DIAGNOSTIC VALUE OF ANTI-RA33 ANTIBODY, ANTIERKERIN ANTIBODY, ANTIPERINUCLEAR FACTOR AND ANTINUCLEAR ANTIBODY IN EARLY RHEUMATOID ARTHRITIS: COMPARISON WITH RHEUMATOID FACTOR

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SUMMARY

The goal of this prospective longitudinal study was to determine the serological profile of early rheumatoid arthritis (RA), and to test whether antikeratin antibody (AKA), antiperinuclear factor (APF), anti-RA33 antibody and antinuclear antibodies (ANA) had an additional diagnostic value when prescribed after rheumatoid factor (RF)-detecting methods. Sixty-nine patients with early polyarthritis suggestive of RA, seen between 1991 and 1993, were included. Five autoantibodies (i.e. RF, AKA, APF, RA33, ANA) were looked for at regular intervals. After 24 months follow-up, patients were classified as having RA (n = 49), unclassified polyarthritis (UP; n = 15) or other rheumatic diseases. Among patients with early RA, the sensitivity of these markers was 40.8% for RF, 36.7% for AKA, 28.6% for APF and 28.6% for anti-RA33. Among RF-negative RA patients, 51.7% were positive for AKA, APF, anti-RA33 antibodies and/or ANA. Positivity of the three recent markers usually persisted throughout follow-up, whereas RF was lost by 58% of patients with early, RF-positive, treated RA. Using multivariate analysis, only latex, RF test and AKA or APF had an independent and statistically significant diagnostic value for early RA. Our data suggest that RF and AKA (or APF) should be concomitantly determined for diagnosis in patients with suspected early RA.

KEY WORDS: Early rheumatoid arthritis, Anti-RA33 antibody, Antikeratin antibody, Antiperinuclear factor.

The diagnosis of rheumatoid arthritis (RA) rests on American Rheumatism Association (ARA) criteria [1], which are clinical, radiological and immunological (rheumatoid factor, RF). During the first months after onset, the diagnosis is often difficult because of a frequently unsuggestive presentation and a lack of radiological changes. Rheumatoid factor has low specificity and is often negative in early RA. The identification of more specific immunological markers detectable early in the disease would be very useful.

Three markers have been found to be more specific but less sensitive than rheumatoid factor. Two are directed against epithelial cell components: antiperinuclear factor (APF), first described in 1964 by Nienhuis and Mandema [2], and antikeratin antibody (AKA), described in RA patients in 1979 by Young et al. [3]. Recent data indicate that APF and the so-called antikeratin antibodies are in fact the same RA-specific autoantibodies [4]. Anti-RA33 antibody, identified in 1989 by Hassfeld et al. [5], is specific for a nuclear protein from HeLa cells [6].

The usefulness of these three markers for the diagnosis of RA has been demonstrated by many studies, which found high specificity, as well as positivity, in some RF-negative patients.

Disease duration does not seem to influence the rate of occurrence of these antibodies, which have been detected <1 yr after disease onset [7-11]. These markers may, therefore, be particularly helpful for the diagnosis of early disease.

To investigate this hypothesis, we conducted a prospective longitudinal study in patients with suspected early RA. RF, AKA, APF, anti-RA33 antibody and antinuclear antibody (ANA) were looked for at study entry and during the 12–24 month follow-up period. The purpose of the study was to evaluate the diagnostic value of each serological marker and of associations of markers by determining the serological profile of early RA, i.e. the time to the appearance of each serological marker and changes in marker status over time.

Our aim was to test whether AKA, APF, anti-RA33 antibody and ANA had an additional value when prescribed after latex and Waaler–Rose tests.

PATIENTS AND METHODS

Patients

Patients with suspected early RA evaluated between 1991 and 1993 in the rheumatology department or out-patient clinic of our institution were included in the study, provided they had the following inclusion criteria: isolated, bilateral, roughly symmetrical oligoarthritis or polyarthritis of >6 weeks but <12 months duration; absence of monoarthritis, markedly asymmetrical oligoarthritis or polyarthritis; absence of arthritis due to a disease other than RA (e.g. psoriasis, spondyloarthropathies, systemic lupus erythematosus or other connective tissue diseases). Consequently, patients were not required to meet ARA criteria at inclusion.
Sixty-nine patients (52 females and 17 males), mean age 50 yr (range 18–82 yr), were included. Median disease duration at inclusion was 6 months (range 1–15 months) and exceeded 12 months in only one patient.

Before study entry, some of the 45 RA patients with active disease received second-line therapy with methotrexate, gold, hydroxychloroquine or prednisone in dosages >15 mg/day (n = 8); most, however, received only non-steroidal anti-inflammatory drugs or low-dose steroid therapy.

**Methods**

**Diagnostic criteria.** The diagnosis was re-evaluated periodically during the 12–24 month follow-up period. Patients were categorized as having established RA, unclassified polyarthritis or other rheumatic diseases. The diagnosis of RA was based on 1987 ARA criteria [1].

**Biological methods.** Blood specimens for the determination of the serological markers for RA were obtained at study entry and, whenever possible, 6–12 and 12–24 months later. Five markers were looked for: RF, AKA, APF, anti-RA33 antibody and ANA.

To minimize assay error, when results in a given patient changed over time, the sera obtained from that patient at all the study time points were re-examined during a single run.

Anti-RA33 IgG antibodies were detected using a variant [10] of the immunoblot technique developed by Hassfeld et al. with sera diluted 1/20 [5]. AKA IgG were looked for using indirect immunofluorescence as described by Young et al. [3], with a few modifications [9]; sera were diluted 1/20. IgGAM APF were detected using indirect immunofluorescence on human buccal cells; sera were diluted to 1/40 to improve specificity

**Statistical methods.** To assess the diagnostic value of the tests, we used sensitivity, specificity and positive predictive values. The positive likelihood ratio (LR) was calculated as sensitivity/(1 − specificity) and the negative likelihood ratio was calculated as (1 − sensitivity)/specificity. The 95% confidence intervals of likelihood ratios were calculated according to the method of Centor [12]. To determine the independent contribution of each test to the diagnosis, and whether a combination of tests could improve diagnostic accuracy, stepwise logistic regression procedures were used. The status of RA (present or absent) was used as the dependent variable and the values of the dichotomized diagnostic tests as the independent variables.

Logistic regression analysis was performed with the BMDP statistical software (BMDP Statistical Software, Inc., 1441 Sepulveda Boulevard, Suite 316, Los Angeles, CA 90025, USA). We considered a P value of <0.10 as statistically significant.

**RESULTS**

**Description of the study population at the end of follow-up**

Forty-nine of the 69 patients (38 females and 11 males) were diagnosed with RA, among whom four RA patients (8.2%) met ARA criteria for disease remission [13].

Fifteen patients had unclassified polyarthritis (UP). In seven cases (46.7%), a remission occurred before a diagnosis could be established. Five patients had polyarthritis due to a disease other than RA (RS3PE in two cases, psoriatic arthritis in two and spondyloarthropathy in one).

After study entry, two patients with mild RA responded satisfactorily to low-dose steroid therapy and four to hydroxychloroquine. The other RA patients required second-line therapy with gold, D-penicillamine, methotrexate or sulphasalazine.

**Proportions of patients with a positive test in patients with RA, UP and other diagnoses (Table I)**

Among the patients with early RA, the proportion of RF-positive (latex and/or Waaler–Rose) patients was 40.8%. Proportions of patients with the other markers studied ranged from 8.4% for ANA to 36.7% for AKA; APF and anti-RA33 antibody were each found in 28.6% of early RA patients. Proportions of UP patients with positive markers were, respectively, 13.3% for RF, 0% for AKA, 6.7% for APF, 20% for anti-RA33 and 13.3% for ANA. Patients classified as RA were stratified according to RF status. AKA and APF were more frequent in the subgroup with a positive latex test (Table II). Among the 29 early RA

<table>
<thead>
<tr>
<th>Rate of occurrence</th>
<th>RA (%</th>
<th>UP (%</th>
<th>Other diagnosis (%</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF*</td>
<td>20 (40.8)</td>
<td>2 (13.3)</td>
<td>0</td>
</tr>
<tr>
<td>AKA</td>
<td>18 (36.7)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>APF</td>
<td>14 (28.6)</td>
<td>1 (6.7)</td>
<td>0</td>
</tr>
<tr>
<td>RA33</td>
<td>14 (28.6)</td>
<td>3 (20)</td>
<td>0</td>
</tr>
<tr>
<td>ANA</td>
<td>9 (18.4)</td>
<td>2 (13.3)</td>
<td>0</td>
</tr>
</tbody>
</table>

**TABLE I**

Rates of occurrence of serological markers for RA in a group of 69 patients with early polyarthritis.

RA, rheumatoid arthritis; UP, undifferentiated polyarthritis.

*RF positive: latex and/or Waaler–Rose test.

†Latex positive only: 3/20; Waaler–Rose positive only: 1/20.
Follow-up period of 41 RA patients

AKA, APF and ANA

Marker No. (%) of RA patients (n — 41) with delayed appearance of ANAs was 12.2%.

There was one case of delayed seroconversion for each of these three markers. Among the 41 patients with more than one serological evaluation, two developed high titres of RF 12 months after study entry; both were on methotrexate, with satisfactory efficacy against clinical symptoms. Thus, the proportion of RA patients with delayed appearance of RF was 4.9% (Table III).

Variations in study markers over the follow-up period

Ten of the 17 initially RF-positive patients (58%) lost RF during the follow-up period, after 5-11 months. Seven of these 10 patients had other markers, which persisted throughout follow-up.

In each of the 10 patients, RF was lost at a time when the joint disease was well controlled by second-line drug therapy (methotrexate, n = 4; gold, n = 3; sulphasalazine, n = 2; hydroxychloroquine, n = 1) with, in three cases, low-dose prednisone (<10 mg/day).

The mean initial RF titre was 2.5-fold lower in RA patients who became RF negative during follow-up than in those who remained RF positive (P = ns; Wilcoxon test).

At the end of the follow-up period, three of the 13 RA patients who were anti-RA33 antibody positive at study entry were negative for this marker (although one had positive tests at later dates). In two of these patients, the joint disease was well controlled by D-penicillamine or sulphasalazine. The third patient lost anti-RA33 antibody at the time of a flare following a remission.

Positive tests for AKA and/or APF did not become negative in any of the patients during the follow-up period.

Diagnostic value

The diagnostic value of markers for early RA is summarized in Table IV. RF, AKA and APF had the best diagnostic value with specificities >90%, but low sensitivities. RA33 and ANA had the lowest likelihood ratios, explained by the lowest sensitivity for ANA and the lowest specificity for anti-RA33. According to Jaeschke et al. [14], a positive likelihood ratio associated with these various tests could induce only small changes in post-test probabilities (i.e. had a relatively low diagnostic value).

Association of tests

In multivariate analysis with a model considering APF without AKA, only latex and APF had an independent and statistically significant diagnostic value, whereas in a second model considering AKA without APF, only latex and AKA had an independent and statistically significant diagnostic value. The remaining four predictors, including Waaler-Rose, had no additional diagnostic value. The diagnostic value of these two combinations of diagnostic tests is shown in Table IV. The best one is the association of latex with AKA.

| TABLE II |

<table>
<thead>
<tr>
<th>Marker</th>
<th>RA-positive*</th>
<th>RF-negative</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKA</td>
<td>18</td>
<td>11/20-55%</td>
<td>7/29-24.1%</td>
</tr>
<tr>
<td>APF</td>
<td>14</td>
<td>9/20-45%</td>
<td>5/29-17.2%</td>
</tr>
<tr>
<td>RA33</td>
<td>14</td>
<td>6/20-20%</td>
<td>8/29-27.6%</td>
</tr>
<tr>
<td>ANA</td>
<td>9</td>
<td>4/20-20%</td>
<td>5/29-17.2%</td>
</tr>
<tr>
<td>At least one marker</td>
<td>32</td>
<td>17/20-85%</td>
<td>15/29-51.7%</td>
</tr>
</tbody>
</table>

*RF positive: latex and/or Waaler-Rose test.

In all cases of delayed positivity of the serological test.

Includes those patients with transiently positive tests.

†RF: latex and/or Waaler-Rose test.

| TABLE III |

<table>
<thead>
<tr>
<th>Marker</th>
<th>Total positive*</th>
<th>Positive at entry (%)</th>
<th>Delayed positivity (%)</th>
<th>Mean RA duration at first positive test† (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF‡</td>
<td>19 (46.3)</td>
<td>17 (41.5)</td>
<td>2 (4.9)</td>
<td>22</td>
</tr>
<tr>
<td>AKA</td>
<td>17 (41.5)</td>
<td>16 (39.0)</td>
<td>1 (2.4)</td>
<td>11</td>
</tr>
<tr>
<td>APF</td>
<td>13 (31.7)</td>
<td>12 (29.3)</td>
<td>1 (2.4)</td>
<td>31</td>
</tr>
<tr>
<td>RA33</td>
<td>14 (34.1)</td>
<td>13 (31.7)</td>
<td>1 (2.4)</td>
<td>11</td>
</tr>
<tr>
<td>AAN</td>
<td>12 (29.3)</td>
<td>7 (17.0)</td>
<td>5 (12.2)</td>
<td>12</td>
</tr>
</tbody>
</table>

*Includes those patients with transiently positive tests.
†For patients with delayed positivity of the serological test.
‡RF: latex and/or Waaler-Rose test.

| TABLE IV |

<table>
<thead>
<tr>
<th>Marker</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Likelihood + ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latex</td>
<td>38.8</td>
<td>90.0</td>
<td>90.5</td>
<td>37.5</td>
<td>3.88 (0.99-14.99)</td>
</tr>
<tr>
<td>Waaler-Rose</td>
<td>32.6</td>
<td>90.0</td>
<td>88.9</td>
<td>35.3</td>
<td>3.26 (0.82-12.82)</td>
</tr>
<tr>
<td>RF</td>
<td>40.8</td>
<td>90.0</td>
<td>90.9</td>
<td>38.3</td>
<td>4.08 (1.05-15.73)</td>
</tr>
<tr>
<td>AKA</td>
<td>36.7</td>
<td>100.0</td>
<td>100.0</td>
<td>39.2</td>
<td>ns</td>
</tr>
<tr>
<td>APF</td>
<td>28.6</td>
<td>95.0</td>
<td>93.3</td>
<td>35.2</td>
<td>5.71 (0.80-40.1)</td>
</tr>
<tr>
<td>RA33</td>
<td>28.6</td>
<td>85.0</td>
<td>82.3</td>
<td>32.7</td>
<td>1.9 (0.61-5.89)</td>
</tr>
<tr>
<td>ANA</td>
<td>8.4</td>
<td>90.0</td>
<td>81.8</td>
<td>33.9</td>
<td>1.83 (0.43-7.69)</td>
</tr>
<tr>
<td>Latex + AKA</td>
<td>55.1</td>
<td>90.0</td>
<td>93.1</td>
<td>45.0</td>
<td>5.51 (1.44-20.82)</td>
</tr>
<tr>
<td>Latex + APF</td>
<td>51.0</td>
<td>85.0</td>
<td>89.3</td>
<td>41.5</td>
<td>1.16-9.93</td>
</tr>
</tbody>
</table>

PPV, positive predictive value; NPV, negative predictive value.
*RF = latex and/or Waaler-Rose test.
DISCUSSION

This prospective longitudinal study of a cohort of patients with early RA provided data on the serological profile in early RA and on changes in serological markers over time.

Among our patients with suspected early RA at study entry, 49 had confirmed RA, 15 had UP and five had other inflammatory joint diseases. Complete remission occurred in 8.2% of RA patients and 46.7% of UP patients. Similar proportions were found in a prospective study by Wolfe et al. [15].

AKA, APF and anti-RA33 antibodies were detectable in 36.7, 28.6 and 28.6%, respectively, of our patients with early RA. Thus, these markers may be useful for the early diagnosis of polyarthritis. ANAs were positive in only 8.4% of cases. Comparable proportions of AKA- and anti-RA33-positive patients were found in studies that were published after the initiation of our study. Hassfeld et al. [16] found that 29% of patients with RA of <3 months duration had anti-RA33 antibodies. The frequency of AKA positivity was 32.9% in the study by Kurki et al. [17] and 38% in the study by Paimela et al. [18]. However, the frequency of APF was lower in our patients than in those studied by Kurki et al. [17] (55.3% of 146 patients with early RA). There was a similar difference with a study in more remote-onset RA [10]. In our study, dilution of specimens to 1/40 allowed specificity to increase to 93% at the expense of a decrease in sensitivity (44% in our earlier study with long-standing RA patients [10]).

Frequencies of RF, AKA and APF were significantly lower in this group of early RA patients than in previous studies including patients with established RA [9]. No such difference was seen for anti-RA33 antibodies [10]. The low sensitivities of AKA and APF can be ascribed to the fact that 55% of patients were negative for RF. This explanation is corroborated by the incidence of RA33 antibodies which is equivalent in RA patients with or without RF [10].

Few of our patients had delayed appearance of RF (4.9% of the 41 patients with more than one serological evaluation). This is in keeping with most previously published studies [19–21]. Similarly, AKA, APF and anti-RA33 antibodies (but not ANAs) were usually present at the first evaluation in those patients who were positive for these markers.

The early positivity of AKA, APF and anti-RA33 antibodies in the absence of RF is thus useful for the early diagnosis of RA. We compared in this study the diagnostic value of different markers of RA: latex, Waaler–Rose, AKA, APF and anti-RA33 antibodies, using several diagnostic tests. To improve the diagnostic value of these tests in a population of early RA, we evaluated the interest of associating several markers. Associations of markers are rare in the population control (1/20 vs 22/49 in RA), especially as some patients with UP eventually turn out to have RA after the 24 month follow-up. A multivariate analysis showed the identification of two associations of markers which had an independent and statistically significant diagnostic value. The other markers, and in particular the Waaler–Rose test, did not provide any additional information. The association which presented the best diagnostic value is the association of latex and AKA. When AKA are prescribed in addition to latex, the diagnostic value of the test is significantly improved: the sensitivity and NPV are increased without alteration of specificity and PPV. This association of tests could be proposed routinely to improve the diagnosis of early RA.

Over time, changes were more common for RF than for the other markers studied. Fifty-eight per cent of patients with RF at study entry became RF negative during follow-up. These findings differ from those of most previous studies [21, 22]. However, a few studies found similar results. Masi et al. [19] reported loss of RF in 55% of patients with early RA; these patients had similar outcomes to those seen in patients with persistent positivity. Michotte and Vanslype [23] found that 55% of RF-positive patients given gold therapy became RF negative and that RF titres fell by 50% or more in an additional 24.5%; these changes occurred only in patients with a disease duration of <1 yr, who were characterized by the lowest initial agglutination titres, as in our study.

We also found that anti-RA33 antibody was lost by some patients with disease remission, whereas APF and AKA remained positive regardless of disease activity. As multivariate analysis has shown that RF and APF or AKA are independent parameters, these data suggest that the simultaneous determination of both type of autoantibodies is of high practical value for the serological confirmation of clinically suspected RA. Further studies are warranted to determine the articular prognostic value of these different autoantibodies in patients with early RA. Preliminary data based on a retrospective cohort suggest that indeed AKA and HLA DR4 or DR1 are independent prognostic factors for severe RA [24].

ACKNOWLEDGEMENT

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