Clin Rheumatol (1999) 18:279–282

Clinical Rheumatology

Original Article

Clinical Significance of Antiproteinase 3 Antibody Positivity in cANCA-Positive Patients

V. van Pesch[1], M. Jadoul[2], C. Lefèbvre[3], B. R. Lauwerys[1], J-P. Tomasi[4], J-P. Devogelaer[1] and F. A. Houssiau[1]

Departments of [1]Rheumatology, [2]Nephrology, [3]General Internal Medicine and [4]Clinical Chemistry, Cliniques Universitaires Saint-Luc, Louvain Medical School, Brussels, Belgium

Abstract: We addressed the clinical significance of antiproteinase 3 (anti-PR3) antibody (Ab) positivity by reviewing the files of 79 patients whose serum contained antineutrophil cytoplasmic antibodies with a cytoplasmic staining pattern (cANCA) and had been tested for anti-PR3 reactivity. Vasculitis was present in most (22/35) $cANCA^+$ $PR3^+$ patients but in only a few (5/44) $cANCA^+$ $PR3^-$ patients, thereby suggesting that anti-PR3 Ab positivity in $cANCA^+$ patients is more indicative of vasculitis than cANCA positivity alone. Noteworthy, one-third of $cANCA^+$ $PR3^+$ patients – those with anti-PR3 Ab titres lower than 100 U/ml – did not suffer from vasculitis. Anti-PR3 reactivity in vasculitis patients was only weakly associated with Wegener's granulomatosis (WG), as nine out of 22 $cANCA^+$ $PR3^+$ vasculitis patients (41%) did not fulfil the ACR classification criteria for WG. There was no correlation between anti-PR3 Ab titres and disease activity at diagnosis. However, titres measured when patients were in remission were much lower than initial values. Taken together, our results indicate that anti-PR3 Ab positivity should be interpreted in its clinical context.

Keywords: Activity; ANCA; Antiproteinase 3; Serology; Vasculitis

Correspondence and offprint requests to: Frédéric A. Houssiau, MD, PhD, Rheumatology Department, UCL 5390, Avenue Hippocrate 10, B-1200 Bruxelles, Belgium. Tel: 32 2 764 53 91; Fax: 32 2 764 53 94; e-mail: houssiau@ruma.ucl.ac.be

Introduction

The differential diagnosis of systemic vasculitides remains a challenge given their varied modes of presentation and frequently overlapping features [1]. In this respect, identification of antineutrophil cytoplasmic antibodies (ANCAs) as a serological marker for vasculitis and unraveling of their subtypes have been important breakthroughs [2,3].

Antiproteinase 3 (anti-PR3) antibodies (Ab), a subset of cytoplasmic (c) ANCAs, are purported to be strikingly associated with Wegener's granulomatosis (WG) [4], but only a few studies [5,6] have specifically addressed the diagnostic value of serum anti-PR3 Ab positivity in patients with clinically suspected vasculitis, in particular in cANCA-positive patients. Moreover, while some studies have investigated the value of ANCA testing as a marker of disease activity [7–9], only few data are available for anti-PR3 Ab.

We received the files of all cANCA-positive patients evaluated at the time of diagnosis in our University Hospital over the last 10 years whose serum had been tested for anti-PR3 specificity. We addressed whether anti-PR3 reactivity was specifically associated with vasculitis and in particular with WG, and whether anti-PR3 Ab titres paralleled clinical disease activity.

Patients and Methods

Seventy-nine patients whose serum contained cANCA and had been tested, by the time of diagnosis, for anti-PR3 Ab were identified through the files of the Laboratory of Autoimmune Serology. Vasculitides

were defined according to the Chapel Hill Consensus Conference on the Nomenclature of Systemic Vasculitis [10]. Moreover, for classification purposes, WG was defined according to the American College of Rheumatology 1990 criteria for the classification of WG [11]. Disease activity was assessed using the Vasculitis Activity Score [12].

Screening for the presence of ANCA was performed by indirect immunofluorescence microscopy on cytocentrifuged slides of ethanol-fixed human neutrophils, according to the recommendations of the First International ANCA Workshop [13]. Serial dilutions of sera were tested (starting at 1/20) and a cANCA titre $\geqslant$1/40 was considered to be positive. Only typical patterns of cANCA (fine granular staining of the neutrophil cytoplasm, especially close to the nucleus and within the nuclear lobes) were considered as cANCA$^+$.

Anti-PR3 Ab were detected using a commercial ELISA kit (VARELISA PR3 ANCA, Elias, Freiburg, Germany). A cut-off value of 10 U/ml was applied, according to the manufacturer's recommendations and to our own validation study. Of note, anti-PR3 Ab were never detected in patients with perinuclear (p) ANCA except in a few patients with very high serum antimyeloperoxidase Ab titres ($>$500 U/ml) and low anti-PR3 titres ($<$20 U/ml), who were excluded from the analysis.

Results

We identified 79 patients whose serum contained cANCA and had been tested, by the time of diagnosis, for anti-PR3 Ab. As shown in Fig. 1, vasculitis was present in most (22/35) cANCA$^+$ PR3$^+$ patients but in only very few (5/44) cANCA$^+$ PR3$^-$ patients ($p<0.0001$, χ^2 test), suggesting that anti-PR3 positivity in cANCA$^+$ patients is more indicative of vasculitis than cANCA positivity alone. Most cANCA$^+$ PR3$^+$ patients without vasculitis suffered from ulcerative colitis (7/13). By contrast, within the cANCA$^+$ PR3$^-$ group without vasculitis (n = 39), diagnoses were much more heterogeneous and emcompassed numereous infectious, inflammatory and neoplastic disorders. In cANCA$^+$ patients without vasculitis, no correlation was found between cANCA titres and hypergammaglobulinaemia (data not shown) and their cANCA titres were lower than in the 27 cANCA$^+$ patients with vasculitis (17 out of 27 vasculitis patients had a cANCA titre $\geqslant$1/160 versus only 14 out of 52 non-vasculitis patients; p<0.002, χ^2 test).

Within the subgroup of 35 cANCA$^+$ PR3$^+$ patients, one-third did not suffer from vasculitis. Their anti-PR3 Ab titres were however, significantly lower than those measured in patients with vasculitis (Fig. 2). A similar trend was observed for cANCA titres (only five out of 13 non-vasculitis patients had a cANCA titre $\geqslant$1/160 versus 17 out of 22 in the vasculitis group; p = 0.02, χ^2 test). Somewhat surprisingly, in view of the claimed strong association between anti-PR3 Ab and WG, only

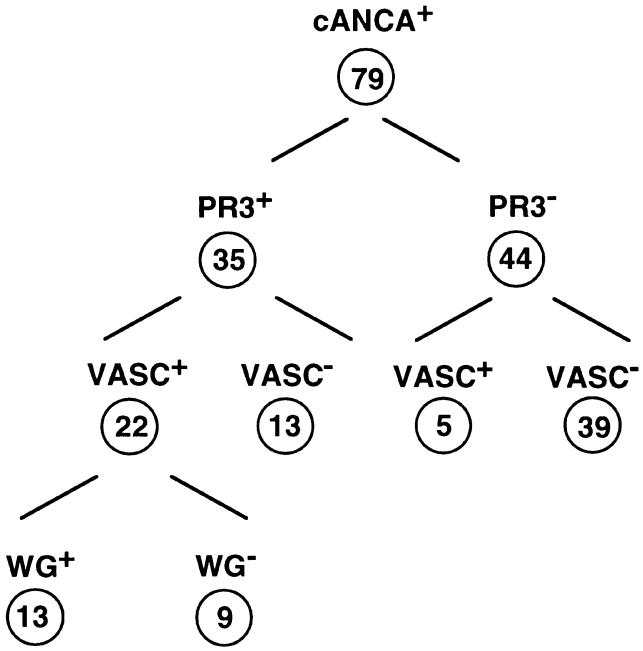

Fig. 1. Subsets of cANCA$^+$ patients, according to anti-PR3 Ab reactivity (PR3$^+$, PR3$^-$), the presence or the absence of vasculitis (VASC$^+$, VASC$^-$) and the presence or the absence of Wegener's granulomatosis (WG$^+$, WG$^-$). Figures are the numbers of patients.

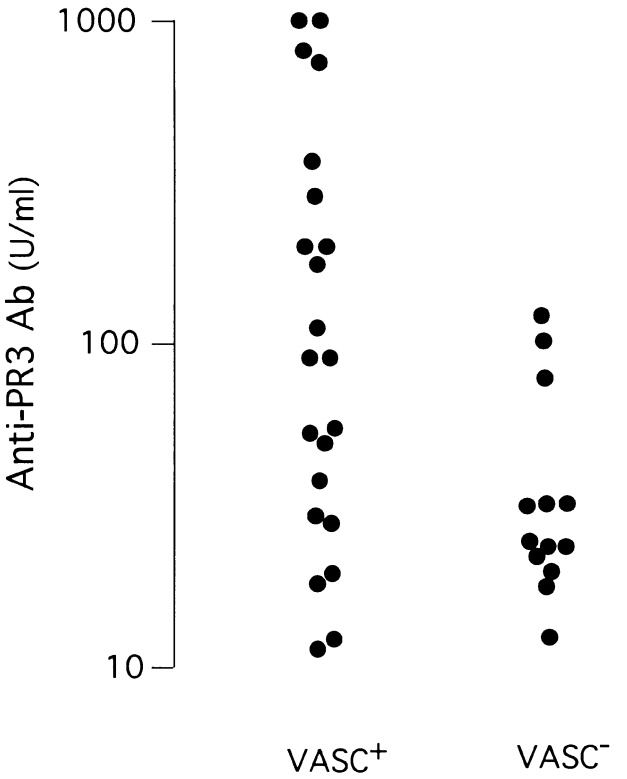

Fig. 2. Anti-PR3 Ab titres in cANCA$^+$ PR3$^+$ patients with (n = 22) and without (n = 13) vasculitis. Figures correspond to anti-PR3 Ab titres measured at diagnosis. Mean ($\pm$ SD) Ab titres were 228 $\pm$ 310 and 41 $\pm$ 35 U/ml in patients with and without vasculitis, respectively (p = 0.004, unpaired t-test). A positive anti-PR3 Ab test was defined by a level $\geqslant$10 U/ml.

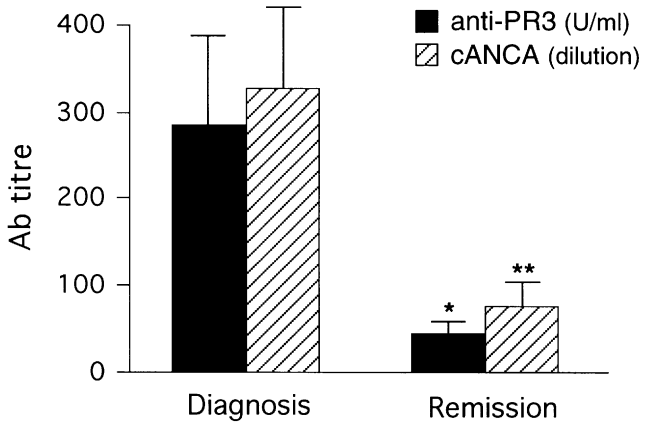

Fig. 3. Evolution of anti-PR3 Ab and cANCA titres in 15 $cANCA^+$ $PR3^+$ vasculitis patients. Figures correspond to mean (± SEM) anti-PR3 (solid columns; U/ml) and cANCA (hatched columns; serum dilution) titres. Patients were evaluated at diagnosis [mean (± SD) VAS: 18 ± 8] and during remission [VAS = 0, by definition]. Results of patients who had been tested more than once while in remission were pooled (arithmetic mean; up to six determinations per patient). The mean (± SD) time interval between diagnosis and last anti-PR3 Ab test performed in remission was 23 ± 18 months. $*p = 0.001$ versus values measured at diagnosis (pairted *t*-test); $**p < 0.005$ versus values measured at diagnosis (paired *t*-test).

13 of the 22 $cANCA^+$ $PR3^+$ patients with vasculitis fulfilled the ACR classification criteria for WG [11] (Fig. 1). The nine remaining patients suffered from microscopic polyangiitis (n = 3), idiopathic rapidly progressive glomerulonephritis (n = 3), classic polyarteritis nodosa (n = 2) or giant cell temporal arteritis (n = 1). The mean (± SD) anti-PR3 Ab titre did not differ between vasculitis patients with or without WG (228 ± 357 versus 267 ± 297 U/ml).

We evaluated whether anti-PR3 Ab titres correlated with disease activity in $cANCA^+$ $PR3^+$ vasculitis patients. At diagnosis, no correlation was found between serum and anti-PR3 Ab titres and the Vasculitis Activity Score (VAS) ($r = -0.29$; n = 17 patients for which the VAS was available) nor between cANCA titres and VAS ($r = +0.22$). Anti-PR3 Ab titres were determined at least twice in 15 patients with vasculitis. As indicated in Fig. 3, a significant decrease in anti-PR3 Ab and cANCA titres was observed when patients went into remission (VAS equal to 0) compared with their initial titre. The retrospective nature of our analysis, together with the small number of patients who relapsed (n = 6; VAS ≠ 0) during follow-up, did not enable us to evaluate whether relapses were, or were not, associated with a rise in anti-PR3 Ab titres.

Discussion

The results of the retrospective analysis presented here indicate that $cANCA^+$ patients without anti-PR3 specificity rarely suffer from vasculitis. On the other hand, however, although anti-PR3 positivity increases the diagnostic value of a cANCA test in a patient suspected of vasculitis, one-third of $cANCA^+$ $PR3^+$ patients – mainly those with low titres of anti-PR3 Ab – did not suffer form vasculitis and should therefore not be misclassified at the bedside. In this respect, it may be argued that the standard cut-off value for anti-PR3 positivity is too low. If a cut-off value >100 U/ml is taken into account, instead of 10 U/ml, only patients with vasculitis will indeed be considered as anti-PR3 positive, with however a considerable loss of sensitivity.

Somewhat surprisingly, anti-PR3 positivity was not restricted to patients suffering from WG: nine $PR3^+$ vasculitis patients, including some with very high serum titres, could not be classified as WG according to the ACR classification criteria [11]. Although four of them had mild, aspecific and transient ENT symptoms (usually one episode of sinusitis), none had evidence of upper airway disease suggestive of WG. Whether additional features of WG will appear in these patients during follow-up remains, however, an intriguing possibility. Owing to the retrospective nature of our analysis, the possibility exists that upper airway involvement and granulomatous lesions may have occurred unnoticed in some patients. However, most $cANCA^+$ $PR3^+$ patients (even those who were asymptomatic) were screened for upper airway disease by careful ENT examination, thereby making this possibility unlikely. Only a few other cases of anti-PR3 Ab positivity in non-WG patients have been reported in the literature [14,15], notably in individuals with microscopic polyangiitis.

The issue whether ANCA testing can be used to monitor disease activity is controversial, because some studies have shown a correlation between ANCA titres and disease activity [7,8], while others did not [9]. Our data suggest that anti-PR3 Ab titres do not correlate with VAS at diagnosis but decrease after treatment, although the predictive value for relapse of a rise in anti-PR3 Ab titres in individual cases could not be demonstrated.

Taken together, our results suggest that anti-PR3 Ab positivity, although indicative of vasculitis, should be viewed in its clinical context to avoid misclassification of patients and thereby inappropriate therapy [16].

References

1. Hoffman GS. Classification of the systemic vasculitides: antineutrophil cytoplasmic antibodies, consensus and controversy. Clin Exp Rheumatol 1998;16:111-5.
2. van der Woude FJ, Lobatto S, Permin H, van der Giessen M, Rasmussen N, Wiik A, et al. Autoantibodies against neutrophils and monocytes: tool for diagnosis and marker of disease activity in Wegener's granulomatosis. Lancet 1985;1:425–9.
3. Niles JL. Antineutrophil cytoplasmic antibodies in the classification of vasculitis. Annu Rev Med 1996;47:303–13.
4. Jenne DE, Tschopp J. Wegener's autoantigen decoded. Nature 1990;346:520.
5. Geffriaud-Ricouard C, Noël LH, Chauveau D, Houhou S, Grünfeld JP, Lesavre P. Clinical spectrum associated with ANCA of defined antigen specificities in 98 selected patients. Clin Nephrol 1993;39:125–36.
6. Hagen EC, Daha MR, Hermans J, Andrassy K, Csernok E, Gaskin

G, et al. Diagnostic value of standardized assays for anti-neutrophil cytoplasmic antibodies in idiopathic systemic vasculitis. Kidney Int 1998;53:743–53.
7. Specks U, Wheatley CL, McDonald TJ, Rohrbach MS, DeRemee RA. Anticytoplasmic autoantibodies in the diagnosis and follow-up of Wegener's granulomatosis. Mayo Clin Proc 1989;64:29–36.
8. Cohen Tervaert JW, Huitema MG, Hené RJ, Sluiter WJ, The TH, van der Hem GK, Kallenberg CGM. Prevention of relapses in Wegener's granulomatosis by treatment based on antineutrophil cytoplasmic antibody titre. Lancet 1990;336:709–11.
9. Kerr GS, Fleisher TA, Hallahan CW, Leavitt RY, Fauci AS, Hoffman GS. Limited prognostic value of changes in antineutrophil cytoplasmic antibody titer in patients with Wegener's granulomatosis. Arthritis Rheum 1993;36:365–71.
10. Jennette JC, Falk RJ, Andrassy K, Bacon PA, Churg J, Gross WL, et al. Nomenclature of systemic vasculitides. Proposal of an international consensus conference. Arthritis Rheum 1994;37:187–92.
11. Leavitt RY, Fauci AS, Bloch DA, Michel BA, Hunder GG, Arend WP, et al. The American College of Rheumatology 1990 criteria for the classification of Wegener's granulomatosis. Arthritis Rheum 1990;33:1101–7.
12. Luqmani RA, Bacon PA, Moots RJ, Janssen BA, Pall A, Emery P, et al. Birmingham Vasculitis Activity Score (BVAS) in systemic necrotizing vasculitis. Q J Med 1994;87:671–8.
13. Wiik A. Delineation of a standard procedure for indirect immunofluorescence detection of ANCA. Acta Pathol Microbiol Immunol Scand 1989;97(suppl):12–3.
14. Gaskin G, Turner AN, Ryan JJ, Rees AJ, Pusey CD. Significance of autoantibodies to purified proteinase 3 in systemic vasculitis. Adv Exp Med Biol 1993;336:287–9.
15. Jennings JG, Chang L, Savige JA. Anti-proteinase 3 antibodies, their characterization and disease associations. Clin Exp Immunol 1994;95:251–6.
16. De Clerck LS, Van Offel JF, Smolders WA, Empsten FA, Bridts CH, Bourgeois N, et al. Pitfalls with anti-neutrophil cystoplasmic antibodies (ANCA). Clin Rheumatol 1989;8:512–6.

Received for publication 3 September 1998
Accepted in revised form 22 February 1999