A 70-Year-Old Man With Pulmonary Infiltrates and a Positive Antineutrophil Cytoplasmic Autoantibody Test Result*

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A 70-year-old white man was referred for evaluation of pulmonary infiltrates and bronchiectases associated with cough and constitutional symptoms. He was in his usual state of health until he acquired B-cell non-Hodgkin lymphoma (NHL) in 1990. The lymphoma was localized to his stomach, and he was treated with surgical resection; chemotherapy with cyclophosphamide, vincristine, prednisone, and hydroxyurea; and radiation to the gastric bed. One year later, left lower lobe pneumonia developed that was treated with antibiotics. Because of persistent infiltrates, bronchoscopy was performed, with biopsy specimens revealing no abnormalities, and bronchiectases was diagnosed based on radiographic features.

Three years prior to presentation, a cough productive of green-to-yellow sputum without hemoptysis developed. One year prior to presentation, progressive fatigue, anorexia, low-grade fevers, significant night sweats, and subsequent 20-lb weight loss developed. He denied other respiratory complaints. CT of the chest revealed patchy areas of consolidation in both lower lungs and in the right middle lobe along with mild mediastinal lymph node enlargement. Multiple sputum samples for acid-fast bacilli, bacteria, and fungi were negative. Endoscopic evaluations of his upper and lower intestinal tracts did not reveal recurrence of the lymphoma.

Established medical problems included hypothyroidism and a previous myocardial infarction with subsequent revascularization surgery. He never smoked, and his family, social, and exposure history were otherwise noncontributory. He was referred to our tertiary care center for evaluation of productive cough, pulmonary infiltrates, worsening fatigue, persistent low-grade fevers with night sweats, and weight loss.

Physical Examination

The patient weighed 143 lb (body mass index of 20.3), down from his baseline of 164 lb. His vital signs were normal, but he appeared to be chronically ill and cachectic. His cardiopulmonary examination was significant for bilateral crackles halfway up his posterior lung fields. There were no other features of heart failure. Examination of lymph nodes revealed nontender lymphadenopathy in the inguinal regions and a soft 1-cm left axillary lymph node. The remainder of his physical examination, including skin, joint, neurologic, abdominal, eye, and ear, nose, throat examination, was unremarkable.

Laboratory Findings

A complete hematology and chemistry panel revealed a mild normocytic anemia with a hemoglobin of 13.2 g/dL, a mildly elevated erythrocyte sedimentation rate (24 mm/h), a moderately elevated C-reactive protein (4.26 mg/dL), and a minimally elevated serum thyroid-stimulating hormone level (5.5 mIU/L). Markers of renal function, liver function, and electrolytes were normal. Fungal serologies for the endemic fungi were negative. His HIV status had already been documented negative.

Spirometry suggested a restrictive process with symmetric reductions of the FEV₁ (1.92 L/s or 58% of predicted) and FVC (2.15 L/s or 50% of predicted) with a normal FEV₁/FVC ratio (89.3%). The

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single-breath diffusing capacity of the lung for carbon monoxide was 9.5 mL/min/mm Hg (37% of predicted after correction for the mild anemia).

The chest radiograph (Fig 1) showed alveolar and interstitial infiltrates in both lower lung fields. A CT scan of the chest (Fig 2) was also performed, revealing subpleural ground-glass opacities in the mid- and upper lung fields in addition to some more coarse infiltrates and areas of frank consolidation and volume loss in the lower lung fields. Mild lymphadenopathy was also seen in the mediastinal, hilar, and axillary regions.

**Figure 1.** Chest radiographs showing bilateral alveolar and interstitial infiltrates, most prominent in the lower lobes. The heart size and pulmonary vasculature are normal. Prior sternotomy had been performed for coronary revascularization.

**Figure 2.** Selected images from the CT of the chest. Areas of frank consolidation and volume loss are apparent in the lower posterior lung fields (long arrows, bottom right). Subtle areas of ground-glass opacities are also apparent in the mid and lower lungs (short arrows, bottom left). Mild mediastinal as well as axillary lymphadenopathy are also present (arrow heads, top left).
Given the systemic nature of the symptoms, concerns about an underlying vasculitic process had been raised by the referring physician. As a result, a series of immunologic studies were ordered to investigate other potential causes of the illness. These investigations revealed strongly positive results for antinuclear autoantibodies (ANAs), antineutrophil cytoplasmic autoantibodies (ANCAs) specific for proteinase 3 (PR3-ANCA), and ANCAs specific for myeloperoxidase (MPO-ANCA). Both ANA and PR3-ANCA levels were beyond the upper limit of detection, being > 12 U for ANA (normal < 1 U) and > 400 enzyme-linked immunosorbent assay (ELISA) units (EU)/mL (normal < 5 EU/mL) for PR3-ANCA. The MPO-ANCA level was also markedly elevated at 132.7 EU/mL (normal < 5 EU/mL). Testing for cytoplasmic ANCA (cANCA) and perinuclear ANCA (pANCA) by indirect immunofluorescence (IIF) was negative.

What is the most likely diagnosis?
Diagnosis: Low-grade B-cell NHL

Recurrence of NHL of low-grade, B-cell type with plasmacytoid differentiation was confirmed by thoracicacoscopic lung biopsy. Serum electrophoresis revealed a small M-spike of 0.7 g/dL, and immunofixation identified this as monoclonal IgG-λ.

Low-grade B-cell lymphoma of mucosa-associated lymphoid tissue type (MALT) is now termed extranodal marginal zone B-cell lymphoma of the MALT type, and accounts for 5% of all NHLs. Of these, 50% are gastric, but dissemination to other MALTs, lymphatics, or the bone marrow is not unusual. When the lung is involved, symptoms may consist of cough with or without hemoptysis, mild dyspnea, or chest pain. Constitutional symptoms, such as fever or weight loss occur in up to 25% of patients. The radiographic appearance is nonspecific. Localized alveolar type opacities are most common (up to 90% of cases). They are usually multiple and bilateral. Air bronchograms are typically present. Bilateral diffuse reticulonodular infiltrates, atelecasis, or pleural effusions occur in <10% of patients. Hilar or mediastinal adenopathy can be detected by CT. Monoclonal gammopathy occurs in up to 60% of cases, particularly in those with plasmacytoid differentiation. Associations with a variety of immune deficiency states and autoimmune disorders have been reported.

Because of the positive ANCA test result, associations between lymphoma and Wegener granulomatosis (WG) deserve further consideration. The radiographic spectrum of WG involving the lungs is variable. The observed bilateral patchy areas of airspace disease including ground-glass infiltrates and consolidation could represent alveolar hemorrhage, one of the typical features of WG or microscopic polyangiitis. The disease defining cavitary nodules of WG resulting from the necrotizing granulomatous inflammation are rare in pulmonary lymphoma. Even though mediastinal, hilar, and peripheral lymphadenopathy may occur in WG, their presence is rare (2%) and should prompt the search for alternative explanations. Lymphomas have long been recognized as complications of cytotoxic therapy for WG. Alternatively, sinonasal NHL and lymphomatoid granulomatosis can mimic WG. Hodgkin lymphoma, T-cell and B-cell NHL, lymphomatoid granulomatosis, and intravascular lymphoma have all been reported as presenting like WG or in association with documented WG. Paraneoplastic vasculitides, myelodysplasia, and other lymphoproliferative disorders have also been associated with ANCA. However, to our knowledge, matching PR3-ANCA with cANCA, which are typical for WG, have not been reported in lymphoma or hematologic disorders.

ANCAs causing a cytoplasmic fluorescence pattern by indirect immunofluorescence (IIF) on ethanol-fixed neutrophils (cANCA) were first reported in necrotizing glomerulonephritis and WG. Proteinase 3 was identified as the target antigen. ANCAs causing a perinuclear fluorescence pattern on ethanol-fixed neutrophils (pANCA) were found in a broader range of autoimmune diseases and react with a variety of neutrophil granule constituents. However, only pANCAs reacting with myeloperoxidase were found to have a specific disease association with the small-vessel vasculitides, microscopic polyangiitis, Churg-Strauss syndrome, and to a lesser extent, WG. WG, microscopic polyangiitis, and Churg-Strauss syndrome are often collectively referred to as ANCA-associated vasculitides (AAVs). The clinical utility of ANCA testing depends on several crucial factors: (1) the analytical accuracy of the ANCA test method applied, (2) the sensitivity and specificity of the test for AAVs, and (3) the pretest probability for AAVs in the particular patient tested.

IIF is a subjective method for ANCA detection, whereas ELISA methods provide a reader-independent, target-antigen specific, semiquantitative determination of PR3-ANCA or MPO-ANCA. Many different ELISA methods are offered commercially. Most are optimized for analytical specificity, often at the cost of analytical sensitivity. The results obtained with different methods correlate poorly and are not directly comparable. Some of the problems inherent to ELISA methods for ANCA detection are related to the preparation of the target antigen. Capture ELISA methods may circumvent these, resulting in better analytical sensitivity and specificity.

Efforts to standardize ANCA testing have not penetrated routine clinical practice widely. The current consensus is that optimal diagnostic accuracy of ANCA testing is achieved by corroborating a positive PR3-ANCA result by ELISA with a positive cANCA by IIF, and a positive MPO-ANCA with a matching positive pANCA IIF result, or vice versa. Incomplete testing or the “wrong pairing” of solid-phase assay (ELISA) and IIF results are likely to be misleading.

The second major factor affecting the clinical utility of a test is the sensitivity and specificity for the disease of an analytically accurate test result. The correctly matching pairs of PR3-ANCA with cANCA and MPO-ANCA with pANCA have a high diagnostic specificity for AAV, even in patients with other autoimmune diseases or pulmonary diseases that can mimic systemic vasculitis or WG. Nevertheless, true false-positive occurrences have been reported. Most of these are related to infections. Reports in amebiasis and hepatitis C have not been corroborated. In contrast, the rare occurrence of PR3-ANCA/cANCA in subacute bacterial endocarditis, which can mimic
small-vessel vasculitis, is well documented. The combination of MPO-ANCA/pANCA is slightly less specific for AAV than PR3-ANCA/cANCA. MPO-ANCA/pANCA may also occur in connective tissue disorders, inflammatory bowel disease, infections, drug-induced vasculitis, and other autoimmune disorders.

The third major factor affecting the clinical utility of a test result is the pretest probability of disease. The positive predictive value of a test depends on the prevalence of true disease being tested. As the test is applied in increasingly prevalent conditions, the positive predictive value also increases, but for rare diseases or in low prevalence conditions, even a highly specific positive test result may have a low positive predictive value.

In our patient, the pretest probability of AAV was moderate, at best. The constitutional symptoms in a progressively ill patient should prompt consideration of a vasculitic syndrome. However, the patient lacked typical extrapulmonary clinical features of AAV such as nose, sinus, or kidney inflammation, palpable purpura, or mononeuritis multiplex. Furthermore, the negative IIF ANCA test results, and the extreme rarity of simultaneous occurrence of PR3-ANCA and MPO-ANCA in AAV, raised suspicions about the validity of the patient’s PR3-ANCA and MPO-ANCA test results. Therefore, the serum sample was further analyzed by PR3-ANCA capture ELISA and by IIF using recombinant proteinase 3 expressing HMC-1 cells. Both methods conclusively indicated that the serum did not contain PR3-ANCA. The capture ELISA method includes a measurement of background reactivity in antigen (proteinase 3) free wells. The serum sample from our patient generated background absorbance values as high as the absorbance generated in antigen-coated wells. The routine direct ELISA method for ANCA detection does not contain background measurements for each serum sample. We subsequently documented that the high reactivity in the background wells of the capture ELISA as well as the highly positive results in the PR3-ANCA, MPO-ANCA, and ANA-ELISAs were caused by nonspecific binding of this serum sample to the plastic ELISA reaction wells. The positive PR3-ANCA and MPO-ANCA test results were thus caused by analytical inaccuracy of the direct ELISA method. We speculate that the B-cell lymphoma with plasmacytoid differentiation led to the secretion of immunoglobulins with a high nonspecific (ie, nonantigen specific) affinity to the plastic reaction wells used in ELISAs causing the initial artifactual false-positive PR3-ANCA and MPO-ANCA results.

All of these considerations lead to the diagnostic lung biopsy. The patient was treated with two cycles of cyclophosphamide, vincristine, prednisone, and five cycles of fludarabine. His symptoms including fevers, night sweats, and cough resolved, and the radiographic abnormalities improved. However, treatment was complicated by significant bone marrow suppression requiring regular transfusion therapy.

**Clinical Pearls**

1. Low-grade B-cell lymphoma of the lung can primarily arise from mucosa-associated lymphoid tissue or represent dissemination from another primary site such as the GI tract.

2. The clinical utility, ie, positive and negative predictive values of ANCA testing for WG and small-vessel vasculitis, are critically dependent on the pretest probability of the disease in the patient tested, as well as on the analytical accuracy of the test method.

3. Maximal diagnostic accuracy of ANCA testing requires corroboration of a positive target antigen-specific test result (PR3-ANCA or MPO-ANCA) by immunofluorescence, and vice versa. Only the PR3-ANCA with cANCA combination, and the MPO-ANCA with pANCA combination are sensitive and specific for ANCA-associated vasculitis.

4. ANCA have been reported in association with lymphomas and other hematologic disorders, but the combination of cANCA and PR3-ANCA has not. Antibodies nonspecifically reacting with a variety of target antigens or plastic of ELISA plates can cause false-positive autoantibody test results in lymphoma patients.

**Suggested Readings**


