Netherlands
The Journal of Medicine

CASE REPORT

ANCA seropositivity in HIV: a serological pitfall

T.L.Th.A. Jansen[1*], D. van Houte[2], T. de Vries[3], A. Wolthuis[3]

Departments of [1]Rheumatology and [2]Internal Medicine, Leeuwarden Medical Centre, Henri Dunantweg 2, Leeuwarden, the Netherlands, e-mail: T.Jansen@znb.nl, [3]Clinical Chemistry, Stichting KCL, Borniastraat 34, Leeuwarden, the Netherlands, [*]corresponding author

ABSTRACT

In systemic vasculitis, cytoplasmic staining in ethanol-fixed neutrophilic granulocytes, i.e. cytoplasmic antineutrophilic cytoplasmic antibody (c-ANCA), is generally considered a highly significant serological marker. When a patient presents with upper airway or renal symptomatology and seropositivity to c-ANCA, a Wegener's granulomatosis is usually easily diagnosed by performing a biopsy of the diseased organ. However, not every ANCA-positive patient with pulmonary inflammation is suffering from Wegener's disease. In some cases of upper airway or pulmonary symptomatology, the *a priori* chance of having Wegener's disease is low despite a positive ANCA. A coincidental positivity of ANCA may then lead to clinicians jumping to conclusions. We present a 40-year-old man who was falsely suspected of having Wegener's disease because of upper airway symptomatology and c-ANCA positivity. Specificity analysis revealed that he was negative to antibodies for proteinase-3, but positive to myeloperoxidase. The potential serological pitfall of the supposedly specific c-ANCA is discussed.

KEYWORDS

ANCA, HIV, pitfall, screening

INTRODUCTION

Wegener's granulomatosis is a necrotising granulomatous vasculitis involving predominantly the upper and lower respiratory tract and kidneys. Other organs may be affected to a variable extent by this small-vessel vasculitis. Some authors have suggested that sensitivity and specificity of antineutrophilic cytoplasmic antibodies (ANCA) are adequate to merit inclusion of ANCA in classification systems of systemic vasculitis. Many clinicians therefore even use ANCA in a similar way to antinuclear antibodies (ANA) early in the diagnostic phase for screening purposes.[1] Positivity of these ANCA may be misleading if clinicians have requested the tests incorrectly in these screening diagnostic situations. They may even become a diagnostic pitfall.[2,3] Such patients enter specialist care via different disciplines, i.e. general internists, pulmonologists, nephrologists, ear, nose and throat (ENT) specialists or rheumatologists, depending on the predominant clinical sign or symptom. In addition to pulmonary and renal involvement, antineutrophilic cytoplasmatic antibodies with a typical cytoplasmic staining pattern on ethanol-fixed neutrophilic granulocytes (c-ANCA) with a proteinase-3 (PR3) specificity has proven to be a valuable serological hallmark in the differential diagnosis of Wegener's disease.[1] The majority of papers focus on ANCA being highly specific, whereas only a minority deal with ANCA positivity as coincidental, i.e. 'false-positive', for example in screening settings. In the presented case we demonstrate the potential of ANCA as a diagnostic pitfall in the screening setting of clinical practice.

CASE REPORT

A 40-year-old male was admitted to the general internal medicine department for analysis of malaise with fever. He had experienced progressive fatigue during the past six months. There was no weight loss or loss of appetite. He did mention having a paronychium with a tendency toward delayed healing for a three-month period. Surgical debridement with amoxicillin/clavulanic acid 500/125 mg four times a day was prescribed, but the problem with wound healing persisted. He also mentioned having a non-productive cough for about a year, with slight progression over the last two months. At rest he experienced slight dyspnoea, and he had nasal symptoms with haemorrhagic crustae. During admission to the ward he had a fever of up to 39°C and chills despite the aforementioned antibiotic regimen. Physical examination revealed a feverish man with red cheeks and normal tension without tachypnoea: respiration frequency was 12/min. On auscultation of the lungs, dry inspiratory crackles were heard in the basal fields. His left digit finger was slightly inflamed but appeared to be healing. Further examination was unremarkable. X-rays of the thorax revealed some interstitial abnormalities in the lower left and right pulmonary lobes, without further abnormalities (*figure 1*). Laboratory investigations were as follows: ESR >90 mm/h, haemoglobin 7.9 mM (normal >8.2 mM), leucocytes 2.2 x 10^9/l (normal 4.0-10.0 x 10^9/l), platelets 143 x 10^9/l (normal 150-300 x 10^9/l), with only 0.5 x 10^9/l lymphocytes. For further data see *table 1*.
In order to rule out the possibility of an autoimmune disorder, blood was drawn for ANA and ANCA. The latter was analysed via an indirect immunofluorescent (IIF) test with serum on activated, ethanol-fixed human granulocytes (prepared within our laboratory). The cytological appearance (*figure 2*) shows the staining pattern, originally interpreted as typical c-ANCA. To determine the specificity of the antibody, a dot blot (BMD) was incubated with the patient's serum. The expected PR3 specificity could not, however, be confirmed; instead the analysis resulted in an myeloperoxidase (MPO)-positive c-ANCA pattern. The discrepancy between the cytological pattern and specificity led us to offer the sample to two other laboratories for reanalysis. The results are given in *table 2*.

Figure 1 *X-ray of lungs: interstitial alveolitis in immunodeficient patient*

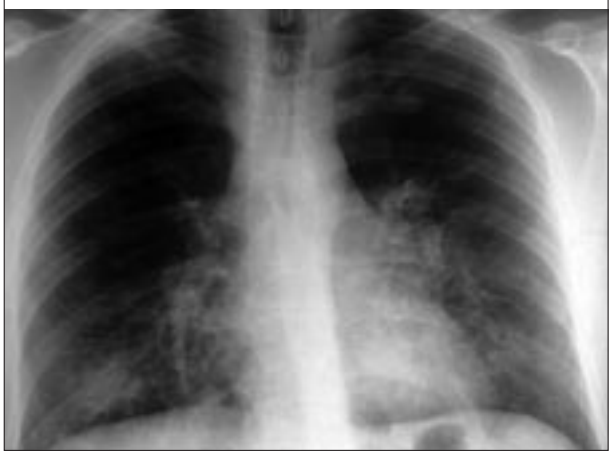

Figure 2 *Cytoplasmic ANCA*

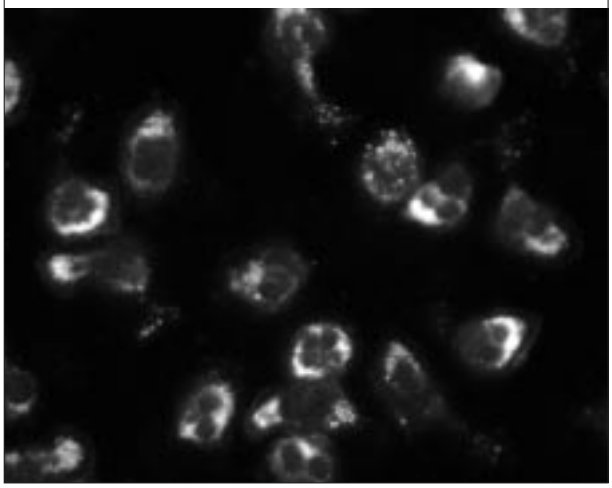

Table 1 *Biochemical findings in the presented patient*

Hb	7.9 (8.0-10.6) mmol/l	**Ht**	0,39 (0.40-0.52) l/l
ESR	>90 (<15) mm/h	**CRP**	10 (<10) mg/l
Leucocytes	2.2 (4.0-11.0) 10^9/l	**Neutrophils**	1.3 (2.0-7.5) 10^9/l
Lymphocytes	0.5 (0.8-3.2) 10^9/l	**Platelets**	143 (150-400) 10^9/l
Sodium	137 (136-146) mmol/l	**Potassium**	3.5 (3.5-5.0) mmol/l
Creatinine	71 (<105) µmol/l	**Urea**	2.5 (2.8-7.5) mmol/l
LDH	540 (<475) U/l	**ASAT**	30 (<45) U/l
ALAT	37 (<45) U/l	**AP**	90 (<120) U/l

Normal values are given between brackets. Hb = haemoglobin; ESR = erythrocyte sedimentation rate; LDH = lactate dehydrogenase; ALAT = alanine aminotranspeptidase; Ht = haematocrit; CRP = C-reactive protein; ASAT = aspartate aminotranspeptidase; AP = alkaline phosphatase.

Table 2 *Serological findings from our laboratory and two reference laboratories*

	Our lab	Lab 1	Lab 2
ELISA technique	Direct	Capture	Direct
ANCA pattern	c-ANCA	Atypical or c-ANCA-like	c-ANCA
Titre	1:80	1:320	1:80
Specificity	MPO	Non	MPO
Interpretation	MPO	Atypical	MPO

Lab 1 = serology laboratory university hospital 1; Lab 2 = serology laboratory university hospital 2.

Table 3 *Differential diagnosis of CD4 lymphocytopenia*[17]

Infection
Retrovirus: HIV-1, HIV-2, HTLV-1
Herpesvirus: varicella zoster virus, human herpes virus type 6, cytomegalovirus
Adenovirus/parvovirus/papillomavirus/rubeolavirus
Hepatitis B virus
Protozoa: leishmania
Ricketsia
Fungals: histoplasma, cryptococcus, coccidiomycosis
Bacterial: tuberculosis, brucella
Autoimmune disease
Lupus, primary Sjögren's syndrome,[18] etc.
Malnutrition
Medication
Corticosteroids
Lymphoreticular malignancy
Lymphoproliferation
Thymoma
Lymphangiectasia
Congenital disorders
Hypogammaglobulinaemia
Di George syndrome
Ageing
Pregnancy

Nasal biopsies were performed by the ENT specialist and the rheumatologist was consulted for confirmation of suspected Wegener's disease and for subsequent treatment options. On consultation no signs or symptoms were seen of arthritis, arthralgia, uveitis or neuropathy. The urine remained unremarkable. Additionally, history was taken focussed on the infectious diathesis and the patient confessed to having had various sexual contacts all over Europe without protection. There was a leucocytopenia, confirmed by a hand differentiation with specifically a CD4-positive lymphocytopenia: CD4-positive lymphocytes 18 x 10^6/l (normal 460-1450 x 10^6/l) and CD8-positive lymphocytes 123 x 10^6/l (normal 210-950 x 10^6/l) with a CD4/CD8 ratio of 0.15 (normal >1.0); see *table 3* for a differential diagnosis. A HIV infection was suspected and, after consent had been obtained from the patient, a rapid HIV test was requested. At the same time the following results came back: bacteriological analysis of the broncho-alveolar lavage revealed *Pneumocystic carinii* and the rapid HIV test was positive; further analysis revealed a high viral load. The patient was concluded to have AIDS category C3 with *Pneumocystic carinii* pneumonitis and a coincidental MPO-positive ANCA without clear signs of vasculitis. He was treated with cotrimoxazole-sulfamethoxazole and subsequently with highly active antiretroviral therapy. The positive ANCA gradually resolved.

DISCUSSION

Tests for circulating ANCA with specificity for MPO or PR3 are of considerable value in the diagnosis of the spectrum of vasculitides including Wegener's granulomatosis, microscopic polyangiitis, the Churg-Strauss syndrome, idiopathic necrotising and crescentic glomerulonephritis, and related overlapping forms of vasculitis.[1] Patients meeting two or more of the four American College of Rheumatology criteria can be classified as having Wegener's granulomatosis with a sensitivity of 88% and a specificity of 92%. The triggers that induce ANCA positivity, however, are largely unknown. Furthermore, in some cases ANCA positivity has been associated with medication: hydralazine,[4,5] minocycline[6,7] and propylthiouracil.[8,9] Cocaine abuse has sporadically been associated with ANCA.[3,10,11] Positivity of ANCA has also been found secondary to HIV infection. HIV infection may evoke ANCA in 20 to 83% of cases,[2,12-15] probably due to polyclonal activation of B cells,[12,15] but not associated with hypergammaglobulinaemia.[2] Savige *et al.* found 44 patients (42%) with ANCA on immunofluorescence testing out of 105 HIV-infected patients, including 26 with MPO specificity,[13] whereas Cornely *et al.* found 40 ANCA-positive patients (20%) out of 199 HIV-infected patients, 67 of whom revealed an atypical pattern and 33% a p-ANCA pattern.[14] Cornely *et al.* found MPO positivity in only one out of 199 HIV patients (0.5%),[14] whereas Koderich *et al.* found a faint c-ANCA positivity in 24 out of 29 HIV-infected patients (83%).[15] The last-mentioned 24 HIV-infected patients had homogeneous cytoplasmic ANCA probably representing nonspecific Fc-receptor binding of immunoglobulin G since this staining pattern was found particularly in 15 hyper-gammaglobulinaemic patients in this group. None of these HIV-infected patients showed p-ANCA, but five patients were repeatedly borderline positive in ANCA-enzyme-linked immunosorbent assays (ELISA) whereas three patients had positive MPO-ELISAs. Clinicians should be aware of the possibility of a false-positive ANCA, particularly in view of the current HIV

epidemiology. This warning may become more and more relevant for clinicians with the increasing application of protocol screening and new ANCA test systems in systemic vasculitis.[15]

Selective reading by young clinicians, as well as premature closure of the diagnostic process, and publication bias on the specificity of ANCA may lead to erroneous interpretation particularly with a confounding clinical copresentation of deformation of the nasal septum,[3] or pulmonary symptomatology as in the presented case. Our patient presented with nasal formation of crustae and an unproductive cough for a period of about a year. Physical examination revealed signs of inflammation with a subfever of up to 39.0°C with certain Wegener-compatible interstitial abnormalities on chest X-rays, which turned out to be due to *Pneumocystis carinii*. At first leucocytopenia and thrombocytopenia were both overlooked and the clinician's attention was drawn to abnormalities in serology, as our laboratory rapidly delivered a positive c-ANCA.

The diagnostic accuracy of ANCA was evaluated in a large multicentre European collaborative study including 169 newly diagnosed and 189 historic patients with idiopathic systemic vasculitis or rapid glomerulonephritis, and 184 disease controls and 740 healthy controls.[16] Both indirect immunofluorescence testing and anti-PR3 and anti-MPO ELISA were evaluated. The sensitivity of c-ANCA for Wegener's disease was 64% using the indirect immunofluorescence test, which could be improved to up to 73% by using a combination, either c-ANCA plus anti-PR3 or p-ANCA plus anti-MPO. In these validation populations no HIV-infected patients were included as far as we know. Even though clear symptoms of Wegener's disease are absent, there seems to be a real risk that clinicians may jump to conclusions in cases with a clinical suggestion of Wegener's granulomatosis. One may be asked for confirmation of the diagnosis and subsequent treatment. This may result in a potentially dangerous situation as cytotoxic therapy for Wegener's disease may well be fatal in AIDS. A similar situation has been described by Rowshani *et al.*, who reported a case with cocaine abuse and a subsequent erroneous interpretation of the clinical status with subsequent erroneous cytotoxic treatment.[3]

Specificity analysis of the antineutrophilic antibodies in the presented case, however, revealed MPO specificity. Others have reported on MPO-positive Wegener's disease; then staining specificity is, however, not cytoplasmic but perinuclear (as is expected with MPO specificity). Sera were sent for a second opinion to two university laboratories. The discrepancy between the results from these two laboratories was striking but this incongruence partly results from the different interpretation strategies and partly from a different laboratory technique: a direct ELISA has a different specificity than a capture ELISA. Capture ELISA is superior to direct ELISA with respect to specificity as it captures the MPO which therefore does not cross-react. The typical ANCA staining pattern shows accentuation of fluorescence intensity in the area within the nuclear lobes. Every positive IIF should be followed by an antigen-specific test for PR3 and MPO. PR3-ANCA is highly specific for idiopathic small-vessel vasculitis, whereas MPO-ANCA have been reported in various conditions not associated with vasculitis, i.e. specific drugs, infections and some connective tissue diseases.

The presented patient was sexually promiscuous and on presentation had a leucocytopenia with specifically a lymphocytopenia. Further analysis even revealed a specific CD4-positive lymphocytopenia. This leads to a differential diagnosis focussed on sexually transmittable diseases including HIV (*table 3*).[17,18] Indeed HIV serology was positive. The load of HIV replicates was determined and found to be sky-high. ANCA disappeared spontaneously during specific anti-HIV therapy. The patient did not develop other rheumatological problems i.e. no clinically apparent vasculitis, arthritis or arthralgia. Monteagudo *et al.* found a systemic necrotising vasculitis in only 1% of 106 cases with drug-induced AIDS.[19] In the presented c-ANCA positive, MPO-positive patient a vasculitis was not, however, found.

We conclude that in view of the increasing screening application of ANCA, one should be aware of false-positive results in all clinical presentations, in cases without specific *a priori* risk, and only vague upper airway/pulmonary symptomatology. History taking in ANCA-positive patients should include data on sexual promiscuity and other risk factors of HIV infection. A careful work-up is mandatory in patients in whom Wegener's granulomatosis is suspected, even when c-ANCA is suggested or even found positive. This report may serve again as a warning for the pitfall of positive ANCA in HIV-infected patients.

REFERENCES

1. Hoffman GS, Specks U. Antineutrophil cytoplasmic antibodies. Review. Arthritis Rheumatism 1998;41(9):1521-37.
2. Klaassen RJ, Goldschmeding R, Dolman KM, et al. Anti-neutrophil cytoplasmic autoantibodies in patients with symptomatic HIV infection. Clin Exp Immunol 1992;87:24-30.
3. Rowshani AT, Schot LJ, Ten Berge IJM. c-ANCA as a serological pitfall. Lancet 2004;363:782.
4. Nässberger L, Sjoholm AG, Johnsson H, et al. Autoantibodies against neutrophil cytoplasm components in systemic lupus erythematosus and in hydralazine-induced lupus. Clin Exp Rheumatol 1990;81:380-3.
5. Short AK, Lockwood CM. Antigen specificity in hydralazine associated ANCA positive systemic vasculitis. QJM 1995;88:775-83.
6. Elkayam O, Yaron M, Caspi D. Minocycline-induced arthritis associated with fever, livedo reticularis and p-ANCA. Ann Rheum Dis 1996;55:769-71.

7. Elkayam O, Levartovsky D, Brautbar C, et al. Clinical and immunological study of 7 patients with minocycline-induced autoimmune phenomena. Am J Med 1998;105:484-7.
8. Dolman KM, Gans RO, Vervaat TJ, et al. Vasculitis and antineutrophil cytoplasmic autoantibodies associated with propylthiouracil therapy. Lancet 1993;342:651-2.
9. Kitahara T, Hiromura K, Maezawa A, et al. Case of propylthiouracil-induced vasculitis associated with antineutrophil cytoplasmic antibody (ANCA); review of literature. Clin Nephrol 1997;47:336-40.
10. Gertner E, Hamlar D. Necrotizing granulomatous vasculitis associated with cocaine use. J Rheumatol 2002;29:1795-7.
11. Armstrong M Jr, Shikani AH. Nasal septal necrosis mimicking Wegener's granulomatosis in a cocaine abuser. Ear Nose Throat J 1996;75:623-6.
12. Kopelman RG, Zolla-Pazner S. Association of human immunodeficiency virus infection and autoimmune phenomena. Am J Med 1988;218:82-8.
13. Savige JA, Chang L, Crowe SM. Antineutrophil cytoplasma antibodies in HIV infection. Adv Exp Med Biol 1993;336:349-52.
14. Cornely OA, Hauschild S, Weise C, et al. Seroprevalence and disease association of antineutrophil cytoplasmic autoantibodies and antigens in HIV infection. Infection 1999;27:92-6.
15. Koderich J, Andrassy K, Rasmussen N, Hartmann M, Tilgen W. "False-positive" anti-neutrophil cytoplasmic antibodies in HIV infection. Lancet 1990;335:1227-8.
16. Hagen EC, Daha MR, Hermans J, et al. Diagnostic value of standardized assays for antineutrophil cytoplasmic antibodies in idiopathic systemic vasculitis. Kidney Int 1998;53:743-53.
17. Soriano V, Hewlett I, Heredia A, et al. Idiopathic CD4+ T-lymphocytopenia. Lancet 1992;340:607-8.
18. Mandl T, Bredberg A, Jacobsson LTH, Manthorpe R, Henriksson G. CD4+ T-lymphocytopenia – a frequent finding in anti-SSA antibody seropositive patients with primary Sjögren's syndrome. J Rheumatol 2004;31:726-8.
19. Monteagudo I, Rivera J, Lopez-Longo J, et al. AIDS and rheumatic manifestations in patients addicted to drugs. An analysis of 106 cases. J Rheumatol 1991;18:1038-41.