What is the significance in routine care of c-ANCA/PR3-ANCA in the absence of systemic vasculitis? A case series

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ABSTRACT

Objective. *ANCA has come to play an important role in diagnosing vasculitis. In selected populations c-ANCA/PR3-ANCA has a high specificity and sensitivity for vasculitis. In clinical practice, how individuals with c-ANCA/PR3-ANCA but without sufficient evidence of systemic vasculitis should be managed is unclear. We therefore retrospectively assessed the disease panorama and outcome in a consecutive series of individuals with c-ANCA/PR3-ANCA, and studied in detail those individuals who turned out not to fulfil criteria for vasculitic disease.*

Methods. *The study population consisted of 74 consecutive patients who all had a positive test for C-ANCA and PR3-ANCA between 1992 and 2002 at the Immunology laboratory at Uppsala University Hospital, Sweden. The patients' medical files were reviewed and their diagnosis re-evaluated through June 2006.*

Results. *18 of the 74 ANCA-positive individuals did not present clinical evidence supportive of, or insufficient to support, a diagnosis of systemic vasculitis, but presented a range of other diseases. During a mean follow-up of 6.8 years, none of these 18 patients developed vasculitis.*

Conclusions. *Individuals with a positive c-ANCA and PR3-ANCA but no vasculitis at the time of testing run an unknown but likely small risk of later developing vasculitis. In this group, a positive ANCA may represent background noise (borderline titres) or be a marker of inflammatory activity rather than of vasculitic disease (high titres).*

Introduction

The presence of ANCA (anti-neutrophil cytoplasmic antibodies) directed against proteinase 3 as tested with immunoflourescence (IIF) (cANCA) and ELISA (PR3-ANCA) has been regarded as specific for certain systemic vasculitides, and especially for Wegener's granulomatosis (WG) (1), although not pathognomonic as such ANCAs have been reported in other conditions (2) and in elderly healthy individuals (3). Conversely, not all patients with systemic vasculitides, or WG, are ANCA positive (4).

Although neither included in the American College of Rheumatology (ACR 1990) – nor in the Chapel Hill Consensus Conference (CHCC 1994) – criteria for the classification of WG or any systemic vasculitis, testing for c-ANCA/PR3-ANCA is an important feature of the work-up for patients with suspected systemic vasculitis. ANCAs should, however, always be used together with clinical assessment tools (5, 6).

The finding of a c-ANCA/PR3-ANCA supportive of a clinical diagnosis of, for example, WG poses little challenge. However, clinicians finding a c-ANCA/PR3-ANCA in individuals whose other investigations eventually provide insufficient evidence of WG or any other vasculitides are faced with three important questions that each prompts a medical decision: First, what is the chance that such a patient actually *suffers* from a systemic vasculitis? Second, what is the chance that such a patient will *go on to develop* a systemic vasculitis? Third, will such patients *develop any other serious disease*?

Just as it is important to be able to correctly diagnose a vasculitic disease in an early stage, and institute adequate treatment, it is vital to avoid erroneous immunosuppressive treatment in patients without vasculitides (7).

To our knowledge, no previous study has assessed the disease spectrum in patients with c-ANCA/PR3-ANCA but with insufficient signs and symptoms of WG or other systemic vasculitis at the time of testing *and* followed such patients over time. Other studies have assessed the disease spectrum *at the time of testing* in ANCA positive

patients without systemic vasculitis, and noted the low predictive value of the test in unselected populations (8).

Patients and methods

The department of clinical immunology at Uppsala University Hospital has served as a catchment area corresponding to Uppsala county and several smaller primary referral hospitals in central Sweden. Between 1992 and 2002, 4,997 tests were performed; of these, 444 tests (in 74 individuals) were positive for IIF /c-ANCA and ELISA/ PR3-ANCA, each individual having one or more positive tests. The indirect immunoflourescence (IIF-ANCA) was performed with human granulocytes as substrate, the result given as positive or negative (Wieslab, Lund, Sweden) (9). The enzyme linked immunosorbent assay (ELISA -ANCA) was performed with a commercial kit (Wielisa ^{R} PR3-102X [9], with a cut-off value of 10 U/ml, sensitivity and specificity of the test for WG, according to the manufacturer, being 92% and 99% respectively. Testing of blood donor sera revealed no positive [>20U] or borderline [10-20 U] tests [9]).

The study population consisted of all individuals (n=74), treated at any hospital in the catchment area, whose blood was sent to Uppsala for testing, and who tested positive for both ELISA and IIF ANCA at least once between 1992 and 2002. Through scrutiny of the medical files of these 74 individuals, one of us (AK) assessed the reason for testing, the underlying medical condition at the time of testing and the possible development of a systemic vasculitis or any other serious disease during the remainder of the study period, which ended in June 2006. The clinical diagnosis was re-evaluated, and patients with findings compatible with vasculitis were assessed and classified according to the ACR- criteria and when biopsy had been performed, also by the CHCC-criteria. Although these criteria primarily are for classification they have been widely to define vasculitides.

Results

Of the 74 patients with c-ANCA and PR3-ANCA, 56 patients had a vasculitic disease (75%); 53 patients (72%) met the ACR criteria for WG and 67% of these cases also had a biopsy-verified diagnosis of WG. When we applied the algorithm for the classification of the ANCA associated vasculitides, as suggested by Watts *et al*. (10), of the 74 patients, 53 patients were classified as WG and one as MPA. The two remaining cases had vasculitis associated with RA and ulcerative colitis.

The remaining 18 (out of 74) patients suffered from a heterogeneous group of medical conditions, signs and symptoms such as other rheumatic diseases, bacterial and viral infections, and inflammatory bowel disease (Table I).

The values of the ELISA tests among the 18 patients without a confirmed systemic vasculitis at the time of testing varied from 10 to 140 U/ml (mean 36, median 15). Although these values were lower than those of the 56 patients with confirmed systemic vasculitis (10 to 910, mean 103, median 28), this difference did not reach statistical significance (Mann Whitney p=0.11).

Eleven of the 8 non-vasculitis patients had only one positive test, all with values in the borderline region (10-20 U). By contrast, 7 out of the 18 non-vasculitic patients, all with more than one positive test, had PR3-ANCA values > 20 in their first test. These 7 cases are more closely presented in Table II.

During the follow-up period from first positive ANCA until 30 June 2006 (range 3 years to 12 years, mean 6.8 years), none of the 18 patients without a diagnosis of systemic vasculitis at the time of testing developed such disease, nor any new signs or symptoms thereof. Four patients died, all above the age of 75, from causes unrelated to vasculitic disease.

Discussion

Our population-based case-series indicates that patients who test positive for c-ANCA/PR3-ANCA but do not have sufficient clinical or other evidence of a systemic vasculitis display a wide array of medical conditions but have a low probability of progressing to systemic vasculitides. Previous studies have pointed out that c-ANCA/PR3-ANCA should not be used to rule in or to rule out a systemic vasculitides (1) and that a false positive may lead to misdiagnosis of vasculitis (7).

In our study, we have approached the clinically important issue of what the presence of c-ANCA and PR3-ANCA in the absence of vasculitis represents. Our results suggest, with the reservation of the limited number of subjects (n=74), that none of the ANCA-positive individuals who did not have vasculitis but presented symptoms that might indicate vasculitic disease (and which prompted ANCA testing) developed WG or any other vasculitic disease during the mean follow-up of 6.8 years.

In WG, overall 75-90% of all patients develop c-ANCA and PR3-ANCA, but whether this antibody may precede clinical symptoms, as is the case with anti-CCP in RA (11) or anti-dsDNA in SLE (12) is still unknown. Accumulating evidence supports the hypothesis that c-ANCA is indeed involved in the pathophysiology of WG, but the finding that none of the 18 c-ANCA positive individuals developed systemic vasculitis may suggest that c-ANCA also reflects neutrophil-activating properties that are not specific to systemic vasculitides. In RA and SLE, ANCA has been found to reflected high inflammatory activity in the disease (13). The pro-inflammatory properties of ANCA may enhance or maintain the inflammatory process, perhaps accounting for the greater relapse risk in WG-patients with a persisting ANCA.

Some of the positive tests reported may have represented analytical false values, especially those with only one test and ELISA values in the borderline positive range.

Our study was designed as a routine care assessment, and as such, a clinical counterpart to studies of ANCA performance in highly selected and clinically typical patients with vasculitides from referral-centres (14). One limitation is our retrospective assessment of the diagnoses and follow-up. We used the ACR criteria to define the systemic vasculitides, but 67% of the patients also had their vasculitis-diagnosis confirmed by biopsy. Since all individuals were followed using their medical charts or with direct contact with their

treating physician, we feel confident that any serious disease during the follow-up period did not pass undetected. The four patients that died were all of high age and none of the deaths were related to vasculitis.

To conclude, our series suggests that in clinical practice, the clinical significance of c/PR3-ANCA in patients

Table I. Characteristics of the 18 patients with a positive c-ANCA/PR3 but no concurrent evidence of Wegener's granulomatosis or other systemic vasculitis.

Patient	Sex	Age at 1st positive PR3 (yrs)	PR3 - ANCA	c-ANCA	Medical condition at 1st positive PR3	Signs and symptoms leading to PR3 testing	Medical diagnosis after re-assessment	Observation time (months)	Vital status at end of follow-up
1	F	38	140	+	HLA B27 related arthritis	Fever, malaise, high ESR, anaemia, arthralgia	Ankylosing spondylitis, possible pneumonia	96	Alive, no vasculitis
2	M	83	22	++	Sarcoidosis + pneumonia	Fever	Sarcoidosis+ pneumonia +fibrosis	36	Deceased, 981027
3	F	66	20	+	None	Acute renal failure, hematuria	Nephropathia epidemica	84	Alive, no vasculitis
4	F	17	127	+++	Ulcerative colitis	Fever, back pain, hematuria, proteinuria, cough, pleuritis	Ulcerative colitis +urinary tract infection	83	Alive, no vasculitis
5	M	31	110	++	None	Fever, exanthema, artritis, elevated inflammatory parameters	Streptococcal infection, rheumatic fever	68	Healthy, no vasculitis
6	M	75	44	+++	Systemic sclerosis	Cutaneous vasculitis	Systemic sclerosis, infection	56	Deceased 060103. No vasculitis
7	M	32	31	+	None	Blocked nose	Observation for possible WG	58	Healthy, no vasculitis
8	M	22	10	++	Ankylosing spondylitis	Renal failure, proteinuria	Ankylosing spondylitis,amyloidosis, kidney transplant	146	Alive, no vasculitis
9	F	35	14	++	Diabetes mellitus	Renal failure, proteinuria	Diabetic nephropathy	147	Alive, no vasculitis
10	M	33	15	+	None	Renal failure,hematuria, proteinuria	Nephropathia epidemica	140	Alive, no vasculitis
11	M	70	14	++	None	Upper respiratory tract symptoms, purpura , liver failure	Liver failure	60	Deceased 2001. No vasculitis at post-mortem
12	M	66	10	+	None	Fever, ESR, malaise, back pain	Gr+ endocarditis	102	Alive, no vasculitis
13	M	54	12	+	Ulcerative colitis, hypertension, diabetes		Ulcerative colitis	100	Alive, no vasculitis
14	F	56	15	+	Primary Sjögren's syndrome	Upper respiratory tract symptoms, malaise	Primary Sjögren's syndrome	84	Alive, no vasculitis
15	M	66	12	++	U.C., hypertension, diabetes	ESR,Creatinine and liverenzymes elevated	Hepatitis C,U.C., diabetes	84	Alive, no vasculitis
16	F	32	12	++	None	Artralgias, fatigue, upper respiratory tract symptoms	Chronic fatigue syndrome	47*	Alive, no vasculitis
17	F	84	11	+++	None	Ear pain, epistaxis	External otitis	39	Deceased 2005, no vasculitis
18	F	84	18	++	None	Fever, cutaneous vasculitis	Infection NUD, erythema multiforme	49	Alive, no vasculitis

*Lost to follow-up after moving abroad after 47 months of observation.

Table II. Description of the investigations performed and treatments given in the seven cases with repeatedly positive PR3-ANCAs >20, but without vasculitis.

No.	Findings indicative of vasculitis	Diagnostic work-up	Treatments	Final diagnosis
1	Pulmonary infiltrates	Repeated lung biopsies negative	NSAID and analgesics	Ankylosing spondylitis
2	Progressive pulmonary infiltrates	ENT incl biopsies negative	Bronchodilators	Sarcoidosis and respiratory insufficiency
3	Acute renal failure	ENT and x-ray lungs negative Hanta-virus serology positive	No specific treatment	Nefropathia epidemica
4	Pleurisy, fever, hematuria	HRCT and angiography of lungs neg.U-culture pos E.coli	Antibiotics, azathioprin mesalazin	Ulcerous colitis
5	Fever, exanthema, arthritis	ENT, x-ray lungs neg. Ekg T-wave inversion, throat swab pos β-strep	Penicillin	β-Strep infection, rheumatic fever
6	Leucocytoclastic rash	ENT and x-ray of lungs neg.	Cortisone short time	Systemic sclerosis
7	Blocked nose	CT sinus, x-ray lungs neg. Repeated blood chemistry neg	None	Healthy

who test positive but do not present sufficient evidence for a systemic vasculitides at the time of testing, is limited. The exact nature of these positive non-vasculitis associated ANCA tests (cross-reactivity, non-specific neutrophil-activating properties, marker of disease activity, analytical false values etc.) remains unknown.

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