ELISA is the superior method for detecting antineutrophil cytoplasmic antibodies in the diagnosis of systemic necrotising vasculitis

Anne Harris, Grace Chang, Matthew Vadas, David Gillis

Abstract

Background—Antineutrophil cytoplasmic antibodies (ANCA) have been used as a diagnostic marker for systemic necrotising vasculitis, a disease classification which includes Wegener granulomatosis, microscopic and classic polyarteritis nodosa, and Churg Strauss disease.

Objective—to compare the diagnostic value of the two methods for detecting these antibodies—immunofluorescence and enzyme linked immunosorbent assay (ELISA)—with respect to biopsy proven active systemic necrotising vasculitis in a clinically relevant population.

Methods—A prospective study to ascertain the patient’s diagnosis at the time of each of the 466 requests for ANCA received at one laboratory over a nine month period, and allocate each to one of five diagnostic groups: active and inactive biopsy proven systemic necrotising vasculitis, suspected systemic necrotising vasculitis, low probability systemic necrotising vasculitis, and not systemic necrotising vasculitis.

Results—ELISA was superior to immunofluorescence in the diagnosis of systemic necrotising vasculitis because it was less likely to detect other diseases. This was reflected in its specificity of 97% and positive predictive value of 73%, compared with 90% and only 50% for immunofluorescence (p = 0.0006 and p = 0.013, respectively). ELISA had a negative predictive value of 98% which was not significantly different to immunofluorescence. ELISA was technically superior.

Conclusions—ELISA is the superior method of ANCA detection in the diagnosis of systemic necrotising vasculitis and should be used in conjunction with a compatible clinical picture and histological evidence.

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Keywords: antineutrophil cytoplasmic antibodies; systemic necrotising vasculitis; ELISA; immunofluorescence

Systemic necrotising vasculitis is a disease classification which includes the syndromes of Wegener granulomatosis, classic and microscopic polyarteritis nodosa (CPAN and MPAN), and Churg Strauss disease. These are differentiated from other vasculitic illnesses because of important common features (table 1). They are characterised by an idiopathic systemic inflammatory illness with evidence of particular system involvement, especially renal and respiratory and less often neurological, rheumatological, dermatological, and gastrointestinal. Histology of renal tissue typically shows a pauci-immune, crescentic, focal segmental glomerulonephritis, while in other organs there is a small to medium size vessel necrotising vasculitis. Some syndromes are associated with granulomatous inflammation. Before treatment with cyclophosphamide or prednisolone most patients died within months, often of renal failure. More detailed descriptions of these syndromes are available elsewhere.

Since the work of Davies et al in 1982 and Van de Woude et al in 1985, antineutrophil cytoplasmic antibodies (ANCA) have become widely used in the diagnosis of systemic necrotising vasculitis and they remain the only serological tests with any specificity for this disease group. Two techniques are used for detecting these antibodies: immunofluorescence and enzyme linked immunosorbent assay (ELISA).

Immunofluorescence uses alcohol fixed human neutrophils as an antigen source. In most patients with systemic necrotising vasculitis, two patterns of immunofluorescence staining are usually seen: cytoplasmic, where the antigen is typically a serine protease, proteinase 3, or perinuclear, where the antigen is typically myeloperoxidase. The antigen involved in each pattern is in the primary granule of the neutrophil; during alcohol fixation the granule membranes are disrupted and the positively charged proteins such as myeloperoxidase move towards the negatively charged nucleus. A nuclear pattern of immunofluorescence staining may be seen in some patients with suspected systemic necrotising vasculitis and usually these patients are antinuclear factor positive. In this case the nuclear staining is so bright that any perinuclear staining is overshadowed, or the nuclear stain may mimic the perinuclear pattern.

The ELISA test for ANCA detection has been available since the early 1990s and uses purified proteinase 3 and myeloperoxidase directly.
ANCA detection in systemic necrotising vasculitis

Table 2  ANCA results according to disease category

<table>
<thead>
<tr>
<th>Disease group</th>
<th>Total ANCA requests</th>
<th>Positive (ELISA and/or IF) (n=135)</th>
<th>Both negative (n=130)</th>
<th>Nuclear IF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ELISA</td>
<td>IF</td>
<td>MPO</td>
</tr>
<tr>
<td>A. Active SNV</td>
<td>26</td>
<td>17</td>
<td>5</td>
<td>17</td>
</tr>
<tr>
<td>B. Inactive SNV</td>
<td>77</td>
<td>18</td>
<td>12</td>
<td>26</td>
</tr>
<tr>
<td>C. Suspected SNV</td>
<td>20</td>
<td>2</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>D. Unlikely SNV</td>
<td>92</td>
<td>1</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>E. Not SNV</td>
<td>250</td>
<td>4</td>
<td>4</td>
<td>15</td>
</tr>
</tbody>
</table>

*See text for explanation of double positive results.

Methods

PATIENT POPULATION AND DIAGNOSTIC CRITERIA

The data were derived from 466 consecutive requests for ANCA received at the Institute of Medical and Veterinary Science, Adelaide, over a nine month period. These referrals came from specialist physicians from a variety of fields in public hospitals and private practices within South Australia and the Northern Territory, from a total of 387 different patients. For each serum specimen, determinations of ANCA were made by both immunofluorescence and ELISA.

Each patient's condition at the time of the ANCA test was classified into one of five diagnostic groups (groups A to E, table 2). To fulfil the diagnosis of systemic necrotising vasculitis each patient had to have compatible clinical features (systemic symptoms and evidence of at least one specific organ or system involved) and documented histology showing a pauci-immune small to medium vessel necrotising vasculitis or a pauci-immune focal segmental necrotising glomerulonephritis. Raised serum inflammatory markers or an active urinary sediment were considered further evidence of a systemic vasculitis.

Patients classified as having active systemic necrotising vasculitis (SNV) required a biopsy confirming the disease at some stage in their illness but not necessarily for each relapse when clinical and biochemical evidence was sufficient.

Group A consisted of cases of “active SNV” and included patients on or off treatment (cyclophosphamide and/or prednisolone) and at or within one month of diagnosis or relapse, or patients remaining active for more than one month from diagnosis or relapse.

Group B consisted of various stages of “clinically inactive SNV” and incorporated treated or untreated patients continuing to improve or in clinical remission and one to six months or more than six months from diagnosis or relapse.

Group C consisted of cases of active or inactive “suspected SNV,” where a patient behaved clinically and biochemically as though having systemic necrotising vasculitis but biopsy was not done or was non-diagnostic.

Patients in group D were considered “unlikely SNV” because systemic necrotising vasculitis was unlikely according to our criteria but a definite alternative diagnosis was not available during the study period.

Finally, group E, “not SNV,” incorporated patients where a clear alternative diagnosis was available to explain their state at the time of the ANCA request.

The subclass of systemic necrotising vasculitis was decided according to the presence or absence of other features. Patients were classified as having Wegener granulomatosis if in addition they had clinical evidence of upper respiratory involvement (sinus or middle ear disease, epistaxis, or nasal bridge destruction) or if their biopsy showed granulomatous vasculitis, or both. Patients were classified as having Churg Strauss disease if asthma with or without a prominent eosinophilia or granulomatous vasculitis was present. Patients with documented aneurysms on angiography in the absence of granulomatous vasculitis on biopsy were classified as having CPAN. Patients without these additional features of Wegener granulomatosis, Churg Strauss disease or CPAN were classified as having MPAN and included isolated idiopathic necrotising crescentic glomerulonephritis. Patients with overlapping features were noted as such.

Information allowing this allocation of the patients’ ANCA results was obtained by reviewing hospital case notes and pathology reports and by using a questionnaire in consultation with the responsible specialist. The information comprised the patients’ signs and symptoms, results of relevant blood tests (other than ANCA), urine analyses, radiology reports, tissue biopsies, and response to any treatment.

METHODS OF ANCA DETECTION

Immunofluorescence

Neutrophil isolation was performed as described previously.11 A fluorescent method was employed to test cell viability.12 Cytospin preparation (Shandon Cyto-2) was performed at 10 g on low acceleration for five minutes. The smears were air dried and fixed in absolute alcohol at 4°C for five minutes. They were then kept at ~20°C in a jar with silicone granules until use. Each batch of slides was checked against a calibrated reference serum, Statens Seruminstitut, for sensitivity. Neutrophil smears were brought up to room temperature and incubated with diluted patient sera at ultra-
tions of 1/10, 1/20, 1/80, and 1/320 for 30 min-
utes. After two five minute washes in phosphate 
buffered saline (PBS) without stirring, a 1/25 
titration of antihuman IgG fluorescein isothio-
cyanate conjugate (FITC) (Dako) was added 
and incubated again for 30 minutes. The slides 
were washed twice and mounted with 50% 
glycerol and the results read by an experienced 
staff member. A cut off point of greater than or 
equal to 1/10 was taken as positive, based on a 
predstudy testing of 50 normal patients.

ELISA
The ELISA assay described previously was 
modified in this laboratory. All incubations 
were carried out in a 37°C Pasteur Diagnostics 
shaker-incubator (as opposed to an ordinary 
37°C incubator) for improved precision and 
sensitivity. All washes were carried out on a 
plate shaker Dynatech microshaker for two 
minutes at low speed. Vinyl microtitre (COS-
TAR) trays were coated with myeloperoxidase 
(Calbiochem) at 0.5 µg/ml or proteinase 3 at 
1/1000 (Statens Seruminstitut) in 0.15 M 
PBS, pH 7.2. Trays were incubated for another 
hour and washed three times with PBS/0.05% 
Tween 20 (PBST). Sera were diluted 1/50 in 
PBST containing 1% albumin (PBST/A) and 
asayed in duplicate. Trays were incubated for 
another hour and washed three times. Alkaline 
phosphatase conjugated antihuman IgG 
(Selinus) was added at a dilution of 1/500 in 
PBSTA and incubated again for one hour, 
followed by three washes. P-nitrophenyl-
phosphate (Sigma), 5 mg/ml in 0.05 M 
carbonate buffer (pH 9.6) containing 0.02% 
MgCl2·6H2O, was used for colour develop-
ment. Trays were incubated for 30 minutes. 
The reaction was then stopped with 50 µl of 3 
M NaOH and read at 405 nm (Pasteur 
Diagnostics).

ELISA ANCA results were expressed as an 
optical density ratio because colour develop-
ment induced by the enzyme linked antibody is 
quantitated and compared to a control. A cut 
off optical density ratio of 1.6 (three standard 
deviations above the mean of five normal 
specimens) was taken as positive for each run. 
Fifty normal serum samples were all below this 
cut off range for both proteinase 3 and 
myeloperoxidase assays.

STATISTICAL METHODS
Comparison of sensitivity, specificity, and 
positive and negative predictive values of the 
immunofluorescence and ELISA methods was 
done using categorical modelling from SAS 
(Statistical Analysis System) to take into 
account the paired and overlapping nature of 
the data. Ninety five per cent confidence inter-
vals (CI) for the probabilities were calculated 
using the normal approximation to the bino-
mial where sample sizes were large enough and 
the tail probability method where sample sizes 
were small. The individual titre and cut off 
values were used to construct a non-parametric 
receiver operating characteristic (ROC) curve 
for the immunofluorescence and ELISA meth-
ods, respectively. Comparison between im-
munofluorescence and ELISA of the area 
under a portion of the ROC curve was 
performed on the log transformed titre values.

Results
Information was available to allow allocation of 
465 of 466 ANCA request results into one of 
the five diagnostic groups, as shown in table 2. 
One ANCA request result could not be 
included because neither the hospital case 
notes nor the doctor involved could be traced. 
The ANCA referrals came from specialist phy-
sicians from a variety of disciplines, particularly 
renal and respiratory medicine, rheumatology, 
immunology, neurology, and general medicine. 
Through data collection and discussion with 
the referring physicians it was found that the 
population studied consisted of patients with 
newly suspected or known systemic necrotising 
vasculitis.

ANCA requests were received from 43 
different patients with biopsy proven systemic 
necrotising vasculitis during the study period. 
There were 20 patients with MPAN, 19 with 
Wegener granulomatosis, two with Churg 
Strauss disease, one with CPAN, and one with 
Wegener granulomatosis/Churg Strauss CS 
overlap. The average age at diagnosis for these 
patients was 57 years, with a range of 12 to 81 
years and a male to female ratio of 27 to 16. 
Sixteen ANCA requests came from patients 
within one month of diagnosis, seven from 
patients within one month of relapse, and three 
from other active cases. There were 77 requests 
from patients considered inactive. These 77 
requests comprised 31 treated patients one to 
six months from diagnosis or last relapse, 42 
treated patients more than six months from 
diagnosis or relapse, and four inactive patients 
off treatment.

Of note, during the study period, two 
patients (indicated by brackets in table 2) 
showed more than one pattern of 
immunofluorescence/ELISA ANCA positivity. 
One patient with Churg Strauss disease was 
positive for perinuclear immunofluorescence 
ANCA (titre 1/20) and at a later stage in his ill-
ness was positive for cytoplasmic immunofluo-
rescence ANCA (titre 1/10). A patient from 
group D (“low probability SNV”) was cyto-
plasmic immunofluorescence ANCA positive 
and both proteinase 3 and myeloperoxidase 
ELISA ANCA positive. Using study and retro-
spective data, three other double positive 
results were seen. One patient with Wegener 
granulomatosis was positive cytoplasmic im-
imunofluorescence ANCA positive (1/80) and 
at a later stage perinuclear immunofluores-
cence ANCA positive (1/80). Another patient 
with Wegener granulomatosis was both protei-
nase 3 and myeloperoxidase ELISA ANCA 
positive at diagnosis but later only myeloper-
oxidase ELISA ANCA positive. A third patient 
with MPAN was myeloperoxidase ELISA and 
borderline proteinase 3 ANCA positive during 
his illness. These double positives could not be 
explained by non-specific adherence of anti-
bodies to the test substrate...

The major disease groups in the low probabili-
ty systemic necrotising vasculitis classification 
included isolated interstitial lung disease,
ANCA detection in systemic necrotising vasculitis

Table 3 Diseases other than systemic necrotising vasculitis with a positive antineutrophil cytoplasmic antibodies (ANCA)

<table>
<thead>
<tr>
<th>No of cases</th>
<th>Immunofluorescence</th>
<th>ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PN</td>
<td>CYT</td>
</tr>
<tr>
<td>Connective tissue disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scleroderma</td>
<td>3</td>
<td>–</td>
</tr>
<tr>
<td>Systemic lupus</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Erythema tosus</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Polyangiitis</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Sarcoid</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Sepsis</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Sepsis/thrombocytopenia/ disseminated coagulation</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Urinary infection</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Inflammatory bowel disease</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Crohn disease</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Nephropathies</td>
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</tr>
<tr>
<td>Mesangial glomerulonephritis</td>
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<td>2</td>
</tr>
<tr>
<td>IgA nephropathy</td>
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<td>–</td>
</tr>
<tr>
<td>Focal segmental glomerulonephritis</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Vascular disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goodpasture syndrome</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Temporal arteritis</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Miscellaneous</td>
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<td></td>
</tr>
<tr>
<td>Stroke</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Myelodysplasia</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Asthma</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Allergic rhinitis</td>
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<tr>
<td>Traumatic nasal ulcer</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Bird/drug related</td>
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<td>–</td>
</tr>
<tr>
<td>Sclerosing cholangitis/ pancreatic cancer</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

CYT, cytoplasmic immunofluorescence; ELISA, enzyme linked immunosorbent assay; MPO, myeloperoxidase; NUC, nuclear immunofluorescence; PN, perinuclear immunofluorescence; PR3, protease 3.

Table 4 Comparison of ELISA and immunofluorescence assays in the diagnosis of biopsy proven systemic necrotising vasculitis (SNV)

<table>
<thead>
<tr>
<th>ANCA result</th>
<th>Immunofluorescence</th>
<th>ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PN</td>
<td>CYT</td>
</tr>
<tr>
<td>Active SNV (group A + E)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELISA</td>
<td>85 (71 to 99)</td>
<td>97 (96 to 99)</td>
</tr>
<tr>
<td>MPO + PR3</td>
<td>65 (47 to 85)</td>
<td>98 (97 to 100)</td>
</tr>
<tr>
<td>MPO only</td>
<td>19 (4 to 34)</td>
<td>98 (97 to 100)</td>
</tr>
<tr>
<td>PR3 only</td>
<td>10 (2 to 27)</td>
<td>98 (97 to 100)</td>
</tr>
<tr>
<td>Inflammatory</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IF</td>
<td>88 (76 to 100)</td>
<td>90 (87 to 94)</td>
</tr>
<tr>
<td>ELISA + PR3</td>
<td>69 (51 to 87)</td>
<td>94 (91 to 97)</td>
</tr>
<tr>
<td>MPO only</td>
<td>30 (25 to 35)</td>
<td>98 (97 to 100)</td>
</tr>
<tr>
<td>PR3 only</td>
<td>17 (9 to 24)</td>
<td>98 (97 to 100)</td>
</tr>
<tr>
<td>All SNV (A + B + E)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ELISA</td>
<td>50 (41 to 60)</td>
<td>97 (95 to 99)</td>
</tr>
<tr>
<td>MPO + PR3</td>
<td>34 (25 to 43)</td>
<td>97 (95 to 100)</td>
</tr>
<tr>
<td>MPO only</td>
<td>17 (9 to 24)</td>
<td>98 (97 to 100)</td>
</tr>
<tr>
<td>PR3 only</td>
<td>10 (2 to 27)</td>
<td>98 (97 to 100)</td>
</tr>
<tr>
<td>Immunofluorescence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IF</td>
<td>70 (61 to 79)</td>
<td>90 (87 to 94)</td>
</tr>
<tr>
<td>ELISA + PR3</td>
<td>83 (74 to 92)</td>
<td>93 (90 to 96)</td>
</tr>
<tr>
<td>MPO only</td>
<td>30 (25 to 43)</td>
<td>97 (95 to 99)</td>
</tr>
<tr>
<td>PR3 only</td>
<td>10 (2 to 27)</td>
<td>98 (97 to 100)</td>
</tr>
<tr>
<td>ELISA + IF</td>
<td>73 (64 to 81)</td>
<td>90 (86 to 93)</td>
</tr>
</tbody>
</table>

Sensitivity (%) Specificity (%) Positive predictive value (%) Negative predictive value (%)

Values are mean with (95% confidence intervals).

Figure 1 Ordinary receiver operating characteristic (ROC) curve using ELISA cut off values and immunofluorescence (IF) titres from groups 1 to 5.

DIAGNOSTIC VALUE OF ANCA IN ACTIVE CASES

To calculate sensitivity, specificity, and positive and negative predictive values with respect to active biopsy proven systemic necrotising vasculitis, group A (“active SNV”) was compared to group E (“not SNV”) (table 2). Patients with a nuclear immunofluorescence result were omitted from calculations.

As can be seen from table 4, both assays were similar with respect to sensitivity, 88% of active systemic necrotising vasculitis cases (23/26) being identified by immunofluorescence and 85% (22/26) by ELISA (p = 0.56). However, the immunofluorescence assay detected a larger proportion of diseases other than systemic necrotising vasculitis compared with ELISA, reflected in a significantly better specificity of ELISA: 97% (242/250) compared with 90% (219/242) for immunofluorescence in the diagnosis of active biopsy proven systemic necrotising vasculitis (p = 0.0006). The superiority of the ELISA assay across the range of cut off values measured was reflected in the significant difference in area under the ROC curve between a specificity of 0.95 and 1.0 (p = 0.0054, fig 1). Neither the immunofluorescence titres nor ELISA cut off values could reliably discriminate between systemic necrotising vasculitis and other diseases.

The ELISA test showed a significantly better positive predictive value, with 73% of patients (22/30) from this population with a positive ANCA by ELISA having active biopsy proven systemic necrotising vasculitis, compared with 50% (23/46) if they were positive by immunofluorescence (p = 0.0013). Both assays had excellent negative predictive values, excluding active biopsy proven systemic necrotising vasculitis with a 98% probability for ELISA (242/246) and 99% (219/222) for immunofluorescence (p = 0.70).

From table 4, the combined diagnostic power of myeloperoxidase and protease 3 was superior to that of separate assays for active
systemic necrotising vasculitis. The positive predictive value of myeloperoxidase was marginally better but this did not reach statistical significance. In general, combining immunofluorescence component assays was more useful than using individual tests. C-ANCA alone was more specific (p = 0.0001) but was of poor sensitivity.

With respect to active biopsy proven systemic necrotising vasculitis, ELISA alone was more useful than combining its diagnostic power with immunofluorescence. ELISA alone had a statistically better specificity (p < 0.001) and positive predictive value (p < 0.001) and there was no statistical difference in sensitivity and negative predictive value.

Of the 43 patients with biopsy proven systemic necrotising vasculitis involved in this series, five were negative at diagnosis for either or both assays, sometimes becoming positive later. Using retrospective data when necessary, three patients were negative for immunofluorescence ANCA when antibodies to proteinase 3 and myeloperoxidase antigens were not routinely sought, and became positive for both assays later in their illness. Another patient was positive for immunofluorescence ANCA at diagnosis but negative for ELISA ANCA. One patient was negative by both assays at diagnosis, and follow up requests for ANCA were not made during the study period.

**Table 5** Subtype and ANCA results for all patients with systemic necrotising vasculitis

<table>
<thead>
<tr>
<th>Subtype</th>
<th>No of cases</th>
<th>ELISA</th>
<th>Immunofluorescence</th>
<th>ANCA</th>
<th>ANCA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cytoplasmic</td>
<td>Perinuclear</td>
<td>Negative</td>
</tr>
<tr>
<td>Wegener</td>
<td>19</td>
<td>PR3</td>
<td>11*</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MPO</td>
<td>4</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Negative</td>
<td>1</td>
<td>2**</td>
<td>3</td>
</tr>
<tr>
<td>Microscopic polyarteritis</td>
<td>20</td>
<td>PR3</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MPO</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Churg Strauss</td>
<td>2</td>
<td>MPO</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Classic polyarteritis</td>
<td>1</td>
<td>PR3</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wegener/Churg Strauss</td>
<td>1</td>
<td>MPO/PR3</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*One of these patients was also perinuclear immunofluorescent ANCA positive; **one of these patients was also PR3 positive.

ANCA, antineutrophil cytoplasmic antibodies; ELISA, enzyme linked immunosorbent assay; MPO, myeloperoxidase; PR3, proteinase 3.

**Discussion**

Our primary aim in this study was to compare the diagnostic value of the original immunofluorescence method of ANCA detection with an ELISA technique in the diagnosis of active biopsy proven systemic necrotising vasculitis, using a clinically suitable population. We showed that ELISA was superior to immunofluorescence with respect to specificity and positive predictive value in the diagnosis of active systemic necrotising vasculitis.

Several previous studies have examined the diagnostic value of ANCA in systemic necrotising vasculitis. Early publications were limited by their study of selected known cases of the disease, especially Wegener granulomatosis,
ANCA detection in systemic necrotising vasculitis

and various selected control groups. These populations were different from those of suspected cases of active systemic necrotising vasculitis seen in clinical practice. These papers quoted high sensitivities and specificities for immunofluorescence and later ELISA ANCA for systemic necrotising vasculitis, in particular cytoplasmic immunofluorescence ANCA with Wegener granulomatosis, as reported in a summary by Rao et al.]

However, Davenport et al. and later Edgar et al. emphasised the limitations of using these variables alone in clinical practice and the usefulness of positive predictive value, which is more dependent on the prevalence of disease in the study population. The positive predictive value tells the clinician the likelihood that a positive result means that the patient has the disease sought. Davenport et al studied 779 samples tested for ANCA at their laboratory with respect to immunofluorescence ANCA and systemic necrotising vasculitis. They reported a positive predictive value of 40% for all cases of systemic necrotising vasculitis and 29% for active cases, and a sensitivity of 59% and a specificity of 82% for all cases of systemic necrotising vasculitis. Most patients had a biopsy to confirm systemic necrotising vasculitis. However, comparisons with ELISA were not made.

Rao et al studied 346 patients with suspected vasculitis prospectively and reported a sensitivity of cytoplasmic immunofluorescence ANCA for Wegener granulomatosis of 28%, a specificity of 96%, a positive predictive value of 50%, and a negative predictive value of 90%. However, American College of Rheumatology criteria, which do not require biopsy confirmation, were used for the diagnosis of Wegener granulomatosis and consequently, as reported by Franssen et al., there is diagnostic uncertainty in the classification of their patients.

Comparisons with ELISA ANCA and other subtypes of systemic necrotising vasculitis were not made.

There have been few direct comparisons of immunofluorescence and ELISA ANCA with respect to systemic necrotising vasculitis. Godbole et al reported specificities for both immunofluorescence and ELISA of 95.7% and sensitivities of 65% and 60% for immunofluorescence and ELISA, respectively. Their analysis was performed on a small subselection of serum samples submitted for ANCA testing at their laboratory, and statistical analysis and predictive values were not documented. Edgar et al determined the positive predictive value in 92% of a series of 327 consecutive ANCA requests at their laboratory, but for a group of vasculitic conditions which included systemic necrotising vasculitis, and biopsy confirmation was not required. They found that myeloperoxidase ELISA ANCA had a better positive predictive value than combined immunofluorescence patterns—62% versus 27%, respectively—suggesting that ELISA ANCA was a better discriminator for this group of vasculitic illnesses. Sensitivities and specificities were not reported and comparisons with proteinase 3 ELISA ANCA were not made.

In this paper, we compared sensitivity, specificity, and positive and negative predictive values of both ANCA techniques in biopsy proven active systemic necrotising vasculitis. Despite previous recognition that ELISA ANCA is often negative in patients with a positive immunofluorescence ANCA, for the first time it has been shown statistically that ELISA is superior to immunofluorescence with respect to specificity and positive predictive value, because of the larger number of patients with non-systemic necrotising vasculitis identified by positive immunofluorescence ANCA. From these data, we do not recommend that the ELISA testing be combined with immunofluorescence, nor is using a component of the ELISA assay in isolation worthwhile.

Technical advantages of ELISA over immunofluorescence have been documented previously. Immunofluorescence is a subjective test and interpretation requires expertise. In addition, there are difficulties associated with nuclear immunofluorescence results, including confusion with perinuclear immunofluorescence ANCA and antinuclear antibodies.

For the clinician, an ANCA test may be considered useful for a patient with improving or quiescent systemic necrotising vasculitis under two circumstances. The first is when the diagnosis is subsequently in doubt. Here immunofluorescence will be more sensitive but this is counteracted by an inferior specificity and positive predictive value and the possibility of a misleading result. The second is when following disease activity. Here it appears that ELISA is the test of choice because it becomes negative more rapidly in patients who are clinically improving yet is sensitive in detecting active disease. However, a prospective study to address this issue is needed. From these data it would seem prudent to follow closely those patients with continuing or rising positive ELISA ANCA results for clinical evidence of relapse, and to remember that very occasionally patients with negative ANCA results relapse.

With respect to ANCA and subtypes of systemic necrotising vasculitis, early reports emphasised a strong association between cytoplasmic immunofluorescence ANCA or proteinase 3 ELISA ANCA and Wegener granulomatosis, and between perinuclear immunofluorescence ANCA or myeloperoxidase ELISA ANCA and other subtypes of systemic necrotising vasculitis. However, we as others have found that neither immunofluorescence patterns nor ELISA antigens reliably distinguish different subtypes of systemic necrotising vasculitis. We therefore feel justified in combining their respective diagnostic yields.

In addition, we have felt justified in incorporating vasculitic syndromes under the title of “systemic necrotising vasculitis” because of the limitations of overlapping clinical features and histological samples which are not always representative of changes elsewhere in the tissues in individual patients. Systemic necrotising vasculitis is a very useful term for highlighting what these syndromes have in common, while allowing for diagnostic limitations in individual patients.
From the results of this paper, we conclude—as have others—that ELISA ANCA results together with a compatible clinical picture are not sufficiently reliable to allow omission of additional supportive histology in the diagnosis of active systemic necrotising vasculitis because of the great importance of a correct diagnosis. ELISA ANCA results may be particularly helpful in cases where the diagnosis is less than clinically obvious, encouraging one to perform a biopsy in these cases, or enabling one to make a presumptive diagnosis when a biopsy is contraindicated. In diagnosing systemic necrotising vasculitis it is also important to actively exclude other diseases such as bacterial endocarditis, tuberculosis, helminthic infections, and connective tissue diseases which can mimic the former disease but where treatment is different and where “false” positive ANCA results are known to occur.

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ELISA is the superior method for detecting antineutrophil cytoplasmic antibodies in the diagnosis of systemic necrotising vasculitis.

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