Antiperinuclear factors are present in polyarthritis before ACR criteria for rheumatoid arthritis are fulfilled

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Abstract

***Objective*—To show that antiperinuclear factor (APF) may be useful for the diagnosis of rheumatoid arthritis at a time when the American College of Rheumatology (ACR) criteria are not yet fulfilled.**

***Methods*—Testing for APF-IgG (1:100 threshold) and rheumatoid factors (RF) was done in 60 patients with polyarthritis of recent onset during a three year follow up.**

***Results*—At the end of the study, 21/40 rheumatoid arthritis were positive for RF and 31/40 for APF, including 18/40 cases (45%) in which ACR criteria were not yet fulfilled.**

***Conclusions*—APF are useful in the diagnosis of early rheumatoid arthritis.**

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It is often observed that no biological gold standard exists for rheumatoid arthritis comparable to anti-native DNA for systemic lupus erythematosus. Indeed, the specificity of rheumatoid factors (RF) is not sufficient to allow a firm diagnosis of rheumatoid arthritis. However, this concept is being reassessed. Several recently reported autoantibodies appear to be more specific for rheumatoid arthritis,[1 2] especially antiperinuclear factor (APF), provided that the immunofluoresence test is correctly carried out.[3] Moreover a combination of these biological markers may be more effective than a single test for a clear diagnosis of rheumatoid arthritis, as suggested by the finding that a positive test for both RF and APF is more specific for long lasting rheumatoid arthritis than an RF test alone.[4] However, such conclusions could be incorrect for polyarthritis of recent onset. Despite the fact that both APF and RF have been found at low titres in the sera of some patients with rheumatoid arthritis before the onset of their first arthritis,[5] it has not been shown that a higher positivity threshold (for example, 1:80 or 1:100)—affording a better specificity for rheumatoid arthritis—would allow detection of APF in early rheumatoid arthritis and thus be useful for diagnosis at a time when ACR criteria have not yet been fulfilled. To address this question, we tested patients with polyarthritis of recent onset both prospectively and blindly for APF-IgG (1:100 dilution) and rheumatoid factors.

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Methods

PATIENTS

We included all patients seen over a two year period in the rheumatology outpatient clinic of our university hospital (serving an area with half a million inhabitants) who had experienced polyarthritis for less than one year (mean six months). Most patients were referred by general practitioners because of active but as yet unclassified polyarthritis, which might partly explain why about 50% of these cases remained seronegative for RF testing. Patients suffering from monoarthritis or tenosynovitis alone were not included, as well as those whose diagnosis of rheumatoid arthritis had already been made, including three cases of erosive polyarthritis. Moreover, the sera of two APF positive patients had not been tested for APF before validation of the ACR criteria. The first sera were collected on average six months after the onset of initial pain, and other samples were obtained during subsequent visits, at which time ACR criteria[6] were also assessed. Sixty patients were enrolled in this study.

ANTIPERINUCLEAR FACTOR TEST

Once a week, buccal mucosal cells were thoroughly scraped from the inner side of both cheeks of a single healthy volunteer "good donor" [that is, one of 10% of the general population having 50% or more buccal mucosal cells with keratohyaline granules (KHG)] for use as substrate. The keratinisation pattern of buccal mucosa differs between subjects, thus affecting the percentage of cells with KHG which are specific for the granular layer of keratinising epithelium. Cells were collected only once a week to allow reconstitution (with KHG positive cells) of the upper layer of the mucosa, as described elsewhere.[7] The cells were washed (900 $g \times 5$ min) once in phosphate buffered saline (PBS), pH 7.4, once in PBS-Triton X-100 (Sigma) 0.5% vol/vol, and then twice in PBS before being resuspended in PBS (3-4 ml for both cheeks) and transferred dropwise to multispot slides [roughly 600 cells (50 ml) per well]. Thus 60 to 80 wells were available each week. After air drying with a fan, the slides were used as substrate within 24 hours. Sera were diluted 1:100 for screening (as previously optimised[7]). If positive, they were further diluted 1:200, 1:500, and 1:1000 to determine the end point titre. They were then applied to the buccal cell smears for 90 minutes in a moisture chamber. After the preparations had been washed three

Table 1 Characteristics of patients diagnosed as having rheumatoid arthritis (n=40) by the end of the study

ACR criteria	Diagnosis (months)	APF + (months)	Difference (months)	APF titre	Latex (IU)	Rose-Waaler (IU)
5	1	-		-	-	-
4	4	-		-	-	-
4	5	-		-	-	-
4	26	-		-	-	-
4	35	-		-	-	-
4	39	-		-	-	-
4	40	-		-	-	-
4	41	-		-	-	-
5	2	-		-	100	64
4	24	26	2	1:5000	100	32
5	11	12	1	1:500	50	64
4	2	2	0	1:1000	50	0
6	2	2	0	1:100	100	32
6	3	3	0	1:10 000	100	16
4	3	3	0	1:100	0	0
4	5	5	0	1:200	25	32
4	5	5	0	1:100	0	0
4	5	5	0	1:2000	100	0
5	6	6	0	1:100	0	0
4	7	7	0	1:1000	0	32
4	10	10	0	1:200	0	0
6	11	11	0	1:500	50	64
5	1	0	−1	1:2000	50	0
4	8	6	−2	1:500	50	64
4	8	5	−3	1:200	50	0
4	8	6	−2	1:100	0	0
4	9	8	−1	1:500	0	0
4	10	6	−4	1:1000	100	32
5	11	0	−11	1:500	0	0
4	12	6	−6	1:10 000	100	64
4	13	6	−7	1:500	50	0
4	14	5	−9	1:100	0	0
4	15	9	−6	1:100	50	32
4	16	12	−4	1:100	0	0
4	17	9	−8	1:1000	0	0
4	17	2	−15	1:500	0	0
4	26	10	−16	1:500	100	64
4	29	20	−9	1:200	50	32
4	40	25	−15	1:2000	100	128
4	41	1	−40	1:100	50	32

ACR, American College of Rheumatology; APF, antiperinuclear factors.

times in PBS, they were incubated for 30 minutes with fluorescein conjugated goat anti-human IgG (Kallestad) (the same antiglobulin batch for all tests) and diluted 1:50 in PBS. At least 300 cells were examined for each well, and the diluted sera were regarded as positive if at least 10% of cells (from the good donor showing 70% positive cells with strongly positive sera) had clearly recognisable and fluorescent KHG. The APF test requires no more than half a day (for up to 80 sera) and can be performed by any laboratory already equipped for immunofluorescence tests. With this technique, our intraobserver reproducibility was always ≤ 1 dilution (50 sera tested blindly at various dilutions).

LATEX AND ROSE-WAALER TESTS

Latex and Rose-Waaler tests were performed using commercial agglutination kits (Rhumalatex and Polyartest; Fumouze Laboratories, Asnières, France). The threshold of positivity was 100 IU for latex and 32 IU for Rose-Waaler.

Table 2 Number of rheumatoid arthritis (RA) sera positive for antiperinuclear factors (APF) and/or rheumatoid factor (RF) by the end of the study

	Number of RA patients
RF+/APF+	20
RF−/APF+	11
RF+/APF−	1
RF−/APF−	8

Results

After 36 months of follow up, eight patients were still unclassified and 52 could be ascribed to definite diseases or syndromes, including three with palindromic rheumatism, three with polymyalgia rheumatica, three with psoriatic rheumatism, one with sicca syndrome, one with thyroiditis, one with hypertrophic osteoarthropathy, and 40 with rheumatoid arthritis. At that time, 36 patients were or had been positive for APF (1:100 dilution) (31 with rheumatoid arthritis and five with other diagnoses) and 24 had remained negative. Twenty of the 24 negative tests were quite negative, and four had titres below 1:100 (that is, less than 10% of cells recognised at the 1:100 threshold). APF titres were 1:100 in 10 cases (nine with rheumatoid arthritis and one with unclassified polyarthritis), 1:200 in four cases (all rheumatoid arthritis), 1:500 in nine cases (eight with rheumatoid arthritis and one with palindromic rheumatism), 1:1000 in four cases (three with rheumatoid arthritis and one with sicca syndrome), 1:2000 in five cases (three with rheumatoid arthritis and two with palindromic rheumatism), and ≥ 1:5000 in three cases (all rheumatoid arthritis). In this group of 60 patients, the sensitivity of APF for rheumatoid arthritis was thus already 0.77 (31/40), with a specificity of 0.75 (5/20): five false positive sera, including three patients with palindromic rheumatism (one at 1:500 and two at 1:2000), one with sicca syndrome with arthritis (1:1000), and one with unclassified polyarthritis (1:100). Interestingly, two cases initially classified as palindromic rheumatism and already positive for APF later fulfilled the criteria for rheumatoid arthritis. APF positivity was noted for the first time at an average of 7.5 months (0-26) after onset of the first arthritis but could have been present before since several patients had not been tested during the first six months. Nineteen of the 36 patients positive for APF were subsequently tested twice or more at least one year after the first test. The titre remained unchanged in 8/19 cases, rose by one dilution or more in four cases, and fell by two dilutions or more in seven cases. However, only 2/19 sera became negative (one case of rheumatoid arthritis and one of palindromic rheumatism). In 18/40 rheumatoid arthritis cases (45%), APF were positive (≥ 1:100) when ACR criteria were not yet fulfilled (table 1). Among the remaining 22 rheumatoid arthritis cases, 11 were also positive for APF when four ACR criteria were present for the first time. Thus, when four ACR criteria were present for the first time, the APF titre was already ≥ 1:100 in 11 + 18 = 29/40 rheumatoid arthritis cases (72%), for an average of 7.1 months (0-40). Conversely, RF were then positive (latex or Rose-Waaler) in only half of cases (21/40) (table 1). APF became positive shortly after the validation of ACR criteria in only two cases, so that at the end of the study 31/40 rheumatoid arthritis sera had been tested as positive for APF at least once (table 2).

Discussion

Recognition of the true value of APF has long been hindered by methodological errors. Sera were only diluted 1:5 or 1:10, even though the APF titre can reach 1:20 000 in rheumatoid arthritis sera and is usually above 1:200. Although a 1:5 threshold for APF gave very good sensitivity (0.86) and specificity (0.96) for rheumatoid arthritis in one study involving 389 patients,[8] disappointing results with this low dilution led other investigators to conclude that APF is not very specific for rheumatoid arthritis.[9] Obviously, if the threshold for anti-DNA had been set too low, it would have been difficult to determine the high diagnostic value of anti-DNA for systemic lupus erythematosus. Two recent studies performed with a 1:80 (606 patients)[7] and a 1:100 dilution (1004 patients)[3] concluded that APF was highly specific for rheumatoid arthritis (0.97 and 0.94 respectively) and still quite sensitive (0.75 and 0.80 respectively), at least in the context of the two rheumatology inpatient clinics in which these studies were conducted (active rheumatoid arthritis versus various rheumatic disorders). It has been claimed that optimal sensitivity can be obtained if the test is performed within 48 hours after fixation of the cells. Storage of the slides, even when frozen, has a dramatic effect on the antigenicity of the substrate.[10] The need to obtain good sensitivity may explain why most investigators have diluted sera only 1:5 and why the best results in previous work were achieved with fresh slides.[8]

In the present study, APF-IgG (at a 1:100 dilution with fresh slides) was detectable early in the disease, generally before the four ACR criteria. This is in agreement with the findings of Aho *et al*, who showed that APF could be present in the sera of rheumatoid arthritis patients several years before the onset of the first clinical signs,[5] and with the conclusions of Saraux *et al*,[11] who reported that APF 1:80 was more useful than rheumatoid factor for the diagnosis of early rheumatoid arthritis.

Conversely, it has been reported that so called "antikeratin" antibodies (AKA) highly specific for rheumatoid arthritis[12] were present in the first six months of rheumatoid arthritis in only 11% of cases.[13] However, the slides used by these authors had been frozen and stored, which could have dramatically lowered the sensitivity of the substrate, as has been shown for APF,[10] especially since both antibodies have similar target antigens. Indeed, the target for APF has recently been identified as profilaggrin by the same team which previously demonstrated that AKA in fact recognised filaggrin.[14] As filaggrin is processed from profilaggrin, these two antibodies might measure different epitopes of a single antigen.[14]

An enzyme linked immunosorbent assay for these antibodies is unlikely to be available in the near future as the antigen is fragile. Thus, the APF test, which is cost-effective, will continue to be quite useful for rheumatologists, especially since APF are present early in the course of polyarthritis of recent onset.

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