

ANCA-ASSOCIATED GLOMERULONEPHRITIS: RELATIONSHIP OF MAIN ANCA SUBTYPES TO RENAL OUTCOME, AGE AND SEX OF THE PATIENTS

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Background: Antineutrophil cytoplasmic antibodies (ANCA) have been proven to be useful diagnostic tools in patients with systemic vasculitis and glomerulonephritis. These antibodies exist in two types, a cytoplasmic pattern (cANCA) and a perinuclear pattern (pANCA). The effect of the main ANCA subtypes on renal outcome and its relationship to demographic findings and clinical features of patients with ANCA-associated glomerulonephritis has not been adequately studied.

Patients and Method: In this prospective study, we compared the clinical features at presentation and the renal outcome after 1 year of follow-up between two groups of patients with cANCA (n=22) and pANCA (n=29) consecutively encountered over a one-year period.

Result: At presentation, rapidly progressive glomerulonephritis (RPGN), and after 1 year of follow-up, end-stage renal disease (ESRD) were seen more commonly in patients with pANCA than cases with cANCA ($P=0.001$ and $P=0.004$, respectively). Seropositivity for cANCA was more common in male and pANCA in female patients ($P=0.005$). Occurrence of the pulmonary-renal syndrome or extra-renal manifestations, such as sinusitis and skin rash, did not differ significantly among the two groups of patients with cANCA and pANCA.

Conclusion: Patients with pANCA present more frequently with RPGN, leading to a poorer renal survival compared to cases with cANCA. RPGN and pANCA are more common in females.

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Key Words: Antimyeloperoxidase, antineutrophil cytoplasmic antibodies (ANCA), antiproteinase 3, glomerulonephritis.

During the past decade, there has been an explosion of data on the new groups of autoantibodies known collectively as antineutrophil cytoplasmic antibodies (ANCA). These antibodies exist in two types, cytoplasmic (cANCA), which correlates mostly with antiproteinase antibodies, and perinuclear (pANCA), which corresponds mostly with antimyeloperoxidase antibodies. ANCA has been accepted both as an attractive diagnostic tool and for follow-up in several forms of systemic vasculitis.¹ Evidence from animal experiments suggests that both cANCA and pANCA may participate in the pathogenesis of at least the group of ANCA-associated vasculitis,² and hence, ANCA are usually determined during the diagnostic evaluation of patients with various types of glomerulonephritis.³ Yet, in previously published series, the effect of the main ANCA subtypes on renal outcome and its relationship to demographic findings and clinical features of patients with ANCA-associated glomerulo-

nephritis has been associated with conflicting results.³⁻⁹ This prospective study was carried out to answer two questions: 1) what is the effect of ANCA main subtypes on the renal prognosis of patients with ANCA-associated glomerulonephritis; and 2) what are the age- and sex-related differences between the two types of pANCA and cANCA-associated glomerulonephritis.

Patients and Methods

In this prospective study, all new consecutively encountered cases presenting with signs and symptoms of acute glomerulonephritis (history <3 months, hematuria, hypertension, generalized edema and/or significant proteinuria) were studied. All the patients were seen in Hasheminezhad Hospital, one of the two main kidney disease centers of Iran and a major referral center for the whole country. Only patients with the following criteria were included in to the study: 1) all new patients who had the clinical features as well as laboratory and histopathological findings of acute glomerulonephritis along with positive tests for ANCA; 2) and those who had negative tests for ANCA but whose serum C3 and C4 were normal.

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Fifty-one consecutive patients (24 female and 27 male) who fulfilled the criteria (with median age of 40 years, range 15 to 70) were enrolled in the study. The patients were divided in two groups: pANCA- (n=29) and cANCA-associated (n=22) glomerulonephritis cases. Clinical features and laboratory findings at presentation and after one year of follow-up were compared between the two groups of patients with pANCA and cANCA.

ANCA was measured by indirect immunofluorescence (IIF) on ethanol-fixed neutrophils, using FITC-conjugated antisera of IgG, IgM, and IgA from Behring Company, as well as ELISA, ANCA quantitative kit (Biocarb Diagnostics AB F-223 70 Lund, Sweden), employing the Wiclander-Rasmussen-Bygren method. Anti-PMO and -PR3 were used and titers less than 10^{EU} were considered normal.

A signed consent was obtained from each patient before performing kidney biopsy. Renal biopsy specimens were fixed in 10% phosphate-buffered formalin dehydrated with ethanol, embedded in paraffin, cut at three microns and then stained with hematoxylin and eosin, periodic acid-Schiff, periodic acid-methenamine silver and Masson's trichrome. The kidney biopsy specimen was cut into 1-2 mm cubes and immediately "snap frozen" in pre-cooled (-20°C) solutions and processed as soon as possible for immunofluorescence studies. Tissue sections were stained by a direct immunoperoxidase method using antisera for IgG, IgA, IgM, C3, C4 and fibrin. Renal tissues with less than seven non-sclerosed glomeruli were considered inadequate and were discarded.

Rapidly progressive glomerulonephritis (RPGN) was defined as the abrupt onset of acute renal failure along with progressive glomerulonephritis, as well as histopathological evidence of extensive crescent formation (>70% of glomeruli) with little or no tendency for spontaneous recovery. The diagnosis of crescentic glomerulonephritis was based on the presence of extensive crescent formation (>75% of glomeruli) with minimal or no immune deposits irrespective of the clinical course. Acute renal failure (ARF) was defined as the rapid and persistent accumulation of waste products (i.e., creatinine, urea nitrogen, and uric acid) with and without oliguria. None of the patients with ARF had pre-renal azotemia, obstructive uropathy or chronic renal failure.

Following kidney biopsy, all patients were treated with pulse therapy (methyl prednisolone intravenously 1000 mg/day for three consecutive days). If no response was observed after a week, intravenous cyclophosphamide (500-1000 mg/m², body surface) was given and, thereafter, all patients received prednisolone (1 mg/kg of body weight) and cyclophosphamide 1000 mg intravenously monthly. Cyclophosphamide in full dose was continued until the one-year follow-up period, but prednisolone was tapered off to <20 mg/day after six months. After discharge from

hospital, all patients were followed carefully in the Nephrology clinic for at least a year, and their kidney function was measured frequently. Patients who developed small-sized kidneys and a serum creatinine >5 mg/dL during the follow-up period were considered to have end-stage renal disease (ESRD).

Statistical analysis was done by a statistical computer software program, using chi-square and Fisher's exact tests.

Results

Of 51 patients with ANCA-associated glomerulonephritis, 22 (43%) had cANCA and 29 (57%) had pANCA by the IIF method.¹⁰ All 29 pANCA cases and 18 of the 22 patients who had cANCA by IIF also tested positive for anti-MPO and anti-PR3 antibodies, respectively, by the ELISA method. The remaining four patients with cANCA neither tested anti-PR3 nor anti-MPO positive, suggesting that the antigen might have been CAP57.¹¹ Clinical manifestations in the 51 patients with ANCA-associated glomerulonephritis are shown in Table 1.

Twelve patients had normal kidney function (serum creatinine <2 mg/dL associated with blood urea nitrogen <20 mg/dL) with normal urine output at presentation (10 patients had cANCA and two cases pANCA pattern), associated with hypertension, isolated hematuria and edema. In all 12 patients, renal function remained within normal range and was stable during the follow-up period. Table 2 shows the renal outcome and clinical findings in relation to the ANCA subtypes.

Of the 39 patients with RPGN, all had crescentic glomerulonephritis involving >75% of the glomeruli, with minimal or no immune deposits in their biopsy specimens. Oliguria leading to the end-stage renal disease (ESRD) was seen in 21 (54%) of them. In 29 cases with pANCA, renal histopathology showed 19 cases of crescentic glomerulonephritis, two of necrotizing glomerulopathy, and in eight cases, crescentic and necrotizing histopathology coexisted. In 22 patients with cANCA, renal histopathology showed 13 cases of necrotizing glomerulopathy, six of crescentic glomerulonephritis, and in three cases, both crescentic and necrotizing lesions overlapped. Immunofluorescent studies revealed no or minimal deposits of immunoglobulins C3, C4 and fibrin without significant difference between the two groups of patients.

The pattern of immunoglobulin deposit was linear in a young girl with pANCA in whom antiglomerular basement membrane antibodies were found both along the glomerular basement membrane and in the patient's serum, and who developed ESRD during the follow-up. Table 3 shows the main subtypes of ANCA in patients with

glomerulonephritis in relation to their age and gender. No significant difference in the ANCA pattern was found among the different age groups. All patients had a corrected erythrocyte sedimentation rate over 39 mm/hr, and this was more than 100 mm/hr in 51.5% of cases. Overall, three patients had eosinophil count more than 500 per mm³ (range, 500-4000 per mm³) which was associated with asthma in two of them.

Discussion

Over the past decade, ANCA has been the subject of intensive investigation. These antibodies are present in a large number of diseases.⁹⁻¹⁸ Using IIF, which is the method of choice for ANCA detection, either of two major patterns can be seen—a cytoplasmic pattern (cANCA) or a perinuclear pattern (pANCA). The cANCA pattern is most often caused by anti-PR3, and in rare cases by anti-MPO.^{10,11} These autoantibodies have been recognized in patients with different forms of glomerulonephritis, especially those with crescentic glomerulonephritis and without immune deposits in their kidney biopsy specimens.¹⁹⁻²⁰ Likewise, in the present series, most patients presented with crescentic or rapidly progressive glomerulonephritis (RPGN), an active urine sediment and oliguria with minimal or no immunoglobulin deposits in their renal tissues.

Of 51 patients with ANCA-associated glomerulonephritis, 21 cases (41%) developed ESRD. This finding is in agreement with results obtained by some investigators^{3,5,20} and in contrast to others.^{1,8,22} However, all our patients who developed ESRD during the follow-up had crescentic glomerulonephritis, presenting initially with RPGN, oliguria and an elevated serum creatinine, factors which are generally accepted to be the worst prognostic markers. Also in this series, RPGN and eventually ESRD were seen more commonly with pANCA than with cANCA. Therefore, it seems wise to assume that pANCA is a risk factor for development of ESRD in patients with ANCA-associated glomerulonephritis. In contrast to our findings, it has been reported that cases with cANCA-associated glomerulonephritis are at a higher risk for development of ESRD than those with pANCA.⁵ Nonetheless, cANCA pattern is highly specific for Wegener's granulomatosis, whereas pANCA is found in the sera of patients with vasculitis, glomerulonephritis and several other diseases.^{6,23} Also, race is an important prognostic factor for renal survival.⁵

The poor renal survival in our patients with pANCA compared with those with cANCA might have stemmed from disparities in genetic factors and/or spectrum of underlying disease associated with the main subtypes of ANCA in different nations.^{5,6,8,9} Clearly, more exploration is needed to determine the extent of significance of each of these aforementioned factors.

It has increasingly been recognized that the majority of

TABLE 1. *Clinical manifestations in 51 patients with ANCA-associated glomerulonephritis.*

Clinical finding	Number (%)
Significant proteinuria and hematuria	39 (76.5)
Rapidly progressive glomerulonephritis	39 (76.5)
Flu-like syndrome	31 (60.7)
Cough, sputum and/or hemoptysis	29 (56.8)
Fever	15 (29.4)
Skin rash	12 (23.5)
Hypertension	12 (23.5)
Isolated hematuria	12 (23.5)
Sinusitis	11 (21.5)
Otitis	3 (6)
Scleritis	3 (6)
Stridor	2 (4)
Nephrotic syndrome	2 (4)
Asthma	2 (4)
Polychondritis	1 (2)

TABLE 2. *Clinical manifestations and renal outcome in 51 patients with ANCA-associated glomerulonephritis and its relation to the seropositivity for the two main subtypes of ANCA.*

Parameter	pANCA (n=29)	cANCA (n=22)	P-value
RPGN	27	12	0.001
End-stage renal disease	17	4	0.004
Pulmonary involvement	16	13	NS
Sinusitis	3	7	NS
Skin rash	4	8	NS

RPGN=rapidly progressive glomerulonephritis; NS=not significant.

patients who present with pulmonary-renal syndrome have ANCA and not antiglomerular basement membrane antibodies.^{6,24} Yet, the significance of the main subtypes of ANCA on the pulmonary manifestations is poorly understood. In the present series, in accordance with previously published series,^{6,8} a significant proportion of our patients had lung and renal involvement at presentation. However, in agreement with one report⁵ and contrary to another one,⁶ neither pulmonary involvement nor other extra-renal manifestations showed a positive correlation with the main ANCA patterns in this study. However, in the previously published series of ANCA-associated glomerulonephritis, correlation between the extra-renal manifestations and main ANCA patterns have varied among different nationalities.^{5,6,8,9} This might suggest that diseases underlying ANCA-associated glomerulonephritis might be different in various nations.

The sex and age of patients have been implicated in the development of certain types of ANCA-associated glomerulonephritis. The relationship between the patterns of ANCA and the sex and age of patients with ANCA-associated glomerulonephritis has not been adequately studied. In one report, the yearly incidence of pauci-immune necrotizing and crescentic glomerulonephritis

TABLE 3. *The seropositivity for the two main subtypes of ANCA in 51 patients with ANCA-associated glomerulonephritis in relation to their age and gender.*

Age group	pANCA (n=29)		cANCA (n=22)		P-value
	Female	Male	Female	Male	
15-25 years	5	1	2	3	NS
21-50 years	8	5	3	12	NS
50-70 years	6	4	0	2	NS
Total	19	10	5	17	0.005

NS=not significant.

TABLE 4. *Pulmonary involvement and renal outcome in 51 patients with ANCA-associated glomerulonephritis in relation to their age and gender.*

Age group	Lung disease		RPGN		ESRD	
	Female	Male	Female	Male	Female	Male
15-25 yrs	4	2	6	3	4	2
21-50 yrs	6	10	5	14	3	5
50-70 yrs	5	2	6	5	6	1
Total	15	14	17	22	13	8

RPGN=rapidly progressive glomerulonephritis; ESRD=end-stage renal disease; P-value was not significant.

was highest among those over 65 years of age, but the pattern of ANCA was not known in all of the patients.⁴ Overall, we found pANCA more commonly in females and cANCA more commonly in males, but no correlation between the different age groups and ANCA patterns was found in this study.

We conclude that ANCA-associated glomerulonephritis is common in patients of Iranian origin with a poor renal prognosis, especially for those with pANCA pattern, which is found more frequently in female patients.

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