AKA was found with the presence of rheumatoid hand deformity. Some correlation to positive SCAT titre and s-Haptoglobin was observed. Our study suggests that determination of AKA will be of value in the diagnosis of RA, especially in rheumatoid factor negative cases and that the presence of AKA indicates a more aggressive form—or results of an aggressive course—of the disease.

Key words: antikeratin antibodies, AKA, rheumatoid arthritis.

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Rheumatoid arthritis (RA) is characterized by the presence of circulating autoantibodies. In 1979, Young et al. (1) described serum antibodies to the keratin layer of rat esophagus in RA. Experimental evidence of the antigenicity of keratins was first demonstrated by Pillemer et al. in 1939 (2). Keratin consists of a heterogeneous group of fibrous soluble proteins, that are found in the epidermis and epidermal appendages (3). It is a major constituent of the stratum corneum of the skin and is present in the superficial layers of the esophageal epithelium in the rat (4).

The sensitivity of AKA in RA has been reported with values ranging from 36% to 69% (4, 5, 6, 7, 8). It has been suggested that the presence of the antibody may be a useful marker for the disease, because AKA appeared to be highly specific for RA. Specificity of more than 90% has been reported (1, 5, 9). The diagnosis of the early stages of RA and the rheumatoid factor (RF) negative RA patients may be difficult. According to the criteria of the American Rheumatism Association (ARA), rheumatoid factor positivity supports the diagnosis of RA (10), but RF lacks specificity.

Several authors (1, 4, 5, 7, 8, 9, 11, 12, 13) have reported correlations of AKA with various clinical and laboratory observations, but few have used a larger group of SCAT/Waaler-Rose negative patients. The purpose of the present investigation was to adapt the indirect immunofluorescence technique in order to demonstrate AKA with the highest possible specificity and sensitivity. Other intentions were to estimate the value of AKA in the diagnosis of RA, to estimate the clinical significance of AKA in RA, and to summarize the literature on correlations.

MATERIALS AND METHODS

Patients

Sera from 297 persons in four groups were tested.

Group A: 68 patients, 41 females and 27 males, with definite RA according to the American

Rheumatism Association (10) were tested and titrated for AKA. Their age range was 18–80 (mean 56) years. 50 of the patients were SCAT-positive and 18 were SCAT-negative.

Group B: 61 control patients, 42 females and 19 males, had non-RA rheumatic diseases. Their diagnoses are shown in Table I. Ages ranged from 20 to 87 (mean 57) years.

Group C: 80 blood donors, 32 females and 48 males, were tested as a healthy control group. Ages ranged from 23 to 65 (mean 41) years.

Group D: 88 sera, from another group of definite RA patients coded with clinical features, were tested for AKA. The 88 patients, 62 women and 26 men, had a mean age of 59 years (range 27–84 years). 43 of the patients were SCAT-positive and 45 were SCAT-negative.

Antikeratin antibodies

AKA were detected by Coons' indirect immunofluorescence technique (14), as described by Young et al. (1). Fresh middle third rat esophagus as antigen substrate was collected and snap-frozen in liquid nitrogen. The tissue was kept at −80°C and was fit for use within half a year. Unfixed 5 μm thick cryostat sections were mounted on glass slides and kept at −20°C for up to one month. All sera were screened after predilution 1 : 10 in phosphate-buffered saline, pH 7.2. Sera were applied to the tissue and incubated at 20°C for 30 min. The slides were washed three times in phosphate-buffered saline.

Fluorescein-conjugated rabbit anti-human immunoglobulin (IgA, IgG, IgM, κ, λ; FITC, Dakopatts, F200) was then added at previously determined optimal dilution (1 : 80). Incubation in darkness at 20°C for 30 min and washed another three times. FITC (fluorescein isothiocyanate) conjugated rabbit anti-human γ, μ and α chains antisera (IgG-γ chains, F202; IgM-μ chains, F203; IgA-α chains F204 all FITC, Dakopatts) were used to determine the Ig class of antibodies in positive sera, and the titres of AKA were determined.

All sections were assessed by two observers, one of whom was the same during all observations. Positive sera gave a fluorescent staining of the superficial layer of the esophageal epithelium. Only distinctive laminar (Fig. 1) or speckled fluorescence of the keratin layer was considered positive. Negative reaction with no fluorescence of the keratin layer is shown in Fig. 2. A Leitz Orthoplan fluorescence microscope with darkground illumination and a 200 W quartz–halogen lamp was employed.

Serological and clinical assessment

All sera from RA patients were titrated for rheumatoid factor as described by Roitt & Doniach (15). A SCAT titre of 1 : 40 or more was considered positive. The scores for functional inability was assessed by the Steinbrocker score (16) and by the Activity of Daily Living (ADL) (17). X-ray erosions in hands were scored positive if one joint had a Larsen score (18) of grade II or more.

Statistics

The differences between mean values of unpaired parametric observations were tested with the two-tailed Student's *t*-test. For qualitative unpaired non-parametric observations, a χ^2-test was used, with

Table I. *The diagnosis of 61 control patients (Group B)*

Diagnosis	Number of patients
Sarcoidosis Boeck (lymphogranulomatosis benigna)	1
Morbus Reiter (syndroma urethro-oculo-articularis)	2
Arthritis urica	1
Morbus Raynaud	1
Arteritis temporalis et polymyalgia arteritica/rheumatica	7
Psoriasis arthropatica	9
Morbus Bechterew (spondylarthritis ankylopoietica)	10
Osteoarthrosis	12
Arthritis postinfectiosa	1
Polymyositis	1
Myosis, myofibrositis	1
Sclerodermia non-specificata	2
Lupus Erythematosus Disseminatus (SLE)	9
Syndroma Sjögren (keratoconjunctivitis sicca)	2
Mixed connective tissue disease (MCTD)	2

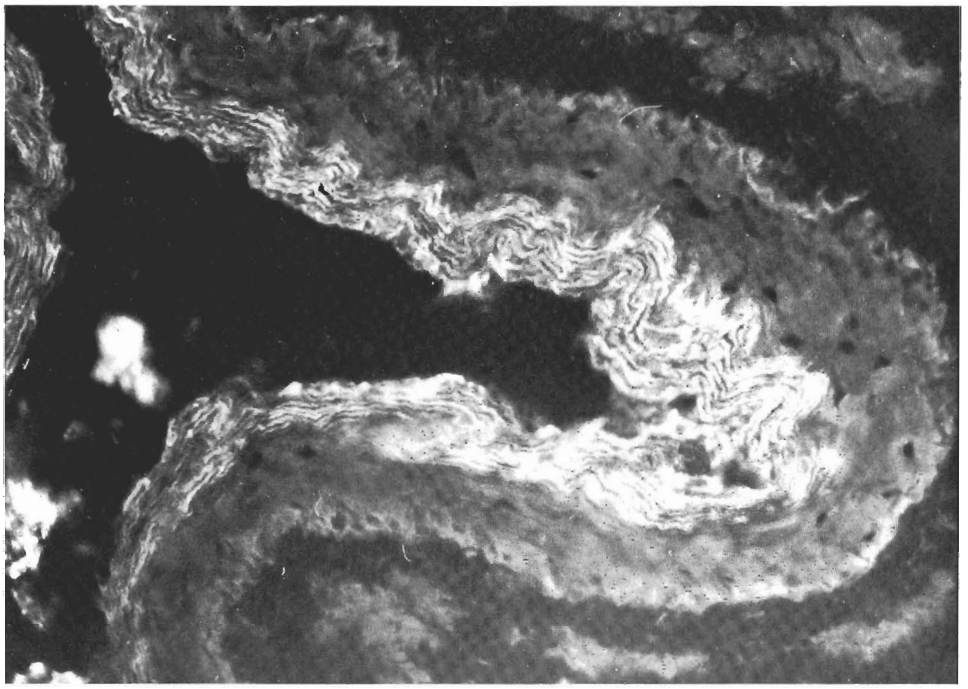

Fig. 1. Positive laminar immunofluorescence of keratin fibres in cross-section of rat esophageal mucosa.

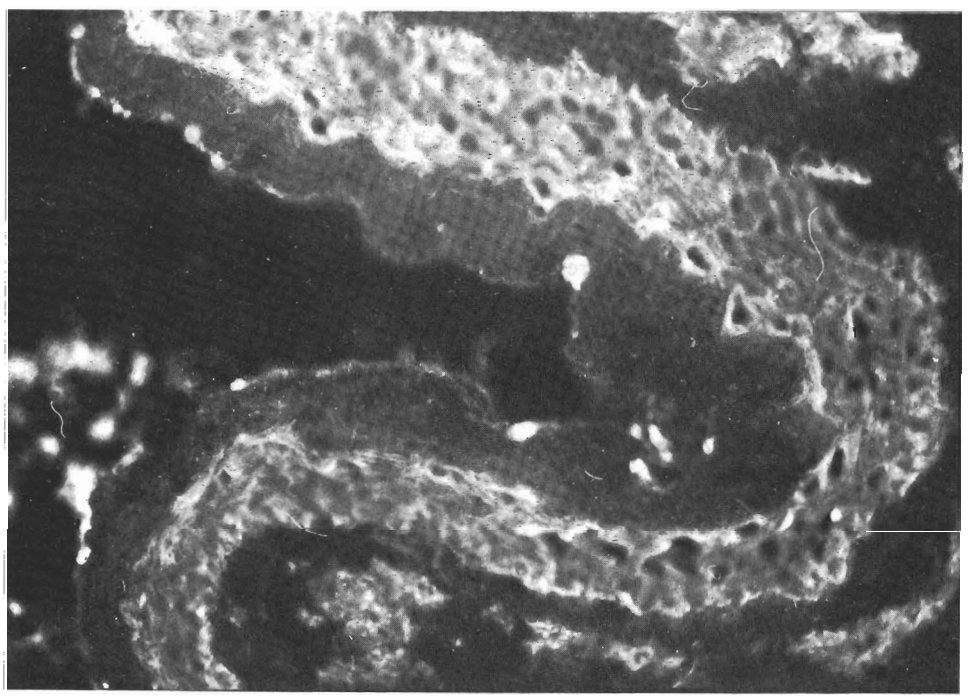

Fig. 2. Negative immunofluorescence of rat esophageal mucosa with normal human serum.

Table II. *Incidence of antikeratin antibodies (AKA) in 209 persons*

Group	AKA positive *n* (%)	AKA negative *n* (%)	Number tested *N*
A) Rheumatoid arthritis	37 (54.4)	31 (45.6)	68
B) Control patients	0 (0)	61 (100)	61
C) Healthy controls	2 (2.5)	78 (97.5)	80

$\chi^2=75.16$, $0.0001>p$.

Yates' correction. With unpaired observations of a quantitative nature, we used a rank-sum test with corrections for tied ranks.

RESULTS

In group A, AKA were found in 37 of 68 (54.4%) patients with rheumatoid arthritis (Table II). The frequency of AKA in rheumatoid factor (RF)-positive RA was 64% (32 of 50 patients), and in RF-negative RA, 27.8% (5 of 18 patients) (Table III).

In group B, none of the 61 control patients with non-RA rheumatic diseases were AKA-positive. Among the healthy controls in group C, 2 of the 80 blood donors (2.5%) were AKA-positive (Table II) and one of these was RF-positive, SCAT titre 1:40.

No AKA of IgM or IgA class were identified. All AKA belonged to the IgG class of immunoglobulins. The AKA titres in the 37 RA patients of group A ranged from 1:10 to 1:2560 (mean 1:160), with no difference of the mean between RF-positive and RF-negative patients. The 2 positive healthy controls both showed a titre of 1:20. Of the 37 AKA sera in group A, 27 showed laminar fluorescence (Fig. 1) and the rest showed speckled fluorescence of the keratin fibres. Only one of the 5 AKA-positive, RF-negative RA patients showed speckled fluorescence staining. The day-to-day and person-to-person variations in our results differed only one titre step.

The 88 RA patients in group D, with precoded clinical features, were tested 18 months later, using older sera which had been thawed several times. There were 56% (24/43) AKA-positive of the RF-positive RA patients and 27% (12/45) AKA-positive of the RF-negative patients.

There was a very high significant correlation (less than 1% level) with the presence of rheumatoid hand deformity. At the 5% level there was a correlation with s-Haptoglobin and with a positive SCAT-titre. There were no correlations with age, sex, erosions, nodules, ADL, Steinbrocker score, duration of disease, morning stiffness, synovitis, or ESR (Tables IV, V, VI). Table VII shows the AKA correlations in the literature.

Table III. *Incidence of antikeratin antibodies (AKA) in 68 patients with rheumatoid arthritis (RA) in relation to the presence of rheumatoid factor*

Group A	AKA positive *n* (%)	AKA negative *n* (%)	No. of RA patients *N*
SCAT positive	32 (64)	18 (36)	50
SCAT negative	5 (27.8)	13 (72.2)	18

$\chi^2=5.62$, $0.02>p>0.01$.

Table IV. *AKA in relation to non-parametric observations in 88 RA patients (Group D)*

AKA	SCAT		Erosions		Nodules		Sex	
	Pos.	Neg.	Yes	No	Yes	No	Male	Female
AKA positive	24	12	31	5	15	21	13	23
AKA negative	19	33	43	9	11	41	13	39
Chi^2	6.57		0.018		3.37		0.78	
Odds-ratio	3.47		1.29		2.66		1.70	
p-value	$0.02>p>0.01$		$p=0.90$		$0.10>p>0.05$		$0.50>p>0.30$	

DISCUSSION

We used rat esophagus as antigen substrate because AKA have been reported to react more specifically for fully differentiated keratins (1). We found a very high specificity of AKA for RA. None of the 61 control patients in group B were AKA-positive. Only 2 out of 80 healthy controls in group C were AKA-positive and with a low titre (1 : 20); one of these was found SCAT-positive. In this material, AKA titres higher than 1 : 20 we found to be 100% specific for RA. Johnson et al. (11) reported a higher incidence of AKA-positive control patients when using tissue from cardia (56%) than from middle third esophagus (5%). The reduced specificity for RA obtained with sections of cardia is unexplained, but could possibly be due to varying differentiation of keratin according to the anatomical site. This could be one explanation for the different results reported by some investigators. The differences in the pattern of immunofluorescent staining could also be responsible for the variable results. In our study we have restricted the designation of a positive reaction for AKA to those sera showing a distinctive laminar or speckled pattern of staining.

Our results of 54% AKA positivity in sera from RA patients accord with the original observations of Young et al. (1). Decreased sensitivity has also been reported (7, 8, 19). The difference in sensitivity for the SCAT-positive RA patients in group A and group D (64% AKA positive versus 56%) may be due to the fact that group D sera were much older and had been thawed and frozen several times. More than 26% of the SCAT-negative RA patients from both groups (17/63) had AKA in serum. The sensitivity for AKA in RF-negative RA patients has been reported to vary between 18 and 38% (5, 8), but it has been in groups of about 30 patients only.

Table V. *AKA (mean values) in relation to parametric observations in RA patients (Group D)*

Observation	AKA-pos ($n=36$)	AKA-neg ($n=52$)	*p*-values
Age (years)	58.69	59.08	$0.9>p>0.8$
Duration of disease (years)	12.17	9.65	$0.2>p>0.1$
ADL score (0.00–3.00)	1.38	1.24	$p=0.40$
Morning stiffness (hours)	1.27	1.19	$0.7>p>0.6$
ESR (mm/h)	37.19	28.23	$0.1>p>0.05$
s-Haptoglobin (g/l)	3.18	2.55	$0.02>p>0.01$

Student's *t*-test (two-tailed).

Table VI. *AKA in relation to non-parametric observations in 88 RA patients (Group D)*

	Synovitis in hands			Hand deformity			Steinbrocker score			
AKA	Both	Single	None	Both	Single	None	4	3	2	1
AKA positive	10	9	17	21	1	14	2	12	16	6
AKA negative	17	2	33	14	4	34	0	15	23	14
Rank sum test	$p=0.41$			$p=0.0062$			$p=0.36$			

Our results showed that all AKA were of the IgG class of immunoglobulins. Ordeig & Guardia (9) have found RA patients with both IgG and IgM class AKA, while in one positive healthy control, AKA was found to be of the IgM class alone. Further investigations will be necessary to explain these results.

All previous studies claim 5% level correlations between AKA and rheumatoid factor. Several authors (4, 7, 13) have found a high significant correlation with positive SCAT titres with p-values between 0.001 and 0.005. Thus there is no doubt about the correlation between AKA and rheumatoid factor. We have found a 5% significance level with positive SCAT titre, high s-Haptoglobin and with the presence of rheumatoid hand deformity, but only the hand deformity can meet a 1% criteria. The differences in duration of disease exceeded 25% (12 versus 9.5 years), but not significant due to a large standard deviation.

We could not show any correlation between AKA and the presence of nodules, even though we did find a positive odds-ratio of 2.66. Due to the size of our material this does not contradict the positive correlations claimed in the earlier literature (5, 8, 9). We did not find significant correlations with signs of inflammatory activity such as the presence of synovitis in hands, long duration of the morning stiffness, or high ESR. AKA seems independent of the present activity of RA. Hajiroussou et al. (5) claimed a correlation to male sex, as they found 48% AKA-positivity in male RA patients versus 28% in female

Table VII. *AKA correlations in the literature*

1st author Publishing year	Young 1979	Scott 1981	Johnson 1981	Miossec 1982	Mallya 1983	Quismorio 1983	Youinou 1983	Ordeig 1984	Hajiroussou 1985
No. of RA patients	129	99	102	96	98	80	178	131	204
Sensitivity, in %	58	36	51	40	69	58	39	54	59
Correlations									
Rheumatoid Factor	Yes	Yes	Yes	Yes	Yes	–	Yes	Yes	Yes
Other anti-tissue antibodies	–	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
Extra-articular features	–	–	–	–	Yes	–	–	Yes	Yes
Disease activity	–	No	–	Yes	Yes/No	–	–	–	–
Functional class	–	–	–	–	Yes	–	–	Yes	Yes
Anatomical class	–	–	–	(Yes)	–	–	–	Yes	Yes
Nodules	–	Yes	–	–	–	–	–	Yes	Yes
Male sex	–	–	–	–	–	–	–	No	Yes
Duration of RA	–	–	–	No	–	–	–	No	–
Other features	–	–	–	–	Platelets	–	–	Age	Erosions

RA patients. We can support this correlation to male sex with a positive but non-significant odds-ratio of 1.70. By contrast Ordeig & Guardia (9) reported 84.5% women among AKA-positive RA patients and 80% women among AKA-negative RA patients. This calls for further studies in larger materials.

In conclusion, we have found AKA to be highly specific for rheumatoid arthritis and our study suggests, that determination of AKA will be of value in the diagnosis of RA, especially SCAT-negative RA. The AKA-positive RA patients were more severely deformed. It seems that the presence of AKA in RA indicates a more severe degree of disease, either as a result of the severity or as an indication of a more severe form of RA.

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