ANTICYTOPLASMIC ANTIBODIES AND WEGENER’S GRANULOMATOSIS

Background

Wegener’s granulomatosis, a disease of unknown etiology, is characterized by a granulomatous vasculitis of the upper and lower respiratory tracts. Varying degrees of systemic vasculitis of small arteries and veins may also be seen (1). Renal disease in the form of focal or diffuse necrotizing glomerulitis is often present. Until recently, the disease was uniformly fatal. Today, immunosuppressive and cytotoxic therapy are used with up to 90% of patients responding (2).

The disease is diagnosed by biopsy of affected tissue, preferably lung. Unfortunately, obtaining a good specimen is often difficult or impossible. Even when a biopsy is obtained, a definitive diagnosis cannot always be made. In many cases, diagnosis and disease activity must be based on clinical impression and nonspecific lab tests such as ESR and CRP (3).

In 1985, Van Der Woude, et al., first described an anticytoplasmic antibody (ACPA) which they felt to be an autoantibody specific for Wegener’s granulomatosis (4). Since then, numerous reports have validated the association between Wegener’s granulomatosis and ACPA. Although both RIA (7,10) and ELISA (12,13,20) methods have been developed, ACPA is most commonly detected by indirect immunofluorescent assays.
Methodology

ACPA measurements are not yet commercially available. Thus, there are many variations on the test methodology depending on the group that performs them. However the basic principle of the assays remains the same. These tests allow for detection of ACPA by applying patient serum to a preparation of granulocytes. Fluorescent anti-human IgG is added, allowing visualization of antibody within the granulocytes. The following is the method of Van Der Woude, et al., the group that first developed the assay (6).

Cytocentrifuge slides of granulocytes are prepared from peripheral blood of normal human controls. Dextran sedimentation and lysis of red blood cells with distilled water yield a clean preparation of cells. These cells are fixed to glass slides with 95% ethanol at 4 C. The slides are incubated with patient sera for 30 minutes, then washed with phosphate buffered saline (PBS). Next, the slides are incubated with Fluorescein Isothiocyanate conjugated sheep anti-human IgG for 30 minutes, then washed. Slides are evaluated under a fluorescent microscope. ACPA is characterized by strong, diffuse staining of the entire cytoplasm, leaving the nucleus unstained.

Discussion

A number of studies have now been published proposing ACPA as a marker for Wegener’s Granulomatosis (4,5,6,11,20). Most claim a very high specificity. The largest study, including 1657 controls, claims a specificity of 99.4% for IFA. Most of the false positives in the majority of these studies were due to other unclassified vasculitides. The high specificity is highly dependent on good technique and experienced interpretation of the fluorescent patterns. Several studies, showing considerably lower specificity, counted perinuclear staining as well as the diffuse cytoplasmic staining said
to be specific for Wegener's granulomatosis (13,14,17,18). It is only when positives are restricted to the diffuse cytoplasmic staining that high specificity is obtained. A number of case reports are now in the literature supporting ACPA as a specific marker of Wegener's granulomatosis (15,16,19). Other case reports, contradict these findings (9,11,18). However, many of these latter cases involve diseases, such as microscopic polyarteritis, which are ill-defined entities. These may in fact be related to Wegener's granulomatosis.

The sensitivity of the ACPA test varies considerably from 56% to 96% depending on what group of patients is included (4,5,6,11,20). When all patients with Wegener's granulomatosis are counted, the sensitivity ranges from 56% to 76%. The sensitivity rises to 96% when restricted to patients with active generalized disease. This fact has led some people to conclude that the test can be used as a marker of disease activity.

Several points weaken this conclusion. In most of the studies, the majority of patients were defined by biopsy diagnosis. However, a number of patients were defined by clinical parameters alone. This casts some doubt on the reliability of this test's sensitivity. In addition, most of these studies used nonspecific markers such as CRP and ESR to define disease activity.

It is not yet clear what the anticytoplasmic antibody is directed against. Researchers have noted an association with alkaline phosphatase (7) and with myeloperoxidase (13). However, both of these claims have been disputed (8,12,14). The antibody has most consistently been associated with the specific granule of the neutrophil, but a specific protein has not been identified (8,12). Indeed, some feel that the antibody is actually directed against a heterogeneous mix of proteins (17). This would account for the overlap in specificity noted by some groups.
Most groups have used the IFA technique to study the ACPA. This is the easiest and most reproducible method. In an effort to overcome the problems inherent to this method, a number of groups have developed RIA and ELISA assays (7,10,12,13,20). However, these methods have their own shortcomings. Most important is the varying specificity of these tests. This is due to the differing methods of preparing the antigen extract used by each group. As yet none of these assays is widely available.

In summary, Wegener's granulomatosis is a uniformly fatal disease without treatment. The diagnosis is often quite difficult, even with a tissue biopsy, and patients are often treated without a definitive diagnosis. Unfortunately, some of the diseases in the differential diagnosis are treated quite differently. Thus a test specifically for Wegener's granulomatosis would be highly desirable. The anticytoplasmic antibody (ACPA) is an intriguing possibility. It appears to be quite specific, though not very sensitive for Wegener's granulomatosis. The ability of this assay to follow disease activity is still unclear. The antigen measured by this assay remains unknown. Although ACPA is clearly not ready to replace tissue biopsy as the gold standard for diagnosis of Wegener's granulomatosis, it clearly deserves further study.

References


3. Sack,K., "Wegener's granulomatosis", Western J of Medicine, 1989;150:329-333.


