

Clinical Evaluation of a New Quantitative Enzyme-Linked Immunosorbent Assay for Detection of Double-Stranded DNA Autoantibodies

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ABSTRACT: The measurement of autoantibodies specific for double-stranded DNA (anti-dsDNA) is a useful tool for the diagnosis and the prognosis of systemic lupus erythematosus (SLE). A new quantitative enzyme-linked immunosorbent assay (ELISA), ORG anti-dsDNA, is recently available for the determination of anti-dsDNA antibodies. The aim of this study was to evaluate the clinical performance of this new assay in a cohort of SLE patients. Seventy-five sera from SLE patients were tested by two methods for anti-dsDNA determination, ORG anti-dsDNA, and EliA anti-dsDNA. Normal controls were 60 sera from healthy subjects. Moreover, 37 sera from patients with non-SLE connective tissue diseases were tested in parallel. The levels of complement components (C3, C4, CH50) were measured by nephelometry. From SLE patients, 91% were positive against 9% in non-SLE patients and 2% in healthy subjects. The sensitivity, specificity, and Youden test for SLE were 90%, 98%, and 88%, respectively. The Yule test (1%) indicated a close association with the disease. The comparison with EliA anti-dsDNA showed a moderate concordance between the two tests in the group of SLE ($\kappa = 0.51$) and a good concordance in the non-SLE group ($\kappa = 0.89$). A significant inverse correlation was found with complement components levels, biological markers associated with disease activity. Our results show this new assay as sensitive and specific for the diagnosis of SLE. Moreover, the correlation with markers associated with disease activity makes it promising for clinical use.

KEYWORDS: autoantibodies anti-dsDNA; systemic lupus erythematosus; ELISA

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INTRODUCTION

The measurement of autoantibodies specific for double-stranded DNA (anti-dsDNA) is a useful tool for the diagnosis of patients with systemic lupus erythematosus (SLE).^{1,2} Anti-dsDNA antibodies are included in the criteria for the classification of SLE.³ Once the clinical diagnosis of SLE was established, anti-dsDNA antibodies became a valuable tool for monitoring the progression of the disease.⁴ Indeed, increasing anti-dsDNA antibody levels may herald exacerbation of lupus nephritis or other organ involvement. However, the absence of the antibodies does not rule out SLE in any case.⁵

There are several methods used to detect anti-dsDNA antibodies, such as the Farr assay, the *Crithidia luciliae* immunofluorescence technique (CLIFT), and the enzyme-linked immunosorbent assay (ELISA).⁶ More recent methods include techniques, such as bead-based immunoassays (luminex) or fluorescent enzyme immunoassay (EliA).^{7,8}

The Farr assay, referred to as the gold standard, uses radiolabeled dsDNA incubated with serum containing anti-dsDNA antibodies. Immune complexes of dsDNA/anti-dsDNA antibodies are precipitated with ammonium sulfate. *Crithidia luciliae* is a protozoan monoflagellate organism containing a giant kinetoplast packed with mitochondria, in which circular dsDNA is concentrated. Anti-dsDNA antibodies, generally of IgG class, bind dsDNA in the kinetoplast, and are detected by the use of fluorescein conjugates. Labeled kinetoplasts are viewed using fluorescence microscopy. ELISA uses colorimetric reaction to detect antibodies bound to dsDNA. ELISA is easy to perform, gives quantitative results and does not involve the use of any radiolabels. The main difference between these three methods resides in their ability to detect antibodies to dsDNA with varying degrees of avidity and subclass.⁹ In particular, the Farr assay detects high-avidity antibodies believed to be more closely associated with SLE activity, while CLIFT and ELISA detect both high- and low-avidity antibodies.¹⁰ The CLIFT is considered to be the most specific of these three assays, since the antigen includes only dsDNA, which is not contaminated with single-stranded DNA. ELISA measures levels of IgG anti-dsDNA antibodies, which are thought to be more important pathogenically than IgM.² Moreover, the Farr method has become less available because it requires the use of radioactive material and cannot be automated. Therefore, there is increasing reliance on the ELISA for anti-dsDNA antibodies measurement because it is widely available, easy to perform, and spares the use of radiation. However, it tends to produce more false positive results compared to Farr and CLIFT. The reasons for these false positives include an insufficient purification of the antigen employed or dsDNA alteration during the coating of the microtiter plates. The employment of recombinant dsDNA for the coating of the plates should guarantee greater performance.¹¹

A new quantitative ELISA, ORG anti-dsDNA, which uses human recombinant dsDNA bound to microwells, recently became available for the determination of anti-dsDNA antibodies.

The aim of this study was first to test performances of “orgentec” anti-dsDNA ELISA for diagnosis of SLE and second to analyze a potential correlation with markers associated with the disease activity.

MATERIALS AND METHODS

Patients

Seventy-five serum samples from SLE patients were retrospectively analyzed. All patients had at least four ACR criteria for SLE classification.³ The M/F ratio was 10/65 and mean age was 40 ± 13 . Among them two patients were followed for the duration of 1 year.

The control population, age and sex matched, consisted of 60 healthy subjects (blood donors) and 37 non-SLE patients presenting other diseases. This last group, called non-SLE patients, was composed by 30 patients with connective tissue diseases (9 Sharp syndrome, 5 Sjögren's syndrome, 5 scleroderma, 5 CREST syndrome, 4 dermatomyositis, 1 sarcoidosis, 1 Raynaud), 2 patients with autoimmune hepatitis, 1 patient with Crohn's disease, 2 patients with autoimmune thyroid diseases, 1 patient with infectious disease, and 1 patient with Waldenstrom's macroglobulinemia.

Laboratory Measurements

Orgentec (ORG) Anti-dsDNA Measurements (Orgentec, Plaisir, France)

One hundred microliter per well of diluted serum samples (in 1:100 in PBS BSA), controls and standard solutions were added to microwells coated with human recombinant dsDNA and incubated for 30 min at room temperature (RT). After three washes with PBS, horseradish peroxidase (HRP)-conjugated anti-human IgG was incubated for 15 min at RT. After washes, 100 μ L per well of TMB (3,3',5,5'-Tetramethyl-benzidine) substrate were added and incubated for 15 min at RT. After incubation of 100 μ L per well of a stop solution (1 M hydrochloric acid) for 5 min at RT, the reaction was read at 450 nm. Results (IU/mL) were calculated using calibrators provided by Orgentec.

Phadia (EliA) Anti-dsDNA Measurements (Phadia, Freiburg, Germany)

This method used circular recombinant plasmid dsDNA. Single polystyrene wells were automatically filled and processed in the UNICAP 100 instrument (Phadia) at 37°C. Values higher than 16 IU/mL were considered positive.

Levels of Serum Complements

C3 and C4 were measured by nephelometry.¹²

Statistical Analysis

The concordance between the methods was assessed by the coefficient κ determination.¹³ The diagnostic value of the test was assessed by the Yule and Youden coefficients determination.¹⁴ Correlation of variables was assessed using Spearman's rank correlation. A *P*-value <0.05 was considered as statistically significant.

RESULTS

Analytical Parameters of ORG Anti-dsDNA Assay

The cutoff value was assessed by the method of percentile (99th) using 60 sera from normal controls and was determined at 20 IU/mL. This result agrees with the cutoff value recommended by the manufacturer. The sensitivity, specificity, PPV, and NPV were 90%, 98%, 99%, 88%, respectively. Youden and Yule coefficients, reflecting the diagnostic value of the test, were 88% and 1%, respectively.

Analytical parameters were also calculated with a larger control group including both normal subjects and non-SLE patients (described in the "Materials and Methods" section). The sensitivity, specificity, PPV, NPV, Youden and Yule coefficients were then 90%, 88%, 85%, 92%, 78%, and 97%, respectively. The intra- and interassay CV, calculated from six positive samples, ranged from 1% to 3% and from 3% to 10%, respectively.

Distribution of Anti-dsDNA Antibodies and Correlation between Assays

Sera samples from SLE ($n = 75$), non-SLE ($n = 37$) and healthy subjects ($n = 60$) were analyzed. Anti-dsDNA antibodies were found in 91% of SLE patients, in 9% of non-SLE patients, and in 2% of healthy subjects (TABLE 1).

A comparison was made with EliA anti-dsDNA assays (TABLE 1). Good concordance was found between the two methods for non-SLE patients ($\kappa = 0.89$) whereas moderate concordance was found for SLE patients ($\kappa = 0.51$). In SLE patients group, 12% of results were discordants. Among them, most of positive results detected using ORG anti-dsDNA assays were found close to the cutoff value with EliA anti-dsDNA assays.

TABLE 1. Prevalence (%) and concordance of anti-dsDNA detection by two techniques

Patients (<i>n</i> =)	Orgentec anti-dsDNA		Phadia anti-dsDNA		Concordance (κ)
	Positive (%)	Negative (%)	Positive (%)	Negative (%)	
SLE (<i>n</i> = 75)	91	9	81	19	<i>0.51</i>
Non-SLE (<i>n</i> = 37)	36	64	33	67	<i>0.89</i>
Healthy Subjects (<i>n</i> = 60)	2	98	2	98	<i>1</i>

***Relationship between Anti-dsDNA Antibody Levels and Markers
Associated with the Disease Activity***

We examined the correlation between ORG anti-dsDNA antibody levels and C3, C4, and CH50 levels, markers associated with clinical activity of the disease.¹³ A significant inverse correlation was found with C3 ($P = 0.0005$), C4 ($P = 0.02$), and CH50 levels ($P = 0.02$).

To further analyze these results, two patients were followed over 1 year (FIG. 1). Three samples were collected from each patient at different times. For patient 1, a progressive augmentation of anti-dsDNA antibody levels was inversely associated with a gradual reduction of CH50, C3, and C4 levels. In contrast, for patient 2, the levels of anti-dsDNA antibody were dramatically decreased from the second sample. This decrease was associated with an upregulation of the complement components levels.

DISCUSSION

The measurement of anti-dsDNA antibodies is largely used for the diagnosis and monitoring of SLE. The ELISA is becoming the prevalent method used in routine laboratory practice because it is automated and it expresses a quantitative result. The type of assay used influences the results in terms of sensitivity and specificity.

We evaluated the analytical performances of ORG anti-dsDNA, a new ELISA for the measurement of anti-dsDNA antibodies. Because it employs recombinant plasmid dsDNA bound directly to the plastic surface, it should guarantee a greater analytical specificity than traditional ELISA, which used purified DNA. Our findings showed that ORG anti-dsDNA had good analytical performances in terms of sensibility and specificity. For diagnosis, specificity is most important whereas for monitoring the disease, sensitivity is most useful. The diagnostic value of the assay was assessed by the high levels of Youden and Yule coefficients.

In this study, anti-dsDNA antibodies were found with a higher prevalence in SLE patients by using ORG anti-dsDNA compared to EliA anti-dsDNA

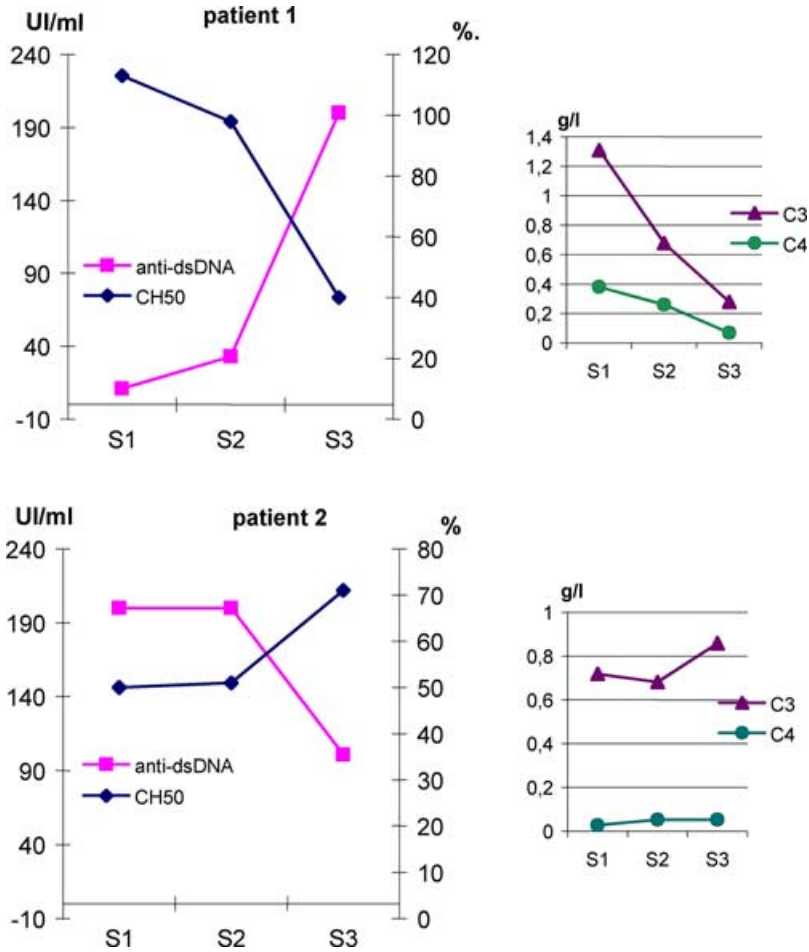


FIGURE 1. Anti-ds DNA antibody levels over time in two systemic lupus patients. For each patient three samples (S1, S2, S3) were collected at different times over 1 year. Analyses were performed in comparison with CH50, C3, and C4 levels.

assays (91 versus 81%). Nevertheless, discrepant results were close to the cutoff value with EliA anti-dsDNA assays. Discrepancies in the results could be due to difference in avidity of anti-dsDNA antibodies detected. Each assay has its advantages and there is no single test to cover both sensitivity and specificity simultaneously. In practice, the combined use of the two tests could be helpful in some cases to confirm results that are not in agreement with the clinical state of the patient.

Consumption of complement proteins due to activation of the classical pathway by immune complexes accounted for the hypocomplementemia noted in

active disease.¹⁵ Patients with active extrarenal or renal diseases were more likely to demonstrate the lowest levels of CH50, C3, and C4. Our study showed that ORG anti-dsDNA antibody levels were inversely correlated with the serum levels of CH50, C3, and C4. Interestingly, the follow-up was performed for two patients and we found, as reported in the literature,¹⁶ that single point determinations of complement components are less important than the trend of serial measurements. Indeed, the fluctuations of ORG anti-dsDNA antibody levels remained associated with inverse variations of complement proteins levels over time. Altogether our results showed that ORG anti-dsDNA assay is reliable for monitoring the disease activity. In conclusion, good analytical performances of ORG anti-dsDNA ELISA demonstrated that it is a useful test for diagnosing and monitoring SLE.

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