

Opportunistic Infections Are Preceded by a Rapid Fall in Antineutrophil Cytoplasmic Antibody (ANCA) Titer in Patients with ANCA Associated Vasculitis

RUNOLFUR PALSSON, HYON K. CHOI, and JOHN L. NILES

ABSTRACT. Objective. To study the clinical course and changes in antineutrophil cytoplasmic antibody (ANCA) titers in patients with ANCA associated vasculitis (AAV) who developed opportunistic infections.

Methods. Among the patients with AAV tested in the Immunopathology Laboratory at the Massachusetts General Hospital between 1989 and 1998, all patients who experienced opportunistic infections (n = 16) were included. We retrospectively studied their clinical features and examined the relationship between changes in ANCA titer and the onset of the opportunistic infections. ANCA titers were measured by antigen-specific ELISA.

Results. Of the 16 AAV patients with opportunistic infection, 15 had no evidence for active vasculitis at the time of the infection. Among these 15 patients, opportunistic infections were associated with a steep fall in ANCA titers. There was no consistent pattern of change in C-reactive protein levels. In 7 patients, the immunosuppressive regimen was increased for new clinical findings shortly before the diagnosis of an opportunistic infection, despite the absence of histologic documentation of active vasculitis. Three of these 7 patients died. One patient, who did not experience a significant fall in ANCA titer, i.e., less than 4-fold from his prior peak, was simultaneously found to have *Pneumocystis carinii* pneumonia and biopsy proven active vasculitis.

Conclusion. Our data strongly suggest that opportunistic infections in patients with AAV are associated with negative or rapidly falling ANCA titers. Therefore, changes in ANCA titer can help distinguish opportunistic infections from vasculitis flares when patients with AAV present with indeterminate clinical findings. (J Rheumatol 2002;29:505–10)

Key Indexing Terms:

OPPORTUNISTIC INFECTION

ANTINEUTROPHIL CYTOPLASMIC ANTIBODIES
VASCULITIS

Antineutrophil cytoplasmic antibodies (ANCA) are associated with the spectrum of vasculitis that includes Wegener's granulomatosis (WG), microscopic polyangiitis, the Churg-Strauss syndrome, and primary pauciimmune necrotizing and crescentic glomerulonephritis¹⁻³. Two specific types of ANCA have been shown to be useful in the diagnosis of this disease spectrum: antiproteinase 3 antibodies (anti-PR3), which produce a cytoplasmic pattern of staining by indirect

immunofluorescence (C-ANCA), and antimyeloperoxidase antibodies (anti-MPO), which produce a perinuclear pattern of staining (P-ANCA)⁴⁻⁶.

Studies suggest that serologic tests for ANCA can be useful in longterm management of patients with this group of diseases⁷⁻¹³. Longitudinal studies have shown a positive correlation between ANCA titers and disease activity in patients with WG^{7-9,13}. However, other investigators have not confirmed this correlation^{12,14}.

Immunosuppressive therapy with the combination of cyclophosphamide and glucocorticoids has markedly improved the outcome of ANCA associated vasculitis (AAV)¹⁵⁻¹⁸. Unfortunately, this therapy is often associated with major side effects¹⁸. Among the most serious adverse effects are opportunistic infections, which result from the immunosuppressed state¹⁸⁻²⁰.

The clinical presentation of an opportunistic infection may mimic that of a vasculitis flare and it may be difficult to distinguish between these 2 disorders. Not uncommonly, physicians pursue invasive biopsies in this high risk setting in an effort to make the correct diagnosis and avoid the serious consequences of mismanagement. Thus, there is an obvious need for a noninvasive tool to help make this

From the Renal and Arthritis Units, Department of Medicine, and Department of Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA.

Supported in part by a Physician-Investigator Fellowship grant to Dr. Palsson from the American Heart Association, Massachusetts Affiliate. R. Palsson MD, Renal Unit and Department of Medicine, Landspítali University Hospital and Assistant Professor of Medicine, University of Iceland, Reykjavik, Iceland (current position); H.K. Choi, MD, MPH, Arthritis Unit, Department of Medicine, Massachusetts General Hospital, Instructor in Medicine, Harvard Medical School; J.L. Niles, MD, Renal Unit, Department of Medicine, Department of Pathology, Massachusetts General Hospital, Assistant Professor of Medicine, Harvard Medical School.

Address reprint requests to Dr. J.L. Niles, CPZ 701, Massachusetts General Hospital, 55 Fruit Street, Boston, MA 02114. E-mail: jlniles@partners.org

Submitted April 23, 2001; revision accepted August 17, 2001.

distinction. To evaluate the value of ANCA in this setting, we retrospectively reviewed the clinical course and ANCA titers of 16 patients with ANCA associated vasculitis who developed opportunistic infections.

MATERIALS AND METHODS

Study patients. Among the patients with AAV tested at the Immunopathology Laboratory at the Massachusetts General Hospital between 1989 and 1998, we identified 16 who experienced an opportunistic infection. We retrospectively studied their clinical features and ANCA titers. Clinical information was obtained from medical records, including the mean duration of vasculitis, organ involvement, the time interval from the most recent episode of active disease (original or relapse) until the detection of the opportunistic infection, type and duration of immunosuppressive therapy, chemoprophylaxis, white blood cell count and absolute lymphocyte count, cause of infection, diagnosis, and treatment of infection and outcome. Any increase in immunosuppressive therapy prior to the diagnosis of opportunistic infection was also noted. The Institutional Review Boards at Massachusetts General Hospital and Brigham and Women's Hospital approved the study.

ANCA testing. The presence of anti-PR3 and anti-MPO antibodies was determined by combining the results of indirect immunofluorescence assay for cytoplasmic (C-ANCA) and perinuclear (P-ANCA) patterns with the results of antigen-specific ELISA. Indirect immunofluorescence was performed as described²¹. Quantitative ELISA were performed during the clinical course as described^{21,22}. Interpretation of ANCA test results was determined for each patient as described²³.

C-reactive protein (CRP) testing. CRP was measured in serum samples that had been stored at -20°C by nephelometry (Beckman Instruments Inc., Galway, Ireland). Normal range was 0.08 to 0.8 mg/dl. Sera from 11 of the 16 patients were tested for CRP.

Presentation of ANCA titer and CRP level changes. To illustrate changes in anti-MPO and anti-PR3 titers, the titer values of anti-MPO and anti-PR3 were divided by their cutoff levels (2.8 and 5, respectively) and presented in a log scale. ANCA titers are presented at the onset of immunosuppressive therapy for the most recent episode of active vasculitis and at the time of the opportunistic infections. CRP values are presented for the same 2 time points.

RESULTS

Clinical characteristics of the 16 patients. Patients' clinical characteristics are shown in Table 1. The mean age was 56.2 ± 18.0 (SD) years. There were 11 men and 5 women. No patient was known to have human immunodeficiency virus infection. All had clinicopathologic features considered diagnostic of AAV. In 6 patients, the diagnosis of pauciimmune small vessel vasculitis was histologically proven, whereas in 10 patients the diagnosis of vasculitis was based on clinical features and a positive ANCA test. Nine patients had anti-PR3 and 7 had anti-MPO antibodies. One patient had antiglomerular basement membrane antibodies in addition to anti-MPO. Ten patients had evidence for nephritis during their most recent flare of vasculitis, including 9 with renal insufficiency. Three patients required dialysis and 2 of these patients did not recover their renal function.

The median duration of AAV was 4.5 months (range 2–228 mo). Eleven of the 16 patients were being treated for their first episode of vasculitis and the remaining 5 were receiving treatment for a relapse. One patient (Patient 12)

had multiple relapses during a 19 year period. At the time of the opportunistic infection, all patients were undergoing immunosuppressive therapy for a recent episode of active vasculitis. All patients were receiving daily glucocorticoids and 15 of the 16 patients were also receiving a cytotoxic agent. Twelve patients were taking daily oral cyclophosphamide, one patient had received 2 doses of intravenous cyclophosphamide 2 weeks apart, and 2 patients had recently (within one month) been converted from oral cyclophosphamide to azathioprine. One patient (Patient 15) had received a single 15 mg dose of subcutaneous methotrexate but was subsequently treated with high dose glucocorticoids alone.

Opportunistic infections. The data are summarized in Table 1. Eight patients had *Pneumocystis carinii* pneumonia (PCP); 5 had cytomegalovirus (CMV) infection including 3 with CMV pneumonitis, one had both PCP and CMV pneumonitis, one CMV pneumonitis and invasive pulmonary aspergillosis, and one disseminated cutaneous zoster. Only 3 patients were taking prophylactic therapy with trimethoprim-sulfamethoxazole (TMP-SMZ) preceding the infection. One of these patients developed PCP and the other 2 CMV infection and disseminated zoster infection, respectively. The opportunistic infections occurred a median of 2 months (range 1–10 mo) following the last episode of active vasculitis. Diagnosis of infection was obtained by open lung biopsy in 4 patients, bronchoscopy and bronchoalveolar lavage in 6 patients, and detection of CMV in a buffy coat sample in 4 patients. In one patient, the diagnosis was made during autopsy (Patient 10) and in one by clinical examination of skin findings (Patient 13). Seven patients were leukopenic at the time of diagnosis of their infection. Data on absolute lymphocyte counts were available for 13 patients and of these, 11 had profound lymphopenia. There was no obvious difference in leukocyte counts or lymphocyte counts between those with CMV infection and those without.

In 7 patients, the immunosuppressive regimen was initially increased for new findings shortly before the diagnosis of an opportunistic infection and without histologic documentation of active vasculitis. After identification of the opportunistic infections, all patients received antimicrobial therapy directed against the offending agent, except one patient (Patient 10) who did not receive treatment against CMV because the infection was not detected prior to death. PCP was treated with high dose TMP-SMZ or pentamidine and CMV was invariably treated with ganciclovir. Cytotoxic agents were eventually discontinued in 13 of the 16 patients during management of the infection. However, cyclophosphamide was continued in 2 patients and azathioprine was continued in another patient. Glucocorticoid therapy was maintained in all patients. Four patients died from their infection, one with PCP, one CMV, one PCP and CMV, and one with CMV and pulmonary aspergillosis. Three of the 4

Table 1. Characteristics of 16 patients with ANCA associated vasculitis and opportunistic infection.

Patient	Age, Sex	Type of ANCA	Organ Involvement at Dx or Re	Therapy of Last Episode of Active Disease	Duration from Dx or Last Re to Dx of Infection	Type of Infection	WBC Count (Lymphocytes)	Outcome
1	65 F	Anti-PR3	L (Re)	Cs, Cyc	8 wks	PCP/CMV	2.2 (Ly0)	Died
2	68 M	Anti-MPO	K (Dx)	Cs, Cyc	7 wks	CMV	2.7	Survived
3	60 F	Anti-MPO	K, PNS, H (Dx)	Cs, Cyc	8 wks	CMV	8.8 (Ly1)	Died
4	66 M	Anti-PR3	L, K (Re)	Cs, Cyc	4 wks	PCP	3.4	Survived
5	49 M	Anti-PR3	E, K, PNS (Dx)	Cs, Cyc	8 wks	PCP	9.2	Survived
6	27 M	Anti-PR3	L, K, J, PNS (Dx)	Cs, Cyc	8 wks	PCP	6.9 (Ly3)	Survived
7	25 F	Anti-PR3	E, L, J (Dx)	Cs, Cyc	10 mo	PCP	11.7 (Ly3)	Survived
8	66 M	Anti-MPO	E, J (Re)	Cs, Cyc-Aza	8 wks	PCP	6.9 (Ly14)	Survived
9	70 M	Anti-PR3	L, K (Dx)	Cs, Cyc	8 wks	CMV	1.5 (Ly3)	Survived
10	65 F	Anti-MPO	L (Dx)	Cs, Cyc	8 wks	CMV/Asp	7.5 (Ly3)	Died
11	63 M	Anti-MPO	L, K, CNS (Dx)	Cs, Cyc	6 mo	PCP	1.2 (Ly4)	Died
12	33 M	Anti-PR3	E, L, K (Re)	Cs, Cyc	4 mo	CMV	3.1 (Ly49)	Survived
13	82 M	Anti-PR3	K (Re)	Cs, Cyc-Aza	9 mo	VZV	8.1 (Ly9)	Survived
14	70 M	Anti-MPO	K, S (Dx)	Cs, Cyc	10 wks	PCP	7.0 (Ly2.4)	Survived
15	63 M	Anti-MPO	E, PNS, Orbit (Dx)	Cs*	10 wks	PCP	14 (Ly0)	Survived
16	27 F	Anti-PR3	K, L (Dx)	Cs, Cyc	12 wks	CMV	2.5 (Ly2)	Survived

Anti-MPO: antimyeloperoxidase antibodies; anti-PR3: antiproteinase 3 antibodies; CNS: central nervous system; E: ear, nose and throat or upper airway; H: heart; J: joints; K: kidney; L: lung; PNS: peripheral nervous system; S: skin; Dx: diagnosis; Re: relapse; Aza: azathioprine; Cyc: cyclophosphamide; Cs: corticosteroids; Asp: aspergillus; CMV: cytomegalovirus; PCP: *Pneumocystis carinii* pneumonia; VZV: varicella zoster virus; WBC: White blood cells (normal range $4.5-11.0 \times 10^9/L$); Ly: lymphocytes (normal range $1.0-4.8 \times 10^9/L$). * Patient had received a single dose of methotrexate around the time of PCP.

patients who died were among those whose immunosuppressive regimen was initially increased.

In 15 of the 16 patients, it was concluded that there was no active vasculitis present at the time of the opportunistic infection. However, one patient (Patient 15) was simultaneously found to have PCP and active vasculitis that was detected by a maxillary sinus biopsy showing necrotizing granulomatous vasculitis. This patient had been treated with steroids alone in high doses, off and on for 4 months, without a cytotoxic agent except for a single 15 mg dose of methotrexate.

ANCA titers and CRP values. Figure 1 shows the changes in ANCA titers in 15 of the 16 patients at the onset of therapy for the initial course or the most recent relapse of vasculitis and at the time of diagnosis of opportunistic infection. All 15 patients had steeply falling or negative ANCA titers immediately preceding the development of evidence of the infection. Among these patients, the mean ANCA titer at the time of the last episode of active disease was 75 times the upper limit of the normal range as defined by our laboratory. At the time of diagnosis of the opportunistic infection, the

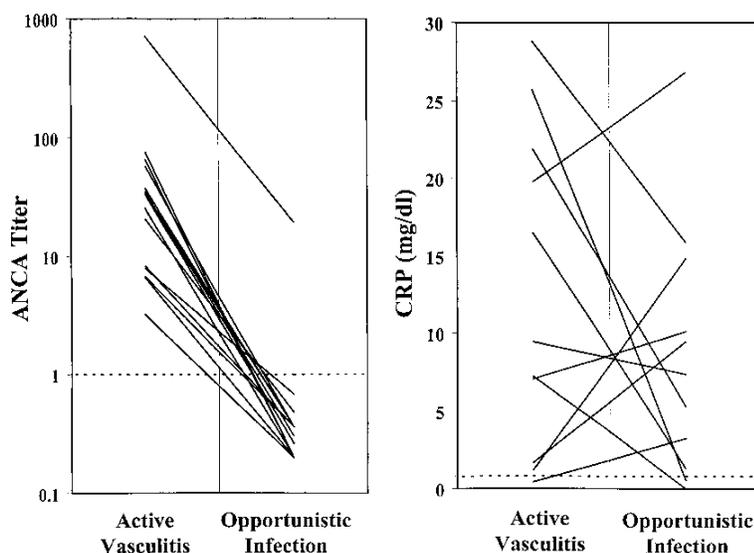


Figure 1. ANCA titers and CRP levels at the onset of immunosuppressive therapy for the most recent episode of vasculitis and at the time of opportunistic infection. Broken lines denote normal cutoffs of ANCA and CRP (0.8 mg/dl).

ANCA titer in 14 of the 15 patients had fallen to less than the cutoff value representing the upper limit of the normal range, while in the remaining patient the titer fell to less than 3% of the former value. Figure 1 also displays the CRP values at the same time points. There was no consistent pattern of change in CRP levels. Since the 16th patient had active disease at the time of his opportunistic infection, his values are not included in the figure.

DISCUSSION

We describe the relationship between changes in ANCA titers and opportunistic infections in patients with AAV. In all of our 15 patients with opportunistic infections and no evidence of active vasculitis, the infection was associated with a dramatic fall in ANCA titer. One patient who did not experience a rapid fall in his ANCA titer (less than 4-fold from his prior peak) had biopsy proven active disease at the same time he developed PCP. These data strongly suggest that opportunistic infections during immunosuppressive treatment for AAV are associated with rapidly falling or negative ANCA titers. Thus, in a clinical setting that could be the result of either a vasculitis relapse or an opportunistic infection, a high or rising ANCA titer weighs against the possibility of an opportunistic infection and a rapidly falling or negative ANCA titer suggests that clinical deterioration is due to infection or other comorbidities rather than exacerbation of AAV.

Several studies have looked at the usefulness of changes in ANCA titer as a marker for disease activity of AAV⁷⁻¹². Active disease has been shown to be generally associated with high or rising titers, making ANCA titers useful in the diagnosis of a flare^{7,8,10,11}. In contrast, negative or falling titers are rarely found during flare^{7,8,24,25} and are usually not immediately followed by a flare²⁴⁻²⁷. Others have not observed a similar usefulness of ANCA titers, and therefore the value of ANCA titers in monitoring disease activity has remained controversial^{12,14}. Lack of standardized definitions of disease activity and different methods of ANCA testing may explain some of the variability in the results of these studies. The most conclusive study on this issue is the recent, prospective study by Boomsma, *et al*¹³. They measured serial ANCA titers in 100 patients with Wegener's granulomatosis and blinded the clinicians to the results. Among 37 patients who developed relapses of disease, 34 had a rise in ANCA titer by indirect immunofluorescence or ELISA prior to the relapse. Of the remaining 3 patients who experienced a relapse with no increase in ANCA titer, 2 had a persistently elevated titer. The positive predictive value of a rise in anti-PR3 antibodies detected by ELISA for a flare of vasculitis was 71%. When these results are combined with those of our study, a falling or negative ANCA titer indicates a decreased probability of developing or having a flare of vasculitis, but remains consistent with the presence of an opportunistic infection. In contrast, a persistently high

or rising ANCA titer suggests an increased probability of a vasculitis flare and appears inconsistent with an opportunistic infection.

In our study, the subjects were not selected based on the changes in ANCA titers. Instead, we examined the ANCA titers among patients with proven opportunistic infections. Thus our results cannot be applied to all instances of a falling or negative ANCA titer. Indeed, in most patients this change in ANCA titer reflects the desired clinical response of the vasculitis to immunosuppressive treatment. The main importance of our findings is to provide physicians with an additional tool for distinguishing between vasculitis flare and opportunistic infection, which is among the greatest clinical challenges encountered in the care of patients with vasculitis.

Immunosuppressive treatment was intensified prior to the diagnosis of the opportunistic infection in 7 of the patients due to the belief that the vasculitis was recurring. The decision to increase the therapy was made despite the lack of documentation of an actual vasculitis relapse. The clinical features that led the clinicians to suspect a flare proved to be consistent with the opportunistic infections that were subsequently documented. Three of these 7 patients died. These cases illustrate the diagnostic challenge arising from the similarity of the clinical presentations of opportunistic infections and flares of AAV, and the potential consequences of erroneous treatment of such cases. It is possible that management guided by ANCA titers could have improved the outcome of these cases.

Obviously, the usefulness of serial ANCA titers depends on the ability of the ANCA assay to quantify the difference in titer between samples. Thus, a reproducible and fully quantitative method of titration is necessary. Most laboratories use indirect immunofluorescence assays, which have an appreciable subjective component resulting in difficulty in reproducing modest differences. Others use semiquantitative antigen-specific ELISA. However, an ELISA at any one dilution is only quantitative over a narrow range. To be fully quantitative, testing each sample at multiple dilutions is required. In our ANCA laboratory, we test all positive samples over a full range of dilutions to assure fully quantitative results.

Leukocyte and lymphocyte counts are other useful measures in monitoring the state of immunosuppression among patients undergoing treatment for AAV²⁸. Leukopenia was common in our patients and profound lymphopenia was present in 11 out of 13 patients for whom data were available. This has been observed by other investigators²⁰. While cyclophosphamide and other cytotoxic agents can cause lymphopenia²⁹, functional abnormalities of lymphocytes and monocytes may also be induced by glucocorticoids.

Other potential markers of disease activity that have been proposed for guiding management of vasculitis include

erythrocyte sedimentation rate (ESR) and CRP^{28,30}. While CRP levels have been suggested as a useful marker of active disease³⁰, we did not find this marker of inflammation useful for distinguishing between active vasculitis and infection.

The mortality rate in our patients was substantial and emphasizes the seriousness of the infectious complications of immunosuppressive therapy. Therefore, it is critically important to monitor patients closely during this form of treatment. Prophylactic antimicrobial therapy for PCP seems advisable during periods of heavy immunosuppression, as supported by a recent cost effectiveness analysis³¹. Our current practice is to administer TMP-SMZ to all patients who are receiving treatment with high doses of immunosuppressive drugs.

We conclude that opportunistic infections during treatment for AAV are usually associated with rapidly falling or negative ANCA titers but not with rising ANCA titers, whereas previous studies suggest that flares of vasculitis are usually associated with rising or persistently elevated titers. Therefore, when the clinician encounters a patient undergoing immunosuppressive treatment for vasculitis with clinical features that could be due to either a flare of the disease or an opportunistic infection, a negative or sharply falling ANCA titer should increase the suspicion of an opportunistic infection and decrease the suspicion of a vasculitis relapse.

ACKNOWLEDGMENT

The authors thank Dr. Peter H. Schur for his assistance in performing tests for C-reactive protein.

REFERENCES

1. Davies DJ, Moran JE, Niall JF, Ryan GB. Segmental necrotising glomerulonephritis with antineutrophil antibody: possible arbovirus aetiology? *BMJ* 1982;2:606.
2. van der Woude FJ, Rasmussen N, Lobatto S, et al. Autoantibodies against neutrophils and monocytes: tool for diagnosis and marker of disease activity in Wegener's granulomatosis. *Lancet* 1985;1:425-9.
3. Falk RJ, Jennette JC. Anti-neutrophil cytoplasmic autoantibodies with specificity for myeloperoxidase in patients with systemic vasculitis and idiopathic necrotizing and crescentic glomerulonephritis. *N Engl J Med* 1988;318:1651-7.
4. Goldschmieding R, van der Schoot CE, ten Bokkel Huinink D, et al. Wegener's granulomatosis autoantibodies identify a novel diisopropylfluorophosphate-binding protein in the lysosomes of normal human neutrophils. *J Clin Invest* 1989;84:1577-87.
5. Niles JL, McCluskey RT, Ahmad MF, Arnaout MA. Wegener's granulomatosis autoantigen is a novel serine protease. *Blood* 1989;74:1888-93.
6. Ludemann J, Utecht B, Gross WL. Anti-neutrophil cytoplasm antibodies in Wegener's granulomatosis recognize an elastolytic enzyme. *J Exp Med* 1990;171:357-62.
7. Nölle B, Specks U, Ludemann J, Rohrbach MS, DeRemee RA, Gross WL. Anticytoplasmic autoantibodies: their immunodiagnostic value in Wegener granulomatosis. *Ann Intern Med* 1989;111:28-40.
8. Cohen Tervaert JW, van der Woude FJ, Fauci AS, et al. Association between active Wegener's granulomatosis and anti-cytoplasmic antibodies. *Arch Intern Med* 1989;149:2461-5.
9. Cohen Tervaert JW, Huitema MG, Hene RJ, et al. Prevention of relapses in Wegener's granulomatosis by treatment based on antineutrophil cytoplasmic antibody titre. *Lancet* 1990;336:709-11.
10. Egner W, Chapel HM. Titration of antibodies against neutrophil cytoplasmic antigens is useful in monitoring disease activity in systemic vasculitides. *Clin Exp Immunol* 1990;82:244-9.
11. Fienberg R, Mark E, Goodman M, McCluskey RT, Niles JL. Correlation of antineutrophil cytoplasmic antibodies with the extrarenal histopathology of Wegener's (pathergic) granulomatosis and related forms of vasculitis. *Hum Pathol* 1993;24:160-8.
12. Kerr GR, Fleischer THA, Hallahan CW, Leavitt RY, Fauci AS, Hoffman GS. Limited prognostic value of changes in antineutrophil cytoplasmic antibody titer in patients with Wegener's granulomatosis. *Arthritis Rheum* 1993;36:365-71.
13. Boomsma MM, Stegeman CA, van der Leij MJ, et al. Prediction of relapses in Wegener's granulomatosis by measurement of antineutrophil cytoplasmic antibody levels: a prospective study. *Arthritis Rheum* 2000;43:2025-33.
14. Davenport A, Lock RJ, Wallington T. Clinical significance of the serial measurement of autoantibodies to neutrophil cytoplasm using a standard indirect immunofluorescence test. *Am J Nephrol* 1995;15:201-7.
15. Novack S, Pearson C. Cyclophosphamide therapy in Wegener's granulomatosis. *N Engl J Med* 1971;284:938.
16. Fauci AS, Katz P, Haynes BF, Wolff SM. Cyclophosphamide therapy of severe systemic necrotizing vasculitis. *N Engl J Med* 1979;301:235-8.
17. Falk RJ, Hogan S, Carey TS, Jennette JC. Clinical course of antineutrophil cytoplasmic autoantibody-associated glomerulonephritis and systemic vasculitis. The Glomerular Disease Collaborative Network. *Ann Intern Med* 1990;113:656-63.
18. Hoffman GS, Kerr GS, Leavitt RY, et al. Wegener's granulomatosis: an analysis of 158 patients. *Ann Intern Med* 1992;116:488-98.
19. Jarrousse B, Guillevin L, Bindi P, et al. Increased risk of pneumocystis carinii pneumonia in patients with Wegener's granulomatosis. *Clin Exp Rheumatol* 1993;11:615-21.
20. Ognibene FP, Shelhamer JH, Hoffman GS, et al. Pneumocystis carinii pneumonia: a major complication of immunosuppressive therapy in patients with Wegener's granulomatosis. *Am J Respir Crit Care Med* 1995;151:795-9.
21. Niles JL, Guo Li P, Collins AB, et al. Antigen-specific radioimmunoassays for anti-neutrophil cytoplasmic antibodies in the diagnosis of rapidly progressive glomerulonephritis. *J Am Soc Nephrol* 1991;2:27-36.
22. Hellmark T, Niles J, Collins A, McCluskey R, Brunmark C. Comparison of anti-GBM antibodies in sera with or without ANCA. *J Am Soc Nephrol* 1997;8:376-85.
23. Merkel P, Polisson R, Chang Y, Skates S, Niles J. Prevalence of antineutrophil cytoplasmic antibodies in a large inception cohort of patients with connective tissue disease. *Ann Intern Med* 1997;126:866-73.
24. De'Oliviera J, Gaskin G, Dash A, Rees AJ, Pusey CD. Relationship between disease activity and anti-neutrophil cytoplasmic antibody concentration in long-term management of systemic vasculitis. *Am J Kidney Dis* 1995;25:380-9.
25. Jayne DR, Gaskin G, Pusey CD, Lockwood CM. ANCA and predicting relapse in systemic vasculitis. *Q J Med* 1995;88:127-33.
26. Stegeman CA, Cohen Tervaert JW, Kallenberg CG. Anti-neutrophil cytoplasmic antibodies: tools for diagnosis and follow-up in systemic vasculitis. *Ann Med Interne (Paris)* 1994;145:523-32.
27. 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.
28. 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.
29. Dale DC, Fauci AS, Wolff SM. The effect of cyclophosphamide on leukocyte kinetics and susceptibility to infection in patients with Wegener's granulomatosis. *Arthritis Rheum* 1973;16:657-64.
30. Hind C, Winearls C, Lockwood C, Rees A, Pepys M. Objective monitoring of activity in Wegener's granulomatosis by measurement of serum C-reactive protein concentration. *Clin Nephrol* 1984;21:341-5.
31. Chung JB, Armstrong K, Schwartz JS, Albert D. Cost-effectiveness of prophylaxis against *Pneumocystis carinii* pneumonia in patients with Wegener's granulomatosis undergoing immunosuppressive therapy. *Arthritis Rheum* 2000;43:1841-8.