The prognostic value of the antiperinuclear factor, anti-citrullinated peptide antibodies and rheumatoid factor in early rheumatoid arthritis

C.H.M. van Jaarsveld[1], E.J. ter Borg[2], J.W.G. Jacobs[1], G.A. Schellekens[3], F.H.J. Gmelig-Meyling[4], C. van Booma-Frankfort[5], B.A.W. de Jong[3], W.J. van Venrooij[3], J.W.J. Bijlsma[1]

[1]Department of Rheumatology and Clinical Immunology, University Medical Center Utrecht;
[2]Department of Rheumatology. Sint Antonius Hospital, Nieuwegein;
[3]Department of Biochemistry. University of Nijmegen, Nijmegen;
[4]Department of Immunology, University Medical Center, Utrecht;
[5]Department of Rheumatology, Diakonessen Hospital, Utrecht, The Netherlands.

Abstract

Objective

To study the prognostic value of the antiperinuclear factor (APF), determined by an indirect immunofluorescence test (IIF) and a recently developed anti-citrullinated cyclic pepide (CCP) ELISA, in combination with rheumatoid factor (RF) status, in early RA (< 1 year).

Methods

A total of 249 participants in a randomized trial of treatment strategies were divided into 4 groups according to their APF (or CCP) and RF status at baseline. Differences in disability, joint involvement and radiological damage over a 3-year period were analysed.

Results

APF-IIF results differed from CCP-ELISA in 42 cases (17%); 38 of the 42 had a positive IIF and negative ELISA value. Disability after 3 years did not differ significantly between the RF and APF groups. APF^- patients had significantly lower Thompson joint scores compared to APF^+ patients (6 vs 24 for CCP-ELISA; 2 vs 24 for IIF). RF^+APF^+ patients exhibited more radiological damage compared to RF^-APF^- patients. RF^+APF^- and RF^-APF^+ patients had intermediate scores. Within the RF^+ and RF^- groups, APF^+ was associated with more radiological damage and thus yielded prognostic information in addition to RF. In this respect, the results of ELISA and IIF were comparable. Thirty percent of the RF^+APF^+ patients had a radiological score higher than 45, compared to 13% of the RF^+APF^-, none of the RF^-APF^+, and 2% of RF^-APF^- patients ($p < 0.001$). In addition, more large joints were affected in APF^+ than in APF^- patients, while no difference was observed between RF^+ and RF^- patients.

Conclusion

APF has prognostic value in addition to RF for joint involvement and radiological damage in early RA. The CCP-ELISA technique for APF assessment may facilitate its use in clinical practice. However, the prognostic value of the two tests lies in their ability to predict mild disease. Reliable identification at baseline of individual patients with progressive disease is still not possible.

Key words
Antiperinuclear factor, early RA, indirect immunofluorescence, ELISA.

C.H.M. van Jaarsveld, PhD, Epidemiologist; J.W.G. Jacobs, MD, PhD, Rheumatologist; J.W.J. Bijlsma, MD, PhD, Professor of Rheumatology; E.J. ter Borg, MD PhD, Rheumatologist; G.A. Schellekens, PhD, Biochemist, B.A.W. de Jong, Biochemist; W.J. van Venrooij, PhD, Professor of Biochemistry; F.H.J. Gmelig-Meyling, PhD, Immunologist; C. van Booma-Frankfort, MD, Rheumatologist.

On behalf of the Rheumatic Research Foundation Utrecht, The Netherlands.
Supported by a grant from the Dutch League against Rheumatism (Het Nationaal Reumafonds). The work of G.A. Schellekens, B.A.W. de Jong and W.J. van Venrooij was supported by The Netherlands Foundation for Chemical Research with financial aid from The Netherlands Technology Foundation (grant 349-3159).

Please address correspondence to: J.W.G. Jacobs, Department of Rheumatology and Clinical Immunology, University Medical Center Utrecht, PO Box 85500, 3508 GA Utrecht, The Netherlands. E-mail: J.W.J.Bijlsma@digd.azu.nl

Received on January 11, 1999; accepted in revised form on June 2, 1999.

Introduction

Treatment strategies for rheumatoid arthritis (RA) are moving towards a more aggressive approach early in the course of disease (1). It is important to be able to predict long-term disease outcome at the individual level, however, in order to choose the optimal treatment. A good set of prognostic markers - or even just one marker - would allow a clinician to choose a more powerful (although potentially more toxic) slow-acting antirheumatic drug (SAARD) early on, even when clinical judgement might not yet indicate such a need. An ideal prognostic marker should be reliable, present in early disease, simple, valid and accurate (2).

At the group level, a positive rheumatoid factor (RF) test and female gender are known to be prognostic markers for severe disease (3). The antiperinuclear factor (APF) has been suggested both as a diagnostic tool and as a prognostic marker for severe disease. APF is the term used for a group of autoantibodies which react with 'keratohyaline granules' around the nucleus of human buccal mucosa cells (4). The pathophysiological mechanisms that lead to the expression of APF in RA patients are not clear as yet. The major APF antigen migrates as a diffuse 200-400 kD protein band in an immunoelectrophoretic blot; it is closely related to human epidermal (pro)filaggrin (5-8). APF and RF are different autoantibodies (9), but the incidence of these autoantibodies in RA appears to be correlated (10, 11).

There is substantial evidence for the diagnostic value of APF. APF has been found to have better specificity, sensitivity, and positive and negative predictive values for the diagnosis of RA than either the latex agglutination or Rose Waaler tests for RF (12-15). Disagreement exists regarding the extent to which APF positivity predicts a more severe course of the disease, however. Kerstens *et al*. found low but significant correlations between APF titres and the Ritchie joint score and pain, albeit at only one of three time points (16). Other studies have shown that APF positivity indicates a poor prognosis, i.e. it is linked to the extent of erosions (10, 17) and to a patient classification in functional class III (14). In addition, Westgeest *et al*. found APF to be associated with progressive disease, especially in RF-negative patients (18).

Classically, APF is detected by indirect immunofluorescence (IIF). It has never become a popular test, which might in part be due to difficulties in its conduction (19). Recently, a peptide-based enzyme linked immunosorbent assay (ELISA) was developed using citrullinated cyclic peptides (CCP) as a substrate. It was convincingly shown that antibodies detecting such substrates also recognise the perinuclear factor and (pro)-filaggrin (20, 21). As such, this ELISA detects a set of antibodies directed to a subset of APF-determinants and therefore can be considered as a functional replacement of the APF-IIF test. The ELISA technique has clear advantages over IIF, since its execution and the interpretation of the results are much more straightforward.

In the present study, APF test results determined by IIF and the single peptide-based CCP-ELISA are compared and interpreted. The main objective was to study the prognostic value of APF in combination with RF for disease severity as measured by functional disability, joint involvement and radiological damage over a 3-year period in patients with recent-onset RA.

Patients and methods

Patients

Since 1990 all patients with recent-onset RA who met the 1987 ACR criteria at six rheumatological centres in the Utrecht region of The Netherlands have been asked to participate in a randomized, controlled trial to compare therapeutic strategies (1, 22). Data have also been collected from patients who refused to be randomized; therefore this represents a population-based study.

Disease duration had to have been less than one year; most patients enrolled shortly after diagnosis. Included in the present study were only those patients for whom serum samples (stored at -20° C) taken during the first 6 months after enrollment in the clinical trial were available and patients who were not lost to follow-up within the first 2 study years (n = 249).

Materials

Functional disability and joint scores were assessed at the start of the trial, every 3 months during the first 2 years and subsequently every 6 months. Functional disability was measured using a validated Dutch version of the Health Assessment Questionnaire (HAQ): the questionnaire score may vary from zero to three, zero representing the best (no problems) and three representing the worst score (23). The joint score according to Thompson assesses the simultaneous presence of joint tenderness and swelling in a selection of joints weighted according to joint size; range 0 - 534 (24, 25). Joints that had received a corticosteroid injection in the 2 months before an evaluation were not included in the joint score.

Radiographs of the hands (including the wrists) and feet were made at the start of the trial and were repeated every year. A modified version of the method of Sharp was used to score radiological abnormalities (26). According to this method, erosions and joint space narrowing are scored and added to obtain a total radiological damage score (range 0 - 448). RF status was determined at baseline and was considered positive if the qualitative latex fixation test at a dilution of 1:1 was positive and/or the Rose-Waaler test was positive (titre ≥ 40 IU/ml).

APF was measured in the earliest available stored frozen serum sample, within 6 months after enrollment. The serum samples were thawed on ice and divided into two series of 100 µl for the determination of APF by IIF and by CCP-ELISA. None of the sera had been previously thawed. The conventional immunofluorescence assay was performed by the Department of Immunology of the University Medical Center. Determination by means of the newly developed CCP-ELISA was conducted by the Department of Biochemistry of the University of Nijmegen.

APF by indirect immunofluorenscence

The indirect immunofluorescence method is based on a polyvalent antiglobulin which involves an IgG preparation with reactivity to human IgG and IgM (and presumably also to IgA). Cell preparation was done according to the study protocol of Hoet *et al.* (7) with only minor modifications; a fixed patient serum dilution of 1:10 was used. The APF readings were based on the assessment of at least 100 buccal mucosa cells. The APF test was considered positive if in at least 30% of the inspected cells one or more fluorescent keratohyalin granules were clearly visible. A standard healthy control serum pool was used as a control, which exhibited no fluorescence in the granules or at most dull background.

Positivity was graded arbitrarily as weakly positive (+), positive (++), or strongly positive (+++). This reading was usually based on the fluorescence intensity rather than on the numbers of positive cells and of keratohyalin granules per cell as these were fairly constant. The study was conducted using buccal mucosa cells from one single donor, selected for adequately reactive cells. Two well-trained observers assessed all the fluorescent patterns independently, i.e. they were unaware of each other's results. Readings differing by more than one grading (which occurred in less than 3% of the cases) were reviewed together to reach agreement. In all instances, the observers were not informed about the disease parameters of the patients and about the ELISA results. For the analysis, dubious results were regarded as negative and all positive gradations as positive.

The anti-citrullinated cyclic peptide ELISA

The ELISA was performed as described previously (20). A CCP variant of cfc-1, formed by substituting serine residues by cysterine, was used as a substrate. The CCP-ELISA detects a set of antibodies directed to a subset of APF-determinants and can therefore be considered as a functional replacement of the APF-IIF test. In this paper, APF^+ patients can refer to patients with either a positive APF-IIF test or a positive CCP-ELISA test.

The CCP ELISA proved to be extremely specific for RA (98%) with a moderate sensitivety of 65-70% depending on the cohort of patients studied. A detailed account of this test will be published elsewhere (Schellekens *et al.*, submitted paper). The CCP-ELISA test will become commercially available in 1999 via Euro-Diagnostica (Arnhem, The Netherlands). Sera were tested in duplicate at a dilution of 1:200 and the results were averaged. Sera were considered positive if the optical density at a wavelength of 450 nm (OD_{450}) resulted in a signal ≥ 0.11.

Statistical analysis

The statistical analyses were performed using the SPSS 6.1 statistical package (27). Both joint scores and radiological damage scores had a skewness towards high scores. Non-parametric Mann-Whitney U tests were used for comparison of the medians between two groups of patients, and the Kruskall-Wallis test was used in case of more than 2 groups. The independent two-sample T-test was used for the comparison of means, i.e. for the functional disability scores.

Patients were divided into four groups according to their APF and RF. Differences between these groups in mean disability, median joint scores and the median radiological damage score over a 3-year period were analysed. In addition, differences in involvement of the large versus small joints were assessed by comparing knee, ankle, wrist and elbow involvement with small hand/foot joint involvement for the joint score and the wrist versus small hand/foot joints for the radiological damage score.

Results

Patient selection

At the time of analysis, 577 patients had entered the study, of whom 404 had completed at least two years of follow-up. A total of 249 of these 404 patients had sera available for the baseline, 3-month and 6-month time points. Due to organisational difficulties unrelated to the study objectives, sera were not collected on one of these first visists for 155 patients. The patients with available sera (n = 249) did not differ from the patients without available sera (n = 155) on the following baseline characteristics: RF status, gender, age, ESR, disability, pain and radiological damage. However, patients with available sera had statistically significant lower median joint scores than the patients without sera (114 vs 138, p = 0.02). In most cases (n = 157) serum was available from baseline, prior to treatment with a SAARD. In a minority

Table I. Patient characteristics at baseline *

Parameter	%	Median	Mean	S.D.	Range
Female	71				
Positive RF test	61				
Positive APF according to IIF	66				
Positive CCP ELISA	52				
Age in years			56	14	18 - 83
ESR in mm/1st hour			30	31	0 - 140
Disability score †			1.3	0.8	0 - 2.9
Joint score †		114			0 - 506
Radiological damage †		2			0 - 73

*12 patients (4.8%) had rheumatoid nodules. No other extra-articular manifestations were found at baseline.
† Functional disability was assessed by means of a validated Dutch version of the HAQ (range 0-3) (23). The joint score according to Thompson (range 0-534) was used (24, 25).
A modified method of Sharp was used to score radiological damage (26). According to this method erosions and joint space narrowing in the hands, wrists and feet are scored (range 0-448).
High values of all variables indicate more disease activity. Median scores are also presented for the joint score and radiological damage because of skewed distributions.

of cases serum obtained after 3 months (n = 60) or 6 months (n = 32) was used. The baseline clinical and socio-demographic data as mentioned above, and the RF status did not differ between these groups.

Patient characteristics

Table I shows the baseline demographic and clinical parameters for the study cohort, which are quite characteristic of a recent-onset RA population. The follow-up of these patients varied from 1 to 5.5 years (mean 3.2 years). At baseline, 31 patients (12%) were randomized to receive non-steroidal anti-inflammatory drugs (NSAIDs) only; 71 patients (29%) a mild SAARD with an expected, relatively long delay of treatment effect (i.e., hydroxychloroquine, replaced by auranofin in cases of toxicity or insufficient effect); 56 (23%) a more potent SAARD with an expected, relatively long delay of treatment effect (i.e., intramuscular gold / D-penicillamine); and 63 (25%) a more potent SAARD with an expected, short delay of treatment effect (i.e., methotrexate / sulphasalazine). Twenty-seven patients (11%) refused to be randomized.

In the literature female gender has been found to be a prognostic marker for a severe course at the group level. However, in our cohort the parameters of disability, joint scores and radiological progression in the first three years after diagnosis did not differ between males and females and therefore gender was not considered in the analyses. Moreover, gender was not related to the RF or APF status.

Comparison of APF-IIF with CCP-ELISA

APF-IIF was positive for 164 RA patients (sensitivity 66%), while the CCP-ELISA was positive for 130 patients (sensitivity 52%). Table II shows that only 4 sera were positive according to CCP-ELISA and negative according to IIF, whereby 2 were classified as dubious by means of IIF. In total 38 sera were APF-positive in IIF while negative in CCP-ELISA, 18 being classified as clearly positive in IIF. The agreement was 0.83 and the Kappa statistic, which takes into account the proportion of agreement which would be expected due to chance alone, was 0.66, indicating reasonable to good agreement. In Figure 1 the semi-quantitative results of the tests are compared. These results are not discussed in detail, since it is generally accepted that the semi-quantitative results of IIF should be dichotomized and interpreted as such. Similarly, at this stage of test development the quantitative results of the ELISA test should also be dichotomized, with the cut-off point at $OD_{450} \geq 0.11$ indicating positivity.

APF and RF were both positive or both negative in 192 cases (77%) using IIF, and in 190 cases (76%) using the CCP-ELISA; in a minority of cases one of the APF or RF tests was positive. Treatment according to randomization was not significantly different between the groups separated according to RF and APF. Therefore, the grouping by randomization was assumed not to have biased the results.

Prognostic marker for functional disability

The functional disability score was available for 193 patients (78%) after 2 years of follow-up and for 159 patients (64%) after 3 years of follow-up. Mean functional disability assessed after 3 years of follow-up did not differ significantly between the RF and APF groups (Table III); the same applied for the baseline, 1 year and 2 year scores (data not shown).

Prognostic marker for joint involvement

Joint scores varied considerably over time per individual and did not progress linearly, as was the case with radiological damage, in our study. Therefore, 3 assessments (i.e. at 2, 2.5 and 3 years) were averaged to obtain a more reliable indication of joint involvement after 2 to 3 years of follow-up. For 17 patients, follow-up assessments were not avail-

Table II. Agreement of APF values obtained by indirect immunofluorescence* (IIF) and CCP-ELISA.

	IIF negative	IIF positive	Statistics
CCP-ELISA negative	81	38	Agreement = 0.83
CCP-ELISA positive	4	126	Kappa = 0.66

* IIF results were dichotomized: negative and dubious results were regarded as negative and all positive gradations as positive.

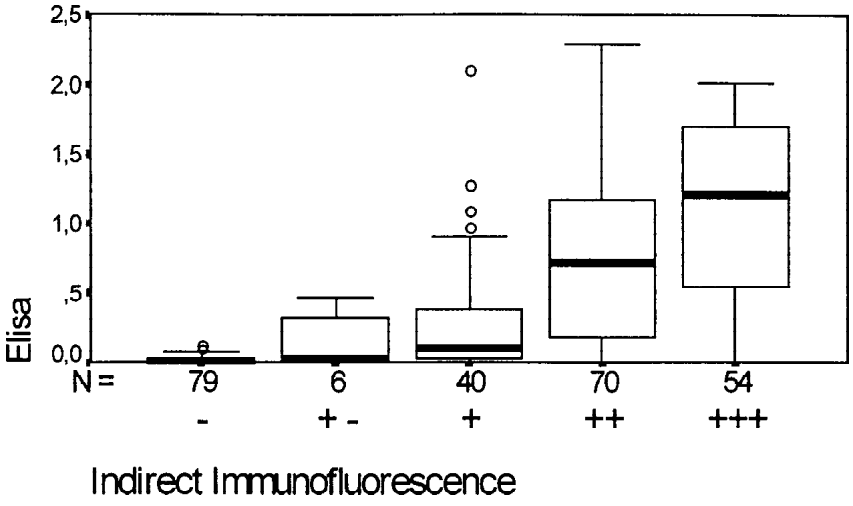

Fig. 1. CCP-ELISA values related to APF scores obtained by indirect immunofluorescence for 249 patients (thick line represents the median, box: 25-75 percentiles, whiskers: 10-90 percentiles, o: outliers). CCP-ELISA ≥ 0.11 was considered positive.
Indirect immunofluorescence: negative (-) and dubious (+/-) results were considered negative; weakly positive (+), positive (++) and strongly positive (+++) results were considered positive.

Table III. Mean functional disability score after 3 years according to the RF test; APF was assessed by CCP-ELISA and IIF.

	Negative test			Positive test			p-value
	Mean	(SD)	No.	Mean	(SD)	No.	T-test
RF	0.82	(0.7)	59	1.00	(0.8)	100	0.07
CCP-ELISA	0.94	(0.8)	72	0.98	(0.8)	87	0.75
APF-IIF	0.86	(0.8)	51	1.00	(0.8)	108	0.26

able after 2 years, leaving 232 patients for analysis.
There was no statistically significant difference in the median joint scores between RF$^-$ (16.0) and RF$^+$ (19.0) patients (data not shown). However, APF$^-$ patients had a significantly lower joint score compared to APF$^+$ patients (i.e., 6.0 vs 23.5 for CCP-ELISA and 2.0 vs 24.0 for IIF). The median joint scores decreased over time, as shown in Figure 2 for both groups (APF$^+$ and APF$^-$, determined by CCP-ELISA). The results for APF assessed by IIF were comparable, indicating a more rapid decrease in the joint score for APF$^-$ patients compared to APF$^+$ patients. RF status was not included in Figure 2, since this did not significantly affect the results.

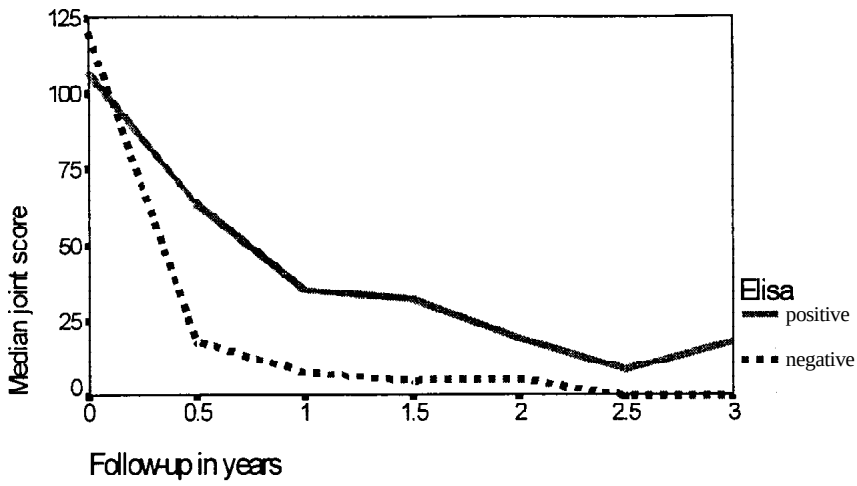

Fig. 2. Median joint scores (theoretical range 0-534) in the first three years of follow-up for 249 patients according to APF assessed by CCP-ELISA.

The median joint scores after 2-3 years of follow-up for the four groups divided according to RF and APF status showed significantly lower scores for RF$^-$APF$^-$ and RF$^+$APF$^-$ patients compared to the RF$^+$APF$^+$ group, also indicating that APF is better in predicting joint involvement than RF.
Involvement of the large joints (i.e., knee, ankle, wrist, elbow) and the small joints (i.e., hand and foot) was analysed separately. APF$^+$ patients suffered significantly more involvement of the large (0.3 vs 0.0, $p = 0.01$) and small joints (1.3 vs 0.0, $p < 0.01$) compared to APF$^-$ patients; the results were comparable for IIF and CCP-ELISA. RF status was not significantly associated with the number of affected large joints, but RF$^+$ patients exhibited slightly more involvement of the small joints compared to RF$^-$ patients ($p = 0.06$). The percentages of patients with at least one affected large joint in the first three years of follow-up are presented in Figure 3 for the APF$^+$ and APF$^-$ groups assessed according to ELISA. Of the CCP-ELISA-positive patients 42% had at least one affected joint after 3 years of follow-up compared to 23% of the CCP-ELISA-negative patients ($p = 0.01$). The results for APF-IIF were comparable, indicating a more rapid decrease of large joint involvement in APF$^-$ patients than in APF$^+$ patients. The decrease in large joint involvement did not differ significantly between RF$^-$ and RF$^+$ patients (data not shown).

Prognostic markers for radiological damage

The median radiological damage scores after 2 to 3 years are presented in Table IV. Radiographs after 3 years of follow-up were available in 179 cases; in 56 cases radiographs after 2 years of follow-up had to be used while in 14 cases radiographs after 2 or 3 years of follow-up were not available. The median radiological damage scores for patients with a negative RF, CCP-ELISA and

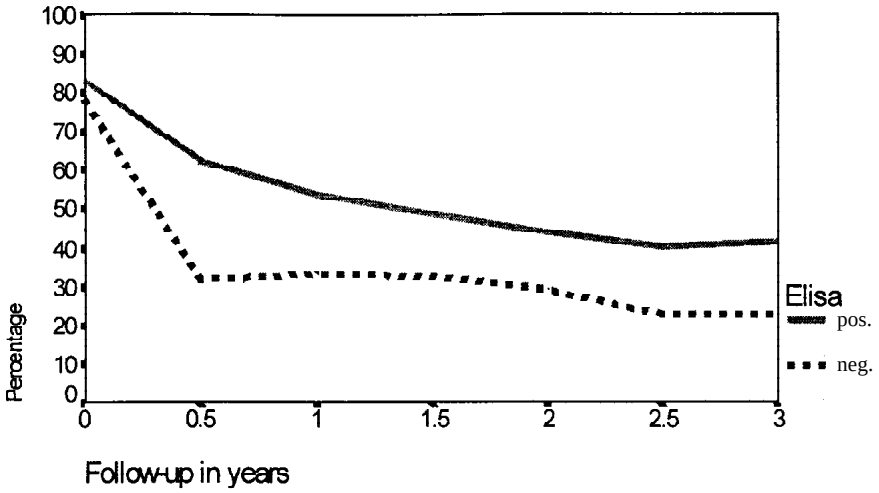

Fig. 3. Percentage of patients with at least one affected large joint in the first three years of follow-up among 249 patients grouped according to APF as assessed by CCP-ELISA. After 3 years of follow-up, 42% of the CCP-ELISA positive patients had at least one affected large joint (i.e., knee, ankle, wrist, elbow) compared to 23% of the CCP-ELISA negative patients (χ^2 test: p = 0.01).

APF-IIF were 10.0, 10.5 and 9.5, respectively, while patients with a positive RF, CCP-ELISA and APF-IIF scored 21.0, 22.0 and 21.0, respectively. The differences between the positive and negative patients were statistically significant for all tests and were of the same magnitude.

Table IV presents the results for four groups based on a combination of RF and APF, using CCP-ELISA or IIF. Statistically significant differences were found between some groups, with high radiological damage scores for RF^+APF^+ patients, intermediate radiological damage for RF^+APF^- and RF^-APF^+ patients, and low radiological damage scores for RF^-APF^- patients. For RF^+ and RF^- patients, APF^+ was significantly associated with more radiological damage. There were no significant differences between the RF^+APF^- patients and the RF^-APF^+ patients nor between the RF^-APF^+ patients and the RF^+APF^+ patients. APF assessment by means of CCP-ELISA and IIF gave comparable results. The only disagreement between IIF and CCP-ELISA was the observation that RF^+ APF^- patients had more radiological damage than RF^-APF^- patients when APF was assessed by CCP-ELISA (p = 0.04); this difference was not significant when IIF was used (p = 0.39).

Figures 4a and 4b show the median radiological damage scores over a 3-year period for groups separated according to their RF and APF as assessed by CCP-ELISA and IIF, respectively. It is clear that patients with a positive RF and APF exhibited more radiological damage compared to patients with negative results for both tests. Patients with one positive test (RF^+APF^- and RF^-APF^+ patients) had intermediate scores.

The range of the radiological damage score is 0 to 448. In the four groups defined according to RF and APF status, the percentage of patients exceeding 10% of this range (i.e., a score > 45) was studied. According to the CCP-ELISA, 30% of the RF^+APF^+ patients exceeded a radiological score of 45 compared to 13% of the RF^+APF^- patients, none of the RF^-APF^+ patients and 2% of the RF^-APF^- patients (χ^2 test, p < 0.001). Comparable results were found using IIF for the APF assessment. The positive prognostic value (PPV) for a high radiologically assessed damage score (score > 45) of CCP-ELISA alone was 26%, while the negative prognostic value (NPV) was 94%.

The percentage of patients with obvious radiological damage in the wrist (score > 2) was similar for the RF^+ and RF^- patients (49 vs 42%, p = 0.41); however, the wrist was more frequently involved in APF^+ patients compared to APF^- patients (55 vs 36%, p = 0.02 for CCP-ELISA and 53 vs 34%, p = 0.03 for IIF). Radiographic damage in the small hand and foot joints was found significantly more often in the RF^+ and APF^+ patients than in the RF^- and APF^- patients, respectively (89 vs 73% for RF, 91 vs 73% for CCP-ELISA and 90 vs 71% for APF-IIF; p < 0.01).

Table IV. Radiological damage after 2 to 3 years for groups classified according to their RF and APF, using CCP-ELISA (upper panel) or IIF for the APF assessment (lower panel).

	Median	(SD)	Range	N	Missing*	Difference†		
CCP-ELISA								
RF^+ APF^+	23	(30)	0-166	106	6	p = 0.04		
RF^+ APF^-	19	(36)	0-223	41	0		p = 0.05	p < 0.0001
RF^- APF^+	17	(12)	5- 37	15	3	p = 0.02		
RF^- APF^-	9	(26)	0-193	73	5			
APF-IIF								
RF^+ APF^+	22	(29)	0-166	124	6	p = 0.02		
RF^+ APF^-	10	(47)	0-223	23	0			p < 0.0001
RF^- APF^+	17	(35)	1-193	31	3	p = 0.01		
RF^- APF^-	9	(14)	0 - 63	57	5			

* Radiological scores were missing for 14 patients
† Significant differences in the median radiological score between groups according to RF and APF are presented (Mann-Whitney U Test: p < 0.05).

Discussion

The comparison of APF scores obtained by IIF with those measured by CCP-

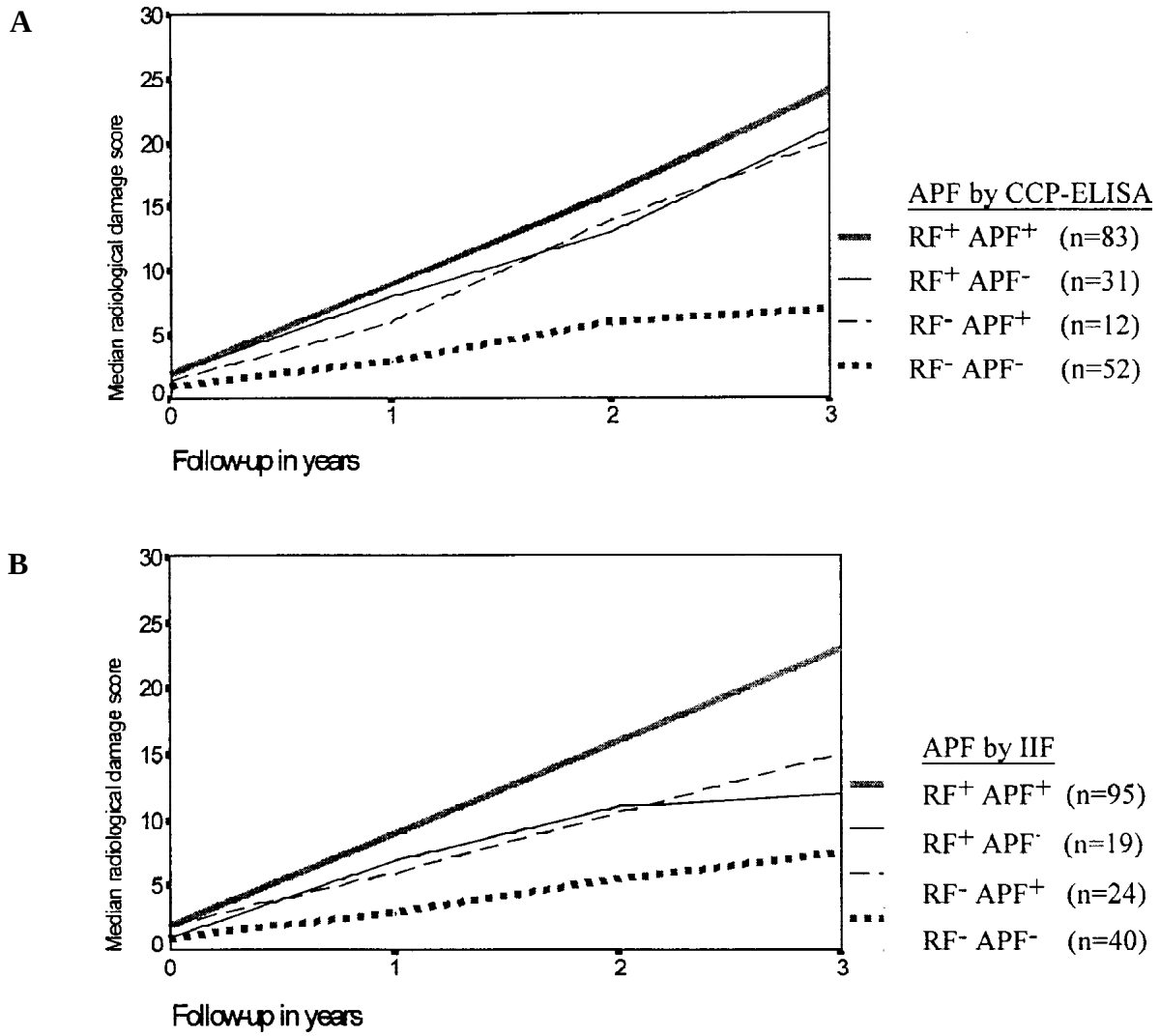

Fig. 4. Radiological damage in the first three years of follow-up for 178 patients with available radiographs on all four occasions: APF determined by CCP-ELISA (**A**) and by IIF (**B**). After 3 years of follow-up (see Table IV), RF^+APF^+ patients differed significantly from RF^- APF^- and from RF^+APF^- patients; RF^-APF^- patients differed significantly from RF^+APF^+ and from RF^-APF^+ patients for both methods.

ELISA revealed a difference for 42 sera (17%). In total, 38 sera were positive according to IIF and negative according to CCP-ELISA. This agrees with our previous observations, in which it was shown that the CCP-ELISA detects antibodies which recognise a subset of APF determinants (20). Recently, others have also stated that e.g. APF and AKA, which were previously considered to be two different RA-associate antibodies, are actually largely the same autoantibodies, referred to as antifilaggrin autoantibodies (8, 21).

There are at least three possible explanations for these discrepancies. Firstly, some incorrectly positive IIF results are inevitable, due to the difficulty of discriminating the fluorescence of keratohyaline granules from background fluorescence. However, in the present study the percentage of incorrect results was decreased by the use of duplicate assessments by independent trained observers and by the use of a single selected donor for the buccal cell substrate. Moreover, the observation that 18 of the 38 sera which were negative by CCP-ELISA were classified as strongly positive by IIF also suggests another explanation. Secondly, it has been shown that the reactivity of RA sera to different citrullinated peptide variants is highly diverse (20). Therefore, the peptide used here may represent a set of antigenic determinants that largely, but not entirely, overlaps with the antigenic determinants presented by the keratohyalin granules in the APF-IIF test. Lastly, the cut-off point at 0.11 for a positive CCP-ELISA, as determined in a previous study, might be too high, resulting in 'incorrect' negative ELISA results (20). However, a lower cut-off point would increase the number of 'incorrect' positive ELISA results. The cut-off point chosen seemed to be the optimal one for our data.

The mean functional disability at several time points did not differ in the RF and APF groups. Others have also stated that the predictors for joint involvement might differ from the predictors for disability (2). The best suggested predictor for functional disability later in the disease course is functional disability at an early stage (28).

Our results favour APF over RF as a prognostic marker for joint involvement. APF^+ patients clearly exhibited higher median joint scores compared to APF^-

patients. Moreover, the percentage of patients with at least one affected large joint was increased in the APF^+ group, while no difference was observed between the RF groups. Similar results were found for radiological damage of the wrist. Radiological damage of other 'large' joints was not scored. APF positivity was associated with higher median damage scores for the wrist, while no difference was found between the RF groups. This suggests that APF could be particularly useful for identifying patients with large joint involvement in RA.

As for the radiological damage scores, our results show that the combination of RF and APF is a better prognostic marker than the single tests alone. The progression of radiological damage showed a linear trend. Radiological progression in the first year(s) has predictive value for long-term damage (3). However, this does not constitute an optimal prognostic marker since it is not available early in the course of disease, when it would be most useful in terms of treatment options. Thirty patients had a radiological damage score higher than 45, 25 of whom (83%) were RF^+APF^+.

The positive prognostic value (PPV) for a high radiologically assessed damage score (score > 45) of APF alone was 26%; the negative prognostic value (NPV) was 94%. The NPV is superior to the PPV. This indicates that the likelihood that APF^- patients will indeed develop less radiological damage (score ≤ 45) is higher than the likelihood that APF^+ patients will develop more damage (score > 45). However, PPV and NPV are interrelated and depend on the cut-off point used. Using the median score for the total population (18) as the cut-off point, the PPV of APF is 59% and NPV is 63%. Still, patients with considerable damage were mainly RF^+APF^+. However, not all of the RF^+APF^+ patients exhibited high scores. In fact, the majority (70%) had scores ≤ 45. Therefore, we conclude that the combination of RF and APF can identify individual patients with a less severe prognosis (i.e., RF^-APF^-). It can also identify the group of patients with a more severe prognosis (i.e., RF^+APF^+), but this group includes both individuals with a less severe (70%) prognosis and those with a more severe (30%) prognosis; these two groups could not be differentiated. Other markers are needed to distinguish the subgroup of individuals with the worse prognosis within the group of RF^+APF^+ patients.

Most studies have reported associations between APF positivity and relatively severe disease. Some investigators concluded that APF had greater specificity (i.e., defining subgroups with less severe disease) rather than an ability to define a subgroup of RA patients with particularly severe disease (6, 29). Our results support this view. Our study design fulfilled the most essential criteria for predictive analyses, i.e. the inclusion of early cases of RA prior to the start of second-line therapy, regular and adequate assessments, and a follow-up of at least 2-3 years for the majority of patients.

The present study presents data based on a recently developed ELISA technique for APF assessment. APF assessment by means of ELISA or IIF revealed comparable results with respect to its prognostic value for severe disease. The ELISA technique has clear advantages over IIF, since its performance and the interpretation of the results are straightforward. APF assessment by IIF requires a specialised laboratory and experienced personnel. Finding a suitable donor of buccal mucosal cells is notoriously difficult. Only about 10% of the general population has 50% or more buccal mucosal cells with keratohyaline granules suitable for use as substrate (12). Variations exist between different donors, between different samples from one donor, and even between the cells within one single sample (30, 31). In addition, there are various criteria for positivity, ranging from a single stained cell (5), a cut-off point at 6 positive cells (16) to a minimum of 10% of cells showing immunofluorescent granules (12, 32). The serum dilution is also critical. Dilution of serum to 1:5 or 1:10 increases the sensitivity of the APF test by almost 30%, while loss of specificity is only 5% (33). In spite of these problems with the IIF technique, a comparative study involving five European laboratories showed only a small inter-laboratory variation when the results were expressed in International Units determined with a WHO standard serum (19).

We conclude that the combination of RF and APF has prognostic value for the course of RA. It can identify individuals with less severe radiological damage in the first years of disease (i.e., RF^-APF^- patients). At the group level, RF^+APF^- and RF^-APF^+ patients have intermediate disease, while RF^+APF^+ patients have the worst prognosis. However, the combination of RF and APF status cannot reliably identify individuals with severe radiological damage. Within the RF^+APF^+ group only a subgroup has severe disease. The combination of RF and APF with other factors must be investigated.

Acknowledgements

The authors thank H. Wolters-Dicke for her expert APF-immunofluorescence determinations, and G.A. van Albada-Kuipers, A.A.M. Blaauw, H.L.M. Brus, H.C.M. Haanen, A.H.M. Heurkens, D.M. Hofman, A.A. Kruize, Y. Schenk, M. van der Veen for their contributions to this article.

References

1. HEIDE A VAN DER, JACOBS JWG, BIJLSMA JWJ *et al.*: The effectiveness of early treatment with "second-line" antirheumatic drugs: A randomized, controlled trial. *Ann Intern Med* 1996; 124: 699-707.
2. YOUNG A, HEIJDE DMFM VAN DER: Can we predict aggressive disease ? *Clin Rheumatol* 1997; 11: 27-48.
3. HEIDE A VAN DER, REMME CA, HOFMAN DM, JACOBS JWG, BIJLSMA JWJ: Prediction of progression of radiological damage in newly diagnosed rheumatoid arthritis. *Arthritis Rheum* 1995; 38: 1466-74.
4. NIENHUIS RLF, MANDEMA E: A new serum factor in patients with rheumatoid arthritis the antiperinuclear factor. *Ann Rheum Dis* 1964; 23: 302-5.
5. SEBBAG M, SIMON M, VINCENT C *et al.*: The antiperinuclear factor and the so-called antikeratin antibodies are the same rheumatoid arthritis-specific autoantibodies. *J Clin Invest* 1995; 95: 2672-9.
6. AHO K, PALUSUO T, KURKI P: Marker antibodies of rheumatoid arthritis: diagnostic and pathogenetic implications. *Semin Arthritis Rheum* 1994; 23: 379-87.
7. HOET RMA, BOERBOOMS AMT, ARENDS M, RUITER DJ, VENROOIJ WJ VAN: Antiperinuclear factor, a marker autoantibody for rheumatoid arthritis: Colocalisation of the perinuclear factor and profilaggrin. *Ann Rheum Dis* 1991; 50: 611-8.
8. VINCENT C, KEYSER F DE, MASSON-BESSIÈRE C, SEBBAG M, VEYS EM, SERRE G: Anti-perinuclear factor compared with the so called "antikeratin" antibodies and antibodies

to human epidermis filaggrin, in the diagnosis of arthritides. *Ann Rheum Dis* 1999; 58: 42-8.

9. BERTHELOT JM, BENDAOUD B, MAUGARS Y, AUDRAIN M, PROST A, YOUINOU P: Antiperinuclear factor of the IgA isotype in active rheumatoid arthritis. *Clin Exp Rheumatol* 1994; 12: 615-9.
10. VON ESSEN R, KURKI P, ISOMAKI H, OKUBO S, KAUTIAINEN H, AHO K: Prospect for an additional laboratory criterion for rheumatoid arthritis. *Scand J Rheumatol* 1993; 22:267-72.
11. MUNOZ S, BALSA A, HALL ND *et al*.: Antiperinuclear factor in early synovitis. *Br J Rheumatol* 1997; 36: 710-2.
12. BERTHELOT JM, MAUGARS Y, AUDRAIN M, YOUINOU P, PROST A: Specificity of antiperinuclear factor for rheumatoid arthritis in rheumatoid factor-positive sera. *Br J Rheumatol* 1995; 34: 716-20.
13. MANERA C, FRANCESCHINI F, CRETTI L, BRAGA S, CATTANEC R: Clinical heterogeneity of rheumatoid arthritis and the antiperinuclear factor. *J Rheumatol* 1994; 21: 2021-5.
14. JANSSENS X, VEYS EM, VERBRUGGEN G, DECLERCQ L: The diagnostic significance of the antiperinuclear factor for rheumatoid arthritis. *J Rheumatol* 1988; 15: 1346-50.
15. CORDONNIER C, MEYER O, PALAZZO E *et al*.: Diagnostic value of anti-ra33 antibody, antikeratin antibody, antiperinuclear factor and antinuclear antibody in early rheumatoid arthritis: Comparison with rheumatoid factor. *Br J Rheumatol* 1996; 35: 620-4.
16. KERSTENS PJ, BOERBOOMS AM, JEURISSEN ME *et al*.: Antiperinuclear factor and disease activity in rheumatoid arthritis. Longitudinal evaluation during methotrexate and azathioprine therapy. *J Rheumatol* 1994; 21: 2190-4.
17. MEYER O, COMBE B, ELIAS A, BENALI K, CLOT J, SANY J, ELIAOU JF: Autoantibodies predicting the outcome of rheumatoid arthritis: evaluation in two subsets of patients according to severity of radiographic damage. *Ann Rheum Dis* 1997; 56: 682-5.
18. WESTGEEST AAA, BOERBOOMS AMT, JONGMANS M, VANDENBROUCKE JP, VIERWINDEN G, PUTTE LBA VAN DE: Antiperinuclear factor: Indicator of more severe disease in seronegative rheumatoid arthritis. *J Rheumatol* 1987; 14: 893-7.
19. FELTKAMP TE, BERTHELOT JM, BOERBOOMS AM *et al*.: Interlaboratory variability of the antiperinuclear factor (APF) test for rheumatoid arthritis. *Clin Exp Rheumatol* 1993; 11: 57-9.
20. SCHELLEKENS GA, JONG BAW DE, HOOGEN FHJ VAN DEN, PUTTE LBA VAN DE, VENROOIJ WJ VAN: Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis specific antigens. *J Clin Invest* 1998; 101: 273-81.
21. GIRBAL-NEUHAUSER E, DURIEUX JJ, ARNAUD M *et al*.: The epitopes targeted by the rheumatoid arthritis-associated antifilaggrin autoantibodies are post-translationally generated on various sites of (pro)filaggrin by determination of arginine residues. *J Immunol* 1999; 162: 585-94.
22. ARNETT FC, EDWORTHY SM, BLOCH DA *et al*.: The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; 31: 315-24.
23. BIJLSMA JWJ, OUDE HEUVEL CHB, ZAALBERG A: Development and validation of the Dutch questionnaire capacities of daily life (VDF) for patients with rheumatoid arthritis. *J Rehabil Sci* 1990; 3: 71-4.
24. BRINK HR VAN DEN, HEIDE A VAN DER, JACOBS JWG, VEEN MJ VANDER, BIJLSMA JWJ: Evaluation of the Thompson articular index. *J Rheumatol* 1993; 20: 28-32.
25. THOMPSON PW, SILMAN AJ, KIRWAN JR, CURREY HLF: Articular indices for joint inflammation with rheumatoid arthritis. Correlation with the acute-phase response. *Arthritis Rheum* 1987; 30: 618-23.
26. SHARP JT, YOUNG DY, BLUHM GB *et al*.: How many joints in the hands and wrists should be included in a score of radiologic abnormalities used to assess rheumatoid arthritis ? *Arthritis Rheum* 1985; 28: 1326-35.
27. NORUSIS MJ: *SPSS Advantaged Statistics User's Guide*. Chicago, SPSS Inc. 1990.
28. HEIDE A VAN DER, JACOBS JWG, HAANEN HCM, BIJLSMA JWJ: Is it possible to predict the first year extent of pain and disability for patients with rheumatoid arthritis? *J Rheumatol* 1995; 22: 1466-70.
29. MEYER O, TAUXE F, FABREGAS D *et al*.: Anti-RA 33 antinuclear autoantibody in rheumatoid arthritis and mixed connective tissue disease: Comparison with antikeratin and antiperinuclear antibodies. *Clin Exp Rheumatol* 1993; 11: 473-8.
30. YOUINOU P, LE GOFF P: The reliability of the antiperinuclear factor test despite the inconsistancy of the targeted antigens. *J Rheumatol* 1994; 21: 1990-1.
31. YOUINOU P, SEIGNEURIN JM, GOFF LE P, DUMAY A, VICARIOT M, LELONG A: The antiperinuclear factor. II. Variability of the perinuclear antigen. *Clin Exp Rheumatol* 1990; 8: 265-9.
32. YOUINOU P, GOFF LE P, DUMAY A, LELONG A, FAQUERT P, JOUQUAN J: The antiperinuclear factor. I. Clinical and serologic associations. *Clin Exp Rheumatol* 1990; 8: 259-64.
33. VEYS EM, KEYSER F DE, VLAM K DE, VERBRUGGEN G: The antiperinuclear factor. *Clin Exp Rheumatol* 1990; 8: 429-31.