Rheumatology 2000;39:1082–1088

Association of rheumatoid factors and anti-filaggrin antibodies with severity of erosions in rheumatoid arthritis

S. Bas, T. V. Perneger[1], E. Mikhnevitch, M. Seitz[2], J.-M. Tiercy[3], P. Roux-Lombard[4] and P. A. Guerne

Division of Rheumatology, Department of Internal Medicine, University Hospital, Geneva, [1]Quality of Care Unit and Institute of Social and Preventive Medicine, University Hospital, Geneva, [2]Department of Rheumatology and Clinical Immunology/Allergology, Inselspital, Bern, [3]Transplantation Immunology Unit, Department of Internal Medicine, University Hospital, Geneva and [4]Division of Immunology and Allergology, Department of Internal Medicine, University Hospital, Geneva, Switzerland

Abstract

Objectives. To evaluate and to compare the association of two types of autoantibodies—rheumatoid factors (RF) and anti-filaggrin antibodies (AFA)—with clinical severity and joint damage progression in rheumatoid arthritis (RA) patients.

Methods. In a cross-sectional study, we determined RF and AFA titres in 199 RA patients and 65 controls. Erosions apparent on X-rays were quantified using the Larsen score in 143 patients, and the distribution of these scores was studied according to disease duration in patients who were positive and negative for RF and AFA.

Results. RF were detected in 72% and AFA in 47% of RA patients. AFA were highly specific for RA (100%). RF positivity was correlated with the presence of subcutaneous nodules, sicca syndrome and the severity of erosions for a given disease duration. AFA positivity was correlated only with the presence of the HLA-DRB1 shared epitope.

Conclusions. Since no significant correlation was observed between joint damage progression and AFA positivity, the determination of AFA does not appear to be useful in assessing the prognosis of RA. However, AFA, which appear early in RA, could be helpful for the diagnosis of RA in patients who do not fulfil four American College of Rheumatology criteria.

KEY WORDS: Rheumatoid factors, Anti-filaggrin antibodies, Rheumatoid arthritis, Erosion, Larsen score, Prognosis.

The so-called antikeratin antibodies described by Young *et al.* in 1979 [1] label the stratum corneum of the epithelium of the rat oesophagus when detected by indirect immunofluorescence on cryosections. Simon *et al.* [2] demonstrated later that they recognize epitopes on a neutral/acidic variant of filaggrin, a well-known cytokeratin filament-aggregating protein of the epidermis. These anti-filaggrin antibodies (AFA) have been found to be highly specific for rheumatoid arthritis (RA) [1, 3–11] but have also been observed in some sera from patients with juvenile chronic arthritis [12], systemic sclerosis [1, 5, 6, 13, 14], systemic lupus erythematosus [3, 6, 14] and ankylosing spondylitis [5, 14]. However, Vincent *et al.* [15] showed recently that the frequency of their presence in these other diseases was threshold-dependent: when a high cut-off point was chosen a diagnostic specificity for RA of more than 99% was reached.

In RA, the appearance of AFA may precede disease onset [16], and it has been suggested that AFA correlate with disease expression (activity, severity or outcome) [4, 6, 7, 9–11, 14, 16–22]. These associations, however, were often weak and not confirmed by other groups [23, 24], so that the place of these autoantibodies in the clinical management of RA is still uncertain. These controversies probably arise largely from the difficulty of accounting for disease duration when analysing the progression of erosions.

Submitted 12 November 1999; revised version accepted 17 March 2000.

Correspondence to: S. Bas, Research Laboratory, Division of Rheumatology, University Hospital, 1211 Geneva 14, Switzerland.

With the present tendency to earlier and more aggressive treatment of RA patients who have more severe disease, the availability of reliable prognostic markers of progressive and destructive disease is increasingly important. In this study, we investigated the association of rheumatoid factor (RF) and AFA with the progression of joint damage, determined with the Larsen score adjusted for disease duration. We also evaluated the association of these autoantibodies with the number of ACR (American College of Rheumatology) criteria [25] fulfilled and with extra-articular manifestations. Furthermore, since the critical genetic element in RA is now believed to be constituted by the shared amino acid residues QK/RRAA at positions 70–74 of the HLA-DRB1 chain [26], we investigated potential associations between this sequence and the presence of AFA.

Patients and methods

Patients

We studied 199 consecutive, unselected patients with RA, diagnosed according to the revised criteria formulated by ACR [25], recruited in outpatient clinics of the five university hospitals of Switzerland (Basel, Bern, Geneva, Lausanne, Zurich) and in five non-university primary referral centres in Switzerland between 1992 and 1998. For all patients, we obtained blood samples and detailed questionnaires that included age, sex, disease duration, questions on joint destruction and erosion, and extra-articular manifestations. Nodules and serositis were defined as the presence of either of these manifestations at any time during evolution of the disease. Vasculitis was defined as any clear manifestation of rheumatoid vasculitis during evolution of the disease. For sicca syndrome, information was requested about anamnestic xerophthalmia or xerostomia and performance or not of a Schirmer's test and of a lachrymal or salivary gland biopsy with their results. A sicca syndrome was taken into account in the presence of either anamnestic xerophthalmia or xerostomia, a positive Schirmer's test or a positive biopsy. Since data on joint destruction and erosions proved difficult to interpret, we subsequently requested recent hand X-rays (made less than 2 yr previously) for all patients, and obtained them for 143 patients. Joint damage progression was assessed with the standardized Larsen score as a function of disease duration at the time the X-ray was taken. X-rays were analysed blindly with respect to clinical and laboratory data, by the same reader (rheumatologist). The Larsen score was established using standard reference films [27]. Wrists, metacarpophalangeals 2–4 and proximal interphalangeals 2–4 were scored on a five-point scale: 0 = no abnormalities, 1 = slight abnormalities (joint space narrowing or band like osteoporosis), 2 = small but definite erosions, 3 = medium erosions, 4 = severe destructive abnormalities, 5 = mutilating abnormalities. The score for the wrist was then multiplied by 2, so that the total score ranged from 0 to 100.

Most patients had been treated with at least one DMARD and/or corticosteroid. However, the treatments were so heterogeneous in terms of drug, dosage and duration that it was impossible to classify or describe them in a synthetic and useful manner.

To assess their specificity, RF and AFA were determined in 65 consecutive sera from non-RA patients that had been sent for latex testing during January 1997 in the routine laboratory of the Division of Rheumatology (University Hospital, Geneva). After subsequent consultation of the medical records 1 yr after sample collection, it was established that none of these patients had developed RA. Diagnoses were transient hip synovitis, psoriasis arthritis, septic arthritis, Lyme arthritis, polymyalgia rheumatica, osteoarthritis, low back pain, ankylosing spondylitis, mixed connective tissue disease, scleroderma, polymyositis, fibromyalgia, and some non-rheumatic diseases (stroke, deep venous thrombosis, urticaria, dermohypodermitis, autoimmune thyroiditis, uveitis, hepatitis and glomerulonephritis). A group of 20 serum samples from healthy blood donors with age and gender distributions as close as possible to the RA patient group was also used as a negative control. The median age (yr), range and percentage of females in the three groups are given in Table 1. The serum samples were aliquoted and stored at -80°C until use.

Rheumatoid factor test

Sera were tested for RF by agglutination of latex particles coated with human Ig (Difco Laboratories, Detroit, Michigan, USA). Titrations were performed in tubes. Agglutination titres $\geqslant 1/80$ were regarded as positive.

Anti-filaggrin antibody test

Serum samples were tested for the presence of AFA by an indirect immunofluorescence technique using unfixed cryostat sections of the middle third of the rat oesophagus as the antigen source (slides purchased from Alphadia Diagnostics Products, Belgium). Patient serum samples were diluted 1:10 in phosphate-buffered saline containing 0.1% Tween-20, and were incubated on the slides for 30 min. The slides were rinsed twice with buffer for 5 min and incubated for 45 min with a polyvalent anti-human immunoglobulin fluoresceinated conjugate (Kallestad, Minnesota, USA). The slides were then rinsed twice for 5 min, mounted with glycerol, and viewed with a Zeiss microscope (Oberkochen, Germany) with ultraviolet epi-illumination optics. Only laminar staining of the stratum corneum was interpreted as positive. A negative and a positive control were included in each series. Positive sera were titrated and the last serum dilution exhibiting evidence of fluorescence was considered the titration end-point.

Immunogenetic analysis

HLA-DR generic typing and DRB1*01 and DRB1*04 subtyping were performed as described elsewhere [28].

Statistical analysis

The sensitivity and specificity of RF and AFA with respect to the diagnosis of RA were calculated [29], together with exact 95% confidence intervals (CI). We did not compute positive and negative predictive values for these tests, since control patients were sampled independently of RA patients.

Subgroups of RA patients who were positive and negative for RF and/or AFA were compared by means of Student's *t*-test (for continuous variables) and the χ^2 test (for discrete variables). To examine associations between serological markers and clinical manifestations of RA, we used adjusted regression models in which RF and AFA were independent variables and each clinical variable in turn was the dependent variable. For dichotomous dependent variables (such as the presence of subcutaneous nodules), we used logistic regression models in which both RF and AFA were included as predictors, and expressed associations in terms of odds ratios (OR), together with the 95% CI. For continuous dependent variables (age and duration of RA), we used two-way analysis of variance, again with both RF and AFA included as factors, and used *F*-tests to evaluate statistical significance.

To examine the natural progression of disease by serological status, we plotted the Larsen score as a function of disease duration, stratifying by RF and AFA status. We then used non-parametric regression (Lowess regression) [30] to explore the patterns of associations. This analysis includes only a subset of the observations at a given time, and consists in scanning the scatterplot from left to right (from short to long disease durations), data point after data point, each time recomputing a weighted average of the Larsen scores in the selected subset (the procedure is slightly more complex than using a moving average to provide a smooth function). This method allows the average estimate to move up or down without imposing a functional shape, such as a linear relationship. Based on this exploratory analysis, we selected the most appropriate linear regression model for testing of statistical significance: either a simple linear model or a model in which the slope was allowed to change at a point in time suggested by the non-parametric regression plot [31]. Regression slopes were expressed in Larsen units per year of disease duration.

Results

Sensitivity and specificity of RF and AFA determinations

When assessed with our 199 RA patient samples and 65 consecutive and unselected non-RA control sera, sensitivity was better for RF (72%, 95% CI 66% to 78%) than for AFA (47%, 95% CI 40% to 54%), but specificity was better for AFA (100%, 95% CI 94% to 100%) than for RF (89%, 95% CI 79% to 96%) (Table 1). When both positive RF and AFA were considered as detecting RA, the sensitivity was increased (77%) without decreasing specificity (89%).

Characteristics of RA patients according to their RF and AFA status

The presence of AFA showed a marked association with RF ($\chi^2 = 29.0$, $P < 0.0001$, OR 6.9, 95% CI 3.1 to 15.1).

There were no significant associations of serological markers with patient sex, age, duration of RA or the presence of serositis or vasculitis (Table 2). In contrast, after adjustment for AFA status, RF-positive patients fulfilled more ACR criteria at diagnosis (+ 0.6 criteria, 95% CI 0.3 to 0.9, $P < 0.001$) and were more likely to have subcutaneous nodules (OR 3.4, 95% CI 1.3 to 8.8, $P = 0.012$) and sicca syndrome (OR 5.0, 95% CI 1.6 to 15.5, $P = 0.005$). After adjustment for RF status, AFA-positive patients were more likely to express the HLA-DRB1 70–74 shared epitope (OR 2.6, 95% CI 1.2 to 5.8, $P = 0.015$).

When the total number of extra-articular manifestations (subcutaneous nodules, serositis, sicca syndrome and vasculitis) was considered, a significant difference was observed between RF-positive and negative patients (0.46, $P < 0.001$, 95% CI 0.21 to 0.71) but not between AFA-positive and -negative patients ($P = 0.77$). When the presence of at least one extra-articular manifestation was compared with its absence, again a significant difference was observed for the presence of RF (OR 4.2, 95% CI 1.9 to 9.2, $P = 0.0004$) but not for the presence of AFA ($P = 0.72$).

Characteristics of nine AFA-positive but RF-negative RA patients

Eight (89%) of these patients were women. A wide range of age (28–75 yr), disease duration (0.5 yr to 38 yr 5 months), ACR criteria (4–6) and AFA titres (1/20–1/640) was observed (Table 3). Except for two patients with subcutaneous nodules, none of them had extra-articular manifestations such as serositis, sicca syndrome and vasculitis. When the presence of erosions was established from hand radiographs (if the Larsen score was $\geqslant 9$ for the fingers of at least one hand or $\geqslant 2$ for at least one wrist), 4/6 (67%) were found to be positive (87% of the 143 RA patients with hand radiographs were also positive according to these criteria, 78% of these RA patients without RF, and 85% of those without AFA).

Analysis of RF and AFA status by regression of Larsen score as a function of disease duration. Graphical exploratory analysis suggested that the Larsen score increased approximately linearly with disease duration among patients who were AFA-positive or RF-positive (Fig. 1A, B). The trend was similar among AFA-negative patients who had had RA for less than 15 yr, but the severity of erosions increased only moderately thereafter (Fig. 1A). The contrast was even sharper for RF-negative patients, in whom the severity of erosions was not associated with disease duration beyond 12 yr of RA (Fig. 1B). Because of the association between positive AFA and RF, we further stratified the sample into four subgroups of patients who were negative for both markers or either one of the markers, or positive

TABLE 1. Characteristics of the three groups

	RA patients ($n = 199$)	Non-RA control patients ($n = 65$)	Healthy blood donors ($n = 20$)
Women	149 (75)	40 (62)	12 (60)
Age (yr) (median, range)	62, 22–86	50, 4–87	59, 51–69
RF-positive (RF titre $\geqslant$ 1/80)	144 (72)	7 (11)	0
AFA-positive	93 (47)	0	0

Except where stated otherwise, values are numbers (percentages) of subjects.

TABLE 2. Characteristics of patients with rheumatoid arthritis according to RF and AFA positivities

	RF^+, AFA^+ ($n = 84$)	RF^+, AFA^- ($n = 61$)	RF^-, AFA^+ ($n = 9$)	RF^-, AFA^- ($n = 45$)
Women	56 (67)	49 (80)	8 (89)	36 (80)
Age (yr) (mean $\pm$ S.D.)	63 $\pm$ 11	60 $\pm$ 14	56 $\pm$ 18	60 $\pm$ 16
Disease duration (yr.months) (mean $\pm$ S.D.)	13.2 $\pm$ 9.1	15.4 $\pm$ 13.6	13.7 $\pm$ 12.1	12.5 $\pm$ 11.4
Number of ACR RA criteria fulfilled	5.6 $\pm$ 1.0	5.4 $\pm$ 1.0	4.8 $\pm$ 1.0	4.9 $\pm$ 0.8
Extra-articular manifestations				
Subcutaneous nodules	30 (36)	18 (30)	2 (22)	4 (9)
Serositis	5 (6)	4 (7)	0	3 (7)
Sicca syndrome	17 (20)	18 (30)	0	4 (9)
Vasculitis	6 (7)	4 (7)	0	1 (2)
HLA-DRB1 70–74 shared epitope	73 (87)	44 (72)	8 (89)	32 (71)

Except where stated otherwise, values are numbers (percentages) of subjects.

TABLE 3. Characteristics of rheumatoid arthritis patients without serum RF but with AFA

Patients:	1	2	3	4	5	6	7	8	9
Sex	F	F	F	F	F	F	F	F	M
Age (yr)	59	32	28	64	75	61	55	85	48
Disease duration (yr.months)	38.5	9	1.11	7.5	11.8	0.5	20.7	28.5	4.7
Number of ACR RA criteria fulfilled	5	4	4	4	6	4	6	6	4
AFA titre	1/40	1/160	1/20	1/40	1/40	1/80	1/80	1/640	1/40
Subcutaneous nodules	–	+	–	–	–	–	+	–	–
Dose of HLA-DRB1 70–74 shared epitope	0	1	1	1	1	1	2	2	2
Erosions[a]	+	–	–	+	+	n.d.	n.d.	n.d.	+

n.d. = not determined.
[a]A positive result was obtained when the Larsen score was 9 for the fingers of at least one hand or 2 for at least one wrist.

for both (Fig. 1C). The resulting analysis strongly suggested that RF, but not AFA, is associated with the severity of erosions.

In a linear regression model which allowed a change in slope at 15 yr, as suggested by the graphical analysis, the Larsen score increased on average by 1.6 points per year (95% CI 1.1 to 2.2) between diagnosis and year 15 in both AFA-positive and AFA-negative patients; thereafter, the slope declined to 1.0 point per year among AFA-positive patients and to 0.5 per year among AFA-negative patients, the difference between the two subgroups being non-significant (0.5 point per year, 95% CI -0.4 to 1.3, $P = 0.26$).

A similar regression analysis was performed for RF-positive and RF-negative patients, but the change in slope was located at 12 yr of disease duration. Between 0 and 12 yr, the Larsen score increased by 2.0 points per year (95% CI 1.3 to 2.6) in both subgroups. Beyond 12 yr, the slope declined to 0.9 point per year among the RF-positive patients, and to -0.5 point per year among the RF-negative patients. The difference between the groups was significant (1.4 points per year, 95% CI 0.6 to 2.1, $P = 0.001$).

Discussion

From a diagnostic point of view, AFA determination was more specific (100%) but less sensitive (47%) for RA, whereas the latex test appeared more sensitive (72%) but less specific (89%). In this study, these parameters were determined with control sera corresponding to consecutive and unselected samples sent to a routine laboratory for the latex test. Thus, in actual conditions of use, AFA were undetectable in non-RA patients, and thus appear to be of great diagnostic value compared with RF. These results are very similar to those reported by other groups (specificity of AFA was generally greater than 90% [6, 10, 21] and sensitivity was between 33 and

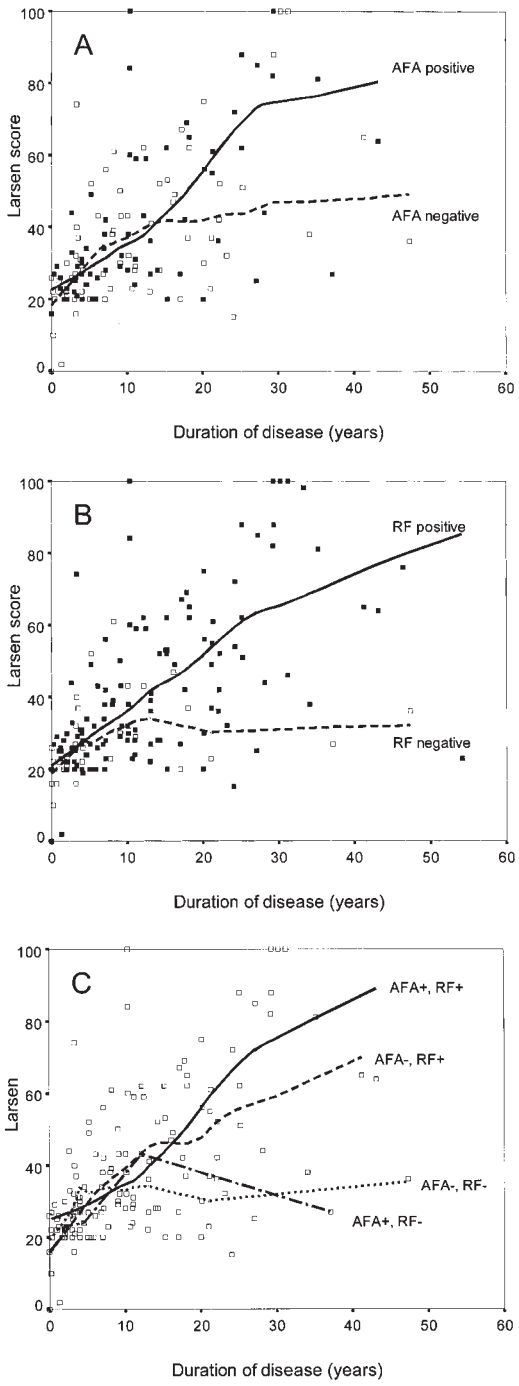

FIG. 1. Plots of Larsen score (severity of erosions on X-ray) against duration of disease (interval between onset of rheumatoid arthritis and X-ray) in patients who were positive and negative for AFA (A, 143 patients) or RF (B, 166 patients) and in patients with different combinations of AFA and RF phenotypes (C, 143 patients) at the time of X-ray. The lines represent non-parametric regression lines (similar to a moving average).

69% for patients with RA [1, 3–7, 9, 10, 13, 14, 17, 21, 32]). AFA were also reported in 5–47% of seronegative patients with RA [3, 6, 9, 10, 20], which is consistent with AFA in 16% of RF-negative patients in this study.

With regard to disease severity, only RF correlated with erosions. The fact that the difference in Larsen scores between RF-positive and -negative patients was detectable only for patients with more than 12 yr of disease duration could be due to several reasons. The negative effect associated with the presence of RF may not have been sufficient to be detectable earlier with the number of patients recruited in our study. Additionally, recent progress in treatment interventions may have prevented detection of this effect in patients with more recent disease onset. Indeed, the increasing use of methotrexate, at higher doses, and low-dose prednisone [33, 34] could markedly reduce the power of such studies when performed on patients with more recent disease.

Concerning joint damage progression, our results do not agree with those of other groups [20, 22] who have suggested that the presence of AFA may constitute a predictor of severe radiographic damage. This divergence might be explained by the different statistical analysis used in the present study. Indeed, we analysed a cross-sectional sample of RA patients with the non-parametric method of Lowess regression, considering the progression of joint damage as a function of time. This approach takes into account the fact that the Larsen score is widely dependent on disease duration; it probably represents a good alternative insofar as it is easier to perform than a prospective study. In addition, with the availability of new and more effective treatments that are adjustable to disease severity, prospective studies are confronted either with ethical problems or with a possible loss of sensitivity.

Only patients who were hospitalized or who consulted a specialized rheumatology clinic were included in our study. It is therefore possible that our estimates of disease progression were exaggerated, but the comparison of subgroups who were positive or negative for serological markers would remain valid.

Characterization of patients who were RF-negative but AFA-positive showed that the majority of these individuals were positive for the shared epitope and had erosive disease and, sometimes, nodules (2/9). AFA probably define the same RA subgroup as RF, and can be considered as a substitute marker when RF are negative.

The prevalence of the HLA-DRB1 shared epitope, itself considered to be a parameter of disease severity [35], was higher in patients with AFA than in AFA-negative patients. An association between HLA-DR and AFA positivity has been established in a Greek RA patient population [23], but other studies have failed to show any association [5, 14, 20, 36]. However, molecular subtyping was not performed in these studies and can explain this discrepancy. The various genetic backgrounds of the populations studied might also contribute to these different findings.

In conclusion, our results do not show any significant independent association between AFA positivity and signs of disease severity. Consequently these autoantibodies do not have a better value than RF for prognosis in RA. The larger multicentre study that is in progress

in Switzerland might make it possible to define the place of AFA in prognosis in association with other parameters, such as the shared epitope or RF. Nevertheless, AFA can be useful in the clinical management of rheumatic diseases since they appear at the beginning of the disease [16, 20], are present in some RA-seronegative patients and appear to be more specific than RF. Since the current treatment of RA requires prompt diagnosis, AFA might find an important place, particularly in patients with suspected early RA but fulfilling fewer than four ACR criteria.

Acknowledgements

The technical assistance of Régine Edelman, Martine Rodier, Ursula Spenato and Madeleine Vuillet is gratefully acknowledged. This study was supported by the Subvention fédérale pour la lutte contre le rhumatisme de l'Office Fédéral de la Santé Publique and a grant from Novartis.

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