Antineutrophil Cytoplasmic Autoantibody in the Absence of Wegener's Granulomatosis or Microscopic Polyangiitis: Implications for the Surgical Pathologist

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Antineutrophil cytoplasmic antibodies (ANCA) are useful serologic markers for the diagnosis and management of patients with Wegener's granulomatosis (WG) and microscopic polyangiitis (MPA). However, problems in diagnosis and classification may occur when patients with other disorders develop ANCA. A 7-year review (1993–1999) disclosed 247 patients whose sera tested positively for ANCA by an indirect immunofluorescence method: 166 patients for cytoplasmic-ANCA (C-ANCA) and 81 patients for perinuclear-ANCA (P-ANCA) Twenty-seven patients had active pulmonary disease and underwent open-lung biopsy or transbronchial biopsy. Eight patients (30%) had a disease other than WG or MPA, and their clinical, pathological, and serological findings were reviewed. The patients, all women, ranged in age from 28 to 77 years (median, 37 y). Dyspnea (n = 6), cough (n = 6), chest pain (n = 2), and/or hemoptysis (n = 2) were present. The duration of symptoms lasted from 3 weeks to 6 years (median, 6 mo). ANCA titers were C-ANCA (n = 4; range, 1:40–1280) or P-ANCA (n = 4; range, 1:40–640). The lung biopsies disclosed nonspecific interstitial pneumonia (n = 4), bronchiolitis obliterans organizing pneumonia (n = 1), diffuse alveolar damage (n = 1), organizing diffuse alveolar hemorrhage without capillaritis (n = 1), and necrotic granuloma (n = 1). No cases showed characteristic histology for WG or MPA. The final diagnoses were various connective tissue disorders (n = 5), chronic hypersensitivity pneumonia (n = 1), postinfectious bronchitis/bronchiectasis (n = 1), and ulcerative colitis-related lung disease (n = 1). Surgical pathologists should be aware that significantly elevated ANCA titers may be associated with diverse forms of pulmonary disease. ANCA positivity alone, in the absence of appropriate clinical or pathologic findings, should not be used to substantiate a diagnosis of WG or MPA.

KEY WORDS: Antineutrophil cytoplasmic antibodies, Microscopic polyangiitis, Vasculitis, Wegener's granulomatosis.

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Since the initial discovery by Davis *et al.* (1) of antineutrophil cytoplasmic antibodies (ANCA) in 1982, ANCA have become useful serological markers for the evaluation of systemic vasculitic disorders. The pulmonary diseases that are most frequently associated with ANCA include Wegener's granulomatosis (WG), microscopic polyangiitis (MPA), Churg Strauss syndrome, and other disorders (2–5). Elevated ANCA titers particularly occur during disease exacerbation but may be low or absent in remission (3, 6, 7).

By indirect immunofluorescence (IIF), two well-recognized staining patterns of ANCA tend to show associations with specific diseases (7, 8). In active WG, the majority of ANCA demonstrate a cytoplasmic pattern (C-ANCA) on IIF tests, although 5–20% of ANCA may exhibit a perinuclear pattern (P-ANCA; 4). Enzyme-linked immunosorbent assays (ELISA) detect specific antigens directed to proteinase-3, which is a 29-kDa serine proteinase in neutrophil primary granules (9, 10). In MPA, ANCA are present in ≤80% of sera, and the staining pattern tends to be perinuclear (P-ANCA; 4). ELISA assays in P-ANCA+ sera detect myeloperoxidase but occasionally identify other antigens (2–5, 11).

Although ANCA have become useful serological markers for diagnosis and disease monitoring, false-positive and false-negative ANCA occur for a variety of reasons (12-14). During a review of ANCA-positive patients who underwent lung biopsies at our institution, we identified a group of these

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patients who did not have a systemic vasculitic syndrome. In this report, we present the lung biopsy findings in a group of ANCA-positive patients who lacked clinical, serological, and pathological criteria for WG or MPA. Moreover, we would like to review some reasons why ANCA occurs in other disorders and propose guidelines for surgical pathologists who may be confronted with this problematic situation.

MATERIALS AND METHODS

A retrospective review was performed of ANCA testing from 1993 to 1999 at Emory University Hospital. The majority of patients were evaluated for active renal disease or vasculitic syndromes. There were 247 patients whose sera tested positively for ANCA by an IIF test (ANCA-IIF; Inova Diagnostics, San Diego, CA). There were 166 patients whose sera were C-ANCA+ and 81 patients whose sera were P-ANCA+. If the IIF demonstrated a P-ANCA pattern, differential fixation with formalin was used to discriminate between P-ANCA and antinuclear antibodies (15). ELISAs for ANCA were performed only if specifically ordered by the referring clinician.

All ANCA-IIF+ patients underwent a retrospective review of anatomical pathology records. An open-lung ($n = 26$) or transbronchial ($n = 1$) biopsy was performed in 27 patients. Using pathological criteria for WG or MPA proposed by the Chapel Hill Consensus Conference (16), 19 patients had pathologic findings of WG (necrotizing granulomatous inflammation with vasculitis and/or alveolar hemorrhage with capillaritis) or MPA (alveolar hemorrhage with capillaritis) in their biopsies. The patients with vasculitis were excluded from this study.

Eight patients with open-lung biopsies who lacked clinical and pathologic criteria of WG or MPA formed the basis of this study. An additional review was undertaken of relevant clinical, radiological, and laboratory data including microbiological cultures and other serological tests. Follow-up information was obtained in all patients.

RESULTS

All patients in this study were women, ranging in age from 28 to 77 years, with a median of 37 years. At presentation the pulmonary findings included dyspnea ($n = 6$), cough ($n = 6$), chest pain ($n = 2$), and hemoptysis ($n = 2$). Chest radiographs and computer tomographic imaging detected bilateral pleural effusions ($n = 4$), bilateral interstitial infiltrates ($n = 4$), or bilateral pulmonary nodules ($n = 2$). The duration of illness lasted from 3 weeks to 6 years, with a median of 6 months. One patient had chronic renal failure, noted at admission, caused by hypertensive nephropathy. The patients were treated with corticosteroids ($n = 4$), antibiotics ($n = 3$), or bronchodilators ($n = 1$).

The ANCA, other serological tests, histopathological findings, final clinical diagnoses, and follow-up data are summarized in Table 1. By ANCA-IIF testing, four patients were C-ANCA+ and four patients were P-ANCA+. The titers ranged from 1:40 to 1280. ELISA-ANCA was performed in two patients: one patient was borderline for PR-3 (Patient 1, 12 units; negative $\leq$10 ELISA units) and another was negative for PR-3 and myeloperoxidase (Patient 4).

Four patients had multiple autoantibodies present in their sera at the time of a positive ANCA: antinuclear antibodies (ANA; $n = 2$), rheumatoid factor ($n = 2$), anti-DNA ($n = 2$), anti–smooth muscle antibody ($n = 1$), and lupus anticoagulant ($n = 1$). All of these other autoantibodies were not considered to be atypical, aberrant, or variant ANCA.

The open-lung biopsies showed various patterns of tissue injury (Table 1). The most common histological pattern was a nonspecific interstitial pneumonia (NSIP; $n = 4$) associated with ill-defined granulomas (Patient 3; Fig. 1), fibrosis (Patient 6; Fig. 2), acute fibrinous pleuritis (Patient 7; Fig. 3), or eosinophilia. Bronchiolitis obliterans organizing pneumonia (BOOP) was the dominant pattern in Patient 4 (Fig. 4) and coexisted with NSIP in Patient 5. Other histological patterns of lung injury in the remaining patients included diffuse alveolar damage (DAD; Patient 1), organizing alveolar hemorrhage without capillaritis (Patient 2), and necrotic granulomas (Patient 8).

The final diagnosis correlated the pathologic findings with the subsequent clinical course. Five patients had a rheumatologic disorder with pulmonary involvement: systemic lupus erythematosus (Patient 7), rheumatoid lung (Patient 5), dermatomyositis (Patient 1), probable antiphospholipid antibody syndrome (Patient 2), and an unclassified connective tissue disorder (Patient 6). The remaining patients were diagnosed with chronic hypersensitivity pneumonia (Patient 3), postinfectious bronchitis/bronchiectasis (Patient 4), and ulcerative colitis–related granulomatous lung disease (Patient 8).

In follow-up evaluations, none of the patients have developed renal disease or systemic vasculitis. No specific antigen was identified in Patient 3 with chronic hypersensitivity pneumonia. The necrotizing granulomas identified in Patient 8 were thought to be a manifestation of her underlying inflammatory bowel disease. Four patients are alive and well with resolution of their pulmonary disease, but four patients have died, three deaths of which could be directly attributed to lung disease.

TABLE 1. Summary of ANCA, Serological Tests, and Other Histopathological and Clinical Findings

Patient No.	IIF-ANCA	ELISA-ANCA	Anti-Nuclear Antibodies	Rheumatoid Factor	Anti-DNA	Other Antibody	Histologic Pattern	Final Diagnosis	Therapy	Follow-Up	Pulmonary Disease	Outcome
1	C-40	PR3-12	Neg	Neg	—	—	Diffuse alveolar damage	Dermatomyositis	Antibiotics	3 days	Progression	Died
2	C-40	—	Neg	—	156	Lupus anticoagulant	Organizing alveolar hemorrhage	?Anti-phospholipid syndrome	Corticosteroids	25 months	Resolution	Alive
3	C-80	—	—	—	—	—	NSIP, ill-defined granulomas	Chronic hypersensitivity pneumonia	Bronchodilators	65 months	Resolution	Alive
4	C-128	PR3-Neg MPO-Neg	Neg	Neg	Normal	—	BOOP	Postinfectious bronchitis/ bronchiectasis	Antibiotics	60 months	Progression	Died: colonic cancer
5	P-40	—	Neg	1:5120	—	—	NSIP, BOOP, eosinophils	Rheumatoid lung	Corticosteroids	11 months	Progression	Died
6	P-320	—	1:1280 nucleolar	1:160	Normal	—	NSIP, fibrosis	Unclassified connective tissue disorder	Corticosteroids, plasmapheresis	3 months	Progression	Died
7	P-640	—	1:640 centromere	—	538	Smooth muscle antibody	NSIP, acute pleuritis	Systemic lupus erythematosus	Antibiotics	52 months	Resolution	Alive
8	P-640	—	Neg	Neg	—	—	Necrotizing granuloma	Ulcerative colitis-lung disease	Corticosteroids	36 months	Resolution	Alive

IIF, indirect immunofluoresecence; Neg, negative; NSIP, nonspecific interstitial pneumonia; BOOP, bronchiolitis obliterans organizing pneumonia; ANCA, antineutrophil cytoplasmic antibodies.

FIGURE 1. Cytoplasmic antineutrophil cytoplasmic antibodies 1–80: Nonspecific interstitial pneumonia (**top**) associated with ill-defined granulomata (**bottom**). Final diagnosis: chronic hypersensitivity pneumonia. Patient 3.

DISCUSSION

In the beginning of our study, we considered that the lung biopsy findings represented unusual manifestations of ANCA-related lung disease. This was understandable, given the broad spectrum of "typical" and "atypical" histological lung injury patterns that may occur in WG or MPA (Table 2; 17–30). Some of these "atypical" patterns (*i.e.*, NSIP, BOOP, DAD, or pleuritis) overlapped with the findings that were seen in our patients, but no vasculitic changes were evident. Only after correlating the histopathological results with the clinical, radiographic, serological, and other laboratory data were we assured that none of our patients had WG or MPA.

Although ANCA have become useful serological markers for diagnosis and monitoring of WG and MPA, they can be present in other diseases. In 1989, DeClerck *et al.* (31) reported a C-ANCA+ patient with active pulmonary tuberculosis who had been misdiagnosed with limited WG. Davenport (12) presented four patients with nonvasculitic diseases who had false-positive ANCA. A review of the literature reveals that ANCA may occur in various clinical settings unrelated to WG or MPA (Table 3; 3–5, 11, 13, 14, 32–80). Many of these diseases have

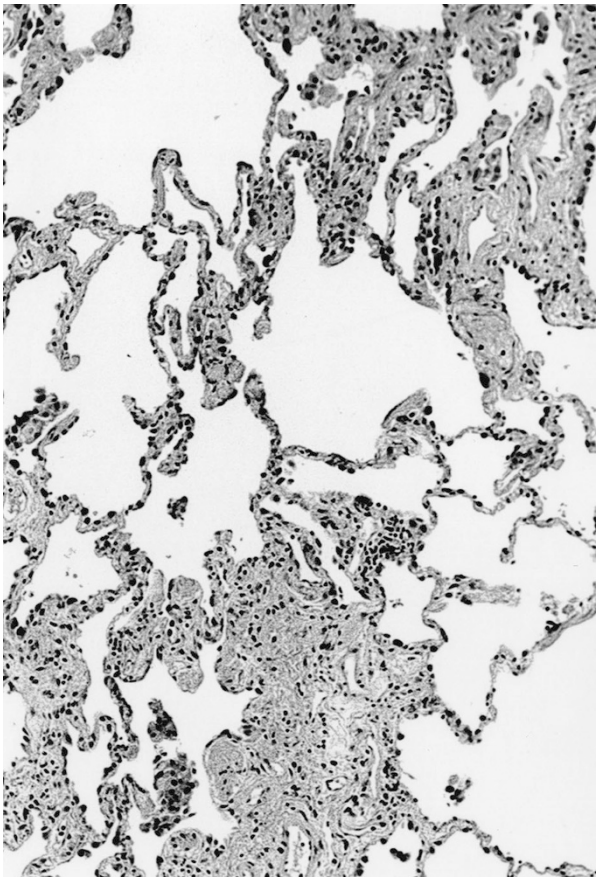

FIGURE 2. Perinuclear antineutrophil cytoplasmic antibodies 1–320: Nonspecific interstitial pneumonia with focal fibrosis. Final diagnosis: unclassified connective tissue disorder. Patient 6.

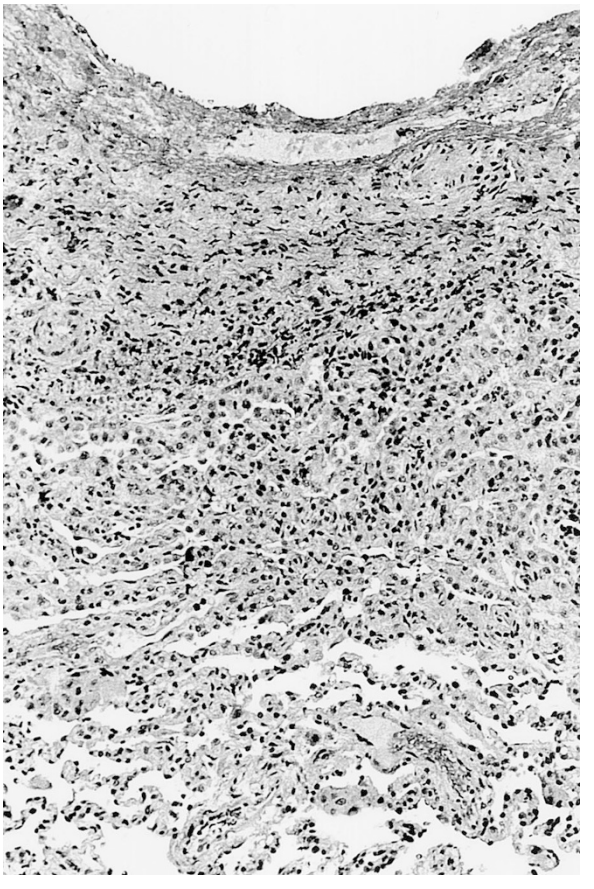

FIGURE 3. Cytoplasmic antineutrophil cytoplasmic antibodies 1–640: Nonspecific interstitial pneumonia with acute fibrinous pleuritis. Final diagnosis: systemic lupus erythematosus. Patient 7.

clinical presentations that can closely resemble systemic vasculitis (33, 34).

There are several reasons that ANCA occur in other disorders. Although PR-3 and myeloperoxidase are the most frequent determinants, ANCA can be directed to lactoferrin, enolase, cathepsin-G, elastase, lysozyme, and other antigens (3–5, 11, 81–83). Some patients with connective tissue disorders have an atypical ANCA in which the IIF staining pattern does not resemble typical C- or P-ANCA (11, 82). By IIF, P-ANCA may be identified in patients with systemic lupus erythematosus and may be difficult to distinguish from ANA (2, 3, 15, 35, 36, 83). P-ANCA can be seen in various connective tissue diseases, such as in rheumatoid arthritis, inflammatory arthritis, progressive systemic sclerosis, and other disorders (4, 33, 38, 84). In rheumatoid arthritis, granulocyte-specific ANA can closely resemble P-ANCA in IIF tests (3, 5, 38, 82). Finally, novel or uncharacterized antibodies could conceivably give rise to ANCA.

Interpretative and reproducibility issues may occur in the evaluation of sera by the IIF method (33, 82, 83). There may be considerable interinstitutional variation in performing ANCA (4, 9, 85). Among various commercial assay kits for ANCA, Lim *et al.* (86) showed that the INOVA IIF kit, which was used in our study, had a sensitivity of 91% but a specificity of 61%. This low specificity approximates our finding that 30% (8/27) of those ANCA positive did not have WG or MPA but another disorder. There can be differences in technologist test performance and interpretation of IIF-ANCA tests, based on individual ability and experience (9, 33, 83). A lack of standardized reporting of ANCA results by some laboratories further complicates this situation.

In a literature review and meta-analysis of the utility of ANCA testing in WG, Rao *et al.* (87) found that the sensitivity of ANCA is higher in active disease (91%) than in inactive disease (63%) but that the specificity was similar (98.6% *versus* 99.5%, respectively). However, in low-prevalence situations, such as when ANCA is not expected, a substantial number of false-positive tests occurs. These authors recommended "judicious ordering of C-ANCA testing and cautious interpretation of the test results (87)."

For surgical pathologists, the implications of our study are clear. ANCA positivity alone, in the absence of appropriate clinical and pathologic find-

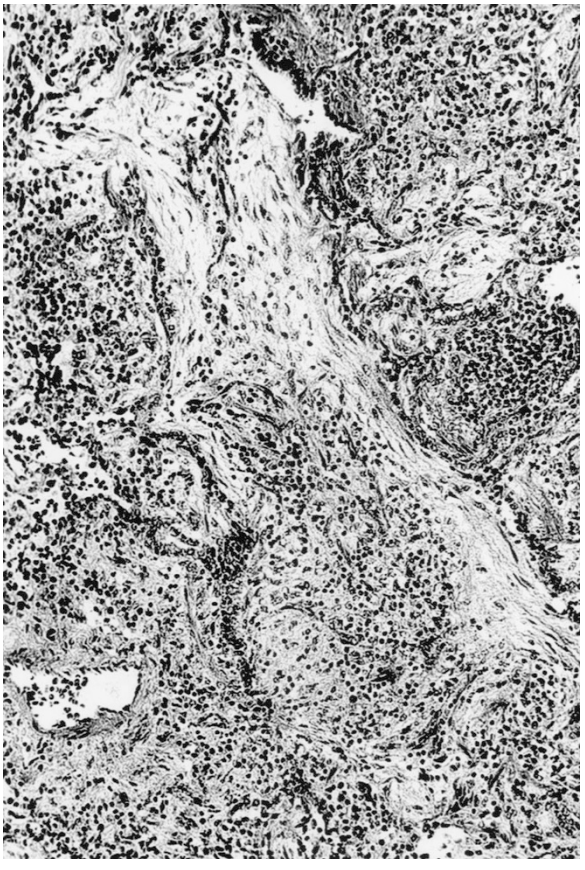

FIGURE 4. Cytoplasmic antineutrophil cytoplasmic antibodies 1–1280: Bronchiolitis obliterans organizing pneumonia. Final diagnosis: postinfectious bronchitis/bronchiectasis. Patient 4.

TABLE 2. Atypical Histological Patterns in Wegener's Granulomatosis and Microscopic Polyarteritis

Eosinophilia (18, 19, 21, 27–29)
Bronchiolitis obliterans organizing pneumonia (21–23, 26–29)
Acute or chronic bronchiolitis (21)
Bronchocentric injury (18, 21–23, 26, 27, 29)
Bronchopneumonia (21)
Follicular bronchiolitis (21)
Bronchial stenosis (21, 29)
Squamous metaplasia (21)
Organizing pneumonia (18)
Interstitial fibrosis (20, 21, 26, 29)
Fibrous scar (17, 18, 21)
Lymphoid aggregates (18, 21)
Diffuse alveolar damage (26, 29)
Lipoid pneumonia (21, 23, 29)
Xanthogranulomatous lesions (21, 23)
Acute fibrinous pleuritis (18, 21, 23, 26)
Chronic fibrosing pleuritis (18, 21, 26)

TABLE 3. Other Disorders that May Be Associated with Antineutrophil Cytoplasmic Antibodies

Connective tissue disorders
Systemic lupus erythematosus (2, 35, 36)
Rheumatoid arthritis (37, 38)
Felty's syndrome (39)
Progressive systemic sclerosis (38, 40)
Sjogren's syndrome (41, 42)
Ankylosing spondylitis (43)
Reactive arthritis (43)
Juvenile rheumatoid arthritis (44)
Takayasu's vasculitis (6, 7)
Dermatomyositis (33)
Antiphospholipid syndrome (33)
Gastrointestinal disorders
Ulcerative colitis (45–47)
Crohn's disease (45–47)
Sclerosing cholangitis (45, 47)
Autoimmune liver disorders (47, 48)
Primary billary cirrhosis (48)
Other disorders
Idiopathic necrotizing and crescentic glomerulonephritis (2)
Churg-Strauss syndrome (34, 49, 50)
Goodpasture's disease (51, 52)
Post-streptococcal glomerulonephritis (53)
Giant cell arteritis (54)
Kawasaki's disease (55)
Sarcoidosis (56)
Sweet's syndrome (57)
Classic polyartertitis nodosa (49)
Infections
Mycobacterial (12, 31, 58)
Leprosy (59)
Aspergillus (60)
Sporotrichosis (61)
Paracocciodomycosis (62)
Chromomycosis (63)
Amoebiasis (64)
Subacute bacterial endocarditis (65)
Malaria (58)
Leptospirosis (66)
Influenza virus (67)
Human immunodeficiency virus/AIDS (12, 68–70)
Cystic fibrosis (71)
Bacterial septicemia (72)
Other pulmonary infections (13, 61, 72)
Neoplasia
Lymphoid neoplasia (12, 73)
Lymphomatoid granulomatosis (6, 7)
Monoclonal gamopathies (73, 74)
Myeloproliferative disorders (73, 75)
Carcinomas (76)
Miscellaneous
Bone marrow transplantation (77)
Hemodialysis (78)
Drugs (79)
Anticytokeratins (80)

ings, should not influence the pathologist to render a diagnosis of WG or MPA. In the setting of a positive ANCA, caution should be exerted when a lung biopsy does not show necrotizing granulomatous inflammation and vasculitis or alveolar hemorrhage with/or without capillaritis. When another pattern of injury such as NSIP, BOOP, or pleuritis predominates, there must be a careful search for subtle histological features of WG or MPA. For example, a small focus of vasculitis or capillaritis could be overlooked when another pattern predominates. Although many hospital laboratories only perform IIF testing, it is highly recommended that a positive ANCA result be followed by an ELISA for PR-3 and/or myeloperoxidase (3, 5, 11, 14, 33, 82, 83, 86, 88). The pathologist should inquire as to what type of ANCA test was performed (IIF versus ELISA), where it was performed (hospital versus reference versus research laboratory), and whether multiple autoantibodies (antinuclear antibodies, rheumatoid factor, antiglomerular basement membrane

antibody, or others) are present. Finally, the results of ANCA testing must always be viewed in the context of the clinical picture, with correlation of the histological findings with clinical, radiographic, and other serological tests (24, 87). An inappropriate diagnosis based solely on the results of ANCA testing could have serious consequences, particularly if the patient is unnecessarily treated with glucocorticoids and cytotoxic agents.

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