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Original Article

The clinical utility of anti-chromatin antibodies as measured by BioPlex 2200 in the diagnosis of systemic lupus erythematosus versus other rheumatic diseases

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Abstract: Background: The detection of autoantibodies is indispensable to systemic lupus erythematosus (SLE). Bioplex 2200 ANA screen is a multiplex immunoassay system that allows simultaneous determination of autoantibodies to extractable nuclear antigens (ENA) including anti-chromatin antibodies (ACAs). However, the clinical significance of the ACAs by this new method in SLE patients has not been studied in comparison with other rheumatic disorders. We performed a retrospective study of patients with rheumatic diseases to assess the diagnostic value of the ACAs by Bioplex 2200 method in SLE. Methods: Adult patients with rheumatic complaints seen by rheumatologists at the Cleveland Clinic between January 2008 and February 2010 were screened for positive anti-ENA antibodies by the Bioplex 2200. Patients with positive anti-ENA antibodies were classified into two populations based upon the presence and absence of the ACAs. We retrospectively studied the clinical and laboratory data of these patients. Results: A total of 764 subjects with positive anti-ENA antibodies were screened, including 115 with positive ACAs. There were 93 SLE patients consisting of 58 with positive ACAs and 35 with negative ACAs. The sensitivity, specificity, positive predictive value and negative predictive value of the ACAs in SLE were 62.4%, 91.5%, 50.4% and 94.6% respectively. Apart from SLE, positive ACAs were associated with mixed connective tissue disease (MCTD)/undifferentiated CTD (UCTD) and other autoimmune diseases. No correlation was found between the ACAs and lupus glomerulonephritis or anti-dsDNA antibodies. Conclusions: Measurement of the ACAs by the Bioplex 2200 is specific for diagnosing SLE but not useful for differentiating between SLE and MCTD/UCTD.

Keywords: Systemic lupus erythematosus, anti-chromatin antibodies, anti-ENA antibodies, BioPlex 2200

Introduction

The detection of autoantibodies is indispensable to the diagnosis of systemic lupus erythematosus (SLE). Chromatin consists of double stranded DNA linked with multiple nucleosomes which comprise histones and 164 base pairs of DNA [1]. In recent years, anti-chromatin antibodies (ACAs) have come into the limelight as a marker for diagnosing SLE. In the past, ACAs were clinically measured by traditional methods, notably enzyme-linked immunosorbent assay (ELISA). The BioPlex 2200 multiplexed system has been developed for high throughput analysis of 13 autoimmune analytes simultaneously in a single tube in a timely manner [2]. Even if the clinical significance of the ACAs were studied and characterized by the traditional methods, it has not been studied by the new BioPlex system in SLE as compared to other rheumatic disorders. Therefore, we performed a retrospective chart review of patients with rheumatic diseases to assess the diagnostic value of the ACAs in SLE.

Patients and methods

Patient selection

We conducted a retrospective study of adult patients with rheumatic complaints who were seen by rheumatologists at the Cleveland Clinic between January 2008 and February 2010. Since we intended to explore the clinical signifi-

Table 1. The clinical utility of ACAs in the diagnosis of SLE

ACAs	SLE	Non-SLE	Total	SN	SP	PPV	NPV
Positive	58	57	115	62.36%	91.50%	50.43%	94.60%
Negative	35	614	649				
Total	93	671	764				

ACAs, anti-chromatin antibodies; SN, sensitivity; SP, specificity; PPV, positive predictive value; NPV, negative predictive value

cance of positive ACAs as measured by the Bioplex 2200 system described below, the electronic medical records of these patients were screened for positive anti-extractable nuclear antigens (anti-ENA) antibodies containing the ACAs. Patients with any positive anti-ENA antibodies were then stratified into two populations based upon the presence and absence of the ACAs. A total of 649 patients with positive anti-ENA but negative ACAs were found and manually screened for SLE. Thus, the patients with positive anti-ENA patients were further subgrouped into SLE and non-SLE. The demographic, clinical and laboratory data of these patients were recorded, and the diagnoses documented by the treating rheumatologists were verified according to the American College of Rheumatology (ACR) criteria for the various rheumatic diseases. For example, the ACR 1982 criteria for SLE were used to verify the disease.

BioPlex method

Bioplex 2200 ANA screen is a fully automated system that determines levels of a panel of autoimmune autoantibodies (Sm, RNP, SSA, SSB, centromere, Scl 70, Jo 1, ribosomal RNP, and chromatin), and it involves multiplexed bead technology to simultaneously perform measurement of the autoantibodies in a single tube [2]. The testing was performed in the Central laboratory at the Cleveland Clinic as per the manufacturer's instruction (BioRad). Anti-dsDNA antibodies were measured by a quantitative ELISA (Binding Site Inc., CA).

Statistical analysis

To determine the diagnostic utility of the ACAs, we utilized the sensitivity, specificity, positive predictive value and negative predictive value. Continuous variables are expressed as mean±SD. Fisher's exact tests are used to examine the significance of the association for categorical variables. Two-sample t-test is used to examine the significance of the age difference in two groups. The correlation coefficient (Phi method) between the ACAs and anti-dsDNA antibodies was determined as a secondary analysis. All analyses were conducted in statistical software R V2.11 (R Foundation for Statistical Computing, Vienna, Austria), with a significance level of 5%.

Results

The clinical utility of ACAs in the diagnosis of SLE

Of a total of 764 patients with rheumatic complaints and positive anti-ENA antibodies, 115 were identified to have positive ACAs, and 93 were diagnosed with SLE. The sensitivity, specificity, positive predictive value and negative predictive value for the ACAs in SLE were 62.4%, 91.50%, 50.4% and 94.6% respectively (**Table 1**). Apart from SLE, other rheumatic diseases associated with positive ACAs included mixed connective tissue disease (MCTD) 12.2% (14/115), undifferentiated CTD(UCTD) 11.3% (13/115), scleroderma 5.2%(6/115), Sjogren's syndrome 4.3%(5/115), rheumatoid arthritis 4.3%(5/115), polymyalgia rheumatica 3.5% (4/115), polymyositis/dermatomyositis 3.4%, drug induced lupus 0.86% and other disorders with unclear diagnoses 4.3% (5/115).

Demographic, clinical and laboratory features of 93 SLE patients

The demographic, clinical and laboratory features of 93 SLE patients in our study are summarized in **Table 2**. The frequency of leucopenia and anti-Sm antibodies was significantly higher in SLE patients with positive ACAs than those without. Neurologic manifestation was less prevalent in SLE patients with positive ACAs. There was no significant difference in the frequency of lupus glomerulonephritis, proteinuria,

Table 2. Demographic, clinical, and laboratory features of 93 SLE patients

	ACAs (+)	ACAs (-)	P value
No.of SLE patients	58	35	
Age(mean+SD) years	46.22±16.33	45.17±16.16	0.763
Female/Male	47/11	32/3	0.237
Race/ethnicity			
White	32	23	
African American	20	10	
Others	6	2	
Malar rash	6(10.3%)	7(20%)	0.226
Alopecia	18(31.0%)	12(34.3%)	0.820
Discoid rash	4(6.9%)	1(2.9%)	0.647
Photosensitivity	10(17.2%)	7(20%)	0.786
Oral ulcers	14(24.1%)	11(31.4%)	0.476
Arthritis	20(34.5%)	13(37.1%)	0.826
Serositis	10(17.2%)	7(20%)	0.786
Nephritis	9(15.5%)	5(14.3%)	1.000
Proteinuria	12(20.7%)	8(22.9%)	0.801
Hematuria	13(22.4%)	7(20%)	1.000
Neurologic	1(1.7%	5(14.3%)	0.027
Leucopenia	16(27.6%)	2(5.7%)	0.013
Thrombocytopenia	8(13.8%)	7(20%)	0.562
Anti-dsDNA	28(48.3%)	11(31.4%)	0.132
Anti-Sm	30(51.7%)	6(17.1%)	0.001

ACAs, anti-chromatin antibodies; (+), positive; (-), negative

or hematuria between the two groups. There was no significant difference in the anti-dsDNA antibodies between the two groups nor positive correlation between the ACAs and anti-dsDNA antibodies.

Discussion

This current retrospective analysis of the study population has shown that 50.4% (58/115) patients with positive ACAs had SLE. The sensitivity and specificity of the ACAs for SLE versus other rheumatic diseases were computed to be 62.3% and 91.5%, and the positive predictive value and negative predictive value were 50.4% and 94.6% respectively. In the past, anti-ENA autoantibodies including the ACAs were measured by such traditional methods as indirect immunofluorescence and ELISA [3]. There were several studies to address the clinical utility of the ACAs measurement in SLE; however, these studies were fully based upon the data obtained using ELISA [3-6]. In these studies, the sensitivity and specificity of the ACAs with ELISA in SLE varied from 64.1 to 93.7% and 97 to 99.2% respectively as opposed to the healthy control and other systemic autoimmune diseases [3, 7].

Since the introduction of the BioPlex 2200 ANA screen, there have been several studies to address its clinical validity [8]. For example, in an evaluation of the clinical value of this method, an analysis of 510 healthy subjects for incidence of natural/predictive autoantibodies concluded that the specificity of the BioPlex 2200 ANA screen analysis of 13 different analytes is comparable to the ELISA ANA screening test [2]. In a study of frozen blood specimens of 192 SLE patients, Hanly and colleagues [9] compared the data on autoantibody profiles obtained using the BioPlex system to the historical data using ELISA, and found reasonable agreement between the detection of lupus autoantibodies by both ELISA and BioPlex. In their study, the frequency of the ACAs by the BioPlex in SLE was 33.9% although no historical data on the antibodies was available to compare with [9]. Apparently the prevalence of the ACAs in SLE in their study is lower than our results. This variation could result from the long-term frozen blood samples. It should be reminded that these two aforementioned studies failed to set up control subjects. In order to address the prevalence of the ACAs by the BioPlex method in SLE, Bardin and colleagues [10] studied 105 patients with

SLE and compared with 96 healthy control subjects. In their study, the prevalence of the ACAs in SLE was 70% which edged higher than our results and 1% in the control. Given the lack of the data on the specificity of the ACA measurement by the BioPlex method in SLE as opposed to other rheumatic diseases, our study aimed to address this issue. As a result, the sensitivity and specificity of the ACAs by the BioPlex method in SLE in our cohort were agreeable with those by traditional ELISA. Our study also demonstrates that the ACAs are not only seen in SLE but also in other rheumatic disorders. Our study agrees and extends the notion that ACAs may not be useful in differentiating between SLE and MCTD [7]/UCTD as a result of the high frequency of the ACAs in these diseases. In another study to examine the specificity of the ACAs, Amoura and colleagues [11] screened 13 different connective tissue diseases and hepatitis C for anti-nucleosome autoantibodies, and detected the antibodies in SLE, scleroderma, and MCTD only but this study was based on the traditional ELISA. The results of our current study are in line with and extend their findings, and demonstrate the wider existence of the ACAs by the BioPlex method in systemic autoimmune disorders other than SLE, scleroderma, and MCTD.

Concerning the relationship between the ACAs and clinical/laboratory features in SLE, most studies have favored the positive correlation between the ACAs and lupus glomerulonephritis [4, 7, 11, 12-14]. However, we found no existence of the reported relationship, being in keeping with other reports [15]. This discrepancy between our results and the other reports could be due to the lower prevalence of lupus glomerulonephritis in our cohort. Interestingly, leucopenia and anti-Sm antibodies were significantly more prevalent in SLE patients with positive ACAs than those without. This observation may deserve further investigation given little data available in the medical literature. Nevertheless, we found no statistically significant correlation between the ACAs and anti-dsDNA antibodies as previously reported, yet they are complementary [7]. Hence, simultaneously measuring anti-dsDNA and anti-chromatin can increase sensitivity in the diagnosis of SLE [3, 10].

Limitations of this study

We must admit that we were unable to address the questions whether there may be a correlation between the ACAs and lupus disease activity, severity, or early SLE as our study aimed to explore the specificity of the ACAs by this new method. In addition, there might be a possibility of selection bias solely based upon the positive anti-ENA antibody screening but its influence if any would be estimated to be minimal given the large sample size. Despite the weaknesses, we can conclude from the current study that measurement of the ACAs by the BioPlex method is as useful as traditional ELISA, and thus of diagnostic value for SLE, although it may not be useful for distinguishing between SLE and MCTD/UCTD.

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