Letters to the Editor

CORRESPONDENCE RE: GAL AA, VELASQUEZ A. ANTINEUTROPHIL CYTOPLASMIC AUTOANTIBODY IN THE ABSENCE OF WEGENER'S GRANULOMATOSIS OR MICROSCOPIC POLYANGIITIS: IMPLICATIONS FOR THE SURGICAL PATHOLOGIST. MOD PATHOL 2002;15:197–204.

The report by Gal and Velasquez (1) analyzes the value of tests for anti-neutrophil cytoplasmic antibodies (ANCA) in the diagnosis of Wegener's granulomatosis (WG) and microscopic polyangiitis (MPA) in 27 patients with pulmonary disease who had open lung or transbronchial biopsies. The authors conclude that "significantly elevated ANCA titers may be associated with diverse forms of pulmonary disease." However, their study is seriously flawed because of their sole reliance on indirect immunofluorescence for the detection of ANCA. Only two forms of ANCA are associated with the spectrum of disease that includes WG, MPA, Churg-Strauss syndrome, and primary pauci-immune necrotizing glomerulonephritis, namely antibodies to proteinase 3 (PR3) or myeloperoxidase (MPO). These antibodies can be detected optimally and accurately only with the combined use of antigen-specific immunoassays and an indirect immunofluorescence assay (2). Although PR3-ANCA can be detected with moderate reliability by a characteristic pattern of cytoplasmic staining of neutrophils (C-ANCA), the finding of perinuclear (or nuclear) staining (P-ANCA) is a totally unreliable method of detecting MPO-ANCA and lacks diagnostic specificity. Thus, the P-ANCA pattern may be produced by antibodies to numerous antigens, including antibodies directed at various nuclear antigens. And even though the authors acknowledge this shortcoming in the discussion, they state that "ELISAs for ANCA were performed only if specifically ordered by the referring clinician." Analysis of Table 1 reveals that among the eight patients (30%) with positive ANCA tests who were found on biopsy to have a disease other than WG or MPA, four had P-ANCA (three with antinuclear antibodies) and none had ELISAs to determine whether antibodies to MPO were present. A metaanalysis, based on numerous studies, has shown that properly performed assays for PR3 and MPO-ANCA, which include both indirect immunofluorescence and ELISAs, show a high degree of specificity and sensitivity for the spectrum of disease that includes WG and MPA (2). In Table 3 of the report of Gal and Velasquez (1), a large number of disorders in which ANCA may be found are listed; in nearly all of these the ANCA are not directed against PR3 or MPO and are therefore irrelevant to the diagnosis of WG or MPA.

We agree with the authors that ANCA tests alone should not be relied on for diagnosis, which must always involve consideration of clinical and, when available, histologic findings. Nevertheless, the diagnostic usefulness of tests for PR3 ANCA and MPO-ANCA is so well established that studies performed today based on immunofluorescence alone are unacceptable.

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REFERENCES

1. Gal AA, Velasquez A. Antineutrophil cytoplasmic autoantibody in the absence of Wegener's granulomatosis or microscopic polyangiitis: implications for the surgical pathologist. Mod Pathol 2002;15:197–204.
2. Choi HK, Liu S, Merkel PA, Colditz GA, Niles JL. Diagnostic performance of antineutrophil cytoplasmic antibody tests for idiopathic vasculitides: metaanalysis with a focus on antimyeloperoxidase antibodies. J Rheumatol 2001;28:1584–90.

In reply: We appreciate the comments by Niles *et al.* regarding our recent publication on antineutrophil cytoplasmic antibodies (ANCA) in patients without Wegener's granulomatosis or microscopic polyangiitis (1). Our intent was to alert surgical pathologists about problems and limitations of ANCA testing, relevant to pulmonary diseases, which had not been well-addressed in the histopathologic literature.

There is no question that we agree with their basic premise: that ANCA-immunofluorescence (ANCA-IF) alone is insufficient for the evaluation of vasculitis and that a positive result be followed by further testing for specific ANCA antibodies by enzyme linked immunosorbent assay (ANCA-ELISA). This has been endorsed by an international consen-

sus panel, who recommended that a positive ANCA-IF be followed by ANCA-ELISA for proteinase 3 (PR3) and myeloperoxidase (MPO) (2). It remains to be proven, however, whether initial screening with ANCA-ELISA remains cost-effective.

Niles *et al.* may wonder why we made the statement: "ELISAs for ANCA were performed only if ordered by the referring clinician." At our institution there is no policy for reflex testing for confirmation of ANCA by ELISA because of the low number of positive tests, economical laboratory utilization issues, and other reasons. A commercial kit-based ANCA-IF is currently used for screening and, if positive, we render an interpretation, followed with the specific recommendation to the referring clinician that he or she follow-up with PR3-ANCA and MPO-ANCA. Thus, the additional testing is left up to the discretion of the clinician, who may or may not be aware of the intricacy and nuance of ANCA testing and interpretation. Unfortunately, our experience has shown that many do not follow-up with confirmatory testing with ANCA-ELISA and make therapeutic decisions based solely on the results of ANCA-IF.

This brings up the bigger issue that needs to be addressed: that there is a knowledge gap between the "cognoscenti" (rheumatologists, nephrologists, nephropathologists, and clinical pathologists) and the "non-cognoscenti" (pulmonologists, thoracic surgeons, and surgical pathologists) regarding the many problems and limitations of ANCA testing. This is somewhat surprising, because the latter group is most often involved in the diagnosis and critical management of patients with pulmonary vasculitis. An example of this void is reflected in the paucity of literature among certain medical specialties; a MEDLINE review using the terms "ANCA and ELISA" revealed 443 citations in the English language, of which a few were in the "non-cognoscenti" literature: pulmonologist (three papers), surgical pathology (three papers), thoracic surgery (0 papers). We suspect that a large number of practicing pulmonologists, thoracic surgeons, and surgical pathologists would welcome guidelines for ANCA testing and interpretation.

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