Clinical relevance of testing for antineutrophil cytoplasm antibodies (ANCA) with a standard indirect immunofluorescence ANCA test in patients with upper or lower respiratory tract symptoms

A Davenport, R J Lock, T B Wallington

Abstract
Background — Reports from specialist nephrological centres have suggested that the antineutrophil cytoplasm antibody (ANCA) test is highly specific and sensitive for patients with Wegener's granulomatosis. To determine the usefulness of the ANCA test in everyday respiratory practice the results of the test were audited in all patients in the south west of England with respiratory symptoms who underwent the test.

Methods — The results of all 335 patients who had presented with upper or lower respiratory tract symptoms, or both, and were tested for ANCA by the indirect ANCA test in 1990, as recommended in the broadsheet of the British Association of Clinical Pathologists, were audited. Case notes and necropsy reports were available for review in 231 cases (69%), and in the remainder information was obtained by a standard questionnaire.

Results — There were 106 positive results, 45 (44%) from patients with Wegener's granulomatosis. The sensitivity and specificity of a positive ANCA test result in this study were 65% and 77% respectively. For a diagnosis of Wegener's granulomatosis the sensitivity and positive predictive accuracy of a positive cytoplasmic ANCA (c-ANCA) test were greater than of a positive perinuclear ANCA (p-ANCA) test. There were 61 positive tests in 266 patients who did not have Wegener's granulomatosis (23%); of these 27 were from patients with infection, 10 with fibrotic lung disease, nine with underlying connective tissue disease, seven with malignancy, and five following pulmonary emboli. Most of these positive ANCA results were p-ANCA (69%) rather than c-ANCA (31%). Serial ANCA requests were made in 15 cases of patients without Wegener's granulomatosis who had an initial positive ANCA test result. In all cases the ANCA tests subsequently became negative.

Conclusions — In this study the sensitivity and specificity of a positive ANCA test result were less than that reported from specialised centres. However, the test was found to be useful in clinical practice, especially c-ANCA, in conjunction with clinical symptoms of respiratory pathology and evidence of renal disease.

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The first clinical report of Wegener's granulomatosis was made in 1931,1 and later defined as a distinct clinical and pathological entity by Wegener in 1936.2 Classical or generalised Wegener's granulomatosis is characterised by necrotising granulomatous vasculitis of the upper and lower respiratory tract together with glomerulonephritis.3

More recently van der Woude and co-workers reported an association between the presence of antineutrophil cytoplasm autoantibodies (ANCA) and Wegener's granulomatosis.4 Several studies have confirmed this association and reported that a positive result has a sensitivity and specificity in excess of 90% for Wegener's granulomatosis.5 Most such series have been reported from specialist units, and the high sensitivity and specificity of the test may have been biased by the referral patterns. Other reports have recorded positive ANCA tests in patients with HIV infection,6 pulmonary tuberculosis,7 and lung cancer.8 Wegener's granulomatosis is a rare disease and therefore most UK respiratory physicians only see a small number of cases.9 The largest reported series are from specialist tertiary referral centres in the USA10,11 and it may be that their experience differs from that of UK physicians.

To determine the sensitivity and specificity of the ANCA test in everyday clinical practice we audited the results from all patients from the south west of England who had presented with respiratory symptoms and had an ANCA test requested in 1990. ANCA tests were performed on all requests made and in all cases the ANCA test was performed according to the recommendations set out in the broadsheet of the British Association of Clinical Pathologists.12

Methods

PATIENTS

In 1990 our reference laboratory received one or more requests for ANCA testing on 335 patients who had presented with respiratory
symptoms, 194 of whom were men and 141 women of median age 63 (range 9–87) years.

The diagnosis of Wegener's granulomatosis was made using established criteria after Bar-
low and Faucci,\textsuperscript{16} as an illness with destructive upper or lower respiratory tract disease, or both, accompanied by glomerulonephritis in most cases. Diagnosis was confirmed when a biopsy showed small vessel vasculitis or venu-
litis, or both, or a necrotizing/crescentic glomerulonephritis. If the inflammation was
limited to a single site the additional histologi-
cal documentation of granuloma was required to substantiate the diagnosis.

Wherever possible case notes and necropsy
reports were reviewed, otherwise clinical data
were obtained by questionnaire followed by
consultation with the requesting doctor. Data
were recorded on a standard form document-
ing symptoms and signs, previous medical
history, laboratory investigations, histology,
treatment, and outcome.

STUDY DESIGN
Preparation of neutrophils
Fresh human granulocytes were separated
from heparinised whole blood by Polymyeloprep
(Nycomed, Birmingham, UK) sedimentation and
diluted to a concentration of 0.1 × 10\textsuperscript{6}/l in
phosphate buffered saline (PBS)/bovine serum
albumin. Neutrophil suspension, 300 µl, was
added to the cuvette of the cytocentrifuge slide
and spun on to the slides at 2000 rpm for three
minutes. The slides were air dried and fixed
either directly in cold ethanol (4°C) for 15
minutes or by immersion in formaldehyde/
acetone fixative (9% formaldehyde, 45% acet-
tone and 46% PBS) for 50 seconds before
immersion in cold ethanol for 15 minutes. All
slides were air dried and stored with desiccant
at −20°C.

Indirect immunofluorescence assay
Test or control sera diluted 1/10 in PBS were
added to slides and incubated for 20 minutes
at room temperature. The slides were washed
twice with PBS and bound antibody was
detected by fluorescence microscopy after
incubation with fluorescein-isothiocyanate
coujugated antihuman IgG (Fc piece) (Dako-
patts, Copenhagen, Denmark).

Definition of c-ANCA and p-ANCA
Sera were recorded as c-ANCA positive if
cytoplasmic staining was in a granular pattern
with central accentuation, and p-ANCA posi-
tive if staining was perinuclear on ethanol fixed
slides and cytoplasmic on formaldehyde/ace-
tone fixed slides. Positive sera were retested at
serial twofold dilutions to a titre of 1/320.

Positive nuclear and perinuclear sera were
also tested for other autoantibodies by immuno-
fluorescence against a composite block of rat
liver, kidney, stomach, and oesophagus at a
1/10 dilution. Positive antinuclear antibodies
were titrated. Rheumatoid factor was detected
by latex or the Rose Waaler test, or both.

Negative control sera were obtained from
healthy AB blood donors and positive controls
from patients with biopsy proven Wegener's
granulomatosis.

Quality control
The regional reference laboratory belongs to
the UK external quality assurance scheme for
ANCA. Analysis of internal quality control
samples showed that positive results did not
vary by more than one dilution. In our labora-
tory between batch coefficients of variation for
c-ANCA and p-ANCA were 4.5% and 6.6% 
respectively.

STATISTICAL ANALYSIS
The χ\textsuperscript{2} test with Yates' correction, if appropri-
ate, was used to analyse the data. Statistical
significance was taken at or below the 5%
level.

Specificity is a measure of the incidence of
negative results in subjects known to be free of
the disease and sensitivity a measure of the
incidence of positive results in subjects known
to have the disease. However, a highly specific
and sensitive test may still detect a significant
number of "false positives," especially if the
condition has a low prevalence.\textsuperscript{17} More useful
is the concept of positive predictive value,
which is the percentage of positive tests which
are "true positives.\textsuperscript{17}

Results
Clinical data were obtained from all 335
patients with respiratory tract symptoms for
whom an ANCA test was requested in 1990.
The majority of requests were made by respirat-
ory physicians (168 (50%)\textsuperscript{17}), followed by
nephrologists (79 (24%)\textsuperscript{17}), general physicians
(63 (19%)\textsuperscript{17}), otolaryngologists/ophthalmic sur-
geons (19 (6%)\textsuperscript{17}) and pathologists (six (2%)\textsuperscript{17}).
There were 106 positive ANCA results, 54
c-ANCA and 52 p-ANCA.

The case notes and necropsy reports were
reviewed in 231 cases (69%), including all
those patients with a positive ANCA result. In
the remaining cases information was obtained
by the standard questionnaire and confirmed
where necessary by the attending physician.

The median duration of follow up in the
surviving patients with positive ANCA results
was 31 months (range 25–38), and no patient
with an initial positive ANCA result but with
no supporting evidence of Wegener's granulo-
matosis has gone on to develop the disease.

WEGENER'S GRANULOMATOSIS
A final diagnosis of Wegener's granulomatosis
was made in 69 cases. 45 men and 24 women of
mean age 65 (range 20–83) years; 35 (51%) had
a positive c-ANCA, of which 25 had a titre of
1/80 or greater, and 10 had a positive p-ANCA
(14%), of which nine had a titre of 1/80 or
greater. Twenty four patients with Wegener's
granulomatosis (35%) had a negative ANCA
test result.
Antineutrophil cytoplasm antibody test in respiratory disease

Table 1  Percentage sensitivity and specificity of a positive ANCA result in those patients with Wegener's granulomatosis (WG) at titres of 1/10 and 1/80 or greater

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive predictive accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1/10</td>
<td>1/80</td>
<td>1/10</td>
</tr>
<tr>
<td>All positive ANCA</td>
<td>65</td>
<td>49</td>
<td>77</td>
</tr>
<tr>
<td>c-ANCA</td>
<td>51</td>
<td>36</td>
<td>78</td>
</tr>
<tr>
<td>p-ANCA</td>
<td>14</td>
<td>13</td>
<td>76</td>
</tr>
</tbody>
</table>

Sensitivity = \[ \frac{\text{WG with positive ANCA test}}{\text{all cases of WG}} \times 100. \]

Specificity = \[ \frac{\text{patients without WG with negative ANCA result}}{\text{all patients without WG}} \times 100. \]

Positive predictive accuracy = \[ \frac{\text{WG with positive ANCA test}}{\text{all positive ANCA tests}} \times 100. \]

At the time of ANCA testing 28 (41%) had clinically active disease, of whom 18 (64%) had a positive ANCA test result (15 c-ANCA and three p-ANCA). The remaining 41 cases with Wegener's granulomatosis were in remission, 27 (66%) of whom had a positive ANCA result (20 c-ANCA and seven p-ANCA). The sensitivity and specificity of a positive ANCA result in patients with Wegener's granulomatosis is set out in table 1.

**Positive ANCA results in patients without Wegener's granulomatosis**

There were 61 positive ANCA results in the remaining 266 patients, 149 men and 117 women of mean age 62 (range 9–87) years. Forty-two of these 61 patients had a positive p-ANCA result, with 32 having a titre of 1/80 or greater, and 19 (31%) had a positive c-ANCA result, with a titre of 1/80 or greater. The final diagnosis in these cases is set out in table 2.

Table 2  Patients with positive ANCA results without any evidence of Wegener's granulomatosis (n = 61) compared with those patients with a similar final diagnosis (n = 159) with a negative ANCA test result. Patients with other clinical conditions who did not have a positive ANCA result are excluded

<table>
<thead>
<tr>
<th>Final diagnosis</th>
<th>Positive ANCA</th>
<th>Negative ANCA</th>
<th>c-ANCA</th>
<th>p-ANCA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1/10</td>
<td>1/80</td>
<td>1/10</td>
<td>1/80</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>16</td>
<td>44</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Empyema</td>
<td>3</td>
<td>6</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>1</td>
<td>7</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Bronchiectasis</td>
<td>3</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Viral infection</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>CFA</td>
<td>5</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fibrotic lung disease</td>
<td>5</td>
<td>12</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Pulmonary embolus</td>
<td>5</td>
<td>12</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Pericarditis</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Rheumatoid lung</td>
<td>4</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SLE lung</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MPA</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Primary lung cancer</td>
<td>5</td>
<td>26</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pulmonary metastases</td>
<td>1</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

CFA = cryptogenic fibrosing alveolitis; SLE = systemic lupus erythematosus; MPA = microscopic polyarteritis.

**Discussion**

Wegener's granulomatosis is a disease which commonly affects the upper and lower respiratory tracts and kidneys. Most requests for ANCA tests in this study came from renal and respiratory physicians. Surprisingly few requests were made by oto-laryngologists.

The sensitivity and specificity of the ANCA test for patients with Wegener's granulomatosis was less than that reported in earlier studies. Most are from specialist centres and are likely to be subject to selection bias. By including all referrals for an ANCA test from the south west of England, a population of some 3·4 million, we had hoped to avoid any selection bias that may have been present in previous series from highly specialised centres.

Other workers have suggested that the sensitivity and specificity of the test can be increased only by including those patients with clinically active disease. In this series there was no difference in terms of the number of patients with positive ANCA results or the ANCA titre between those with active disease and those in remission. It has been suggested that the specificity of the test can be improved by restricting analysis to patients in whom there is high clinical suspicion of Wegener's granulomatosis. In our series restricting analysis to patients presenting with respiratory tract symptoms did not result in the sensitivity or specificity of previously published series.

From involvement in external quality assurance schemes we have no reason to believe that our assay performed less efficiently than that used in other laboratories.

In our series the sensitivity and positive predictive value for a c-ANCA result in a patient presenting with respiratory symptoms was much greater than that of a p-ANCA result. This supports some of the earlier studies.

Sixty one patients presenting with respiratory symptoms had a positive ANCA test result compared with 45 patients with proven Wegener's granulomatosis. The eventual diagnosis in these patients fell into five major groups: pulmonary infection, most commonly bacterial and lower respiratory tract; fibrosing lung disease; connective tissue diseases associated with vasculitis; tumours; and pulmonary embolic disease. The clinical presentation and routine haematological, biochemical, immunological, and radiological investigations were similar in those patients without Wegener's granulomatosis who had both positive and negative ANCA results.

More of those patients with a positive ANCA result had increased serum IgG concentrations. One possible explanation for a positive ANCA result is non-specific IgG binding, and this has been suggested in previous studies which have reported positive results in patients with underlying infections. Some tumours such as lymphomas are known to affect the immune system and may affect immunoglobulin synthesis. However, we have not been able to show any relation between serum IgG concentration and ANCA titre. Positive results may be expected in...
Abnormal sinus radiograph did not have Wegener's granulomatosis but either had a positive (n=61) or a negative (n=205) ANCA test result.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Negative ANCA</th>
<th>Positive ANCA</th>
<th>Other Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory symptoms</td>
<td>92</td>
<td>94</td>
<td>84</td>
</tr>
<tr>
<td>Sinusitis</td>
<td>11</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>Abnormal chest radiograph</td>
<td>81</td>
<td>88</td>
<td>100</td>
</tr>
<tr>
<td>Abnormal sinus radiograph</td>
<td>8</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Anaemia (Hb&lt;10 g/dl)</td>
<td>16</td>
<td>28</td>
<td>11</td>
</tr>
<tr>
<td>Neutrophilia</td>
<td>24</td>
<td>30</td>
<td>44</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>22</td>
<td>17</td>
<td>11</td>
</tr>
<tr>
<td>Increased CRP/plasma viscosity</td>
<td>65</td>
<td>73</td>
<td>63</td>
</tr>
<tr>
<td>Positive ANF</td>
<td>93</td>
<td>14</td>
<td>22</td>
</tr>
<tr>
<td>Positive DNA binding</td>
<td>11</td>
<td>23</td>
<td>27</td>
</tr>
<tr>
<td>Positive rheumatoid factor</td>
<td>17</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Increased IgG</td>
<td>18</td>
<td>57</td>
<td>38</td>
</tr>
<tr>
<td>Urine dipstick protein</td>
<td>20</td>
<td>24</td>
<td>22</td>
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<tr>
<td>Urine dipstick blood</td>
<td>16</td>
<td>12</td>
<td>11</td>
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<tr>
<td>Serum creatinine &lt;150 μmol/l</td>
<td>88</td>
<td>96</td>
<td>96</td>
</tr>
<tr>
<td>Serum creatinine &gt;150 to &lt;500 μmol/l</td>
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<td>2</td>
</tr>
<tr>
<td>Serum creatinine &gt;500 μmol/l</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

CRP = C reactive protein; ANF = antinuclear factor; IgG = immunoglobulin G. *p < 0.05 v all patients with Wegener's granulomatosis.

Patients with vasculitis and connective tissue diseases. Both p-ANCA and c-ANCA staining have been reported in association with microscopic polyarteritis, and antineutrophil nuclear antibodies were first described in patients with rheumatoid arthritis. Others have reported atypical ANCA staining in patients with connective tissue diseases. The role of ANCA in Wegener's granulomatosis has yet to be clarified. In vitro experiments suggest that it prevents activated neutrophils from downregulation and thus may allow further endothelial damage. However, the same mechanism may be important in tissue repair designed to remove excess fibrin and other products of tissue inflammation. Those patients with fibrotic pulmonary disease, pulmonary malignancy, respiratory infection, or embolic disease may have suffered some pulmonary vascular damage and thereby developed ANCA as a natural consequence of endothelial damage and repair.

In this study we restricted the audit to the first ANCA test request made in 1990. Of the 61 patients who had an initial positive test result, 15 (26%) had further requests. All ANCA results subsequently became negative, although in some cases this took up to three months. This differs from the results of serial ANCA tests in those patients who presented with active Wegener's granulomatosis where, despite aggressive immunosuppression, most still had positive ANCA titres after three months, albeit at reduced levels.

Those patients who were subsequently diagnosed as having Wegener's granulomatosis differed from those presenting with respiratory tract symptoms by having a greater frequency of nasal, sinus, and aural disease. More patients with Wegener's granulomatosis were anaemic and had greater renal involvement having both positive urine dipstick results and impaired renal function – as expected by the definition of Wegener's granulomatosis. More patients with Wegener's granulomatosis had the c-ANCA staining pattern (78%) than p-ANCA (22%). This is in contrast with the situation in those patients without Wegener's granulomatosis where only 31% of the positive ANCA results had the c-ANCA staining pattern and 69% p-ANCA. The c-ANCA pattern predominantly reflects the presence of antibodies to proteinase III, whereas p-ANCA is more heterogeneous, reacting with several antigens of which myeloperoxidase is the most common.

The indirect immunofluorescence test is the most widely available ANCA test in the UK. It may be that it should be used as a screening test and that positive samples should be further analysed by ELISA for proteinase III and myeloperoxidase. However, recent studies have shown that the indirect immunofluorescence test was more sensitive than the currently available ELISA assays in detecting patients with Wegener's granulomatosis, and that there was no difference in "false positive" results between the methodologies. This is supported by a recent international cooperative study group who suggested that indirect immunofluorescence ANCA tests remain the gold standard for patient with Wegener's granulomatosis who have antibodies for proteinase III.

In this series of patients presenting with respiratory symptoms there were many positive ANCA results using the currently recommended indirect immunofluorescence test in patients who were ultimately found not to have Wegener's granulomatosis. This suggests that the usefulness of a positive indirect immunofluorescence ANCA test is not as great as has been reported, and that a diagnosis of Wegener's granulomatosis should not be made solely on the basis of an ANCA result without other corroborative proof.

In most cases with initial "false positive" ANCA results serial determinations became negative with time, so repetition of the test may be more helpful than a single result. However, the ANCA test was found to be useful in helping to support the diagnosis of Wegener's granulomatosis, especially when of the c-ANCA pattern and in association with the combination of upper and lower respiratory tract symptomatology in a patient with abnormal urine stick testing and raised blood creatinine concentrations.

We wish to thank all our colleagues in the South Western Region for their help and cooperation in this study.

Table 3 Symptoms and test results (expressed as percentages) in those patients who did not have Wegener's granulomatosis but either had a positive (n=61) or a negative (n=205) ANCA test result.

Table 4 Presenting symptoms and initial investigations in those patients with Wegener's granulomatosis (n=69) who had positive (n=45) and negative (n=14) ANCA results and those patients without Wegener's granulomatosis (n=266). Results are expressed as percentages.

<table>
<thead>
<tr>
<th>All Wegener's</th>
<th>Wegener's positive ANCA</th>
<th>Wegener's negative ANCA</th>
<th>Other Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory symptoms</td>
<td>87</td>
<td>96</td>
<td>71</td>
</tr>
<tr>
<td>Sinus/nasal symptoms</td>
<td>51</td>
<td>42</td>
<td>67</td>
</tr>
<tr>
<td>Aural symptoms</td>
<td>26</td>
<td>31</td>
<td>17</td>
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<tr>
<td>Abnormal chest radiograph</td>
<td>80</td>
<td>82</td>
<td>75</td>
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<tr>
<td>Abnormal sinus cavity</td>
<td>25</td>
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<td>17</td>
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<tr>
<td>Abnormal sinus margin</td>
<td>33</td>
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<td>Anaemia (Hb&lt;10 g/dl)</td>
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<td>Thrombocythaemia</td>
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<td>20</td>
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<tr>
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<td>Increased CRP/PV</td>
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<tr>
<td>Positive ANF</td>
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<tr>
<td>Positive DNA binding</td>
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<tr>
<td>Positive rheumatoid factor</td>
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<td>Creatinine &lt;150 μmol/l</td>
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<td>Creatinine &gt;150 to &lt;500 μmol/l</td>
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<tr>
<td>Creatinine &gt;500 μmol/l</td>
<td>18</td>
<td>16</td>
<td>21</td>
</tr>
</tbody>
</table>

CRP = C reactive protein; PV = plasma viscosity; IgG = immunoglobulin G. *p < 0.05 v all patients with Wegener's granulomatosis.
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