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Antineutrophil Cytoplasmic Antibody (ANCA) in Connective Tissue Diseases

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Abstract

***Background:** Antineutrophil cytoplasmic antibody (ANCA) test is frequently used in the diagnostic evaluation of systemic vasculitides, especially Wegener's granulomatosis (WG). The test becomes positive in some other diseases like systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, infectious diseases and drug-induced conditions. Indirect immunofluorescence (IIF) and enzyme linked immunosorbent assay (ELISA) are different techniques to detect ANCA. This study was planned to evaluate the frequency of positive ANCA in connective tissue diseases and the control group.*

***Patients and methods:** The study group was made up of patients with rheumatoid arthritis, systemic lupus erythematosus, dermatomyositis, polymyositis, systemic sclerosis, and those with various vasculitic syndromes. We used the serum of couples that were undergoing pre-marital screening tests as our control group. Both IIF and ELISA techniques were performed on the two groups.*

***Results:** Positive test result was detected in none of the 171 individuals in the control group. However, among 152 patients of the study group, 24 were positive for p-ANCA, one was positive for c-ANCA, 5 were positive for anti-PR3, and 7 were positive for anti-MPO. There was only one c-ANCA positive test result among the anti-PR3 positive cases. This case had a high titer of anti-PR3. We did not find any association between anti-MPO by ELISA and p-ANCA pattern in IIF, instead the association between antinuclear antibodies and p-ANCA positivity was statistically significant. c-ANCA and anti-PR3 tests were most frequently positive in WG patients. Anti-MPO was more frequently positive in patients with systemic lupus erythematosus, dermatomyositis, and polymyositis.*

***Conclusion:** Our data showed that neither p-ANCA nor anti-MPO is specific for the diagnosis of vasculitis and it seems that c-ANCA, though specific for vasculitis, it is not sensitive for the detection of low-to-moderate antibody titres. This alongwith the problem regarding the correlation of p-ANCA and anti-MPO underlines the necessity of using both methods (IIF and ELISA) in the search for antineurophil cytoplasmic antibodies.*

***Key words:** ANCA, Vasculitis, Wegener's granulomatosis.*

Background

Antineutrophil cytoplasmic antibodies (ANCA) are now used as markers for diagnosis and determining flare-up of many systemic vasculitides. ANCA were first reported in 1982 by Davis and his colleagues in a patient with segmental necrotising glumerulonephritis[1]. Later on ANCA were described in patients with systemic vasculitides. Van der Woud and collaborators were the first to suggest that ANCA yielding a diffuse granular cytoplasmic fluorescence pattern on ethanol-fixed neutrophils were a specific marker for Wegener's granulomatosis (WG) and a useful tool to assess disease activity. Subsequently, target antigens for ANCA such as myeloperoxidase (MPO) and proteinase-3 (PR3) have been recognised[1]. When used to stain ethanol-fixed, cytocentrifuged, normal human neutrophils by indirect immunofluorescence, anti-PR3 produce a cytoplasmic pattern of staining (c-ANCA) and anti-MPO produce a perinuclear pattern (p-ANCA).

The number of diseases in which ANCA may be encountered has been increasing. In 1997 Merkel *et al* suggested that p-ANCA may be positive in many connective tissue diseases including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), Sjogren's syndrome, antiphospholipid syndrome, polymyositis (PM) and dermatomyositis (DM)[2].

Since the clinical features of vasculitides may be similar

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to connective tissue diseases, sometimes distinction between them is difficult. Therefore, testing for ANCA – if highly specific – could be of great importance in the initial diagnostic evaluation of patients with a differential diagnosis that includes both connective tissue disease and vasculitis. Determination of the specificity of test for ANCA in the diagnosis of vasculitis is crucial because the decision of whether to pursue biopsies or initiate potentially toxic immunosuppressive therapy may be made on the basis of results of such testing.

We report the results of a descriptive analytical cross-sectional study to determine the frequency of positive ANCA test in a group of patients with various connective tissue diseases. A standard testing system including indirect immunofluorescence (IIF) and enzyme-linked immunosorbent assay (ELISA) for anti-PR3 and anti-MPO was used.

Patients and methods

In this descriptive, analytical cross-sectional study, 152 persons with various connective tissue diseases participated as the patient group, and 171 healthy persons as the control group.

All consecutive out-patients diagnosed as having RA, SLE, PM or DM, systemic sclerosis (SSc), and systemic vasculitis who attended the out-patient clinic of rheumatology (at Hazrat-Rasool hospital) from December, 2001 to May, 2002 were included as "the study group". The diagnoses of connective tissue diseases were determined using American College of Rheumatology (ACR) criteria and vasculitis was proven with a biopsy specimen in all patients with systemic vasculitides. None of the patients had received drugs such as allopurinol, penicillamine and sulphasalazine for possible interference in the results of the ANCA test[3]. We used 171 healthy persons undergoing premarital screening tests as "the control group".

Serum samples were collected from both the groups and stored at minus 20 degrees centigrade. Both IIF and ELISA techniques were performed on all samples. The laboratory investigators who did the ANCA assays were blinded to the diagnosis for each patient's sample. We used Rayan-teb kits for IIF tests and Organtec kits for ELISA. The positive cut-off for anti-PR-3 ELISA was 5 U/ml and for anti-MPO ELISA was 2.8 U/ml. Anti-nuclear antibodies (ANA) were detected in this study by IIF using cultured HEp-2 cells. p-ANCA must be distinguished from ANA. It has been shown that the presence of antinuclear antibodies in the sera can produce a p-ANCA pattern when ethanol-fixed neutrophils are used as a substrate. Indeed, p-ANCA pattern will be positive in the absence of antineutrophil cytoplasmic antibodies if antinuclear antibodies are present in the sera and differentiation between them is difficult by usual immunofluorescence on ethanol-fixed neutrophils.

IIF was done on each sample. The results of staining were classified as having one of four patterns: c-ANCA, p-ANCA, a-ANCA, or negative. Each sample was stained twice and interpreted independently. Results of the first round of staining were interpreted by one observer, and results of the second round were interpreted by this observer and a second observer. Both observers had considerable experience in interpreting the results of immunofluorescence staining of ANCA. If all three readings were the same, the interpretation was considered final. If the interpretation differed, a third slide was prepared and re-examined by two observers. If at least three of the five interpretations matched, the results were considered final.

The ethical committee of Iran University of Medical Sciences and Health Services approved the study. Informed consent was obtained from all the participants.

The statistical procedures were: chi square and Fisher's exact tests. P-value was considered significant at less than 0.05.

Results

To detect the frequency of ANCA positivity in various connective tissue diseases, we collected blood samples of 323 persons, 152 patients and 171 healthy persons. Of them 42 males were in patient group and 52 in control group; 110 and 119 females were in patient and control groups respectively. Mean age for the study group was 32.18 and for control group was 30.35 years. Mean disease duration for study group was 5.49 years.

In the patient group there were 82 cases with RA (53.9%),

32 cases with SLE (21.1%), 20 cases with DM or PM (13.2%), 9 cases with SSc (5.9%), 5 cases with WG (3.2%), and 4 cases with other vasculitides (2.7%).

The final results of IIF and ELISA tests are shown in Table I. None of the control patients had ANCA positivity by both techniques. In the patient group, the frequency of p-ANCA positivity by IIF was 24 (15.8%), 33.3% in SSc, 25% in SLE, 13.4% in RA. The frequency was low in PM and DM (5%). c-ANCA was positive in only one patient (0.6%). Of 24 cases that had positive p-ANCA, 8 (33%) were positive for antinuclear antibodies. The association between antinuclear antibodies and p-ANCA positivity was statistically significant (p value < 0.001).

5 patients (4 with WG, 1 with RA) were found to have anti-PR3 (3.2%) and 7 patients (3 with SLE, 3 with DM, 1 with RA) were found to have anti-MPO by ELISA (6.4%). There were statistically significant relationship between anti-MPO with DM, PM and SLE, and between anti-PR3 with WG (p value< 0.05).Titres of antibodies are shown in Table II. There was no statistically significant association between the positive results by IIF and ELISA. Among five cases with anti-PR3 only one had c-ANCA by IIF. Titre of anti-PR3 was higher in this case in comparison with others (Table II). Among patients with anti-MPO only one had p-ANCA by IIF and among 24 patients with p-ANCA only one had anti-MPO. No study patients with connective tissue diseases who had positive results by ELISA or IIF were found to have evidence of vasculitis. We did not find any atypical ANCA (a-ANCA).

Table II: Titres of anti-MPO and anti-PR3 antibodies.

Sex	Disease	Anti-MPO (U/ml)	Anti-PR3 (U/ml)
M	DM	12.98	–
M	DM	32.45	–
M	DM	9.38	–
F	WG	–	12.86
F	WG	–	10.4
F	WG	–	12.67
M	WG	–	120.4
F	SLE	15.33	–
F	SLE	8.49	–
F	SLE	98.16	–
F	RA	–	6.26
F	RA	10.77	–

Discussion

The aim of the present study was to measure the frequency of ANCA test positivity in patients with various connective tissue diseases. We found a high frequency of antibodies to neutrophils detected by IIF, but a low frequency of anti-PR3 or anti-MPO detected by ELISA. c-ANCA was not found in connective tissue diseases but p-ANCA was found in many serum samples. This was shown by Merkel *et al* in 1997 too. Among five cases with anti-PR3, only one had c-ANCA by IIF. Titre of anti-PR3 was higher in this case in comparison with others (Table

Table I: Results of tests for ANCA in connective tissue diseases.

Test → ↓Disease	No.	Positive c-ANCA	%	Positive p-ANCA	%	Positive anti-PR3	%	Positive anti-MPO	%
SLE	32	0	0	8	25	0	0	3	9.4
RA	82	0	0	11	13.4	1	1.2	1	1.2
SSc	9	0	0	3	33.3	0	0	0	0
PM DM	20	0	0	1	5	0	0	3	15
WG	5	1	20	0	0	4	80	0	0
Other Vasculitides	4	0	0	1	25	0	0	0	0
Total	152	1	0.6	24	15.8	5	3.2	7	4.6

II). So it may be infered that IIF may not be a sensitive technique for detection of low antibody titres, although it is a reliable marker of vasculitis because it was positive only in a case of WG, and we did not find it positive in other diseases.

Although, Hagen and co-workers have shown a positive relationship between p-ANCA and the presence of anti-MPO[4], there was no relationship between them in this study. Maybe it is the result of the presence of cytoplasmic antibodies other than MPO, or antinuclear antibodies, because we know that antinuclear antibodies (ANA) can produce staining that cannot be distinguished from p-ANCA which is produced by anti-MPO[2-5]. We found a strong association between serum samples that were positive for ANA and those that were positive for p-ANCA. 33% of p-ANCA positive sera were ANA positive too in this study. This finding is compatible with Merkel's study.

In this study, we found anti-MPO in connective tissue diseases, especially DM and SLE.

Because of the presence of these problems with the application of ANCA assays, many investigators suggest using ELISA at the initial stage of ANCA detection and IIF for confirmation of positive findings[6].

In conclusion, our data show that neither p-ANCA nor anti-MPO is specific for vasculitis, and it seems that c-ANCA though specific, is not sensitive for the detection of low-to-moderate antibody titres in vasculitis. This alongwith the problem regarding the correlation of p-ANCA and anti-MPO, and the possibility of the interference of ANA on the result of ANCA test by IIF, underlines the necessity of using both methods (IIF and ELISA) in the search for antineutrophil cytoplasmic antibodies.

References

1. Hoffman GS, Specks U. Antineutrophil cytoplasmic antibodies. *Arthritis Rheum* 1998; 41: 1521-37.
2. Merkel PA, Polissin RP, Chang Y *et al*. Prevalence of antineutrophil cytoplasmic antibodies in a large inception Cohort of patients with connective tissue disease. *Ann Intern Med* 1997; 126: 866-73.
3. Chol HK, Merkel PA, Walker AM, Niles JL. Drug-associated antineutrophil cytoplasmic antibody-positive vasculitis. *Arthritis Rheum* 2000; 43: 405-41.
4. Hagen EC, Daha MR, Hermens J *et al*. The diagnostic value for the standardised assay for ANCA in idiopathic systemic vasculitis. *Kidney Int* 1998; 53: 743-53.
5. Homer RJ. Antineutrophil cytoplasmic antibodies as markers for systemic autoimmune disease. *Clinc Chest Med* 1998; 19: 627-39.
6. Schmitt WH, van der Woude FJ, Fokko J. Clinical application of antineutrophil cytoplasmic antibody testing. *Curr Opin Rhuematol* 2004; 16 (1): 9-17.