ORIGINAL ARTICLE

Antibodies in Systemic Lupus Antineutrophil Cytoplasmic Erythematosus: Prevalence, Disease Activity Correlations and Organ System Associations

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Summary

Systemic Lupus Erythematosus (SLE) is a disease with multiorgan involvement and multiple autoantibody production including antineutrophil cytoplasmic antibodies (ANCA). Despite its reported prevalence in more than one third of SLE patients, the role of ANCA in the pathogenesis or otherwise in SLE remains unresolved. 131 SLE patients had been previously studied for various serologic parameters of disease activity. Their cumulative organ involvement in the course of their disease had also been determined and the Lupus Activity Index (LAI) calculated. Their stored sera were then screened for the presence of ANCA by two methods viz Indirect immunofluorescence (IIF) and also enzyme-linked immunosorbent assay (ELISA). ANCA was present in 24.8% of these SLE patients. The atypical ANCA pattern was predominant and accounted for an overall of 20.6%. Anti-MPO and anti-PR3 were detected in 1.5% of patients respectively. No association was found between ANCA positivity and disease activity. There was also no association of ANCA with specific organ involvement. Despite the high prevalence of ANCA especially the atypical variant in SLE, they probably represent only one of the wide repertoire of autoantibodies found in this disease. Routine testing for ANCA in lupus patients is therefore not recommended.

Key Words: SLE, ANCA, Pleuritis

Introduction

Systemic Lupus Erythematosus (SLE) is an autoimmune disease of unknown cause that can affect skin, joints, kidneys, lungs, nervous system, serous membranes and/or other organs of the body. Distinct immunologic abnormalities of SLE include B cell hyperactivity and the production of a widerepertoire of antinuclear. As the clinical course of SLE is characterised by periods of relapse and remission, the presence of the various autoantibodies are useful for the detection of relapses.

Autoantibodies are classified as primary or secondary depending on whether they are recognized as having a primary role or otherwise, in the pathogenesis of the disease. Secondary autoantibodies are more common and despite their lack of a clearly defined role in the causation of disease, they have a valuable role in the diagnosis and classification of clinical conditions. Antineutrophil cytoplasmic antibody (ANCA) is considered a secondary autoantibody as the role of ANCA in disease has not been fully unravelled. However, evidence exists that ANCA plays a role in the pathogenesis of ANCA-associated diseases. The presence of ANCA in SLE had been known for 20 years. However, it's association with disease activity has yet to be understood. The prevalence of ANCA is reported to be as high as 31% in lupus patients [1]. In particular, only the p-ANCA antibodies were present in these patients

This article was accepted: 23 October 2004

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although atypical ANCA, which is fairly common in all connective tissue, was found in 11-30% of patients[1].

Materials and Methods

For the purpose of this study, we used the stored sera of 134 SLE patients who had given informed consent to various serological studies on their blood. The dermographic data and lupus activity of these patients were also documented.

All those patients satisfied the American College of Rheumatology criteria for the diagnosis of SLE. Exclusion criteria included pregnancy, other connective tissue diseases i.e. scleroderma or rheumatoid arthritis and patients on drugs known to induce SLE such as hvdrallazine.

The presence of ANCA was determined by indirect immunofluorescence (IIF) and classified as c-ANCA, p-ANCA or atypical ANCA. Formalin fixation were used to eliminate positivity in sera with high anti-DNA titres. The subspecificities of anti-PR3 (which corresponds to c-ANCA) and anti-MPO (which corresponds to p-ANCA) were determined by the ELISA technique. Other subspecific antigens which also correspond to p-ANCA such as anti-lactoferrin, anti cathepsin G, anti-elastase were not tested.

Results

Of the stored sera from the 134 SLE patients only 131 were sufficient for the determination of ANCA according to the methodology outlined. 119 sera were from female patients and 12 were from male patients giving a female: male ratio of about 10:1. 51.9% of the patients were Malays, 42% Chinese and 6.1% were Indian (including Sikhs). The mean age of these patients was 34.0 ± 11.3 years and their ages ranged from 14 to 69 years. These data are in keeping with most reported series of SLE patients.

Prevalence of ANCA in SLE (Table I)

a) Determination of ANCA by indirect immunofluorescence (IIF)

A positive result was found in 32 patients (24.4%). Eleven (8.4%) of these were positive for cANCA, two (1.5%) positive for pANCA and 27 (20.6%) samples were positive for an atypical ANCA pattern. Eight out of 11 of the cANCA positive sera were also positive for atypical ANCA.

b) Subspecificities of ANCA by ELISA

All 131 samples were also analysed by ELISA for anti-MPO and anti-PR3. Only 2 samples were positive for each target antigen i.e. two positive for anti-MPO and two for anti-PR3. A total of two samples tested positive for both ELISA and IIF. One was positive for atypical ANCA and anti-MPO while the other was positive for atypical ANCA and anti-PR3.

Association of ANCA with anti DNA and Lupus Activity Index

Both the anti-dsDNA and LAI are measures of disease activity in SLE. There was no association of total ANCA or subsets of ANCA with anti-dsDNA (p=0.24). As expected there was also no association between ANCA and the LAI score (p=0.66). ANCA subtypes also did not show any association with the anti-dsDNA.

Correlation of ANCA with Organ System Involvement (Figure 1)

To evaluate whether a positive ANCA was associated with any particular clinical manifestation, the ANCA results for all these patients were correlated with disease in the various organ systems. Although no association was found between the presence of ANCA and serositis as a whole, a trend was observed (p=0.072). However, subanalysis revealed an inversed association between ANCA and pleuritis (Table II). A negative ANCA was associated with a relative risk of 1.17 for pleuritis (p=0.02) suggesting that ANCA positivity may be protective against the occurrence of pleuritis in SLE.

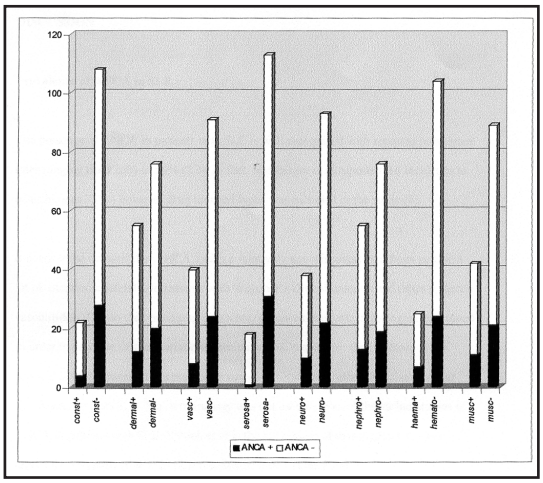

Fig. I: Types and frequency of organ system involvement and ANCA in the study cohort. Const= constitutional, derma= dermatological, vasc= vascular, sero= serositis, neuro= neurology, neph= nephrology, hema= haematology, musc= musculoskeletal - indicates absense, + indicates presence

Table I: Studies on the prevalence of ANCA in SLE.

Reference (year)	No of pts	Total ANCA	p-ANCA	c-ANCA	Atypical ANCA
Nassberger (1990)	96	89 (95%)	NA	NA	NA
Pauzner (1994)	114	41 (36%)	29 (25%)	12 (11%)	NA
Schnabel (1995)	157	40 (25%)	40(25%)	0	NA
Spronk (1996)	25	11 (44%)	4 (16%)	0	7 (28%)
Kabasakal (1999)	117	16 (14%)	16 (14%)	0	NA
Chung (2000)	51	19 (37%)	16 (31%)	3 (6%)	NA
Our study	**131**	**32 (24%)**	**2 (2%)**	**11 (8%)**	**27 (21%)**

Note that only one other investigators (Spronk et al) other than us described atypical ANCA
NA= not available

Discussion

Prevalence of ANCA in SLE

The presence of ANCA in patients with SLE is well recognized with reported prevalence rates ranging from 25% to 56% [2,3]. In fact, Nassberger et al reported the incidence to be as high as 93% - however drug induced lupus was included in the study [4].

It needs to be stressed that ANCA, being a relatively new discovery, has been getting a lot of attention especially in patients with Wegener's Granulomatosis and other systemic vasculitides. Due to the continuing diagnostic advances, newer terminologies are coined in order to describe further variations seen with ANCA staining. One of those is the atypical ANCA pattern that is applied to cytoplasmic staining other than the classical appearance of p-ANCA. This terminology was not used in most of the earlier studies of ANCA in patients with SLE. Spronk et al is the only group of investigators to have reported an atypical ANCA in SLE recently in 1996 (Table III)[5].

Conflicting evidence has been presented concerning the spectrum of ANCA subspecificities in SLE patients and their clinical correlates [6,7] (Table I). Nassberger et al noted pANCA to be positive in 95% of 96 patients with SLE (23,1990). Pauzner et al reported that of 114 SLE patients studied, 10.5% were positive for cANCA and 25.4% positive for pANCA[8]. Spronk et al reported a 9.5% incidence of pANCA positivity in 84 SLE patients but a higher number of his patients, 31%, showed an atypical ANCA pattern[5]. Kabasakal et al reported that 56% of his SLE patients (65 out of 117 patients) were pANCA positive with none of them being cANCA positive [9].

In our study, the prevalence of ANCA (all subtypes) is somewhat similar to those of other earlier studies. 24.4% of patients' sera were positive for ANCA, predominantly of the atypical ANCA variant. As indicated earlier, Spronk et al was the only other group to have reported an atypical ANCA present in 28% of his SLE patients. The prevalence of pANCA was slightly lower at 8.4% of the total number of sera. This is partially contributed by the inherent difficulty due to perhaps the presence of anti-dsDNA antibodies and ANA, as these antibodies were not removed before performing the assays for ANCA. However, we are not aware that this procedure can be routinely performed, outside of specialized research laboratories. The c-ANCA (8.4%) prevalence in our cohort is also higher than studies reported earlier. One possible explanation is that our patients were recruited from the SLE/nephrology unit; thus patients referred to us tended to have a higher incidence of renal involvement. Chin et al, who reviewed patients with lupus nephritis, found cANCA in 6% and pANCA in 32% of his patients[10].

We also found fewer patients (1.5% each) with anti MPO and anti-PR3. However, we were not able to check for the full range of target antigens such as lactoferrin, cathepsin G or elastase due to financial constraint and lack of expertise. This low prevalence of anti-MPO is actually consistent with a few earlier studies [9,11], except that of Nassberger et al [6].

ANCA and disease activity

Spronk et al studied SLE patients with disease relapse and measured fluctuations of serum ANCA levels during relapse and remission[5]. They could not demonstrate the usefulness of serum ANCA titres in determining disease relapse. Kabasakal et al also could show no significant association between disease activity and ANCA[9]. This is in contrast to the usefulness of serum ANCA titres in predicting relapses in patients with WG.

Similarly, we have analysed ANCA positivity with SLE disease activity using the LAI. In this cross sectional study, we too found no correlation between ANCA with various parameters of disease activity using the LAI and anti-dsDNA.

ANCA and organ involvement in SLE

Reports of the association of ANCA in SLE patients with various organ involvement have also revealed conflicting results. Falk Jennette et al found ANCA in five of 11 patients with lupus nephritis[12]. However, they did not clearly differentiate between ANCA and anti-MPO antibodies. Whereas Nassberger et al found no correlation of ANCA with lupus nephritis but found anti-elastase antibodies in 4 patients with idiopathic SLE and 3 of those patients had CNS disease[6]. Nonetheless, the patient numbers are too small for any firm conclusions to be drawn.

Kuster et al reported the prevalence of ANCA in 44 patients with SLE who had been admitted to the renal division[13]. Thirty-three of these patients had renal involvement. cANCA was absent in both groups while pANCA was consistently positive (40 out of 44 patients). Low titer anti-MPO antibodies were present

in 25% of these patients and appeared to be associated with dermal vasculitis and/or glomerulonephritis. Antilactoferrin antibody was found in only one patient with kidney involvement.

Lee et al reported positive IF staining with ethanol fixed neutrophils in a nuclear or perinuclear pattern in the majority of his 87 SLE patients[14]. Anti-lactoferrin antibodies were found in 31(39%) of these patients. Anti-lactoferrin antibodies were associated with clinical activity, as well as with disease duration in their study. There was no significant correlation between WHO Class IV nephropathy and anti-lactoferrin antibodies. However, among the patients with Class IV nephropathy, the presence of crescents was associated with the anti-LF antibodies. Lee et al also found moderate or marginal elevations of anti-MPO in 8 out of 87 patients (9%), of whom six had a history of serositis. A few other studies also revealed the association of pericarditis with anti-LF and anti-MPO antibodies[5,15]. Chin et al also noted the presence of ANCA particularly pANCA, to be associated with nephritis[10]. Nephritis was noted in 35% of ANCA positive patients as compared to none in the ANCA negative group.

Spronk et al noted anti-MPO to be associated with arthritis during relapse[5]. Schnabel et al found no correlation between ANCA with nephritis, vasculitis or any other clinical manifestation of SLE[11]. However, there was a trend for pANCA to accompany a raised anti-dsDNA titre, but this was not statistically significant and he believed that this phenomenon to be simply a reflection of B cell hyperactivity in SLE. Kabasakal found an association of anti-MPO with arthritis, vasculitis and anti-DNA positivity [9].

In our study, we found no correlation of ANCA with organ system involvement and this finding is consistent with some of the earlier findings. In one such study, Schnabel et al[11] found no correlation between the detection of ANCA and organ involvement (as defined by British Isles Lupus Assessment Group Index) or with the occurrence of vasculitis. Another study by Chin et al[10] also found no significant association between ANCA and organ involvement. Thus, the absence of uniformity amongst all these studies indicate that ANCA is not an important autoantibody in SLE as it is for Wegeners granulomatosis and microscopic polyangiitis.

Clinical Implication and Conclusion

Although ANCA plays a pathogenetic role and is an indicator of activity in Wegener's granulomatosis, its role in SLE has no demonstrable significance. Many studies including ours have failed to show ANCA as a marker of disease activity in SLE. All studies to date have not iether demonstrated a uniform association of ANCA with different organ system involvement.

Thus we conclude that although the prevalence of ANCA is high in SLE, it is neither an index of disease activity nor of specific organ involvement. Hence, ANCA is not recommended for the routine serological surveillance of SLE patients.

Acknowledgement

This study was funded by the UKM Research Grant. We thank the Dean of the Faculty of Medicine, the Hospital Director, National University of Malaysia, for their kind permission to publish this article.

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