Rheumatology 2001;40:147–151

Are antineutrophil cytoplasmic antibodies a marker predictive of relapse in Wegener's granulomatosis? A prospective study

T. Girard, A. Mahr, L.-H. Noël[1], J.-F. Cordier[2], P. Lesavre[1], M.-H. André and L. Guillevin

Service de Médecine Interne, Hôpital Avicenne, Université Paris-Nord, 125, rue de Stalingrad, 93009 Bobigny Cedex, [1]Service de Néphrologie et d'Anatomopathologie, Hôpital Necker, Université de Paris, Paris and [2]Service de Pneumologie, Hôpital Louis-Pradel, Université Claude-Bernard, Lyon, France

Abstract

Objectives. To investigate the predictive value of testing for antineutrophil cytoplasmic antibodies (ANCA) in 55 patients with systemic Wegener's granulomatosis (WG) included in a randomized, prospective trial comparing corticosteroids and oral or pulse cyclophosphamide.

Methods. All 55 patients received corticosteroids. A cyclophosphamide pulse of 0.7 g/m^2 was given at the time of diagnosis. After the first pulse, the patients were assigned at random to receive either pulse or oral cyclophosphamide (2 mg/kg/day), independently of ANCA results. ANCA were sought using an immunofluorescence assay and an attempt was made to correlate them with relapse of WG. ANCA were monitored throughout the study.

Results. At the time of diagnosis, ANCA were detected in 48 (87%) patients, with a cytoplasmic labelling pattern in 44 and a perinuclear pattern in four. ANCA follow-up was available for 50 patients. ANCA disappeared in 34 patients and persisted in nine. For 79% of the patients, the clinical course improved with the disappearance of ANCA and deteriorated with their persistence or increased titre. Among the patients who were initially ANCA-positive, 23 relapses occurred. Relapses were more frequent when ANCA remained positive or reappeared [13/19 ANCA-positive patients *vs* 3/29 ANCA-negative patients ($P < 0.01$)]. Nine relapses (39%) occurred in patients with persistent ANCA, and ANCA reappearance preceded relapse in eight (35%). The mean time between inclusion and relapse did not differ between the patients who became ANCA-negative and those who were persistently ANCA-positive (14.6 ± 13.2 *vs* 14.4 ± 8.2 months). The mean time to ANCA disappearance was similar for the patients who relapsed and those who did not. Corticosteroids and pulse or oral cyclophosphamide did not significantly modify the time to ANCA disappearance. Throughout the study, seven patients were ANCA-negative.

Conclusion. Although ANCA positivity was associated with relapse, discordance between cytoplasmic ANCA and disease activity was not unusual. In the absence of clinical manifestations, ANCA titres alone can serve as a warning signal but not indicate whether to adjust or initiate treatment.

KEY WORDS: Antineutrophil cytoplasmic antibodies, Wegener's granulomatosis, Corticosteroids, Cyclophosphamide.

Wegener's granulomatosis (WG) is a rare vasculitis with a prevalence of 3/100 000 in the USA [1]. Kidneys, lungs and upper and lower respiratory tracts are predominantly involved, with inflammatory lesions typically including necrosis, granulomatous changes and vasculitis. Despite treatment with steroids in combination with alkylating agents, relapses occur in half of the cases. The need for long-term therapy increases the risk of side-effects and treatment toxicity must be carefully monitored [2].

Antineutrophil cytoplasmic antibodies (ANCA), discovered in 1985 [3], are now considered to be a useful tool for diagnosis, especially when they have a cytoplasmic labelling pattern (c-ANCA) and when anti-proteinase

Submitted 30 March 2000; revised version accepted 2 September 2000.

Correspondence to: L. Guillevin.

3 (PR3) antibodies are found in enzyme-linked immunosorbent assay (ELISA) [4], and for the classification of vasculitides [5]. ANCA are considered by some authors [6] to be a sensitive marker of disease activity and remission, and might predict relapse. However, their role remains controversial [7] and, in the absence of new studies, ANCA cannot at present be used as a marker indicating that treatment should begin. To clarify the prognostic value of ANCA in WG, we analysed the ANCA test results of patients who participated in a prospective treatment trial [8].

Patients and methods

Fifty-five patients were included in a prospective, randomized, multicentre trial comparing steroids and randomly assigned to treatment with oral or pulse cyclophosphamide (CY). We previously reported [8] the results of the therapeutic trial for the first 50 patients who reached the endpoint. In the present study, we included five patients who did not reach that endpoint.

Only patients with newly diagnosed systemic WG were enrolled. Blood samples were drawn within 1 week after diagnosis and/or prior to treatment initiation. All patients fulfilled the following inclusion criteria: (i) age > 15 yr; (ii) systemic WG diagnosed clinically based on the presence of multiorgan involvement [ear, nose and throat (ENT) and/or lung and/or rapidly progressive glomerulonephritis associated with severe general symptoms] or monovisceral involvement, representing a potential risk of severe morbidity or mortality; (iii) histopathological evidence of necrotizing granulomatous vasculitis, granulomatous inflammation and vasculitis, or segmental necrotizing glomerulonephritis.

Clinical, biological and radiological data were collected during the days preceding inclusion. Symptoms recorded for analysis have been described elsewhere [8]. Biopsy sites were oriented by clinical symptoms.

Treatment protocol

All patients received the same initial regimen, which consisted of i.v. methylprednisolone (15 mg/kg body weight/day) for 3 consecutive days, then oral prednisolone (1 mg/kg body weight/day). One i.v. pulse of CY was administered (0.7 g/m^2) the day after the last methylprednisolone infusion, i.e. concurrently with the first day of oral prednisolone. The patients were randomly assigned to one of the following CY regimens: an i.v. pulse of CY (0.7 g/m^2) administered every 3 weeks, or daily oral CY (2 mg/kg body weight/day), starting on day 10 following the initial CY pulse. The steroid tapering protocol and the adjustment of CY dose as a function of the neutrophil count and renal function are detailed elsewhere [8]. The total duration of CY treatment was 2 yr.

Evaluation of disease activity

Disease activity was assessed at the time of pulse infusion or at comparable intervals for patients taking CY orally. After stopping CY, evaluations were mandatory every 3 months until steroids were stopped, then three times a year until the closing date of 31 December 1995. Disease activity was defined by clinical, radiological and laboratory criteria. The disease was considered to be in complete remission when the patient's general condition improved, no new manifestations of WG appeared and the erythrocyte sedimentation rate returned to normal under treatment. Stabilization or improvement (partial or total) of renal function and other signs present initially was also necessary. The disease was considered to be in partial remission when clinical and radiological symptoms were stable or attenuated and laboratory abnormalities regressed under continued treatment.

Relapses were defined as new major systemic manifestations of WG affecting the same or different organ(s) than initially involved or worsening of the initial symptoms of the disease. ABCA were tested regularly, blood samples being drawn at control visits and when new manifestations occurred. ANCA test results were not taken into consideration for the adjustment of treatment or assessment of disease activity.

ANCA

All sera (diluted 1/10) were subjected to an indirect immunofluorescence assay using a home-made cytocentrifuged neutrophil preparation, fixed in ethanol according to the standard technique proposed by Wiik [9]. Immunofluorescence assays were performed every 3 weeks until ANCA disappeared, then regularly at longer intervals. For five patients, ANCA were not assayed systematically during follow-up. For all patients, we distinguished between no detectable ANCA and perinuclear (p-ANCA) or cytoplasmic (c-ANCA) labelling patterns. When ANCA were positive, sera were tested by ELISA for anti-myeloperoxidase activity. When c-ANCA were found, sera were tested by ELISA for anti-PR3 reactivity. Two assays were used, one home-made [10] (before 1997) and a commercial kit (after 1997; Bioadvance, Emerainville, France). The results of the first assay were verified by the second. Because their results were not sufficiently reproducible and because a centralized analysis of all sera was not available, only the immunofluorescence assays are reported here.

Statistical analysis

Comparisons were made using the χ^2 test, $P < 0.05$ being considered significant. Student's *t*-test was used to evaluate quantitative characters at the time when ANCA titres became negative.

Results

At the time of diagnosis

The clinical characteristics of the 55 patients at the time of inclusion are summarized in Table 1. Their classification according to ANCA status is given in Table 2. Abnormal radiological findings were found

in 43 patients, with masses sometimes excavated in six cases, nodules and, more rarely, infiltrates, interstitial syndrome or pleuritis. At inclusion, among the 43 patients with at least renal involvement, 28 had proteinuria, 34 haematuria and 26 both.

Forty-eight (87%) patients were ANCA-positive by indirect immunoflourescence (44 (91.6%) c-ANCA and four p-ANCA). Seven patients were ANCA-negative.

Forty-one of the 43 patients presenting with renal involvement were ANCA-positive (four p-ANCA, 36 c-ANCA, one c- and p-ANCA). Among the four patients with p-ANCA, one had only kidney involvement, two had lung and kidney involvement, and one had ENT, lung and kidney involvement. Seven of the 12 patients without renal involvement were c-ANCA-positive (not significant).

TABLE 1. Characteristics and organ system involvement (%) at randomization of 55 WG patients

Sex (M/F)	33/22
Mean age	53.4 yr
Kidney ($n = 43$)	78
Lungs ($n = 44$)	80
Ear, nose and throat ($n = 41$)	74
Other organ involvement ($n = 38$)	69
CY regimen (pulse/oral)	30/25

Follow-up

At the end of the study, 34 (62%) patients were in remission, 22 (40%) in complete remission and 12 (22%) in partial remission, and 19 patients had died. For two patients, we did not have any information on follow-up. Twenty-nine patients (including two who were initially ANCA-positive but for whom ANCA follow-up was insufficient) did not relapse and 19 did (25 relapses; 14 once, four twice and one 3 times). Three of the four p-ANCA-positive patients and 16 of the 44 (37%) c-ANCA-positive patients relapsed (NS).

Serial follow-up of ANCA was obtained for 50 patients (43 ANCA-positive and seven ANCA-negative patients). ANCA follow-up data were available for 23 of the 25 relapses. Figure 1 summarizes ANCA follow-up according to the occurrence of relapses. ANCA disappeared in 34 patients and persisted in nine.

ANCA were detected in 13 of the 19 patients who relapsed (17/23 relapses occurred in ANCA-positive patients for whom follow-up was available). Among the 29 patients who did not relapse, three had, from the onset, persistent ANCA positivity (13/19 *vs* 3/29; $P < 0.001$). ANCA-positive patients had an 18-fold higher risk of relapse (odds ratio 18; 95% confidence interval 4–87).

The mean time to ANCA disappearance after entry into the study did differ significantly between patients

TABLE 2. Clinical status according to the E, L, K classification and ANCA status at randomization

Involvement	ANCA+ ($n = 48$)	c-ANCA ($n = 44$)	p-ANCA ($n = 4$)	ANCA− ($n = 7$)
ELK	25	24	1	1
LE	5	5		2
KE	6	6		
KL	8	6	2	
L	2	2		1
K	2	1	1	1
E				2

E, ear; L, lung; K, kidney.

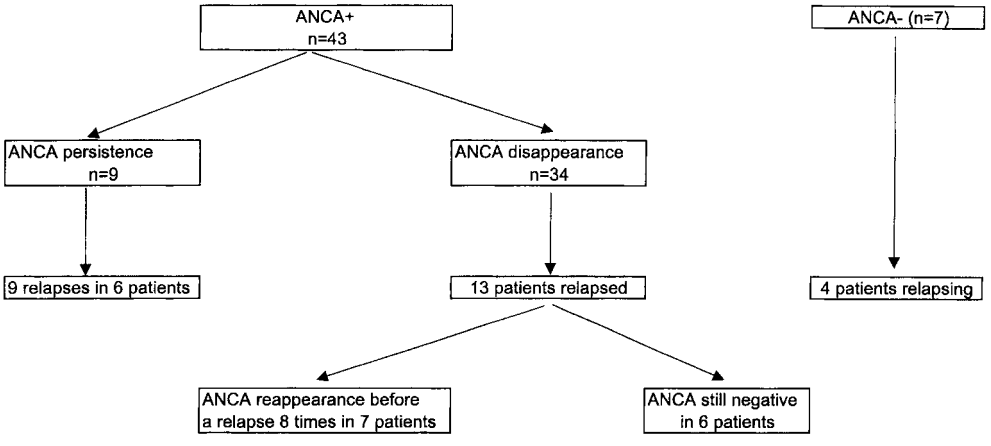

FIG. 1. Wegener's granulomatosis relapses as a function of antineutrophil cytoplasmic antibody (ANCA) evolution. Among the 48 initially ANCA positive patients, sufficient data are available for only 43.

Table 3. Time between ANCA reappearance and relapse in six patients

Patient	ANCA reappearance–relapse interval (months)	ANCA status after relapse	Remission	Follow-up (months)
1	10	Negative	Complete	41
2	1	Negative	Partial	38
3	2	Fluctuating	Partial	23
4	1	Positive	Complete	43
5	15	Negative	Complete	71
6	3	Positive	Complete	51

who relapsed (115.5 ± 86.4 days) and those who did not (82 ± 63.4 days).

ANCA disappeared in 17/26 patients receiving pulse CY and 18/22 treated with oral CY (not significant). The mean time between the onset of treatment and relapse was comparable for patients in whom ANCA disappeared (14.6 ± 13.2 months; $n = 14$) under treatment and those in whom they persisted (14.4 ± 8.2 months; $n = 5$). For the ANCA-positive patients treated with pulse CY and whose ANCA disappeared under treatment, the mean time to ANCA disappearance did not differ significantly regardless of whether the patient relapsed (87.5 ± 46 and 71 ± 48.5 days respectively). Among the patients taking oral CY and whose ANCA disappeared under treatment, the mean time to ANCA disappearance was 199 ± 118 days for patients who relapsed and 87.5 ± 69.5 in those who did not (not significant).

Of the seven patients whose ANCA disappeared during induction treatment and then reappeared before a relapse, five had received pulse CY and two oral CY. ANCA evolution between their reappearance and disease relapse are presented for six of them in Table 3.

Among the 43 initially ANCA-positive patients for whom follow-up data were available, the clinical course paralleled ANCA evolution for 79% of them; i.e. ANCA persistence or reappearance accompanied aggravation, whereas the disappearance of ANCA coincided with attenuation. Among the patients who were ANCA-positive at relapse, 31% had persistent ANCA and 37% experienced reappearance of ANCA before relapse.

During the follow-up of the patients who had been ANCA-negative before treatment, we never detected ANCA, despite the occurrence of four relapses.

No difference was found between the ANCA status of the group of patients who relapsed and/or died (82.3% ANCA-positive) and those who did not relapse and/or die (95.2% ANCA-positive).

Discussion

To date, no markers have been clearly identified as predictive of the occurrence of WG relapses. For many years, the kinetics of ANCA has been studied under treatment, but the results obtained remain controversial and the role of ANCA in patient follow-up has not been definitively established. The first studies on ANCA as a potential marker of relapse showed that relapses were preceded by significant increases in ANCA titres [11, 12]. For some authors [11–14] these results were considered sufficient to start early treatment. In another study, the authors observed that ANCA titres were not correlated in every case with the progression of WG [7]. In that study, ANCA paralleled disease outcome in 64% of patients, 9% of the patients had paradoxical changes between disease activity and the appearance of ANCA, and 9% had persistently negative ANCA during periods of active disease.

In the present study, ANCA sensitivity for active disease was 87% (92% for c-ANCA and 8% for p-ANCA). Relapses were associated with reappearance of ANCA in 37% of the patients and persistence in 31%. As shown in Table 3, among six patients initially ANCA-positive and then ANCA-negative, the time between ANCA reappearance and relapses for four of them was 3 months or less, and for the two others it was 10 and 15 months, demonstrating that ANCA can be positive for several months before relapse.

We also observed that the time to ANCA disappearance in patients who relapsed was longer for those who had received oral CY than for those who had received pulses. Although not significant, this difference could favour the quicker effectiveness of pulses in the short term.

Although the evolution of ANCA under treatment was not correlated with the time to remission or its duration, ANCA reappeared more frequently in patients who relapsed than in those who did not. In addition, the time of occurrence of relapse could not be predicted. These results confirm that discordance between c-ANCA and disease activity is not uncommon. ANCA cannot be considered a predictor of mortality, as the ANCA status did not differ in patients who relapsed and/or died. In this series, deaths were attributed to active vasculitis or infections and not to ANCA positivity. The mortality rate of our patients was higher than those reported for other series. It could be attributed to the method of recruitment used and to infectious side-effects rather than to a different pattern of clinical manifestations or age of the patients compared with the other series [3, 15–17].

Like Kyndt *et al.* [18], we showed that the percentage of patients relapsing was higher for those with persistent ANCA positivity than for those with negative or

declining ANCA titres. On the other hand, Kyndt *et al.* found that one-third of relapses were preceded or accompanied by an increase in ANCA titre.

Owing to the very small number of p-ANCA patients, no conclusions can be drawn concerning this marker for disease monitoring other than to say that p-ANCA can be observed in WG with renal involvement.

In conclusion, we would like to emphasize that ANCA are a marker for monitoring WG and that most patients with persistent or reappearing ANCA are candidates for relapse. Nevertheless, because the correlation between relapse and ANCA is too weak and there are too many major discrepancies, we cannot recommend ANCA monitoring as a tool for treatment decisions and adaptation. ANCA are certainly a warning signal for relapse and their presence indicates that patient monitoring should be intensified.

Further studies are needed to determine whether ANCA monitoring can be used to reduce drug doses more progressively or to maintain the regimen unchanged when the patient is under therapy.

References

1. Cotch MF, Hoffman GS, Yerg DE, Kaufman GI, Targonski P, Kaslow RA. The epidemiology of Wegener's granulomatosis. Estimates of the five-year period prevalence, annual mortality and geographic disease distribution from population-based data sources. Arthritis Rheum 1996;39:87–92.
2. Hoffman GS, Kerr GS, Leavitt RY *et al.* Wegener granulomatosis: an analysis of 158 patients. Ann Intern Med 1992;116:488–98.
3. Van der Woude FJ, Rasmussen N, Lobatto S *et al.* Autoantibodies against neutrophils and monocytes: a new tool for diagnosis and marker of disease activity in Wegener's granulomatosis. Lancet 1985;1:425–9.
4. Niles JL, McCluskey RT, Ahmad MF, Arnaout MA. Wegener's granulomatosis autoantigen is a novel neutrophil serine proteinase. Blood 1989;74:1888–93.
5. Falk R, Hogan S, Carey T, Jennette C. Clinical course of anti-neutrophil cytoplasmic antibody-associated glomerulonephritis and systemic vasculitis. Ann Intern Med 1990;113:656–63.
6. Cohen Tervaert J, Goldschmeding R, Elema JD *et al.* Association of autoantibodies to myeloperoxidase with different forms of vasculitis. Arthritis Rheum 1990; 33:1264–72.
7. Kerr GS, Fleisher TA, Hallahan CW, Leavitt RY, Fauci AS, Hoffman GS. Limited prognostic value of changes in antineutrophil cytoplasmic antibody titers in patients with Wegener's granulomatosis. Adv Exp Med Biol 1993;336:411–4.
8. Guillevin L, Cordier J-F, Lhote F *et al.* A prospective, multicenter, randomized trial comparing steroids and pulse cyclophosphamide versus steroids and oral cyclophosphamide in the treatment of generalized Wegener's granulomatosis. Arthritis Rheum 1997;40:2187–98.
9. Wiik A. Delineation of a standard procedure for indirect immunofluorescence detection of ANCA. APMIS 1989; 6(Suppl.):12–3.
10. Hagen E, Daha M, Hermans J *et al.* Diagnostic value of standardized assays for antineutrophil cytoplasmic antibodies in idiopathic systemic vasculitis. EC/BCR project for ANCA assay standardization. Kidney Int 1998;53:743–53.
11. Cohen Tervaert JW, van der Woude FJ, Fauci AS *et al.* Association between active Wegener's granulomatosis and cytoplasmic antibodies. Arch Intern Med 1989;149:2461–5.
12. Cohen-Tervaert JW, Huitema MG, Hene RJ *et al.* Prevention of relapses of Wegener's granulomatosis by treatment based on antineutrophil cytoplasmic antibodies. Lancet 1990;2:709–11.
13. Egner W, Chapel HM. Titration of antibodies against neutrophil cytoplasmic antigens is useful in monitoring disease activity in systemic vaculitis. Clin Exp Immunol 1990;82:244–9.
14. Specks U, Wheatkey CL, McDonald TJ, Rohrbach MS, DeRemee RA. Anticytoplasmic autoantibodies in the diagnosis and follow-up of Wegener's granulomatosis. Mayo Clin Proc 1989;64:28–36.
15. Fauci AS, Haynes BF, Katz P, Wolff SM. Wegener's granulomatosis: prospective clinical and therapeutic experience with 85 patients for 21 years. Ann Intern Med 1983;98:76–85.
16. Rao JK, Allen NB, Feussner JR, Weinberger M. A prospective study of antineutrophil cytoplasmic antibody (c-ANCA) and clinical criteria in diagnosing Wegener's granulomatosis. Lancet 1995;346:926–31.
17. Reinhold-Keller E, Kekow J, Schnabel A *et al.* Influence of disease manifestations and antineutrophil cytoplasmic antibody titer on the response to pulse cyclophosphamide therapy in patients with Wegener's granulomatosis. Arthritis Rheum 1994;37:919–24.
18. Kyndt X, Reumaux D, Bridoux F *et al.* Serial measurements of antineutrophil cytoplasmic autoantibodies in patients with systemic vasculitis. Am J Med 1999; 106:527–33.