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

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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

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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).[1 2] 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.[3–5] More detailed descriptions of these syndromes are available elsewhere.[3 6 7]

Since the work of Davies *et al* in 1982[8] and Van de Woude *et al* in 1985,[9] 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.[10] 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.

Table 1 Scott's classification of vasculitis.[1 2] Overlap between the groups occurs

1. Systemic necrotising vasculitis (predominantly small and medium sized arteries)
(a) Polyarteritis nodosa group: classic polyarteritis and microscopic polyarteritis nodosa, Kawasaki disease, arteritis of rheumatoid arthritis, systemic lupus erythematosus, Sjögren disease
(b) With granulomas: Wegener disease, Churg Strauss disease, granulomatous angiitis of the central nervous system
2. Small vessel vasculitis (also hypersensitivity or leucocytoclastic or allergic vasculitis): Henoch-Schonlein purpura, essential mixed cryoglobulinaemia, drug induced vasculitis, vasculitis associated with rheumatoid arthritis, systemic lupus erythematosus, and Sjögren disease
3. Giant cell arteritis (predominantly large arteries): temporal arteritis, Takayasu arteritis, aortitis associated with rheumatoid arthritis, ankylosing spondylitis

Table 2 ANCA results according to disease category

Disease group	Total ANCA requests	Positive (ELISA and/or IF) (n=135)				Both negative (n=330)	Nuclear IF
		ELISA		IF			
		MPO	PR3	PN	CYT		
A. Active SNV	26	17	5	17	5 1*	2	0
B. Inactive SNV	77	18	12	26	23	25	1
C. Suspected SNV	20	2	2	5	3	12	0
D. Unlikely SNV	92	1	1 1*	10	4	75	2
E. Not SNV	250	4	4	15	8	216	8

*See text for explanation of double positive results.
ANCA, antineutrophil cytoplasmic antibodies; CYT, cytoplasmic immunofluorescence; ELISA, enzyme linked immunosorbent assay; IF, immunofluorescence; MPO, myeloperoxidase; PN, perinuclear immunofluorescence; PR3, proteinase 3; SNV, systemic necrotising vasculitis.

Despite the pivotal role of these antibody tests in the diagnosis of systemic necrotising vasculitis, previous reports on their diagnostic value are difficult to interpret because of serious limitations with respect to the populations studied and the variables determined. In addition, there have been few direct comparisons of ELISA and immunofluorescence. Our primary aim in this study was to use a population of suspected and known cases of systemic necrotising vasculitis to compare sensitivity, specificity, and positive and negative predictive values of immunofluorescence and ELISA ANCA prospectively in the diagnosis of active biopsy proven systemic necrotising vasculitis. Additional aims were to see how ANCA results change with disease activity and correlate with systemic necrotising vasculitis subtype.

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 titra-

tions of 1/10, 1/20, 1/80, and 1/320 for 30 minutes. After two five minute washes in phosphate buffered saline (PBS) without stirring, a 1/25 titration of antihuman IgG fluorescein isothiocyanate 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 prestudy testing of 50 normal patients.

ELISA

The ELISA assay described previously[13] 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 (COSTAR) 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 (PBSTA) and assayed 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% $MgCl_2$-$6H_2O$, was used for colour development. Trays were incubated for 30 minutes. The reaction was then stopped with 50 µl of 3 M NaOH and read at 405 mn (Pasteur Diagnostics).

ELISA ANCA results were expressed as an optical density ratio because colour development 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)[14] to take into account the paired and overlapping nature of the data. Ninety five per cent confidence intervals (CI) for the probabilities were calculated using the normal approximation to the binomial 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 methods, respectively. Comparison between immunofluorescence 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 physicians 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 illness was positive for cytoplasmic immunofluorescence ANCA (titre 1/10). A patient from group D ("low probability SNV") was cytoplasmic immunofluorescence ANCA positive and both proteinase 3 and myeloperoxidase ELISA ANCA positive. Using study and retrospective data, three other double positive results were seen. One patient with Wegener granulomatosis was positive cytoplasmic immunofluorescence ANCA positive (1/80) and at a later stage perinuclear immunofluorescence ANCA positive (1/80). Another patient with Wegener granulomatosis was both proteinase 3 and myeloperoxidase ELISA ANCA positive at diagnosis but later only myeloperoxidase 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 antibodies to the test substrate.

The major disease groups in the low probability systemic necrotising vasculitis classification included isolated interstitial lung disease,

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

	No of cases	Immunofluorescence			ELISA	
		PN	CYT	NUC	MPO	PR3
Connective tissue disease						
Scleroderma	3	–	–	3	–	–
Systemic lupus erythematosus	1	–	1	–	–	–
Polymyositis	1	–	–	1	–	–
Sarcoid	1	–	–	–	–	1
Rheumatoid arthritis	1	1	–	–	–	–
Sepsis						
Pneumonia	2	1	–	1	–	–
Sepsis/thrombocytopenia/ disseminated coagulation	3	3	–	–	1	–
Urinary infection	1	1	–	–	–	–
Inflammatory bowel disease						
Ulcerative colitis	3	2	1	–	–	1
Crohn disease	1	–	1	–	–	–
Nephropathies						
Mesangial glomerulonephritis	2	2	–	–	–	–
IGA nephropathy	1	–	–	1	–	–
Focal segmental glomerulonephritis	1	–	–	1	–	–
Diabetic nephropathy	2	2	–	–	1	1
Vascular disease						
Goodpasture syndrome	1	–	1	–	–	–
Temporal arteritis	1	–	–	–	–	1
Miscellaneous						
Stroke	1	–	–	–	1	–
Myelodysplasia	1	–	1	–	–	–
Asthma	1	–	1	–	–	–
Allergic rhinitis	1	–	–	1	–	–
Traumatic nasal ulcer	1	–	1	–	–	–
Bird/drug related	1	1	–	–	1	–
Sclerosing cholangitis/ pancreatic cancer	1	1	–	–	–	–
Cystic fibrosis	2	1	1	–	–	–

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

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

	Sensitivity (%)	Specificity (%)	Positive predicitive value (%)	Negative predictive value (%)
Active SNV (group A *v* E)				
ELISA				
MPO + PR3	85 (71 to 99)	97 (96 to 99)	73 (58 to 89)	98 (97 to 100)
MPO only	65 (47 to 85)	98 (97 to 100)	81 (64 to 98)	96 (94 to 99)
PR3 only	19 (4 to 34)	98 (97 to 100)	56 (23 to 88)	92 (89 to 95)
Immunofluorescence				
PN + CYT	88 (76 to 100)	90 (87 to 94)	50 (36 to 64)	99 (98 to 100)
PN only	69 (51 to 87)	94 (91 to 97)	55 (38 to 72)	97 (94 to 99)
CYT only	23 (67 to 89)	97 (94 to 99)	43 (17 to 69)	93 (89 to 95)
ELISA + IF	92 (82 to 100)	90 (86 to 93)	48 (34 to 62)	99 (98 to 100)
All SNV (A + B *v* E)				
ELISA				
MPO + PR3	50 (41 to 60)	97 (95 to 99)	87 (78 to 95)	83 (78 to 87)
MPO only	34 (25 to 43)	98 (97 to 100)	90 (80 to 99)	78 (73 to 83)
PR3 only	17 (9 to 24)	98 (97 to 100)	81 (64 to 98)	74 (69 to 79)
Immunofluorescence				
PN + CYT	70 (61 to 79)	90 (87 to 94)	76 (67 to 84)	88 (84 to 92)
PN only	43 (34 to 53)	94 (91 to 97)	75 (63 to 86)	80 (75 to 84)
CYT only	23 (7 to 39)	97 (94 to 99)	78 (65 to 91)	76 (71 to 81)
ELISA + IF	73 (64 to 81)	90 (86 to 93)	74 (66 to 83)	89 (85 to 93)

Values are mean with (95% confidence intervals).
CYT, cytoplasmic immunofluorescence; ELISA, enzyme linked immunosorbent assay; IF, immunofluorescence; MPO, myeloperoxidase; PN, perinuclear immunofluorescence; PR3, proteinase 3.

chronic renal failure of unknown cause, seronegative polyarthritis, polyneuritis, lung infiltrates, iritis, purpura, urticaria, eosinophilia, haemoptysis, abdominal symptoms, and resolved systemic symptoms. Diseases other than systemic necrotising vasculitis detected by positive ANCA results are shown in table 3.

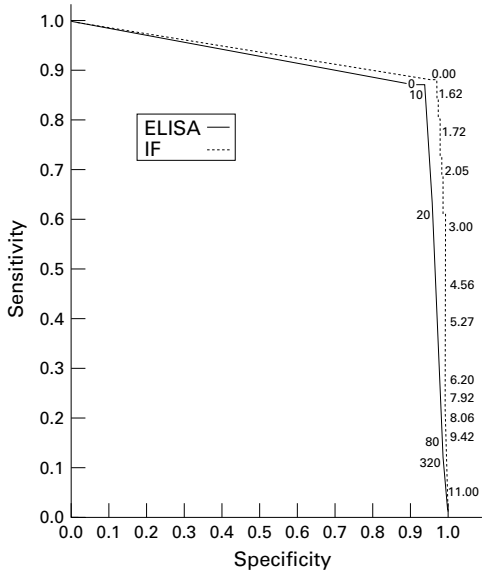

Figure 1 Ordinary receiver operating characteristic (ROC) curve using ELISA cut off values and immunofluorescence (IF) titres from groups 1 to 5.

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 proteinase 3 was superior to that of separate assays for active

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

	No of cases	ELISA	Immunofluorescence		
			Cytoplasmic	Perinuclear	Negative
Wegener	19	PR3	11*	1	
		MPO		6	
		Negative			1
Microscopic polyarteritis	20	PR3	4		
		MPO		8	
		Negative	1	2**	3
Churg Strauss	2	MPO		1	
		Negative			1
Classic polyarteritis	1	PR3		1	
Wegener/Churg Strauss	1	MPO/PR3			1

*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.

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.

DIAGNOSTIC VALUE OF ANCA IN ACTIVE AND INACTIVE CASES

With respect to all cases of biopsy proven systemic necrotising vasculitis, active and inactive, immunofluorescence was more sensitive than ELISA (70% *v* 50%, respectively; $p < 0.001$) with a better negative predictive value (88% *v* 83%; $p < 0.001$). This reflects the fact that ELISA becomes negative more rapidly in patients who are clinically improving or in remission. Importantly, ELISA maintained a higher specificity (97% *v* 90%; $p < 0.001$) and better positive predictive value (87% *v* 76%; $p = 0.02$) in this group. Combining the diagnostic power of ELISA with immunofluorescence added no advantage in this patient group and only detracted from the value of ELISA.

CHANGES IN ANCA WITH DISEASE ACTIVITY

With regard to follow up of patients with biopsy proven systemic necrotising vasculitis, a smaller proportion was still positive by ELISA (16 of 31 requests; 52%) compared with immunofluorescence (28 of 31 requests; 93%) at one to six months after diagnosis or relapse ($p = 0.001$). These patients, the vast majority on cyclophosphamide or prednisolone or both, were continuing to improve or remaining clinically quiescent. This difference was still significant at more than six months but less so with 18 of 42 requests (43%) for patients remaining positive by immunofluorescence and 12 of 42 (29%) by ELISA ($p = 0.02$).

Follow up data were reviewed where ANCA results and coincident clinical details were available following the diagnosis of the 43 patients with biopsy proven systemic necrotising vasculitis involved in this study. It was common to see continuing positive ANCA tests by both methods with clinically improving or quiescent disease; however, negative results in this group were also common. With relapse of vasculitis, most patients showed a preceding rise in titre or continuing highly positive results, usually by both assays. Occasionally a patient had continuing borderline positive or negative ANCA results before or at the time of relapse.

PREDICTION OF THE FORM OF SYSTEMIC NECROTISING VASCULITIS BY ANCA

Table 5 shows the subtype of systemic necrotising vasculitis and the form of ANCA positivity, where applicable, for both assays for all 43 patients with biopsy proven systemic necrotising vasculitis. In cases where a patient had become negative for either assay during the study period, the patient's previous ANCA results were included when available (retrospective data). Some patients, as indicated, lacked any positive ANCA results at this laboratory. Most patients with Wegener granulomatosis and positive ANCA results were cytoplasmic immunofluorescence and proteinase 3 ELISA ANCA positive, and most patients with MPAN were perinuclear immunofluorescence and myeloperoxidase ELISA ANCA positive. However, we found a significant proportion who did not follow these correlations (six of 18 positive results with Wegener granulomatosis (33%) and five of 17 with MPAN (27%).

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,

and various selected control groups.[15–18] 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*.[19]

However, Davenport *et al*,[20] and later Edgar *et al*,[21] 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.[20] 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[22] 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,[23] which do not require biopsy confirmation, were used for the diagnosis of Wegener granulomatosis and consequently, as reported by Franssen *et al*,[24] 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.[25] 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,[21] 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,[21 25–27] 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 experience.[25] 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,[15 16] and between perinuclear immunofluorescence ANCA or myeloperoxidase ELISA ANCA and other subtypes of systemic necrotising vasculitis.[17 18] However, we as others[21 28–30] 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[20–22]—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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