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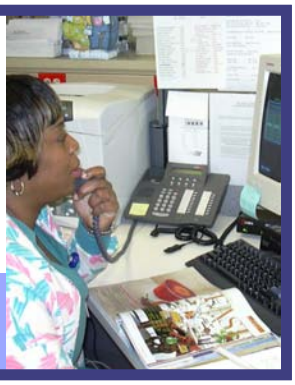

"I Think ANCA, I Think ANCA"[1,7]

(A brief review/update of Antineutrophil Cytoplasmic Antibodies)

Antineutrophil cytoplasmic antibodies (ANCA) are recognized as important laboratory markers for a particularly aggressive category of systemic small-vessel vasculitis and rapidly progressive glomerulonephritis, and may also be observed with inflammatory bowel disease and some rheumatologic diseases. Laboratory evaluation of ANCA has evolved, as understanding of the subclasses and specificity of ANCA has improved. Optimum testing strategies using ANCA require some knowledge of the potential uses and limitations of the test.

Originally described in 1982 as occurring in patients with small-vessel vasculitis and necrotizing glomerulonephritis, ANCA gained early notoriety because of its association with Wegener's granulomatosis.[1,2] In 1994, the Chapel Hill consensus conference suggested a classification scheme of vasculitis based on vessel type, pattern of immune deposition and clinical features; in which Wegener's granulomatosis, Churg-Strauss syndrome, and microscopic polyangiitis were identified as ANCA (+) small-vessel vasculitides.[3] At the same time, it was becoming clear that a (+) ANCA, especially if determined by only indirect immunofluorescence microscopy assay and not confirmed by enzyme immunoassays, did not equate with the presence of a vasculitis. One area where this quickly became apparent was in the arena of pulmonary pathology. Several articles appeared indicating the presence of ANCA in patients with and without the classic histologic findings of pulmonary small-vessel vasculitis (i.e. necrotizing granulomatous inflammation with vasculitis or alveolar hemorrhage with capillaritis).[4-6] Pulmonary lesions observed in addition to or in the absence of vasculitis included diffuse alveolar damage, bronchiolitis obliterans with organizing pneumonia (BOOP), interstitial pneumonitis, alveolar hemorrhage, granulomatous inflammation, eosinophilia, bronchopneumonia, acute or chronic pleuritis, lipoid pneumonia, lymphoid aggregates, follicular bronchitis, and fibrous scar.[4-6] All of these patterns of injury can be caused by ANCA-disease, but they are less definitive than vasculitis or capillaritis. Table 1 lists other diseases that have been reported to be ANCA (+).[6] Clearly, a "(+) ANCA" in and of itself is not diagnostic and must be interpreted in the context of the clinical and pathologic findings in the patient. But all is not lost, read on.

Table 1
ANCA (+) Reported in Diseases other than small-vessel vasculitis [6*]

Other vasculitides	*Infections*
Giant cell arteritis	Mycobacterial
Polyarteritis nodosa	Leprosy
Kawasaki's disease	Fungal (Aspergillus, Sporotrichosis, Paracoccidioides, Chromomycosis)
Rheumatoid diseases	Subacute bacterial endocarditis
Systemic/Drug-induced Lupus	Malaria
Rheumatoid arthritis *(adult and juvenile)*	Leptospirosis
Felty's syndrome	Influenza
Scleroderma	HIV/AIDS
Sjögren's syndrome	Bacterial pneumonia or sepsis
Ankylosing spondylitis	Amoebiasis
Dermatomyositis	
Antiphospholipid syndrome	*Other autoimmune*
	Goodpasture's syndrome
Gastrointestinal	Sarcoidosis
Ulcerative colitis	Sweet's syndrome
Crohn's disease	
Sclerosing cholangitis	*Neoplasia*
Autoimmune hepatitis	Lymphoma
Primary biliary cirrhosis	Carcinoma
	Myeloproliferative disorders
Miscellaneous	Monoclonal gammopathy
Hemodialysis	Bone marrow transplantation
Drugs	
Cystic fibrosis	

* Modified from Gal & Velasquez[6]

What exactly are ANCA?

ANCA were initially recognized and categorized by indirect immunofluorescence (IIF) using test patient serum applied to ethanol-fixed neutrophils. Two major patterns of staining

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were observed – a cytoplasmic pattern (C-ANCA) and a perinuclear (P-ANCA) pattern. In addition two minor patterns were observed – "C-ANCA (atypical)" and "atypical ANCA".[7] With time, some of the antigens responsible for ANCA production were identified and their association with various diseases established. The two major antigens identified in patients with small vessel vasculitis were proteinase 3 (PR3) and myeloperoxidase (MPO). ANCA directed against these antigens, designated PR3-ANCA and MPO-ANCA respectively, are demonstrable by enzyme immunoassays (EIA). In patients with vasculitis, roughly 90% of patients with C-ANCA (by IIF) demonstrate PR3-ANCA specificity (by EIA).[3] Conversely roughly 90% of patients with P-ANCA (by IIF) show MPO-ANCA specificity (by EIA).[3] Occasional vasculitis patients with C-ANCA exhibit MPO specificity, while others with P-ANCA manifest PR3 specificity.[7] As a general rule, most patients with Wegener's granulomatosis have C-ANCA/PR3-ANCA while those with microscopic polyangiitis or Churg-Strauss syndrome have P-ANCA/MPO-ANCA; but either pattern/specificity may occur in the setting of small-vessel vasculitis.[3] *A very important point is that a negative ANCA result does not exclude the possibility of small-vasculitis and occurs in greater than 10% of patients with otherwise typical Wegener's granulomatosis, microscopic polyangiitis or Churg-Strauss syndrome.*[3]

What about "atypical ANCA"?

The atypical ANCA patterns observed by IIF are usually not associated with small vessel vasculitis. Often these patterns result from ANCA directed against antigens other than PR3 or MPO, such as lactoferrin, enolase, cathepsin-G, elastase, lysozyme, bactericidal/permeability-increasing protein or to-be-determined-later.[6,7] These types of ANCA may be observed in patients with inflammatory bowel disease, autoimmune hepatitis, and a variety of rheumatologic diseases.[6,7] Patients with antinuclear antibodies (ANA) may make IIF characterization of P-ANCA difficult.[6] Thus EIA confirmation of (+) ANCA by IIF has been recommended to increase the specificity of C-ANCA or P-ANCA (+) sera (for small-vessel vasculitis).[3]

More "Less Filling"/ "Tastes Great" - Competing Testing Algorithms

With two different methods (IIF & EIA) for evaluating ANCA, it comes as no surprise to students of science and human nature that two schools of thought regarding testing strategies for small-vessel vasculitis have emerged. Everyone agrees that the methods are complementary, and that a positive result by one method should be confirmed by the other method – the question is which assay should be first.[7-9] There is no universally accepted "gold standard" test for ANCA and most commercially available IIF and EIA kits produce "acceptably accurate analytic results…similar to those reported from academic research laboratories."[8] EIA kits often demonstrate superior specificity compared to IIF kits, leading to superior positive predictive values.[8] Nevertheless, many laboratories employ IIF as the initial screening test due to presumed superior sensitivity.[7,8] The Mayo Clinic recently reviewed ANCA results in 615 consecutive samples submitted for ANCA testing over a 10-month period.[9] The patient breakdown was as follows: 86 Wegener's granulomatosis/microscopic polyangiitis, 529 controls (118 other autoimmune disease, and 411 disease not further specified). They found that that PR3-ANCA and MPO-ANCA EIA had the highest sensitivity, while C-ANCA IIF had the highest specificity (Table 2) and subsequently adopted an "EIA first" testing algorithm for screening patients for small-vessel vasculitis.[9]

Table 2
Wegener's Granulomatosis/Microscopic Polyangiitis Comparison of ANCA Detection Levels by Methodology [9*]

C-ANCA or P-ANCA (IIF)	*PR3-or MPO-ANCA (EIA)*
Sensitivity 64.0%	Sensitivity 72.1%
Specificity 91.5%	Specificity 92.8%
PPV 55.0%	PPV 62.0%
NPV 94.0%	NPV 95.3%

PPV = Positive predictive value NPV = Negative predictive value
* Modified from Russell *et al*[9]

ANCA, like *any other laboratory test*, are only as good as the rationale used for ordering the test. *Any test performs poorly when ordered in a patient population with a low prevalence of disease.* The UNC group has found that the PPV of (+) ANCA in a hospitalized patient, not otherwise specified was < 5% (Table 3).[10] Similarly a meta-analysis of 15 suitably screened ANCA studies, which focused only on C-ANCA (the most specific of all recognized ANCA), confirmed that performance deteriorated as disease prevalence fell (Table 4).[11] When ANCA testing (for small-vessel vasculitis) is restricted to patients fulfilling the clinical indications recommended by an international panel of experts (Table 5), the positive predictive value ranges from 50% to >90%.[7]

Table 3
Theoretical Positive Predictive Value of ANCA for any from of ANCA-associated vasculitis– UNC[10]

Clinical Manifestations	PPV %
Necrotizing sinusitis, nodular pulmonary infiltrates, hematuria, and proteinuria	> 90
Hemoptysis, hematuria, and proteinuria	> 90
Palpable purpura, mononeuritis multiplex, hematuria and proteinuria	> 90
Hematuria, proteinuria, and rapidly progressive renal failure	> 90
Hematuria and proteinuria	< 50
Sinusitis	< 5
Pulmonary infiltrates	< 5
Hospitalization for any reason	< 5

from Jennette [10]

Table 4
Positive Predictive Value of C-ANCA for Wegener's granulomatosis (Meta-Analysis)[11]

Prevalence	PPV %	False-Positive Rate%
0.1	3	97
1	26	74
5	63	37
10	78	22
20	89	11

From Rao *et al*[11]

Table 5
Clinical Indications for ANCA – Small Vessel Vasculitis[7]

Glomerulonephritis (esp. rapidly progressive)
Pulmonary hemorrhage (esp. with renal disease)
Cutaneous vasculitis w/ systemic features
Multiple lung nodules
Chronic destructive disease of upper airways
Long-standing sinusitis or otitis
Subglottic tracheal stenosis
Mononeuritis multiplex or other peripheral neuropathy
Retro-orbital mass

Modified from Savige *et al* [7]

ANCA in GI Diseases

The role of ANCA is GI diseases is more complex and controversial. P-ANCA (+) sera have been reported in patients with inflammatory bowel disease (IBD) and autoimmune hepatitis.[12] These sera usually are negative for MPO-ANCA and PR3-ANCA by EIA, and other antigens have been proposed as the targets of the P-ANCA.[13] In the appropriate clinical setting, a (+) P-ANCA, coupled with a (-) MPO-ANCA would be consistent with IBD (and many other autoimmunes disease for that matter).[13] It appears that nuclear histone antigens (sensitive to destruction by DNAase) are required for the ANCA observed in inflammatory bowel disease, particularly ulcerative colitis.[14, 15] No specific EIA for IBD-associated ANCA have emerged.

Some have advocated a serologic panel as a "first step" (screening) approach in the diagnosis and classification of IBD.[14, 15] The rationale is that DNAase sensitive P-ANCA are relatively specific for ulcerative colitis, while antibodies to *Saccharomyces cerevisiae* (ASCA) and *E. coli* outer membrane porin (anti-OmpC) are relatively specific for Crohn's disease, although overlap occurs.[14] However, there is a great deal of skepticism about this approach among gastroenterologists, as well as laboratory scientists, due to concerns about both false-positive and false-negative results (see discussion about positive predictive value above).[16] Further complicating this issue is the fact that some of these tests appear to be proprietary (capitalism trumps science) – and thus not available for independent investigation. However, most agree these tests may be helpful in a select group of IBD patients with "indeterminate colitis", in whom the distinction between ulcerative colitis and Crohn's disease may be difficult by traditional means.

ANCA testing at Rex

All specimens for ANCA testing are referred to Mayo Medical Laboratories. For orders of "ANCA" without any other specification, the MML test #83012 (Antineutrophil Cytoplasmic Antibodies Vasculitis Panel) will be performed. As discussed above, this consists of a MPO-ANCA and PR3-ANCA screen by EIA. Negative results will be reported immediately. A positive result will be reflexed to MML test #9441 (Cytoplasmic Neutrophilic Antibody) for IIF. Positive results for C-ANCA will be titered, while a positive P-ANCA result will simply be reported as positive.

For patients with known small-vessel vasculitis and (+) ANCA, monitoring of MPO-ANCA or C-ANCA may be helpful in monitoring disease activity. These should be ordered as specific tests, rather than just "ANCA". (At the present time, monitoring of PR3-ANCA levels for disease status is not recommended.)

For patients with IBD of "indeterminate" type, an IBD ANCA panel is available from Prometheus Laboratories, by way of Mayo Medical Laboratories. If this test panel is desired, order "Inflammatory Disease Panel – Prometheus Laboratories".

Questions or concerns about ANCA testing are welcome and should be directed to the author.

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Neckties – An Emerging Health Hazard?

Several recent studies have called into question the potential health risks associated with neckties, both to the physician wearing one and those around him/her. Last year ophthalmologists from five New York medical schools reported the effects of neckties on intraocular pressure.[1] 40 eyes of 20 normal subjects and 20 open angle glaucoma patients were subjected to intraocular pressure (IOP) measurements with an open shirt collar (baseline), three minutes after putting on a tight necktie, and three minutes after loosening it. Both groups demonstrated an increase in IOP (2.6 mm +/- 3.9 mm Hg in normal subjects; 1.0 +/- 1.8 mm Hg in glaucoma patients). In normal subjects, 12 eyes had an increase > 2 mm Hg and seven eyes and an increase > 4 mm Hg. In glaucoma patients, six eyes had an increase > 2 mm Hg, while two had an increase > 4 mm Hg. The authors concluded that a tight necktie might cause increased IOP and interfere with accurate measurement of same.

The bacterial flora colonizing physicians' neckties at New York Hospital Center of Queens were compared with those found in security personnel working in non-clinical areas.[2] Nearly half (47.6%) of 42 neckties worn by male physicians, physician assistants and medical students contained potential pathogens including *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. By comparison, only 1 of 10 security personnel yielded a pathogen on culture. While no direct evidence linking neckties to disease transmission was found, the researchers indicated, "…any benefit from the necktie may be offset by their potential risk in spreading disease. This study brings into question whether wearing a necktie is in the best interests of our patients."[2] An earlier study examined the contamination rate of bowties and neckties worn by British obstetricians and gynecologists.[3] Swabs soaked in sterile saline were obtained from specific areas on bowties and neckties on day one and day three of the study. The swabs were plated on choclate blood and MacConkey agar plates with bacterial growth assessed semiquantitatively. The study found a significant difference in contamination rates on day one of the study (neckties were more likely to be contaminated at the end of day one). However, by day three the contamination rates were the same. The authors concluded that there was no association between tie type and bacterial contamination and further stated, "Because of its negative image and difficulty to tie, the bow tie will probably remain a minority fashion."[3]

Neckties have been touted as creating a professional appearance and are *de rigueur* at the Mayo Clinic and other medical centers.[4] However, one study found wearing a necktie "…did not significantly affect patients impression of their physician or the care they received."[5] While patients seemed to prefer physicians who wear neckties, 30% of patients incorrectly perceived that their physician had worn a necktie, when (in fact) that was not the case. In view of the obvious discomfort associated with this accouterment, perhaps it is time for physicians to reconsider their sartorial options.[6] (In a possibly related development, Homeland Security Director Tom Ridge announced that Code Windsor could remain in effect until after the Presidential election. A "no tie zone" perimeter will be maintained around both candidates from now until the election results are finalized to assure safety to them and the general public.)

"Tieless" John Benson

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