INVESTIGATION

Anti-neutrophil cytoplasmic antibodies in new-onset systemic lupus erythematosus*

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Abstract: Background: The clinical significance of anti-neutrophil cytoplasmic antibodies in patients with new-onset systemic lupus erythematosus, especially in systemic disease accompanied by interstitial lung disease remains to be elucidated.
Objectives: This study was designed to investigate the role of anti-neutrophil cytoplasmic antibodies in new-onset systemic lupus erythematosus patients.
Methods: A hundred and seven patients with new-onset SLE were enrolled. Presence of anti-neutrophil cytoplasmic antibodies in the sera was assessed by indirect immunofluorescence as well as enzyme linked immunosorbent assay against proteinase-3 and myeloperoxidase. Clinical features and laboratory parameters of patients were also recorded. All patients were subjected to chest X-ray, chest high-resolution computed tomography and pulmonary function test.
Results: Forty-five systemic lupus erythematosus patients (45/107, 42%) were seropositive for anti-neutrophil cytoplasmic antibodies. Compared with anti-neutrophil cytoplasmic antibodies-negative patients, the anti-neutrophil cytoplasmic antibodies-positive patients had significantly higher incidence of renal involvement, anemia, and Raynaud's phenomenon as well as decreased serum level of complement 3/complement 4 and elevated erythrocyte sedimentation rate. In addition, there was a positive correlation between serum anti-neutrophil cytoplasmic antibodies level and disease activity of systemic lupus erythematosus. Furthermore, prevalence of interstitial lung disease in the anti-neutrophil cytoplasmic antibodies -positive patients (25/45, 55.6%) was obviously higher than that in the anti-neutrophil cytoplasmic antibodies-negative patients (15/62, 24.2%).
Study limitations: The sample size was limited and the criteria for screening new-onset systemic lupus erythematosus patients might produce bias.
Conclusions: The level of anti-neutrophil cytoplasmic antibodies in new-onset systemic lupus erythematosus patients correlates positively with the disease activity and the prevalence of interstitial lung disease.
Keywords: Antibodies, antineutrophil cytoplasmic; Lupus erythematosus, systemic; Lung diseases, interstitial

INTRODUCTION

Systemic lupus erythematosus (SLE) is one of the most complicated autoimmune diseases, which is characterized by the production of clusters of autoantibodies, the involvement of multiple organs, and a broad spectrum of clinical manifestations.[1] Due to the serological hallmark of abnormal appearance of various autoantibodies in SLE patients, increasing evidence has indicated that the evaluation of clinical relevance between these autoantibodies and disease parameters can help clinicians to identify SLE patients at risk for specific complications at the early stage and expand effective therapeutic strategies.[2,3]

Antineutrophil cytoplasmic antibodies (ANCA) are a group of autoantibodies that are directed against specific antigens such as neutrophil cytoplasmic granules and monocyte lysosomes. 4 Since ANCA were first found in the serum from patients with segmental necrotizing glomerulonephritis in 1982, they were soon identified as important serological markers of primary vasculitis.[5] Up to now, the association of ANCA with the small-vessel vasculitis, including Wegener's granulomatosis, Churg-Strauss syndrome and microscopic polyangiitis, has been well reported.[6] In fact, vasculitis is a well-documented clinical manifestation in SLE patients. Although a host of studies have focused on the relationship between ANCA and SLE, the clinical significance of ANCA in SLE patients remains controversial. Moreover, lung involvement is quite common in SLE, and one of the general pulmonary complications of SLE is interstitial lung disease (ILD).[7] It has been suggested that there is a close relationship between ILD and ANCA-associated vasculitis both pathophysiologically and clinically.[8] However, the possible clinical relevance of ANCA with ILD in SLE patients has not been reported.

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In the present study, we investigated ANCA levels of 107 new-onset SLE patients who had never received treatment for SLE. The possible associations between the presence of ANCA and SLE disease activity, as well as ILD in SLE patients, were also evaluated.

Methods

Study subjects

In a cross-sectional descriptive analytical study, 107 new-onset SLE patients (106 women, one man) were screened from a large number of inpatients with SLE at the Department of Rheumatology and Immunology of the First Affiliated Hospital of China Medical University from July 2007 to October 2010 based on the following criteria: 1) first time diagnosis of SLE; 2) disease duration less than six months; 3) no history of corticosteroids or immunosuppressive drugs use before registration.[9] The diagnosis of SLE was established according to American College of Rheumatology (ACR) criteria.[10,11] Mean disease duration of these new-onset SLE patients was 3.7±1.3 months (2 to 6 months) and mean age was 37.5±9.8 years (range from 16 to 60 years). Samples of sera from all patients were collected. Informed consent from each subject was obtained before enrollment into this study, which was conducted according to the ethical standards of China Medical University.

Clinical and laboratory assessments

Clinical features of SLE patients such as oral ulcer, arthritis, renal involvement, Raynaud's phenomenon, rash, anemia and photosensitivity were recorded. Renal involvement of SLE was defined based on the ACR criteria, that is, any of the following: 1) presence of active cellular casts; 2) persistent proteinuria ≥0.5 g/day; or 3) biopsy evidence of lupus nephritis.9 For all the patients, laboratory variables were also determined, including anti ds-DNA antibody (ds-DNA), anti-nuclear antibody (ANA), immunoglobulin levels (IgM, IgG, IgA), serum C3/C4 levels, complete blood cell count, erythrocyte sedimentation rate (ESR) and routine urinalysis. Disease activity was evaluated by SLE disease activity index (SLEDAI) and SLEDAI ≥5 was considered as an active SLE.[12]

Detection of ANCA

ANCA was determined by indirect immunofluorescence (IIF), performed using the Titerplane technique (Euroimmun, Germany). 25µl diluted serum (1:10) was added into each reaction field of reagent tray, incubated for 30 min at room temperature, and then washed with phosphate buffered saline Tween (PBST). Subsequently, cells were labeled with fluorescein isothiocyanate (FITC) conjugated antihuman IgG, washed with PBST and examined using a fluorescence microscope (Leica). The concentrations of antibodies to proteinase-3 (PR3) and myeloperoxidase (MPO) were measured by enzyme linked immunosorbent assay (ELISA) kit according to the manufacturers' instructions (Euroimmun, Germany).

Chest high-resolution computed tomography (HRCT)

HRCT scans of the thorax were performed using a Toshiba spiral CT (scan time 1s, 140mA, 120kV) with images windowed to highlight both lungs and mediastinal structures. Image evaluation was performed by two clinicians from the Department of Radiology blinded to the clinical data of patients. Results were based on consensus agreement. ILD was defined by the presence of characteristic abnormalities. According the clinical background, tuberculosis, virus, bacterial infection and heart failure were excluded by the specialists in the Department of Infection and Department of Radiology.

Statistical analysis

All data were analyzed using Statistical Package for Social Sciences (SPSS) computer software for Windows Version 13. The Student's t-test was used to compare the means between two groups. The Fisher's exact test or Chi-square test was used to assess differences in categorical data between the two groups. In all instances, $P<0.05$ was considered significant.

Results

Among 107 new-onset SLE patients, 45 (42%) were ANCA seropositive. According to the results of IIF and ELISA, all SLE patients were divided into ANCA-positive and ANCA-negative groups. There was no significant difference in age distribution as well as disease duration between the two groups. Of 45 ANCA-positive patients, 40 cases (88.9%) were MPO-ANCA positive. Clinical features and laboratory parameters of the two groups are summarized in table 1. Incidence of rash, renal involvement, Raynaud's phenomenon, ILD, fever, and anemia in ANCA-positive group was obviously higher than in ANCA-negative group. Decreased serum C3/C4 levels, and elevated ESR appeared more often in ANCA-positive cases than in ANCA-negative cases. Additionally, in ANCA-positive group, 32 cases (32/45, 71.1%) presented active SLE, whereas only 20 cases (20/67, 32.3%) had active SLE in ANCA-negative group, which showed a significant difference ($P<0.05$).

In the 62 ANCA-negative cases, 15 patients (24.2%) had SLE accompanied by ILD, which was remarkably lower than in ANCA-positive group (25/45, 55.6%; $x^2=10.95$, $P<0.01$). Moreover, the results of HRCT showed that, compared with ANCA-negative group, ANCA-positive SLE patients had higher incidence of common manifestations of ILD, including interlobular septum thickening, ground-glass density, pleural reaction, small tubercle shadow, pericardial effusion, and pulmonary artery hypertension (Table 2).

Discussion

The present study was conducted to evaluate the clinical significance of ANCA in new-onset SLE patients. We found that there was a positive correlation between serum ANCA level and the SLE disease activity. Furthermore, we also demonstrated, for the first time, that the incidence of ILD in ANCA-positive patients with new-onset SLE was obviously higher than that in ANCA-negative patients.

Up to date, the clinical significance of ANCA in SLE is still controversial. Some studies report that there is an association between ANCA-positivity and SLE. Manolova *et al.* found that ANCA were associated with the particular clinical features of SLE and correlated with disease activity.[13] Pradhan *et al.* also reported that ANCA was positively associated with SLE disease activity, and might be used as a potential complementary parameter to differen-

Table 1. The clinical manifestations in 107 new-onset SLE patients

	ANCA(+) 45	ANCA(-) 62	X^2	P	OR
Oral ulcer	11(24.40%)	14(22.60%)	0.05	>0.05	
Arthritis	34(75.60%)	38(61.30%)	2.41	>0.05	
Rash	23(51.10%)	19(30.60%)	4.58	<0.05	2.36
ILD	25(55.6%)	15(24.20%)	10.95	<0.01	3.92
Renal involvement	31(68.90%)	15(24.20%)	21.25	<0.01	6.31
Raynaud's phenomenon	17(37.80%)	12(19.40%)	4.47	<0.05	2.52
Fever	17(37.80%)	12(19.40%)	4.47	<0.05	2.52
Anemia	13(28.90%)	8(12.90%)	4.22	<0.05	2.74
Photosensitivity	4(0.09%)	4(0.06%)	0.22	>0.05	
C3↓, C4↓	42(93.30%)	48(77.40%)	4.94	<0.05	4.08
ESR↑	44(97.70%)	43(67.74%)	10.01	<0.01	6.48
ds-DNA(+)		55(88.70%)	3.09	>0.05	

Table 2. The manifestations of the HRCT in new-onset SLE patients

Test	ANCA-positive N=45(%)	ANCA-negative N=62(%)
HRCT		
Interlobular septum thickening	16(35.6)	10(16.1)
Ground-glass changes	6(13.3)	2(3.2)
Honeycomb lung	2(4.4)	1(1.6)
Subpleural opacities	8(17.8)	3(4.8)
Hydropericardium	3(6.6)	0 (0)
Pulmonary hypertension	4(8.8)	1 (1.6)

tiate lupus nephritis from SLE without nephritis.[14] On the contrary, others reported no link. Fauzi *et al.* failed to find significant association between ANCA-positivity and SLE disease activity.[15] Nishiya *et al.* reported that ANCA was unrelated to organic involvement.[16] Patient selection criteria might be an important factor responsible for these disparities. These previous studies did not exclude patients with a history of immunosuppressants or corticosteroids use, which should have potential influence on the results. Therefore, in this study, only new-onset SLE patients who had never received any treatment for SLE were enrolled. Our findings demonstrated that serum ANCA level significantly correlated with particular clinical features as well as SLE disease activity, suggesting that it may serve as a potential useful parameter for the diagnosis of new-onset SLE.

In this study, we also found that the incidence of ILD in ANCA-positive SLE patients was significantly higher than in ANCA-negative patients. In ANCA-positive group, ILD was characterized by the thickening of lobular septum, subpleural opacities, and the ground-glass attenuation. These results suggest that ANCA might be used as a complementary parameter of ILD involvement in new-onset SLE. Additionally, it is surprising that among patients with lung involvement, fewer than 50% of patients showed ILD consolidation during the chest X-ray examination. Hence, in order to detect the presence of ILD as early as possible, it may be necessary to use chest HRCT as routine examination for SLE patients, especially ANCA-positive patients with SLE.

Although the mechanisms of pulmonary interstitial lesions in SLE are still undefined, vasculitis has been considered as a basic pathological change in such process. Vasculitis, characterized by the appearance of inflammatory cell infiltration and subsequent blood vessel wall necrosis, is one of the most common complications of SLE. 17 With the discovery of the roles of ANCA in the pathology of vasculitis, ANCA-associated vasculitis (AAV) has been defined as a type of vasculitis, which is characterized by a small-sized vessel vasculitis associated with ANCA positivity. [18] Pulmonary involvement has been considered as one of the hallmark lesions of AAV. [19] Recently, increasing attention has focused on the association between ILD and AAV. Although the pathogenesis of ILD in AAV remains poorly understood, one of the major hypotheses - that MPO-ANCA may play a direct role in the pathogenesis of pulmonary fibrosis - has been demonstrated by several groups.[8,20,21] Consistent with these previous studies, our data showed that 88.9% of ANCA-positive patients were also MPO-ANCA positive. As the target antigen of p-ANCA, MPO forms unspecific ionic bonds with pulmonary capillary wall, and subsequently leads to wall injury through the formation of *in situ* immune complex, which may support a pathogenic role of MPO-ANCA in pulmonary interstitial lesions occurring in ANCA-positive SLE.

Despite these findings, this investigation presented a few limitations. Indeed, as a cross-sectional descriptive analytical study, the sample size was limited and the ANCA level from some control

subjects should be determined to compare with SLE patients. Thus, further research is warranted to evaluate the role of ANCA in SLE patients as well as the relationship between ANCA and SLE-associated ILD using a large number of subjects and a multicenter design. In addition, although new-onset SLE patients were screened based on three criteria, some information related to the criteria (e.g., duration of disease) was collected from the patients' chart. Therefore, we cannot rule out the bias of new-onset definition.

CONCLUSION

The present study demonstrated that serum ANCA level has a positive correlation with SLE disease activity in new-onset SLE patients. Additionally, we also demonstrated that ANCA-positive SLE patients exhibit a higher incidence of ILD than ANCA-negative SLE patients. Our findings suggest that ANCA may serve as a useful marker of SLE disease activity, or even a complementary parameter for the presence of ILD in new-onset SLE patients. Further research will be needed to confirm the utility of ANCA as a diagnostic or prognostic marker. ❑

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