

Proteinase-3 Antineutrophil Cytoplasm Antibody Positivity in Patients Without Primary Systemic Vasculitis

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Objectives: Antineutrophil cytoplasm antibodies (ANCA) are useful diagnostic markers in vasculitis. Historical data have suggested the combination of cytoplasmic (C-ANCA) staining with antibodies specific for proteinase 3 (PR3-ANCA) is 99% to 100% specific for granulomatosis with polyangiitis. We aimed to establish the frequency and associations of PR3-ANCA in patients without primary systemic vasculitis using current methods in our laboratory.

Methods: This was a retrospective review of all patients identified as C- and PR3-ANCA positive as determined by indirect immunofluorescence and Luminex testing, respectively, in our laboratory over a 6-month period.

Results: One hundred ninety-four patients were positive for both C- and PR3-ANCA. One hundred seventy-five patients had primary ANCA-associated vasculitis (AAV). Nineteen patients (9.7%) were C- and PR3-ANCA positive but without AAV. Clinical associations included infections, other autoimmune disorders, and malignancy. PR3-ANCA titer ranged from 31 to 278 U/mL (reference range, 0–25 U/mL). Three patients became PR3-ANCA negative after treatment of associated conditions. One patient went on to develop AAV 6 months after the study period.

Conclusions: We detected a higher than expected frequency (9.7%) of “incidental” C- and PR3-ANCA. Several factors may be contributing, including the occurrence of ANCA in other inflammatory states, the increased use of ANCA testing in unselected populations with low clinical suspicion of AAV, recent changes to detection methods for ANCA, and the probability that circulating ANCA predate onset of clinical disease. Our data underscore the need to secure tissue diagnosis in AAV and to exclude other underlying conditions such as infection. In addition, patients with unexplained ANCA should be followed up because they are at risk of developing disease over time.

Key Words: antineutrophil cytoplasm antibody (ANCA), vasculitis, ANCA-associated vasculitis (AAV), enzyme-linked immunosorbent assay (ELISA), Luminex

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Antineutrophil cytoplasm antibodies (ANCA) are useful diagnostic markers in a range of systemic small vessel vasculitides

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and glomerulonephritides, including granulomatosis with polyangiitis (GPA; formerly Wegener granulomatosis), microscopic polyangiitis, Churg-Strauss syndrome, and renal-limited vasculitis. Antineutrophil cytoplasm antibodies were first demonstrated in the early 1980s by indirect immunofluorescence (IIF) of human neutrophils and classically give rise to 2 distinct patterns of cytoplasmic and perinuclear fluorescence (C-ANCA and P-ANCA, respectively). They have since been shown to be directed against antigens in the granules of neutrophils and lysosomes of monocytes, the 2 major specificities being for proteinase 3 (PR3) and myeloperoxidase (MPO).

Although a variety of atypical fluorescence patterns and “nonclassic” antigens have been described, which may be associated with a range of inflammatory and infectious disorders, the combination of a C-ANCA immunofluorescence pattern with antibodies specific for PR3 (PR3-ANCA) has been reported to be highly specific (99%–100%) for GPA,¹ a syndrome of primary systemic vasculitis and granulomatous tissue inflammation. On this basis, it is the recommendation of the International Consensus Statement on Testing and Reporting of ANCA^{2,3} that, in suspected vasculitis and glomerulonephritis, IIF is used as a screening test, followed by an antigen-specific assay, such as direct or capture enzyme-linked immunosorbent assay (ELISA), as a guide to diagnosis and disease activity.

Our local observation, however, suggests that “incidental” PR3-ANCA are increasingly being reported in patients without primary systemic vasculitis and in association with other autoimmune, inflammatory, or infectious diseases. The aim of this study was to gain a clinical perspective on the frequency and associations of PR3-ANCA positivity in patients without primary systemic vasculitis in our laboratory using current detection methods.

METHODS

Our immunology laboratory receives requests from 4 large teaching and general hospitals in West London, United Kingdom, and processes approximately 500 requests for anti-MPO, anti-PR3, or anti-glomerular basement membrane antibodies per month. Over a 6-month period, we retrospectively identified all patients who were found to be positive for the combination of C-ANCA staining by indirect immunofluorescence and anti-PR3 antibodies as determined using the BMD FIDIS Vasculitis Luminex system (BioMedical Diagnostics, Marne-la-Vallée, France).

For immunofluorescence testing, patient sera (dilution 1:20) were incubated with ethanol-fixed human neutrophil slides (INOVA Diagnostics, San Diego, CA), washed, and then incubated with a fluorescein isothiocyanate-labeled anti-human total immunoglobulin G (IgG) secondary antibody (INOVA Diagnostics). The Luminex assay was conducted according to the manufacturer’s specification. Briefly, patient samples (dilution 1:201) were mixed with microspheres covalently coupled to each of the antigens of interest (PR3, MPO, and glomerular basement membrane), washed, and then incubated with a phycoerythrin-labeled anti-human total IgG conjugate. Following a further wash step,

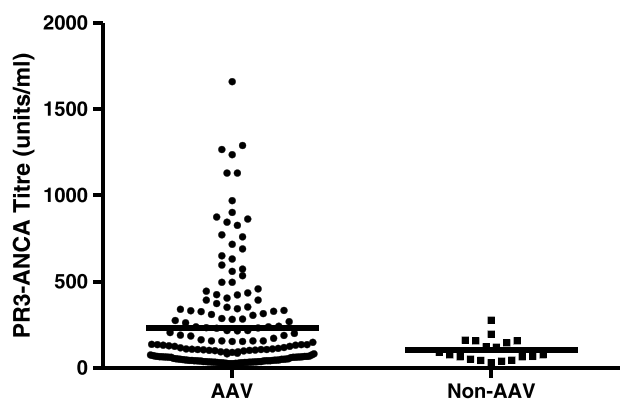


FIGURE. Individual and mean (horizontal bar) PR3-ANCA titers for patients with (n = 175) and without (n = 19) a diagnosis of primary AAV.

the reaction was directly measured by flow cytometer (which differentiates each set of antigen-specific microspheres according to its fluorescence color and simultaneously measures the average fluorescence emitted by the conjugate). A calibration system allowed the determination of a titer (in units per milliliter). We used the manufacturer’s recommended threshold of 25 U/mL for a positive reaction, which correlates to the fluorescence signal greater than the 98.5th percentile distribution of a normal sample

population. Samples were not heat treated before IIF or Luminex assay, regardless of infection status.

We then reviewed the case notes, electronic records, laboratory results, and available imaging for all patients identified to establish if they had a diagnosis of primary ANCA-associated vasculitis (AAV) or alternative diagnoses.

Statistical analysis was conducted using GraphPad Prism (GraphPad Software, San Diego, CA).

RESULTS

In total, 194 patients were positive for the combination of C-ANCA and anti-PR3-specific antibodies. Of these, 175 were known or new diagnoses of AAV within the 6 months of the study. The remaining 19 patients, equating to 9.7% of the cohort, did not have any evidence of primary systemic vasculitis at the time of the study. All 19 patients were reviewed by consultant physicians in rheumatology, nephrology, or hematology clinics following the finding of positive ANCAs and were found not to have any evidence of primary AAV currently or previously. None of the 19 patients were recorded to be taking medications associated with the development of ANCAs (including hydralazine, propylthiouracil, penicillamine, minocycline, and phenytoin).

The mean PR3-ANCA titer in this group was 105 U/mL (range, 31–278 U/mL) versus 233 U/mL in the group with primary AAV ($P = 0.284$; Mann-Whitney test). The individual PR3-ANCA titers for patients in both groups are shown in the Figure 1. The PR3-ANCA titers, age, and contemporaneous

TABLE 1. PR3-ANCA–Positive Patients Without Primary Systemic Vasculitis; Age, PR3-ANCA Titer, Presence of Other Antibodies, and Clinical Associations

n	Age, y	PR3-ANCA Titer, U/mL	Other Antibodies	Clinical Associations
Infection				
1	66	145	Polyclonal hyper-IgG; RF 1/320	Endocarditis
2	79	120	Polyclonal hyper-IgG	Endocarditis
3	42	158	Polyclonal hyper-IgG	HIV
4	37	90	Not available	HIV
5	52	68	None detected	HIV
6	71	78	Polyclonal hyper-IgG; ANA ⁺	Tuberculosis
7	62	44	Polyclonal hyper-IgG	APKD, tuberculosis
Autoimmunity				
8	45	198	None detected	Multiple sclerosis
9	27	163	ANA ⁺	Psoriasis
10	64	76	ANA ⁺	Immune thrombocytopenia
11	89	46	Polyclonal hyper-IgG; RF 1/5120	Rheumatoid arthritis; cirrhosis
Malignancy				
12	66	123	γ-Paraprotein, ANA ⁺ , RF 1/640	Chronic lymphoid leukemia
13	77	65	None detected	Ovarian Cancer
14	74	52	None detected	Prostate cancer, myelodysplasia
15	69	40	Polyclonal hyper-IgG; RF 1/160	Colonic cancer
Miscellaneous				
16	28	278	None detected	Cyclical neutropenia
17	72	158	Polyclonal hyper-IgG	Osteoarthritis
18	73	65	None detected	Chronic TEPAHT
19	58	31	None detected	Osteoarthritis

PR3 indicates proteinase-3; RF, rheumatoid factor; ANA, antinuclear antibody; APKD, autosomal dominant polycystic kidney disease; TEPAHT, thromboembolic pulmonary arterial hypertension.

clinical associations for the 19 patients who did not have primary AAV are shown in Table 1.

DISCUSSION

The combination of C- and PR3-ANCA is widely considered to be highly specific for GPA. However, given the reported specificity of 99% to 100%,¹ this study demonstrates a higher than expected prevalence (9.7%) of “incidental” PR3-ANCA in patients who do not have a primary systemic vasculitis. We suggest that several factors may be contributing to the prevalence of incidental PR3-ANCA seen in this cohort, including the occurrence of ANCAs as part of a polyclonal B-cell response seen during infection and inflammation, recent changes in detection methods for these antibodies, and the likelihood that circulating PR3-ANCA may predate the development of clinical disease.

Approximately half of the patients in our cohort had PR3-ANCA in association with polyclonal B-cell responses and other autoantibodies, typically in the presence of systemic inflammation associated with autoimmunity, malignancy, and in particular infections. The finding of C-ANCA in hematologic malignancy has been reported previously.^{4,5} The infections seen in our cohort included endocarditis, tuberculosis (TB), and human immunodeficiency virus (HIV). There are a small number of cases of PR3-ANCA positivity in association with endocarditis reported in the literature,^{6,7} although we suspect this finding may be underreported. Similarly, the occurrence of PR3-ANCA in TB is recognized, although the frequency varies dramatically between series from 0% to 33%.⁸⁻¹⁰ The reason for these discrepant rates is not clear, although it may be related to treatment or the effects of drugs, as the higher rates appeared to be seen in those groups undergoing treatment. Geographical differences in the study groups may also be playing a role. In HIV, the other infection seen in our cohort, nonspecific ANCAs are reported at rates between approximately 20% and 40%.^{11,12} PR3-ANCA are much less frequent, with 1 series of 200 patients reporting only 2 patients with this specificity.¹¹ PR3-ANCA are rarely reported with a range of other infectious diseases, including amoebiasis,¹³ leptospirosis,¹⁴ parvovirus,¹⁵ chronic hepatitis C,¹⁶ and malaria.¹⁷

That these infections may mimic the presentation of AAV resulted in diagnostic uncertainty, and this was often the indication for determining an ANCA result in our patient cohort. Endocarditis, for example, often presents with features of small vessel vasculitis attributable to microembolism and the effects of immune-complex deposition on the vascular endothelium. Tuberculosis may present constitutional symptoms and pulmonary features such as nodules and hemoptysis; HIV may result in both renal impairment and proteinuria through a variety of mechanisms, including immune-complex glomerulonephritis and classic collapsing glomerulopathy (as opposed to pauci-immune crescentic glomerulonephritis seen in AAV). These cases demonstrate the clear need for securing tissue diagnosis if possible in AAV.

Several mechanisms have been postulated for the development of ANCAs in infection, including nonspecific polyclonal B-cell activation, and there was evidence of this phenomenon in our study group. A role for bacterial superantigens directly activating autoreactive B-cell or T-helper cells has been suggested.¹⁸ In addition, a process of “molecular mimicry” between microbial proteins and ANCA antigens has been proposed, based on the observed homology between certain *Staphylococcus aureus* peptides and complementary PR3¹⁹ and, more recently, the description of a novel ANCA subtype directed against LAMP-2,

which bears considerable homology to FimH, a protein expressed by fimbriated gram-negative bacteria.²⁰

For the remaining patients in our cohort who did not have a clear infectious association or polyclonal immunoglobulin response, it was often difficult to establish the original indication for ANCA testing, perhaps reflecting inappropriate use of specialist investigations. It has been previously reported that the use of IIF testing in patients with a low pretest probability of vasculitis impairs the predictive value of a positive test result,²¹ and our experience suggests the same is true of antigen-specific assays. The use of laboratory “gating” policies to prevent inappropriate ANCA testing has been proposed,²² although we suggest these would require meticulous implementation to prevent missed or delayed diagnoses of patients with vasculitis.

Interestingly, it has been recently suggested that a significant proportion, if not all, of the population may have low-level natural autoantibodies to PR3 in the circulation.²³ We therefore questioned whether the introduction of potentially more sensitive or less specific methods of antigen-specific ANCA quantification accounted for a higher than expected rate of PR3-ANCA detection. “First-generation” ANCA ELISAs, developed in the late 1990s, used target antigens directly immobilized onto ELISA plates, although these lacked sensitivity, presumed due to disruption of the autoantigen structure. Improved methods of immobilization using capture antibodies have improved sensitivity, and there are now many commercially available capture ELISAs in widespread clinical use. Recent developments, including the use of nondisclosed “anchor” molecules, have further improved ELISA sensitivity for PR3-ANCA in GPA to between 96% and 97.1%.^{24,25} Other novel methods of antigen-specific ANCA testing include dot blots,²⁶ although these are nonquantitative, and bead-based multiplex testing.²⁷ We introduced the latter to our clinical laboratory in 2005 and therefore reviewed the available data on sensitivity and specificity for the BMD FIDIS Vasculitis Luminex System that is in use.

The manufacturer reports 94.5% overall agreement with commercially available ELISA kits on individual patient samples, with both negative and positive disagreement accounting for the difference. When we introduced Luminex to our laboratory, we found overall 97.6% agreement with our previous in-house anti-PR3 assay when tested on individual patient samples, with negative and positive disagreement again accounting for the discrepancy. Two studies have compared the use of the BMD FIDIS Luminex system with standard ELISA techniques in clinical populations. The first compared the sensitivity and specificity of the BMD FIDIS Luminex system with both direct and capture ELISA in a group of 60 patients with histologically proven GPA versus 30 healthy and 30 disease controls (patients with rheumatoid arthritis).²⁷ The second compared 20 patients with histologically

TABLE 2. Sensitivity and Specificity of Various Immunoassays for PR3-ANCA in Patients With Granulomatosis With Polyangiitis Versus Healthy and Disease Controls

	Sensitivity	Specificity
Damoiseaux et al, ²⁷ 2007		
Direct ELISA	64%	99%
Capture ELISA	74.3%	100%
FIDIS Luminex	71.6%	96.7%
Trevisin et al, ²⁸ 2008		
Combined ELISA	90%	94%
FIDIS Luminex	95%	91%

proven disease with 76 disease controls (patients with inflammatory bowel disease, systemic lupus erythematosus, or suspected vasculitis subsequently shown to have nonvasculitic disease) and 33 healthy controls, using the BMD FIDIS Luminex system and 12 different capture or direct ELISA kits.²⁸ Results from these studies are summarized in Table 2. Notably, both these studies found the sensitivity of multiplex testing to compare favorably with standard ELISA methods, but with a marginal loss of specificity. When applied to a large population, this decrease in specificity may account in part for the higher than expected rate of “incidental” ANCAs seen in our cohort.

We noted that the median age of our group of patients without primary vasculitis was 66 years, which is approximately 20 years older than the same group in the study of Hagen et al¹ and 10 years older than the subjects in the first comparative study.²⁷ In addition, the control population used by the manufacturer to determine the threshold value of 25 U/mL had an age range of 10 to 42 years. It is possible that age or other demographic differences may contribute to the frequency of incidental ANCAs seen in our study. Whereas a single small study did not confirm an increase in the incidence of ANCAs with advanced age,²⁹ as is seen with other antibodies such as antinuclear antibodies and rheumatoid factor, no large studies (a necessity given the overall rarity of ANCAs) have investigated this possibility. Similarly, the frequencies of certain autoantibodies have been shown to vary significantly in geographically diverse populations, which may relate to differences in genes or environmental exposures.³⁰ Our local population includes a large proportion of Indo-Asians (6 of 19 patients in this cohort), and it is possible that “incidental” ANCAs occur more frequently in this population than in the groups used in the reported studies. Again, large studies are required to address these specific hypotheses.

The long-term outcomes of our patients are instructive. Three patients became anti-PR3 negative following treatment of associated conditions—specifically, antibiotic therapy and valve replacement for endocarditis (patient 2), medical antituberculous therapy (patient 7), and surgical resection of colonic adenocarcinoma (patient 15), suggesting the finding of PR3-ANCA was a genuine association. Strikingly, patient 13 went on to develop evidence of AAV approximately 6 months after her PR3-ANCA was first reported. It is also noteworthy that since this study we identified 1 further patient during workup for live donor renal transplantation who was PR3-ANCA positive but initially asymptomatic and who has since developed upper respiratory tract disease consistent with a diagnosis of GPA. These observations are in keeping with studies using stored samples from US military recruits, where it was found that PR3-ANCA levels were significantly elevated up to 1.5 years before the diagnosis of GPA.^{31,32} There is mounting experimental evidence that ANCAs have a directly pathogenic role in vasculitis^{33,34}; however, they were not associated with the features of primary AAV in our patients at the time of first detection. Our observations suggest that additional factors are necessary for the persistence of these antibodies over time and the subsequent development of clinical disease. For these reasons, we would suggest that the term “ANCA” (as opposed to, eg, neutrophil-specific autoantibodies or NSA³⁵) should be retained for all autoantibodies directed against cytoplasmic antigens in neutrophils, as the association with primary vasculitis or other inflammatory conditions may not be clear at the time of detection.

As a retrospective review, there were limitations to our study. We were unable, for example, to formally review immunofluorescence patterns, the interpretation of which relies on subjective assessment by laboratory personnel. As such, we were not able to confirm that all patients had the typical granular cytoplasmic

staining pattern associated with GPA, versus smooth or “atypical” cytoplasmic patterns, although we would expect that these variations would have been noted in the original reports from our laboratory. Similarly, we were not able to assess the specificity and sensitivity of Luminex testing versus other antigen-specific assays in this cohort of patients. Nor were we able to examine differences in antibody affinity, avidity, or subclass between the 2 groups of patients. It has previously been reported, for example, that the ANCA IgG subclass distribution of patient sera show relative enrichment for IgG3 and depletion of IgG2.³⁶ In addition, the aforementioned natural autoantibodies identified in normal individuals were restricted to the IgG1 subclass, lacked IgG3, and were also of lower avidity compared with antibodies derived from patients.³⁷ It would be of interest to inspect these characteristics in prospective patients found to have “incidental” ANCA.

In summary, the increasing application of specialist testing to an unselected population, who do not have a high pretest probability of having vasculitis, is likely to yield “incidental” ANCA findings. Contributing to this, changes in test methodology and thus specificity, even if small, may result in further “incidental” findings if applied to large numbers of patients. Since the last international consensus statement, a variety of new immunoassays have become available for detecting ANCAs, and a review of the consensus may be necessary in light of this. The most common association of incidental ANCAs in our study was infection, with a concomitant polyclonal B-cell response. Given that the infections seen, such as endocarditis and TB, can closely mimic the presentation of AAV, efforts should always be made to identify or exclude infection in patients with symptoms and a positive ANCA test. The clear corollary is that tissue diagnosis remains the criterion standard for diagnosing AAV and should always be sought where possible. Overreliance on serological testing, even in patients with clinical features highly suggestive of primary vasculitis, may lead to erroneous diagnoses and detrimental treatment decisions. And finally, where patients are found to have a positive PR3-ANCA in the absence of clinical features of disease, we would advise regular long-term follow-up, as our experience suggests that these may be patients who simply do not yet have primary systemic vasculitis.

KEY POINTS

- (1) The increasing use of ANCA testing in unselected populations results in a higher than previously reported rate of positive findings in patients without primary AAV.
- (2) PR3-ANCA may occur in association with a variety of nonvasculitic conditions, including infection and malignancy, and as such tissue diagnosis remains the criterion standard.
- (3) Because detectable antibodies may predate the onset of disease, unexplained ANCA findings require long-term follow-up.

REFERENCES

1. Hagen EC, Daha MR, Hermans J, et al. Diagnostic value of standardized assays for anti-neutrophil cytoplasmic antibodies in idiopathic systemic vasculitis. EC/BCR Project for ANCA Assay Standardization. *Kidney Int*. 1998;53:743–753.
2. Savage J, Gillis D, Benson E, et al. International Consensus Statement on Testing and Reporting of Antineutrophil Cytoplasmic Antibodies (ANCA). *Am J Clin Pathol*. 1999;111:507–513.
3. Savage J, Dimech W, Fritzler M, et al. Addendum to the International Consensus Statement on testing and reporting of antineutrophil cytoplasmic antibodies. Quality control guidelines, comments, and

- recommendations for testing in other autoimmune diseases. *Am J Clin Pathol.* 2003;120:312–318.
4. Zuckerman KK, Leventhal L, Wynne C. Positive C-ANCA in a patient with lymphoma and without vasculitis. *J Clin Rheumatol.* 1997;3:279–281.
 5. Davenport A. “False positive” perinuclear and cytoplasmic anti-neutrophil cytoplasmic antibody results leading to misdiagnosis of Wegener’s granulomatosis and/or microscopic polyarteritis. *Clin Nephrol.* 1992;37:124–130.
 6. Chirinos JA, Corrales-Medina VF, Garcia S, et al. Endocarditis associated with antineutrophil cytoplasmic antibodies: a case report and review of the literature. *Clin Rheumatol.* 2007;26:590–595.
 7. McAdoo SP, Densem C, Salama A, et al. Bacterial endocarditis associated with proteinase 3 anti-neutrophil cytoplasm antibody. *NDT Plus.* 2011;4:208–210.
 8. Flores-Suarez LF, Cabiedes J, Villa AR, et al. Prevalence of antineutrophil cytoplasmic autoantibodies in patients with tuberculosis. *Rheumatology (Oxford).* 2003;42:223–229.
 9. Teixeira L, Mahr A, Jauregui F, et al. Low seroprevalence and poor specificity of antineutrophil cytoplasmic antibodies in tuberculosis. *Rheumatology (Oxford).* 2005;44:247–250.
 10. Esquivel-Valerio JA, Flores-Suarez LF, Rodriguez-Amado J, et al. Antineutrophil cytoplasm autoantibodies in patients with tuberculosis are directed against bactericidal/permeability increasing protein and are detected after treatment initiation. *Clin Exp Rheumatol.* 2010;28(1 suppl 57):35–39.
 11. Cornely OA, Hauschild S, Weise C, et al. Seroprevalence and disease association of antineutrophil cytoplasmic autoantibodies and antigens in HIV infection. *Infection.* 1999;27:92–96.
 12. Savige JA, Chang L, Horn S, et al. Anti-nuclear, anti-neutrophil cytoplasmic and anti-glomerular basement membrane antibodies in HIV-infected individuals. *Autoimmunity.* 1994;18:205–211.
 13. Pudifin DJ, Duursma J, Gathiram V, et al. Invasive amoebiasis is associated with the development of anti-neutrophil cytoplasmic antibody. *Clin Exp Immunol.* 1994;97:48–51.
 14. Constantin A, Marin F, Oksman F, et al. Antineutrophil cytoplasmic antibodies in leptospirosis. *J Rheumatol.* 1996;23:411.
 15. Chou TN, Hsu TC, Chen RM, et al. Parvovirus B19 infection associated with the production of anti-neutrophil cytoplasmic antibody (ANCA) and anticardiolipin antibody (aCL). *Lupus.* 2000;9:551–554.
 16. Wu YY, Hsu TC, Chen TY, et al. Proteinase 3 and dihydroliipoamide dehydrogenase (E3) are major autoantigens in hepatitis C virus (HCV) infection. *Clin Exp Immunol.* 2002;128:347–352.
 17. Pradhan V, Badakere SS, Shankarkumar U, et al. Anti-neutrophil cytoplasmic antibodies (ANCA) in malaria. *Indian J Malariol.* 2002;39:51–59.
 18. Tervaert JW, Popa ER, Bos NA. The role of superantigens in vasculitis. *Curr Opin Rheumatol.* 1999;11:24–33.
 19. Pendergraft WF 3rd, Preston GA, Shah RR, et al. Autoimmunity is triggered by cPR-3(105-201), a protein complementary to human autoantigen proteinase-3. *Nat Med.* 2004;10:72–79.
 20. Kain R, Exner M, Brandes R, et al. Molecular mimicry in pauci-immune focal necrotizing glomerulonephritis. *Nat Med.* 2008;14:1088–1096.
 21. Rao JK, Weinberger M, Oddone EZ, et al. The role of antineutrophil cytoplasmic antibody (c-ANCA) testing in the diagnosis of Wegener granulomatosis. A literature review and meta-analysis. *Ann Intern Med.* 1995;123:925–932.
 22. Arnold DF, Timms A, Luqmani R, et al. Does a gating policy for ANCA overlook patients with ANCA associated vasculitis? An audit of 263 patients. *J Clin Pathol.* 2010;63:678–680.
 23. Cui Z, Zhao MH, Segelmark M, et al. Natural autoantibodies to myeloperoxidase, proteinase 3, and the glomerular basement membrane are present in normal individuals. *Kidney Int.* 2010;78:590–597.
 24. Hellmich B, Csernok E, Fredenhagen G, et al. A novel high sensitivity ELISA for detection of antineutrophil cytoplasm antibodies against proteinase-3. *Clin Exp Rheumatol.* 2007;25(1 suppl 44):S1–S5.
 25. Roggenbuck D, Buettner T, Hoffmann L, et al. High-sensitivity detection of autoantibodies against proteinase-3 by a novel third-generation enzyme-linked immunosorbent assay. *Ann N Y Acad Sci.* 2009;1173:41–46.
 26. Rutgers A, Damoiseaux J, Roozendaal C, et al. ANCA-GBM dot-blot: evaluation of an assay in the differential diagnosis of patients presenting with rapidly progressive glomerulonephritis. *J Clin Immunol.* 2004;24:435–440.
 27. Damoiseaux J, Vaessen M, Knapen Y, et al. Evaluation of the FIDIS vasculitis multiplex immunoassay for diagnosis and follow-up of ANCA-associated vasculitis and Goodpasture’s disease. *Ann N Y Acad Sci.* 2007;1109:454–463.
 28. Trevisin M, Pollock W, Dimech W, et al. Evaluation of a multiplex flow cytometric immunoassay to detect PR3- and MPO-ANCA in active and treated vasculitis, and in inflammatory bowel disease (IBD). *J Immunol Methods.* 2008;336:104–112.
 29. Maillefert JF, Pfitzenmeyer P, Thenet M, et al. Prevalence of ANCA in a hospitalized elderly French population. *Clin Exp Rheumatol.* 1997;15:603–607.
 30. Shapira Y, Poratkatz BS, Gilburd B, et al. Geographical differences in autoantibodies and anti-infectious agents antibodies among healthy adults. *Clin Rev Allergy Immunol.* 2012;42:154–163.
 31. Owshalimpur D, Arbogast CB, Olson SW. Serum anti-neutrophil cytoplasmic autoantibodies prior to the clinical presentation of Wegener’s granulomatosis (ASN Renal Week Abstract F-P01290). 2009.
 32. Olson SW, Arbogast CB, Baker TP, et al. Asymptomatic autoantibodies associate with future anti-glomerular basement membrane disease. *J Am Soc Nephrol.* 2011;22:1946–1952.
 33. Jennette JC, Falk RJ, Gasim AH. Pathogenesis of antineutrophil cytoplasmic autoantibody vasculitis. *Curr Opin Nephrol Hypertens.* 2011;20:263–270.
 34. McAdoo SP, Tam FW, Pusey CD. Disease models of rapidly progressive glomerulonephritis. *Drug Discov Today.* 2010;7:43–50.
 35. Wiik A. Neutrophil-specific autoantibodies in chronic inflammatory bowel diseases. *Autoimmun Rev.* 2002;1:67–72.
 36. Jayne DR, Weetman AP, Lockwood CM. IgG subclass distribution of autoantibodies to neutrophil cytoplasmic antigens in systemic vasculitis. *Clin Exp Immunol.* 1991;84:476–481.
 37. Xu PC, Cui Z, Chen M, et al. Comparison of characteristics of natural autoantibodies against myeloperoxidase and anti-myeloperoxidase autoantibodies from patients with microscopic polyangiitis. *Rheumatology (Oxford).* 2011;50:1236–1243.