The antiperinuclear factor. 1. The diagnostic significance of the antiperinuclear factor for rheumatoid arthritis


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SUMMARY In 1964 Nienhuis and Mandema reported the presence of antibodies against cytoplasmic granules in buccal mucosal cells in the serum of 50% of patients with rheumatoid arthritis (RA). Although they reported a good specificity for RA of these so-called antiperinuclear antibodies (APF), their results never threatened the monopoly of the rheumatoid factor as a serological tool for the diagnosis of RA. A re-evaluation with improved immunofluorescence methods showed a frequency of the APF of 78% in 103 patients with RA. The latex test and the Waaler-Rose test were positive in only 70% and 58% respectively of these patients. Only 15% of the RA patients were negative for all 3 tests. Thus, 40% of patients who were seronegative by the traditional methods gave a positive result on performance of the APF test. The high sensitivity of the APF test was combined with a good specificity, for the frequency in patients with other autoimmune diseases of degenerative joint disease and in healthy subjects was low. For the serodiagnosis of RA it seems best to combine the use of the APF test with one for rheumatoid factor.

Serological support for the diagnosis of rheumatoid arthritis (RA) is mainly based on the detection of rheumatoid factors (RF). This antiglobulin activity is normally detected in agglutination techniques such as the latex-fixation test and the Waaler-Rose test. Nienhuis and Mandema (1964) described antibodies defined as antiperinuclear factor (APF), which they found in the serum of about 50% of patients with RA.

Using the indirect immunofluorescence technique (IFT) on human buccal mucosa cells as substrate, they showed that this factor consists of immunoglobulins, which react with cytoplasmic spherical granules 0.5–4 μm in diameter with a predominantly perinuclear localisation. The antigenic substrate appeared to be both organ- and species-specific, inasmuch as it was not found in any human epithelial cells other than those from the oral mucosa, nor was it present in the oral mucosa of animals.

The original findings were confirmed by others (Visconti et al., 1964; Cava et al., 1965; del Giacco and Mazzei, 1965; Tursi et al., 1966; Marmont et al., 1967; Roques, 1969). From all these papers, except that of Roques it is evident that the APF is highly specific for RA with a sensitivity of about 50%.

It seemed possible that improvements of immunofluorescence techniques and reagents might remove the major drawback of the method: the moderate sensitivity of only 50%. In this investigation we have re-evaluated the diagnostic value of the APF test by comparing its sensitivity and specificity with tests for rheumatoid factor.

Materials and methods

SERA The specificity of the APF test was assessed by testing the serum of 103 patients with classical RA fulfilling the American Rheumatism Association criteria, 94 with systemic lupus erythematosus (SLE), 17 with Sjögren's syndrome, 17 with scleroderma, 30 with an autoimmune liver disease (primary...
biliary cirrhosis, active chronic hepatitis, or cryp-
togenic cirrhosis with autoantibodies), 30 with myas-
thenia gravis, 30 with an autoimmune thyroiditis (thyr-
otoxicosis, Hashimoto’s disease, or myxoedema), 30
with autoimmune gastritis (pernicious anaemia, a
positive parietal cell fluorescence test with or with-
out antibodies to intrinsic factor, or vitamin B_{12}
deficiency), 30 with Addison’s disease, 30 with
autoimmune haemolytic anaemia, 32 with degen-
erative joint disease, 36 with ankylosing spondylitis,
30 with Crohn’s disease, and 30 with ulcerative
colitis. The sera of 111 matched blood donors and
inmates of homes for the elderly were also studied.

The various clinical diagnoses were made by the
following clinicians: Dr A. E. Schipper (Amster-
dam Centre for Rheumatic Diseases), Dr A. J. G. Swaak
(Slotervaart Hospital, Amsterdam), Dr S. G. M.
Meuwissen (University Hospital, Amsterdam),
and Dr L. K. J. van Romunde (University Hospital,
Leiden).

ANTIGENIC SUBSTRATES
Epithelial cells from human oral mucosa were used
as substrate. Normal human buccal epithelial cells
were scraped from the inside of the cheek with a
piece of foam plastic. To obtain a cell suspension, the
foam plastic was rinsed in phosphate-buffered saline
(PBS), pH 7–4. The cell suspension was washed 3
times with PBS in a table centrifuge (5 min, 1500
rpm). A cell suspension was made in PBS, and the
cells were transferred dropwise to microscope slides,
100–200 cells per slide. After air drying at room
temperature for 15 min the cell preparations were
used as a substrate in the IFT.

IMMUNOFLUORESCENCE TECHNIQUE (IFT)
The slides with buccal cells were incubated with the
serum (diluted 1:5 in PBS) of the patients or healthy
controls for 90 min in a humid atmosphere. After
the preparations had been washed in PBS (3 × 10
min), they were incubated for 30 min in the presence
of horse antibodies to human Ig that had been label-
led with fluorescein isothiocyanate (FITC) and
prepared in our laboratory (lot no. PH-17-4-F8).
Another washing with PBS, 3 × 10 min, preceded a
final incubation with a solution of ethidium bromide
(100 μg/ml in PBS) as a nuclear counter-stain. The
preparations were washed with PBS for 10 min and
mounted in a glycerol PBS solution (1:1). The slides
were read under a Leitz Orthoplan fluorescence
microscope.

RHEUMATOID FACTOR DETERMINATIONS
A modified Waaler-Rose test (van Loghem-Lan-
gereis 1952; Feltkamp and van Rossum, 1968) was per-
formed on human group O erythrocytes coated with
rabbit antihuman erythrocyte immunoglobulins
in a subagglutinating concentration. For testing a
serial dilution of patients’ sera a final concentra-
tion of 2 × 10^{8} cells/ml was used. Agglutination
was observed macroscopically after a 2 h incubation
period at 37°C. A titre of 1 in 16 or more was con-
sidered to represent a positive reaction.

Latex agglutination test. The slide test for the
detection of conventional RF was obtained from
Hyland Division, Travalen Laboratories SA, Brus-
se. The IgG used to coat the polystyrene latex
was of human origin. A titre of minimally 1 in 20
was considered to be a positive result.

Results
The serum from 103 patients suffering from definite
RA was examined for the presence of APF. Table 1
shows that 80 of the 103 sera (78%) gave positive
results. RF was detected in 72 of the 103 sera (70%)
by the latex fixation test and in 60 of the 103 sera
(58%) by the Waaler-Rose test. APF was found in
the serum of only 4 of 111 healthy controls, matched
for age and sex. Two of these 111 sera were positive
in the latex-fixation test and 2 others in the Waaler-
Rose test. In our hands, therefore, more sera were
found to be APF-positive than RF-positive, and
there was not a high number (4%) of positive
reactions among a population of normal persons.

To verify the specificity for RA of a positive
APF the sera were examined from 13 groups of
patients suffering from degenerative joint disease
and various autoimmune diseases. The frequency of
APF was not significantly increased in patients suffering
from degenerative joint disease, ankylosing spondyl-
itis, Crohn’s disease, ulcerative colitis, SLE,
scleroderma, myasthenia gravis, autoimmune liver
disease, autoimmune Addison’s disease, autoimmune
gastritis, or autoimmune haemolytic anaemia
(Table 2).

However, there was a significantly increased fre-
quency of APF in patients with Sjögren’s syndrome
and in patients with autoimmune thyroiditis. An
increased occurrence of APF in Sjögren’s syndrome
was not unexpected, as the diagnosis of this syn-
drome is based on a triad, one of which is the pre-
ence of RA. Three patients with autoimmune

<table>
<thead>
<tr>
<th></th>
<th>Number tested</th>
<th>APF</th>
<th>Latex</th>
<th>Waaler-Rose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheumatoid arthritis</td>
<td>103</td>
<td>78</td>
<td>70</td>
<td>58</td>
</tr>
<tr>
<td>Healthy subjects</td>
<td>111</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
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</table>
thyroiditis, whose serum gave a positive APF reaction, proved to suffer from RA when further examined.

The specificity of APF in the serological diagnosis of RA compared with that of the latex-fixation test is shown in Table 3. The frequency of APF in the serum from patients with SLE and autoimmune liver diseases was low compared with that of the latex-fixation test.

Table 4 shows the correlation between the results of the APF and the two RF tests on RA sera. In 77 out of 103 RA sera an RF was detected, either by latex or Waaler-Rose agglutination. The group of 26 RF-negative patients (seronegative) declined to 15 when APF was used as an additional serological parameter. Therefore the gain in diagnostic information was about 40% when the APF was used as an additional serological tool.

In only 8 out of 103 sera tested was APF negative, whereas RF appeared to be detectable by one of the 2 methods applied. When the 3 tests were used, only 15% of the RA sera tested appeared to be seronegative. This combination of APF and RF tests is therefore recommended.

Table 2  Frequency of occurrence of APF in sera of various groups of patients

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Sera tested</th>
<th>APF-positive Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheumatoid arthritis</td>
<td>103</td>
<td>80</td>
<td>78</td>
</tr>
<tr>
<td>Sjögren's syndrome</td>
<td>16</td>
<td>5</td>
<td>31</td>
</tr>
<tr>
<td>Degenerative joint disease</td>
<td>32</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Ankylosing spondylitis</td>
<td>36</td>
<td>2</td>
<td>5.5</td>
</tr>
<tr>
<td>Crohn's disease</td>
<td>30</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>30</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>SLE</td>
<td>94</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Scleroderma</td>
<td>17</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Myasthenia gravis</td>
<td>30</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Autoimmune liver disease</td>
<td>30</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Autoimmune gastritis</td>
<td>30</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Autoimmune haemolytic anaemia</td>
<td>30</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Autoimmune thyroiditis</td>
<td>30</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td>Healthy persons</td>
<td>111</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 3  Comparison of specificity between APF and RF determination by latex-fixation

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Sera tested</th>
<th>APF-positive Number</th>
<th>%</th>
<th>Latex-positive Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheumatoid arthritis</td>
<td>103</td>
<td>80</td>
<td>78</td>
<td>72</td>
<td>70</td>
</tr>
<tr>
<td>SLE</td>
<td>94</td>
<td>8</td>
<td>9</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>Autoimmune liver diseases</td>
<td>30</td>
<td>2</td>
<td>6</td>
<td>13</td>
<td>43</td>
</tr>
</tbody>
</table>

Discussion

Our re-evaluation of the diagnostic value of the APF test for RA surprisingly revealed in our hands a somewhat higher sensitivity than the well-known RF tests. Moreover, the extent of false positivity was obtained in a group of healthy blood donors and patients suffering from autoimmune diseases.

When APF was used together with both RF tests, the number of seronegative RA patients decreased in this study by at least 40%. When as a routine only one RF test is applied, the serological gain will be even greater. Therefore a combination of APF with an RF test (latex or Waaler-Rose) seems the most profitable procedure.

The high sensitivity of the test, as described by Visconti et al. (1964), was attained after a special treatment of buccal epithelial cells to increase the susceptibility of the perinuclear antigen. However, our data indicate a similar high sensitivity without this special treatment of the cells.

It is probable that the laborious character of the APF test presents the major drawback to its popularity in rheumatic-serology. Difficulties in obtaining a satisfactory substrate are a serious disadvantage in the performance of this test on a routine basis. In our experience only a small percentage of potential cell donors (10%) have the perinuclear antigen in a satisfactory amount. Small variations in antigen content of the cells of one and the same donor and between different donors have stimulated us to search for other sources of antigen (Smit et al., in preparation, 1).

Because studies on the morphology and the chemical properties of the antigen by Smit et al. (in preparation, 2) have shown that the antigen is not species- or organ-specific, we have tried to use human and animal tissues as a more constant substrate for the test. A lower sensitivity and a higher number of positive reactions in the sera of healthy controls were observed, which have so far made this alternative material unsuitable as a substrate.
In conclusion, we have shown the high value of the APF for rheumatic serology, especially for differential diagnosis between RA and diseases such as SLE.

The stimulating discussions with Dr Thea M. Feltkamp-Vroom are gratefully acknowledged. The study was financially supported by the Netherlands League against Rheumatism.

References


