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Titration of antibodies against neutrophil cytoplasmic antigens is useful in monitoring disease activity in systemic vasculitides

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SUMMARY

Titration of antibodies against neutrophil cytoplasmic antigens (cANCA), as detected by indirect immunofluorescence, is shown to be clinically useful for monitoring disease activity in Wegener's granulomatosis and microscopic polyarteritis. Ten patients were followed (eight from presentation) prospectively for up to 2 years; during this time there were six episodes of vasculitic relapse in four patients and five infective episodes and one pulmonary embolus in four patients. Titres of cANCA were markedly raised, both at presentation (1/32–1/2048) and at vasculitic relapse (1/125–1/1048) but not in infection or embolism (negative, 1/16). Thus the titre of these antibodies can distinguish non-vasculitic illness from vasculitic relapse, in contrast to C-reactive protein levels which were raised in both. Titres of cANCA fell gradually after vasculitic relapse, in keeping with the half-life of IgG (3 weeks). C-reactive protein is a better measure of recovery.

Keywords anti-neutrophil cytoplasmic antibodies Wegener's granulomatosis monitoring

INTRODUCTION

Systemic vasculitis is used to denote a variety of overlapping multi-system disorders involving necrotizing vascular inflammation (Lie, 1988; Dahlberg, Lockhart & Overhold, 1989). The term encompasses a variety of clinical and pathological diagnoses that are difficult to differentiate. Involvement of lungs and kidneys may be life threatening (Fauci, Haynes & Katz, 1978; Savage *et al.*, 1985) and the emergence of tests for antibodies to neutrophil cytoplasmic antigens (ANCA) has provided a means of rapid diagnosis in some patients. The classical anti-neutrophil cytoplasmic antibody (cANCA), detected by indirect immunofluorescence, has been shown to be a useful diagnostic tool in Wegener's granulomatosis and microscopic polyarteritis (MPA) and is positive in almost all cases at presentation (van der Woude, Daha & van Es, 1989).

Only a rise in the level of C-reactive protein (CRP) (Hind *et al.*, 1984a, b) has been shown to predict an increase in disease activity; steroid and immunosuppressive effects on leucocyte and platelet counts reduce the value of these tests for monitoring disease activity. Since CRP levels rise in infection too, differentiation between infection in an immunosuppressed patient and recrudescence of disease activity can be difficult. The relationship of the titre of cANCA to disease activity and prediction of relapse by an increase in titre is not established. This study examined the serial relationship between clinical disease activity, cANCA titre, CRP, erythrocyte sedimentation rate (ESR) and other appropriate laboratory indices. Since the relationship between the classical immunofluorescence pattern, other immunofluorescence patterns (such as the peripheral pattern) and the results of ELISA and solid-phase radioimmunoassay (RIA) assays is not clear (van der Woude *et al.*, 1989), only the classical pattern of immunofluorescence was considered.

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PATIENTS AND METHODS

Patients

Sera from ten patients (eight men and two women), mean age 44 years (range 27–68), presenting with a clinical syndrome of systemic vasculitis were collected over a 2-year period (June 1987 to July 1989). Six patients were subsequently diagnosed as having Wegener's granulomatosis, three as having MPA and one had an unclassified systemic vasculitis. Samples were collected prospectively and stored at minus 20°C.

Disease activity was assessed by the clinician's assessment as recorded in the notes; raised laboratory indices (these included CRP, ESR, urinary sediment or red cells on microscopy); biopsy evidence of vasculitis where available; and creatinine clearance. Intention to treat with increased immune suppression by the clinicians, who did not know the ANCA results, was the primary criterion.

Disease activity at the time of serum sampling was categorised by the following criteria:

Active vasculitis. Clinical diagnosis of active vasculitis accompanied by institution of appropriate immunosuppressive treatment, and supported by laboratory indices of disease activity excluding ANCA. There were six episodes of vasculitic

Table 1. The clinical features and modes of treatment in the 10 patients who were followed serially

Patient no.	Age (years)	Sex	Diagnosis	System involvement								Treatment						Remission achieved?	Number of vasculitic relapses	Number of non-vasculitic illnesses	Positive tissue biopsy	Follow-up (months)	Outcome
				URT	LRT	Renal	Skin	Gut	Joints	Eyes	NS	i.v. MePred.	i.v. cyclo.	Oral pred.	Oral cyclo.	Oral AZA	Plasma phoresis						
1	47	M	MPA		+	+	+	+	+		+	+	+	+	+	+	×5	Yes	2	3	Renal	19	Well
2	34	M	MPA		+	+			+	+		+		+	+			Yes	1	—	Renal	21	Well
3	41	M	WG	+		+			+	+	±	+		+	+			Yes	1	—	Nasal	12	Well
4	45	M	MPA		+	+	+		+					+	+		×5	Yes	—	—	—	12	Well
5	34	M	WG	+	+	+	+		+					+				Yes	—	—	Nasal	10	Well
6	27	F	WG	+	+		+	+	+			+	+					Yes	—	—	Nasal	8	Well
7	68	F	VASC		+	+					+			+		+		Yes	—	1	—	12	Well
8	56	M	WG	+	+	+	+	+	+			+	+	+	+			Yes	—	1	Renal	11	Well
9	53	M	WG	+	+				+	+	+			+	+			Yes	2	1	Nose	24	Well
10	33	M	WG	+	+	+	+	+		+	+	+	+	+	+			Yes	—	—	Skin	1	Well

URT, upper respiratory tract; LRT, lower respiratory tract; NS, nervous system; MePred., methyl prednisolone; Cyclo., cyclophosphamide; AZA, azathioprine; MPA, microscopic polyarteritis; WG, Wegener's granulomatosis.

relapse in four patients (Table 1); samples were also available at the time of initial presentation from eight patients.

Inactive vasculitis (remission). There was no clinical or laboratory evidence of active vasculitis (excluding ANCA titres). Patients with Wegener's granulomatosis who had mild persistant upper respiratory tract symptoms but without systemic symptoms and who were otherwise feeling well, were included in this category. Treatment had either not been started or was being reduced in this group.

Non-vasculitic illness (infections and pulmonary embolus). Institution of appropriate therapy for presumed or proven non-vasculitic complications leading to clinical resolution; clinical assessment that vasculitis was inactive with a resultant reduction of immunosuppressive therapy; there were six such episodes in four patients; four were proven infections, one pyrexia of unknown origin and one pulmonary embolus. All resolved in such a way as to suggest that the diagnosis was accurate. CRP and ESR (laboratory indices of inflammation) were raised in five of these six episodes (four infections and one pulmonary embolus).

Methods

Indirect immunofluorescence on freshly prepared, fixed human polymorphonuclear neutrophils (PMN) was performed using a modification of the method of van der Woude *et al.* (1985).

PMN from a single source were separated by dextran sedimentation for 30 min at 37°C and erythrocytes were lysed by distilled water, with rescue by 1·8% saline. This leucocyte preparation was washed three times and resuspended in phosphate-buffered saline (PBS) with 5% bovine serum albumin (BSA). Cells were cytospun onto glass slides and fixed with 95% ethanol for 5 min at 4°C. Sufficient cells were deposited to form a monolayer with non-overlapping cells. A single source of neutrophils was used for all the assays.

Slides were incubated with doubling dilutions of sera (from 1/2) for 1 h at 4°C, washed in buffer for 20 min, and further incubated with the fluorescein-conjugated second antibody (goat anti-human IgG Fc-specific) (Atlantic Antibodies). Slides were mounted and read immediately. Only classical cytoplasmic staining (cANCA) was reported; other staining patterns were disregarded. All assays were performed and read blind, and all sera from a single patient were assayed in a single batch. The specificity of the conjugated second antibody was validated using a panel of bone marrow slides from patients with multiple myeloma. Optimal dilution of this antiserum has been established using a chequer-board technique (Thompson, 1981).

Each batch of tests included positive and negative control sera as well as controls without test sera. The positive control had been validated by two other laboratories using immunofluorescent and other assays, and was titrated on each occasion. The assay itself had been validated by exchange of 42 blind sera with a reference centre using immunofluorescent techniques.

No cANCA was detected in the sera (at a dilution of 1/8) from 21 patients with systemic lupus erythematosus (SLE), 25 people with bacterial infections (whose sera also gave high CRP levels), (mean 25·7 mg/dl, range 13·7–49· 5), from three patients with Kawasaki disease or from 10 patients with autoimmune thyroid disease. Sera from 30 elderly controls without vasculitis (mean age 77·5 years, range 61–98), 40 healthy blood transfusion donors (20 men and 20 women; mean age 37·4 years; range 19–56), 15 patients with polyarteritis nodosa and 10 with giant cell arteritis were also negative. Anti-nuclear antibodies (ANA), C3, C4, and repeated blood cultures (when relevant) were negative.

Statistical analysis

Student's *t*-test was used to analyse the significance of the CRP and ESR data and the Mann–Whitney *U*-test was used for ANCA data.

RESULTS

The clinical details of the 10 patients in the study are listed in Table 1. A wide variety of organ systems were involved and the

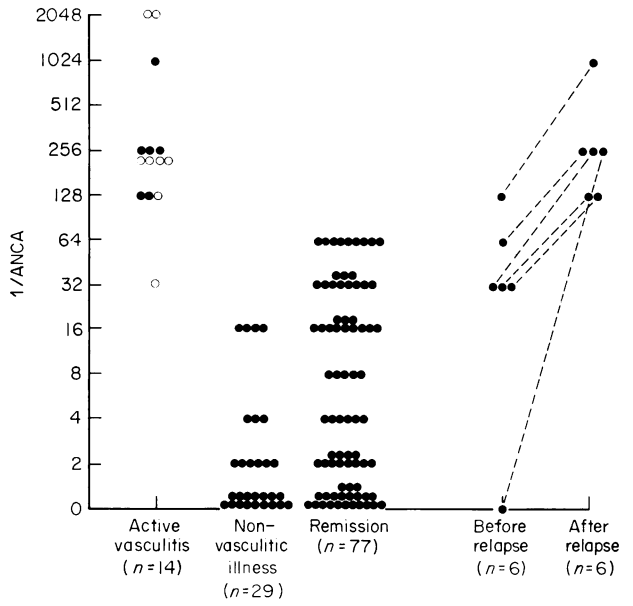

Fig. 1. cANCA titres in patients with active vasculitis, remission and non-vasculitic illness; paired samples (pre- and during relapse) from individual patients are also shown. ●, Relapses; ○, at presentation.

patients were initially seen by specialists in rheumatology, neurology, nephrology, chest and general medicine. All patients were alive at the end of the follow-up period; all but one were in remission at the end of the study. Patient 2, though asymptomatic, had recurrent microscopic haematuria at his last visit but without other laboratory indicators of relapse.

One-hundred and sixty-two serum samples were collected from these 10 patients and ANCA titres were determined. Eight samples were from the time of initial presentation and six were the initial samples at the time of vasculitic relapse; there was no evidence of infection at these times and the patients had not yet been started on treatment. These titres are shown in Fig. 1, as are the titres for the previous sample for each patient in the latter group (i.e. prerelapse samples). Twenty-nine further sera were taken during non-vasculitic illnesses and 82 samples from patients during clinical remission. Five samples from the latter patients were excluded from initial analysis (Fig. 1) for the following reasons: two sera were within 4 weeks of treated relapses and CANCA titres were therefore still elevated in keeping with the half-life of immunoglobulin (3 weeks); three were excluded because they were subsequently shown to be in the pre-relapse phase of vasculitis. In order to examine the rate of fall in cANCA titre following an episode of active vasculitis, 116 samples were used.

ANCA titre at presentation

cANCA titres, levels of CRP and ESR were available at the time of diagnosis for eight patients at first presentation (Table 2). All had evidence of an acute phase response with raised CRP levels (2·5–17·6 mg/dl) and raised values of ESR (75–110 mm/hr). Leucocyte and platelet counts, however, were not raised in all patients. cANCA titres were high in all these patients (1/32– > 1/2048), but there was no relation to disease severity as judged by the need for intensive therapy support. The three patients requiring intensive therapy support (for pulmonary involvement with hypoxaemia) did not have significantly elevated titres compared with the five who did not need intensive therapy support; neither were levels of CRP, leucocyte counts or platelet counts significantly different between these two groups of patients.

Table 2. Laboratory indices at presentation in eight episodes (initial samples only)

	CRP (mg/dl)		cANCA (1/titre)		ESR (mm/h)	
	Mean ± s.d.	Range	Median	Range	Mean ± s.d.	Range
Active vasculitis (8 sera)	10·7 ± 4·5	2·5 ± 17·6	256	32– > 2048	92 ± 15 (5 samples)	75–110
ITU admissions (3 sera)	8·1 ± 5	2·5 ± 12·6	256	128– > 2048	108 (1 sample)	108
Non-ITU admissions (5 sera)	12·2 ± 3·8	7·3 ± 17·6	256	32– > 2048	88 ± 14 (4 samples)	75–108

	Leucocyte count ($\times 10^9$/l)		Platelets ($\times 10^9$/l)	
	Mean ± s.d.	Range	Mean ± s.d.	Range
Active vasculitis (8 blood counts)	11·5 ± 3	6·4–16·2	478 ± 198	218–780
ITU admissions (3 blood counts)	13·3 ± 2·6	11·1–16·2	446 ± 295	218–780
Non-ITU admissions (5 blood counts)	10·5 ± 3	6·4–13·9	498 ± 154	266–644

ITU, Intensive Therapy Unit.

Table 3. cANCA titres in relapse (initial samples), inactive vasculitis, and non-vasculitic illness

	CRP (mg/dl)		cANCA (1/titre)		ESR (mm/h)	
Diagnosis	Mean ± s.d.	Range	Median	Range	Mean ± s.d.	Range
Relapse active vasculitis (6 sera from 6 episodes)	8·2 ± 3·0	3·3–11	256	128–1024	92 ± 17 (4 samples)	77–110
Non-vasculitic illness (6 episodes 29 sera)	9·3 ± 10	1–38·7	2	0–16	47 ± 20 (4 samples)	25–63
Clinical remission (77 sera)	0·88 ± 0·5	0·6–2·8	8	0–64	35 ± 23 (22 samples)	3–75

Table 4. Rising cANCA titres in paired samples between remission and relapse in six episodes

	CRP (mg/dl)		cANCA (1/titre)		ESR (mm/h)	
	Mean ± s.d.	Range	Median	Range	Mean ± s.d.	Range
Before relapse (6 sera, 6 episodes)	1·4 ± 0·99	0·6–2·6	32	0–128	28 ± 27 (4 samples)	6–58
In relapse (6 sera, 6 episodes)	8·1 ± 2·9	3·3–10·8	256	128–1024	92 ± 17 (4 samples)	77–110
Mean interval (weeks) 7·7 ± 5 Range 2–15 weeks						

Active vasculitis versus *non-vasculitic episodes*

Comparisons of the CRP, ESR and cANCA values in episodes of active vasculitic *versus* non-vasculitic illness are given in Table 3. cANCA values were significantly different, being raised in all episodes of relapse of active vasculitis and low in non-vasculitic episodes ($P < 0{\cdot}001$) and remission ($P < 0{\cdot}001$, Table 3). There was little difference between the CRP values in these situations ($P > 0{\cdot}5$) but both were significantly different from levels seen in remission ($P < 0{\cdot}001$). ESR values were higher in active vasculitis ($P < 0{\cdot}001$) than in either of the other two groups, but there was little difference between non-vasculitic relapse and remission ($0{\cdot}1 < P < 0{\cdot}5$).

Relapse

The significance of rising titres of cANCA antibodies is illustrated in Fig. 1. Six episodes of disease relapse in four patients were preceded by increased titres of cANCA of greater than four-fold in the preceding 2–15 weeks ($P < 0{\cdot}01$), and accompanied by a significant rise in CRP and ESR values ($P = < 0{\cdot}01$, Table 4).

The relationship of CRP levels and cANCA titres to clinical course and treatment in patient 1, who had two vasculitic relapses, two episodes of infections and a pulmonary embolus during follow up, is shown in Fig. 2. cANCA titres rose at the time of diagnosis of relapse on both occasions. Furthermore, cANCA titres differentiated vasculitic relapse from non-vasculitic illness in three out of three episodes, in contrast to CRP levels which were raised in infection and pulmonary embolus. However, in active vasculitis, CRP titres responded more rapidly to treatment than cANCA titres, in keeping with the rapid turnover of CRP compared with that of antibody molecules.

This is further illustrated by patient 8, who had relapsed and was treated with oral immunosuppression prior to admission for i.v. therapy for persistent symptoms. On admission, 3 weeks after the start of the relapse, CRP was normal but cANCA remained raised; the ANCA fell from 256 to negative over the following 6 weeks, reflecting the longer half-life of immunoglobulins (3 weeks) compared with the CRP.

Figure 3 shows cANCA titres at various times after a period of active vasculitis. The fall of the titres in each 3-week period is compatible with the half-life of IgG. Two patients who were treated with plasma exchange showed rapid falls, as expected as a result of this treatment, and their results are not included in the figure.

In two patients, cANCA titres have risen again, as yet without concurrent rises in CRP or clinical symptoms, although in one case there has been a recrudescence of microscopic haematuria after 9 weeks. The other remains well after 16 weeks.

DISCUSSION

The indirect immunofluorescent assay for ANCA is now well established as a rapid screening test. The classical cANCA

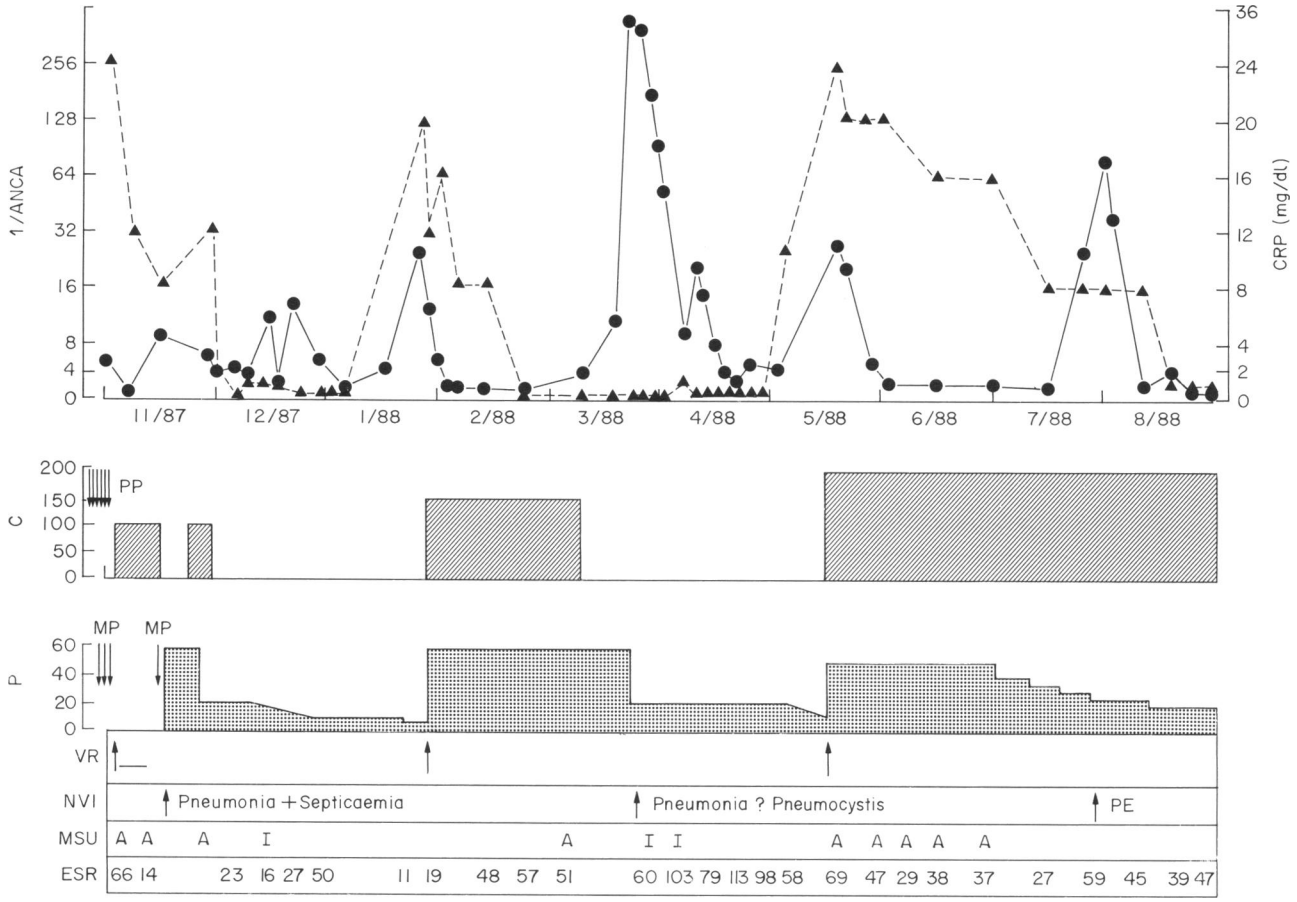

Fig. 2. Serial measurements of cANCA titres (▲ and ▨), C-reactive protein (CRP) (● and ▨) and erythrocyte sedimentation rate (ESR), shown in relation to clinical course and treatment in patient 1. C, dose of cyclophosphamide; PP, plasma exchange; P, dose of prednisolone; MP, infusion of 1 g of methyl prednisolone; VR, vasculitic relapse; NVI, non-vasculitic illness; PE, pulmonary embolus; MSU, urinary microscopic findings; A, erythrocytes/erythrocyte casts; I, no erythrocytes/erythrocyte casts.

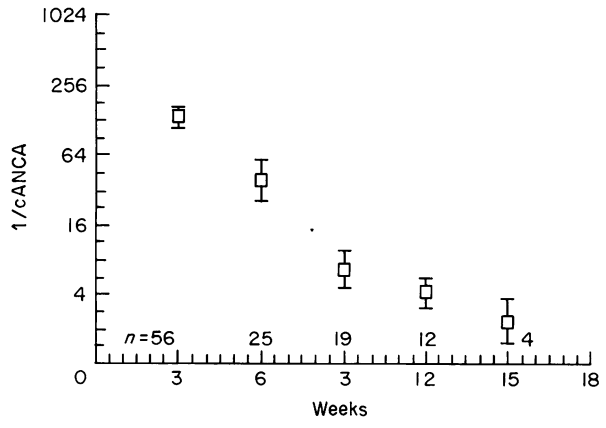

Fig. 3. cANCA titres (mean ± s.e.m.) in relation to time after onset of treatment for an episode of active vasculitis, both at presentation and in relapse, for patients who did not receive plasmaphoresis. (*n*, number of samples in given time period).

immunofluorescent pattern is well described, and rarely found in vasculitis other than that due to Wegener's granulomatosis or MPA. Since the original descriptions of the classic 'c-ANCA' pattern of cytoplasmic fluorescence, other distinct non-classic patterns have been reported in a variety of other conditions. ELISA and solid-phase RIAs have been developed but the exact relationship of these to the classical pattern is uncertain (van de Woude *et al.*, 1989). This study therefore only examined the usefulness of the cANCA pattern in monitoring activity in a group of systemic vasculitides.

All the 10 patients described had elevated titres of cANCA in association with active disease, a finding which confirms suggestions from earlier studies. Titres varied widely between patients and did not correlate with the severity of an individual patient's disease (Specks *et al.*, 1989).

Levels of CRP, ESR and cANCA all rose significantly during relapses; in all six episodes, cANCA rises of greater than four-fold preceded the relapse by 2–15 weeks. Prediction of relapse by ANCA has been commented on by Andrassy *et al.* (1989) but no data have been given; however, these authors did not distinguish between cANCA and the peripheral pattern of ANCA.

Over a total follow-up period of 131 patient-months (including eight patients for at least 10 months each) there were only two episodes when patients were apparently in clinical remission and yet had a greater than four-fold rise in cANCA titres over 9 or 16 weeks. One of these patients has developed microscopic haematuria and this may represent subclinical disease or incipient relapse. The other patient continues to have a raised titre and may yet relapse; a similar patient has been described by Cohen-Tervaert *et al.* (1989). Only the cANCA titres differentiated disease relapse from clinical deterioration due to other causes (Figs 1 and 2). This is important, given the dangers of infection in the immunosuppressed host and the clinical difficulty in differentiating infection from disease relapse in many cases. Leucocyte counts and thrombocytosis are of little use or for monitoring disease activity, due to steroid effects.

This study shows a correlation between disease activity and antibody titres over a reasonable follow-up period, and demonstrates for the first time that cANCA is a useful marker for distinguishing disease relapse from non-vasculitic illness in a group of systemic vasculitides. CRP levels respond more rapidly to disease remission than cANCA but this is not surprising. While IgG has a half-life of 3 weeks, the CRP has a half-life of 8 h, and is therefore a more useful monitor of early response to treatment. Furthermore, these IgG antibodies fall gradually, in keeping with the half-life. Care must be taken in interpretation of cANCA titres within 6–15 weeks of vasculitic relapse (Fig. 3).

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