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ANCA serology in the diagnosis and management of ANCA-associated renal vasculitis

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GUIDELINES

a. Serum anti-neutrophil cytoplasmic antibody (ANCA) measurement should not be used alone in the initial diagnosis of ANCA-associated vasculitis (AAV) but should be used in combination with the gold standard of tissue diagnosis. (Level 1 evidence)

b. Measurement of ANCA by both ELISA and indirect immunofluorescence (IIF) in combination ensures optimal test sensitivity and specificity. (Level I evidence)

c. The use of serial ANCA monitoring alone is insufficient to predict relapse or monitor disease activity. (Level I evidence)

SUGGESTIONS FOR CLINICAL CARE

(Suggestions are based on Level III and IV evidence)

- **Test performance increases with increasing clinical features of disease. Test performance is poor as a screening test in patients with few clinical features of AAV.**
- **Rapid ELISA test may be helpful as an adjunct to urgent therapeutic decisions when formal histological diagnosis is delayed, but should not supplant the need for histological confirmation of disease and ANCA IIF testing.**
- **Serial ANCA testing (ELISA and IIF) to monitor disease activity may be useful in some situations as:**
  - **disappearance of ANCA is associated with disease remission and a lower risk of relapse[1,2] (Level IV evidence),**
  - **reappearance or rising ANCA titre is of greater relevance in the setting of worsening clinical features, and**
  - **the persistence of anti-proteinase 3 (ant-PR3) antibodies is associated with a higher risk of relapse[3] (Level IV evidence).**

BACKGROUND

The primary systemic vasculitides are a group of heterogeneous clinical syndromes that are idiopathic in nature and classified by variable degrees of inflammation of the vessel wall. Classification of systemic vasculitis is complicated, although the most widely accepted of these have usually included consideration of the size of the vessel predominantly involved and the histological appearance on biopsy.

Renal involvement is particularly common in vasculitides of the small vessels; those with paucity or absence of immune deposits (namely Wegener's granulomatosis [WG], microscopic polyangiitis [MPA] and Churg-Strauss syndrome [CSS]) are strongly associated with the presence of anti-neutrophil associated antibodies (ANCA). The utility of diagnostic classifications of these diseases, commonly referred to as the ANCA-associated vasculitides has been contentious, largely due to the lack of incorporation of histological, clinical and serological findings in the two main published classifications. In 1990, the American College of Rheumatology published classification criteria for vasculitis,[4] which demonstrated high sensitivity and specificity for diagnosis of WG and CSS,[5,6] but did not include ANCA or MPA and was based on the presence of clinical symptoms and histopathological findings. In 1994, the Chapel Hill Consensus statement (CHCS)[7] produced histological definitions for vasculitides that were not intended as classification criteria. Anti-neutrophil cytoplasmic antibodies were not included in the definitions, although the authors recognised the importance of ANCA in small vessel vasculitis, as well as the use of surrogate clinical markers of disease in the diagnosis.

A number of studies have demonstrated marked discordance of the two major diagnostic criteria, while others have proposed modifications that attempt to better incorporate histology, surrogate markers and serology[8] that have been evaluated for validity with additional modifications recommended[9]. More recently, a four-step algorithm has been developed[10] as a basis for epidemiological studies and in an attempt to provide standardised criteria for future clinical trials. This algorithm includes the use of histology, surrogate markers and serology, and uses exclusion criteria for other features that may mimic primary vasculitides.

Incidence varies between regions[11] and may also have latitudinal variation. In Australia, AAV accounts for less

than 1% of end-stage kidney disease (ESKD). With the introduction of cyclophosphamide and prednisolone in the 1970s, the long-term prognosis for those with AAV improved dramatically. Due to the relatively high incidence of disease, and the evolution of AAV into a chronic relapsing disease with a 5-year 80% survival, ANCA serology is being increasingly used as a diagnostic marker of active disease.

The purpose of this guideline is to review the available clinical evidence relating to the usefulness of serial ANCA testing to diagnose disease and to predict clinical relapse.

A. ANCA serology in the diagnosis of ANCA-associated vasculitis

It was some time ago that the renal lesion of rapidly progressive glomerulonephritis in the absence of identifiable immune deposits was recognised as a distinct pathology.[12] Although acknowledged to have a variable clinical presentation of systemic necrotising vasculitis or in some cases, a renal limited lesion, it was some time before the Chapel Hill consensus formally defined the clinical subgroups that comprise systemic vasculitis. In the case of the pauci-immune renal lesion, three sub-groups were described, based on clinical and histological variants:[7]

1. Wegener's granulomatosis was defined as a granulomatous inflammation affecting the respiratory tract and necrotising vasculitis affecting small-sized to medium-sized vessels, where necrotising glomerulonephritis is common.
2. Churg-Strauss syndrome was defined as an eosinophil-rich granulomatous inflammation in the respiratory tract, with necrotising vasculitis affecting small-sized to medium-sized vessels and associated with asthma and eosinophilia.
3. Microscopic polyangiitis was also described as a necrotising vasculitis, with few or no immune deposits, affecting small vessels. Necrotising arteritis involving small-sized and medium-sized arteries might be present and necrotising glomerulonephritis is very common. Pulmonary capillaritis frequently arises.

The renal lesion of pauci-immune vasculitides is recognised in the early stages by fibrinoid necrosis of capillary loops, with progression to diffuse proliferative, pauci-immune glomerulonephritis with basement membrane rupture and cells in Bowman's space and an associated interstitial reaction. As with other progressive renal pathologies, the end-stage is that of sclerosed glomeruli.

Anti-neutrophil cytoplasmic antibodies

It was in 1982 that ANCAs were first described in a cohort of patients with segmental necrotising glomerulonephritis. Davies *et al*. described cytoplasmic staining of neutrophils when studying nuclear antibodies using IIF in 8 patients, 7 of whom had positive Ross River virus serology.[13] It was shortly after, that the association of ANCAs with WG[14] and MPA[15] was described.

ANCAs are antibodies directed against primary granules of neutrophil and monocyte lysosomes. Without ethanol fixation, all ANCAs stain in a cytoplasmic distribution. However, following ethanol fixation, two distinct patterns are described, cytoplasmic (cANCA) and perinuclear (pANCA), depending on their relative charge.

1. The staining of ANCA reaction with proteinase (PR3), a weaker cation or neutral protein, will result in a cytoplasmic fluorescent pattern called c-ANCA.
2. When cytoplasmic granules redistribute resulting in a pANCA pattern, a number of antigens have been described, of which myeloperoxidase (MPO) is the only one of clinical importance.

ANCA antigens (PR3 and MPO) are measured in serum using ELISA, freely available in kit form.

The international consensus statement on reporting of ANCA recognised the association of other diseases with ANCAs that have atypical staining patterns on IIF or antigens other than the two described (PR3 and MPO) and recommended both IIF and ELISA testing when seeking to identify ANCA, but also stressed the importance of histological confirmation as the gold standard.[16] The statement proposes screening ANCA by IIF, followed by confirmation by both protease-3 ELISA and myeloperoxidase-ELISA. Furthermore, they recommend that negative results by IIF should also be tested by ELISA, as 5% of samples are only positive by ELISA.

ANCA *test performance*

Although it was noted in the Chapel Hill statement that ANCAs (IIF/ELISA) are useful in the definition of small vessel vasculitis with the renal lesion being that of pauci-immune focal necrotising glomerulonephritis, the presence of ANCAs was not considered in the diagnostic schema. It has since been demonstrated that the use of the Chapel Hill criteria in isolation has poor predictive value,[8] and studies have since focused on the diagnostic utility of the various ANCA markers in comparison with histological diagnosis.

Earlier studies[14,17–21] demonstrated limited sensitivity of ANCA by IIF alone, often examining only one disease group. An early study incorporating ELISA assays by Sinico *et al*.[22] examined a renal specific population of rapidly progressive renal failure and/or disease with systemic vasculitis. Prospective analysis of 1535 serum ANCAs in 920 consecutive patients, using histology as comparator, confirmed a high sensitivity and specificity for ANCA by IIF, with higher sensitivity when used in conjunction with ELISA.

With the development of standardised techniques for IIF and ELISA, collaborative groups have determined the diagnostic value of standardised ANCA assays. The largest of these is from the EC/BR project for ANCA assay standardisation,[23] a multi-centre analysis of ANCA assays compared with histology, which comprised 169 prospective and 189 retrospective biopsies and sera, along with 184 disease controls and 740 healthy controls. When using ANCA IIF and ELISA in combination, the tests were of high specificity (99%), with sensitivity of 73% and 67% for WG and MPA, respectively. The conclusion is that the value of the IIF test for ANCA detection can be greatly increased by the

addition of ELISA. The authors did note, however, that in a significant number of patients with idiopathic small vessel vasculitis, the ANCA results were negative. Similarly, Harris *et al.*,[24] compared the diagnostic performance of ELISA and IIF, and found the highest sensitivity with combined testing (92%) and the highest positive predictive value with combined ELISA and IIF (73%), although the equivalent negative predictive value for each test individually was 99% and 98%, respectively. In a smaller study, dual testing in a single centre study with 123 patients with either WG or MPA demonstrated ANCA positivity in 97%.[25]

There are only two meta-analyses of note. One examined the utility of IIF for cANCA testing alone in the diagnosis of WG, demonstrating a pooled sensitivity of 91% (95% CI, 87%, 95%) for patients with active disease and 63% for inactive disease.[26] The other was slightly larger, examining MPO ELISA testing.[27] Myeloperoxidase performance again demonstrated best sensitivity and specificity with combined testing of IIF and ELISA, 84.7% (70.7%, 98.7%) and 98.6% (97.9%, 99.3%) respectively, encompassing 5 studies that were considered robust.

It has furthermore been demonstrated that the ANCA test is best used in cases with a high index of clinical suspicion, that it is extremely useful in patients with multiple clinical symptoms of WG, where a post-test probability of ~98% was demonstrated, but less useful in patients with only one symptom of WG, in this case with a post-test probability of only 7%-16%. This underlines the problem that ANCA-associated vasculitides are very rare diseases and the ANCA test applied to the general population (low pre-test probability) results in a large number of false positives, irrespective of high test specificity. This was further demonstrated by Mandl *et al.*,[28] who showed that the ordering of ANCA test in patients with a high suspicion of ANCA-associated vasculitis, reduces false-positive rates.

It has been recognised that most published data come from specialised research laboratories, that do not accurately reflect the performance of assays under routine clinical conditions. Russell *et al.* demonstrated on 615 consecutive samples that with the relatively high sensitivity of combination PR3-ANCA and MPO-ANCA ELISA (72%) that significant labour and cost savings could be made by initial ELISA screening and IIF confirmation.[29] Others have demonstrated the superiority of ELISA as a method for detecting ANCA[24] and a more recent study, in attempting to meet the clinical need for urgent ANCA testing when IIF is not available out of hours, compared rapid qualitative ELISA with formal IIF and quantitative ELISA in 103 consecutive samples, 30 of which were positive for MPO/PR3. In this study, Aslam *et al.*[30] showed a high sensitivity of 82%, specificity 97%, PPV 92%, and NPV 93%.

B. ANCA serology in the monitoring of relapse and treatment of ANCA-associated vasculitis

ANCA titres are commonly monitored serially on the grounds that a rise/fall or disappearance may correlate with the clinical course and suggest relapse or remission. Thus, it is thought that timely identification may allow early detection of disease relapse, which can subsequently be confirmed on clinical findings or further investigation and allow prompt treatment.

It has also been suggested that pre-emptive treatment adjustments based on a change in ANCA titre may prevent disease relapse.

SEARCH STRATEGY

Databases searched: MeSH terms and text words for kidney failure were combined with MeSH terms and text words for vasculitis and then combined with MeSH terms and text words for analysis ANCA (immunology, blood, diagnostic use), CD14, ELISA, immunoglobulin and biopsy. The search was carried out in Medline (1966 – August Week 3, 2006).
Date of searches: 22 August 2006.

WHAT IS THE EVIDENCE?

Birck and colleagues[31] performed a systemic review of the literature addressing the clinical value of serial ANCA monitoring in patients with systemic vasculitis. Small studies (fewer than 10 patients), studies where there was no assignation to a particular vasculitic syndrome, and studies where treatment was based on ANCA titre were excluded. From an initial 3611 citations, 22 studies of 950 patients were included. Qualitative analysis showed that only a minority of studies (4) reported consecutive patient enrolment, prospective data collection and independence of both the index and reference tests. Differences in methodology (especially the nature of the ANCA assay and the clinical definition of disease relapse) were sufficient to not allow the authors to draw a firm conclusion as to the usefulness of ANCA serial monitoring.

A number of cohort studies have been performed (often with retrospective data entry) and with differences in the ANCA assay methodology and definition of disease activity.

MacIsaac *et al.*[32] tested over 7500 sera for ANCA-IIF that were referred for ANA testing. Seventeen were found positive and their medical details reviewed; 14 consecutive patients with positive ANCA and glomerulonephritis were followed up for a mean duration of 6.3 years. Eleven patients became ANCA negative during clinical remission and 5 relapses occurred with ANCA changing from negative to positive. Two patients developed a positive ANCA without clinical relapse.

Geffriaud-Ricouard *et al.*[1] studied the ANCA (IIF and ELISA) specificities of 98 patients with various types of systemic vasculitis. Serial ANCAs were obtained in 44 patients – there was no association with relapse, however, disappearance of ANCA was associated with remission.

Kerr *et al.*[33] analysed 106 patients with WG, of whom 68 had serial ANCA titres by IIF every 1–3 months. Active disease was assessed clinically as well as with biopsy confirmation if possible. A total of 23 patients had sustained remission. The 45 remaining patients had active disease.

A subset of 17 patients either in remission or with stable "smouldering" disease had disease flares, of which only 4 had a rise in cANCA titre. Out of all the patients studied, 16 patients had a four-fold rise in ANCA titre of which 7 did not have a relapse.

Gordon *et al.*[34] studied relapse in 150 consecutive patients with systemic vasculitis – 12 with polyarteritis, 95 with microscopic polyarteritis and 43 with WG; ANCA were detected by IIF. Positivity at the time of relapse ranged from 2/5 in polyarteritis to 12/13 with WG. Specificity again was low.

Keogh and Specks[35] studied 91 patients with Churg-Strauss syndrome. Serial ANCA testing was performed in 74 patients and 22/30 tested at the time of diagnosis were ANCA positive. Twelve of 16 patients tested during a disease flare were positive with 8/49 of patients tested during remission being positive. Central nervous system involvement correlated with ANCA positivity.

Jayne *et al.*[36] performed a prospective study of 60 patients with ANCA-positive systemic vasculitis with monthly monitoring including ANCAs by IIF and ELISA. Thirty-eight per cent of patients had relapses, 57% of which were associated with an ANCA-IIF rise (defined as a change from negative to positive or a more than 30% rise in titre) in the preceding 8 weeks and with a positive predictive value of 57%.

Davenport *et al.*[37] performed a retrospective study of 37 biopsy-proven WG patients with a total of 532 serial ANCA assays by IIF. Of 82 four-fold rises in ANCA titre, 19 were associated with clinical relapses, 25 with infection and 32 occurred in well patients (PPV 23%).

Blockmans *et al.*[38] performed a retrospective chart review of 94 patients with a positive ANCA IIF. Anti-PR3 and anti-MPO ELISAs were performed on these patients. No correlation was shown between ANCA titre (by IIF or ELISA) and disease activity.

Tervaert *et al.*[39] performed a prospective study over 16 months involving 35 patients with WG. Seventeen relapses occurred and all were preceded by a rise in the ANCA IIF titre.

Boomsma *et al.*[40] undertook a prospective study of 100 patients with WG over 3 years, with ANCA by both IIF and ELISA measured every 2 months. Disease activity was measured by Birmingham Vasculitis Activity Score (BVAS) by clinicians blinded to the ANCA result. Thirty-four of 37 relapses were associated with a rise in ANCA level (more than 75% rise by ROC) with a positive predictive value of 57% by IIF, 71% by PR3-ELISA and 100% by MPO-ELISA. There was an increase in PPV for both assays when there was a concomitant rise in the PR3-ANCA iGG3 subclass but with a large decrease in sensitivity. However, 43% of patients with a rise in cANCA by IIF and 29% by PR3-ELISA did not have a clinical relapse (sensitivity and specificity for IIF, 52% and 75% and for ELISA, 82% and 79%).

Arranz *et al.*[41] performed a retrospective analysis of 118 serum samples from 11 patients by both anti-PR3 direct ELISA and capture ELISA. Capture ELISA showed a significant association with clinical activity (as assessed by the BVAS and key laboratory parameters). Eight clinical relapses occurred in 5 patients with positive capture ELISA in 7 cases and positive direct ELISA in 3.

Nowack *et al.*[42] performed a retrospective study in 18 patients with ANCA-associated systemic vasculitis, analysing 169 serum samples for ANCA by IIF and ELISA. Clinical activity was assessed by BVAS. Nine major and 26 minor relapses occurred, none of which were predicted by increases in ANCA or other parameters such as IgG subclasses or soluble CD14 (sensitivity range 0.18 to 0.45, specificity 0.69 to 0.86).

Girard *et al.*[43] performed a prospective study of 55 WG patients participating in a randomised prospective trial of oral versus pulse cyclophosphamide. In 19% of patients with a positive ANCA (IIF) at the time of diagnosis, there was a correlation between ANCA titre and clinical course. Nine relapses occurred in persistently positive patients and 8 in patients where ANCA had reappeared. Anti-neutrophil cytoplasmic antibody was detected in 13/19 patients who relapsed versus 3/29 who did not.

Gisslen *et al.*[44] retrospectively compared two ELISA methods (capture and direct) in 10 patients with WG with a mean follow-up of 8 years. Twenty-nine episodes of active disease occurred, detected in 100% of cases by capture ELISA and 79% by direct ELISA. The capture assay had low specificity, however, with high titres in patients without active disease.

Boomsma *et al.*[45] followed a cohort of 16 consecutive PR3-ANCA positive WG patients with a renal relapse. Samples at the time of relapse and historic sera collected every 3 months for the preceding 12 months were subjected to several IIF and ELISA assays. Sixteen patients with WG without relapse were used as controls. PPV ranged from 56% to 75%, depending on the assay, with the capture ELISA performing best; NPV was 63% to 75%, again with capture ELISA being superior.

Han *et al.*[46] undertook a retrospective study of 48 patients with ANCA-associated vasculitis with serial measurement of MPO and PR3 ELISAs and clinical assessment by BVAS. Twenty-three relapses occurred in 16 patients. Fourteen of these episodes were associated with a preceding or concurrent 4-fold rise in ANCA titre. Three relapses had a 3-fold or less rise in titre and 6 had a negative or falling titre. Eight patients in complete remission with a 4-fold rise in titre received no increase in immunosuppression, whereas 11 patients also in remission with a 4-fold rise in ANCA were treated with increases in immunosuppression. The untreated group relapsed at a mean of 5.8 months whereas only 2 in the pre-emptively treated group relapsed.

Damoiseaux *et al.*[47] measured PR3 and MPO ANCA by FEIA (fluorescent-enzyme immunoassay) in several patient groups, including 23 patients with PR3-ANCA positive vasculitis with relapse and 23 matched patients without relapse (16 retrospective from the 2003 paper and 7 additional consecutive patients). Eighteen of 26 had a rise in PR3-ANCA by FEIA associated with relapse and 5/10 without a rise in ANCA had a relapse (PPV 69%, NPV 75%).

The question as to whether pre-emptive treatment on the base of a rise in ANCA titre can reduce the risk of subsequent relapse has also been discussed in the literature.

Tervaert[48] looked at 58 patients with biopsy-proven WG. They were screened every 3 months for clinical activity and had ANCA estimation by IIF performed monthly. Twenty patients had a rise in ANCA titre: 9 patients were treated with an increase in immunosuppression and 11 were treated only if there was a clinical relapse. Nine of the latter group relapsed whereas there were no relapses in the pre-emptively treated group.

SUMMARY OF THE EVIDENCE

Attempted system review[31] demonstrated the shortcomings of the published evidence with methodological heterogeneity precluding firm conclusions. There are differences in ANCA assays (IIF and ELISA) and in patient populations and the definition of active disease. Data has been obtained both retrospectively and prospectively (but not always consecutively).

Studies have suggested that the ELISA and particularly, capture ELISA, is more sensitive and specific, although Nowack *et al.*[42] found capture ELISA no better than other laboratory parameters.

Tervaert's[48] paper from 2000 suggests that pre-emptive treatment in response to a rise in ANCA titre may reduce the risk of relapse and also reduce the total immunosuppression burden. Given the lack of certainty about the association between ANCA and disease activity, this cannot currently be recommended.

Monitoring of serial ANCA titres may be of benefit in individual patients; however, with current evidence this should not lead to pre-emptive treatment but at best, careful patient observation.

WHAT DO THE OTHER GUIDELINES SAY?

Kidney Disease Outcomes Quality Initiative: No recommendation.
UK Renal Association: No recommendation.
Canadian Society of Nephrology: No recommendation.
European Best Practice Guidelines: No recommendation.
International Guidelines: No recommendation.

IMPLEMENTATION AND AUDIT

No recommendation.

SUGGESTIONS FOR FUTURE RESEARCH

1. A prospective trial with both clinical (BVAS) and if possible, histological confirmation of disease relapse, agreed treatment protocols and with ANCA testing blinded to patient status is needed. Due to the small numbers of patients, this will require multiple investigating centres and a long period of recruitment and follow-up.
2. New research focusing on the identification of novel markers of disease activity may also help in determining episodes of relapse and disease activation.

CONFLICT OF INTEREST

Grant Luxton has no relevant financial affiliations that would cause a conflict of interest according to the conflict of interest statement set down by CARI.

Robyn Langham has no relevant financial affiliations that would cause a conflict of interest according to the conflict of interest statement set down by CARI.

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APPENDIX

Table 1 Characteristics of included studies

Author	Year	Study design	Disease	ANCA test	Definition of change in titre	Assesment of disease activity	No. of patients	No. of relapses	No. of relapses with change in ANCA	Comments
MacIsaac *et al.*	1990[32]	Prospective cohort	Glomerulonephritis	IIF	Positivity	Clinical assessment	17	14	5	2 positive ANCA without relapse
Geffriaud-Ricouard *et al.*	1993[1]	Prospective cohort	Systemic vasculitis	IIF and ELISA	Rise in titre	Clinical assessment	98 (44 with serial ANCAs)		No association	Disappearance of ANCA was associated with remission
Kerr *et al.*	1993[33]	Prospective cohort	Wegener's granulomatosis	IIF	4 × rise in titre	Clinical and renal biopsy (if possible)	106 (68 with serial ANCAs)	17	4	
Gordon *et al.*	1993[34]	Prospective cohort	Systemic vasculitis	IIF	Positivity	Clinical assessment	150	18	2/5 polyarteritis, 12/13 WG	
Keogh and Specks	2003[35]	Prospective cohort	Churg-Strauss syndrome	IIF	Positivity	Clinical assessment	91 (74 with serial ANCAs)	16	12	
Jayne *et al.*	1995[36]	Prospective cohort	Systemic vasculitis	IIF and ELISA	Negative to positive or a more than 30% rise in titre	Clinical assessment	60	23		
Davenport *et al.*	1995[37]	Retrospective cohort	Wegener's granulomatosis	IIF	4 × rise in titre	Clinical assessment	37 (522 sera)	19		
Blockmans *et al.*	1998[38]	Retrospective chart review	Not stated	IIF and ELISA	Rise in titre	Clinical assessment	94			
Tervaert *et al.*	1999[39]	Prospective cohort	Wegener's granulomatosis	IIF	Rise in titre	Clinical assessment	35	17		
Boomsma *et al.*	2000[40]	Prospective cohort	Wegener's granulomatosis	IIF and ELISA	Rise in titre	BVAS (Birmingham Vasculitis Activity Score)	100	37		
Arranz *et al.*	2001[41]	Retrospective cohort	Systemic vasculitis	Direct ELISA and capture ELISA	Rise in titre	BVAS and lab parameters	14 (118 sera)	8 relapses in 5 patients		
Nowack *et al.*	2001[42]	Retrospective chart review	Systemic vasculitis	IIF and ELISA	Rise in titre	BVAS	15 (105 sera)	37		
Girard *et al.*	2001[43]	Prospective cohort	Wegener's granulomatosis	IIF	Rise in titre	Clinical assessment	55	19		
Gisslen *et al.*	2002[44]	Retrospective cohort	Wegener's granulomatosis	Direct ELISA and capture ELISA	Rise in titre	Clinical assessment	10	2		
Boomsma *et al.*	2003[45]	Retrospective cohort	Wegener's granulomatosis	IIF and ELISA	Rise in titre	Renal relapse	16	16		
Han *et al.*	2003[46]	Retrospective cohort	Vasculitis	ELISA	4 × rise in titre	BVAS	48	23 relapses in 16 patients		
Damoiseaux *et al.*	2005[47]	Retrospective and prospective cohort	Vasculitis	FEIA	Rise in titre	Clinical assessment	23	23		
Tervaert	2000[48]	Prospective cohort	Wegener's granulomatosis	IIF	Rise in titre	Clinical assessment	58	9		

IFF, indirect immunoflorescence; ELISA, enzyme-linked immunosorbent assay; FEIA, fluorescent enzyme immunoassay; BVAS, Birmingham Vasculitis Activity Score; WG, Wegener's granulomatosis.