Clinical Evaluation of Cobas Core Anti-dsDNA EIA Quant

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The measurement of antibodies to double-stranded DNA (anti-dsDNA) is a useful tool for the diagnosis and monitoring of patients with connective tissue diseases, particularly systemic lupus erythematosus (SLE). The aim of the present study was to compare a new enzyme-linked immunosorbent assay (ELISA) for the measurement of anti-dsDNA antibodies, which uses purified double-stranded plasmid DNA as the antigen (anti-dsDNA EIA Quant; Roche Diagnostics, Mannheim, Germany), with an established ELISA. The clinical usefulness of this new ELISA was also assessed. We measured anti-dsDNA antibodies in 398 serum samples that were divided into four groups: 1) routine samples sent to our laboratory for an antinuclear antibody (ANA) test (n=229), 2) samples from blood donors (n=74), 3) samples from patients

with SLE (n=48), and 4) samples from patients with other autoimmune diseases (n=47). The methods used were the Cobas Core Anti-dsDNA EIA Quant (Roche Diagnostics, Mannheim, Germany) and the Anti-dsDNA test (Gull Diagnostics, Bois d'Arcy, France). We obtained a kappa index and Spearman correlation coefficient in the comparative study, and sensitivity, specificity, predictive values, and likelihood ratios in the clinical study. The results obtained show a good agreement between the two methods in both the qualitative results ($\kappa=0.91$) and the quantitative data ($r=0.854$). The best accuracy, predictive values, likelihood ratios, and correlation with active disease were obtained with the Roche anti-dsDNA assay. *J. Clin. Lab. Anal.* 18:200–205, 2004. © 2004 Wiley-Liss, Inc.

Key words: anti-dsDNA antibodies; clinical sensitivity; clinical specificity; likelihood ratios; predictive values; systemic lupus erythematosus

INTRODUCTION

The measurement of antibodies to double-stranded DNA (anti-dsDNA) is a useful tool for the diagnosis and monitoring of patients with connective tissue diseases, particularly systemic lupus erythematosus (SLE) (1). Autoantibodies to dsDNA were included in *The 1982 Revised Criteria for the Classification of Systemic Lupus Erythematosus* by the American College of Rheumatology (2). Once the clinical diagnosis of SLE based on those criteria was established, dsDNA antibodies became a valuable tool for monitoring the progression of the disease (3,4). However, the absence of these antibodies does not rule out SLE in any case.

The issue of which method is best for detecting anti-dsDNA antibodies remains controversial (5). The most common techniques are the Farr immunoprecipitation assay, *Crithidia lucilae* indirect immunofluorescence, and enzyme immunoassay (EIA). Different methods are able to detect anti-dsDNA antibodies of different affinities (6). The Farr assay detects high-avidity

antibodies, the *Crithidia* assay detects medium-to-high-avidity antibodies, and the EIA detects both high- and low-avidity antibodies (7).

The aim of the present study was to compare two methods: 1) a new enzyme-linked immunosorbent assay (ELISA) for anti-dsDNA antibody measurement, which uses a purified double-stranded plasmid DNA as the antigen (anti-dsDNA EIA Quant; Roche Diagnostics, Mannheim, Germany), and 2) an established ELISA (dsDNA ELISA; Gull Diagnostics, Bois d'Arcy, France). The clinical usefulness of this new ELISA was also assessed.

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MATERIALS AND METHODS

Patient Samples

A total of 398 serum samples were analyzed. The samples were divided into four groups: 1) routine samples sent to our laboratory for an antinuclear antibody (ANA) test ($n=229$), 2) samples from blood donors ($n=74$), 3) samples from patients with SLE ($n=48$), and 4) samples from patients with other autoimmune diseases ($n=47$). In the latter group, the patients' diagnoses included rheumatoid arthritis ($n=5$), Sjögren's syndrome ($n=9$), diffuse systemic sclerosis ($n=2$), CREST syndrome ($n=2$), polymyositis ($n=3$), vasculitis ($n=5$), primary antiphospholipid syndrome ($n=1$), limited scleroderma ($n=2$), discoid lupus ($n=4$), primary biliary cirrhosis ($n=2$), autoimmune hepatitis ($n=2$), primary Raynaud's phenomenon ($n=3$), and undefined systemic autoimmune diseases ($n=7$).

Disease activity in SLE was assessed at the time the subjects were enrolled in the study, and was based on the clinical and analytical criteria set forth in the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) (8).

To study the methodological interferences of high IgG and IgM concentrations on Cobas Core Anti-dsDNA, we used three different sera pools with low, medium, and high anti-dsDNA levels, and mixed them with different amounts of serum containing the interfering substance. We compared the results from each sera pool with those obtained from the same pool with various concentrations of the interfering substance. Serum samples from two patients with multiple myeloma (IgG and IgM) were used. The concentrations of IgG and IgM were 3730 mg/dL and 7830 mg/dL, respectively. Neither of the samples exhibited anti-dsDNA antibodies.

Blood samples were collected in plain tubes from fasting individuals. After the blood coagulated, the sera were aliquotted and stored at -80°C until the measurements were performed. The samples were thawed only once.

Cobas Core Anti-dsDNA

The Cobas Core Anti-dsDNA EIA Quant is an indirect, noncompetitive, solid-phase EIA for the quantitative detection of antibodies against double-stranded DNA in human serum or plasma. It uses purified recombinant plasmid DNA as the antigen. The method is fully automated on the Cobas Core instrument.

The method is briefly described as follows: During the first incubation step, specimens are incubated with recombinant dsDNA immobilized on the solid phase of a polystyrene bead. Antibodies directed against

dsDNA bind to the immobilized dsDNA. The beads are washed to remove unbound material. Bound human IgG antibodies are labeled during the second incubation with mouse (monoclonal) anti-human IgG conjugated with horseradish peroxidase. After a second washing step, the beads are incubated with an enzyme-substrate solution. Then the intensity of the color produced by the enzyme reaction, which is proportional to the concentration of anti-dsDNA in the specimens, is read off. Concentrations in IU/mL are calculated based on the calibrators, which have been calibrated against the International WHO Wo/80 anti-dsDNA reference preparations (9). The linear range is 0–120 IU/mL. The results obtained with the Cobas Core Anti-dsDNA EIA Quant are interpreted as: negative = <25 IU/mL, gray zone = 25–35 IU/mL, and positive = >35 IU/mL.

Gull dsDNA ELISA

The anti-dsDNA test is a quantitative indirect EIA for the detection in human serum or plasma of antibodies to dsDNA, which uses calf thymus DNA as the antigen. Briefly, the wells of microtiter strips are coated with calf thymus DNA. During the first incubation, specific autoantibodies in diluted serum or plasma will bind to the antigen coating. The wells are then washed to remove unbound serum or plasma components. A conjugate of enzyme-labeled monoclonal antibodies to human IgG and IgM binds to surface-bound antibodies in the second incubation. After a further washing step, specific antibodies are traced by incubation with substrate solution. The addition of a stop solution terminates the reaction and provides the appropriate pH for color development. The amount of conjugate bound is measured in absorbance units. In the quantitative protocol, the concentration of anti-dsDNA antibody in an unknown sample is estimated by interpolation from a dose-response curve based on reference standards calibrated against WHO Reference Preparation Wo/80 (8). The results are reported in IU/mL. The asymptomatic reference range has been established by the manufacturer as <30 IU/mL. The gray zone includes 30–50 IU/mL.

Statistical Analysis

Statistical analysis was performed with the use of the SPSS v 10.0 statistical software package (SPSS Inc., Chicago, IL). Concordance between the qualitative results obtained with both methods (Roche and Gull) was determined by means of the κ statistic. We compared the quantitative results obtained with both methods by a nonparametric Spearman correlation. In the clinical evaluation, we determined the sensitivity, specificity, predictive values, and likelihood ratios.

RESULTS

Analytical Characteristics of the Assay

Before we conducted the clinical evaluation, we carried out a short analytical evaluation. We performed precision profiles by analysing 20 aliquots of three samples with low, normal, and high anti-dsDNA concentrations (intra-assay precision), and aliquots of the same samples for 20 consecutive days (interassay precision). The results are shown in Table 1. The detection limit obtained by assaying 20 replicates of calibrator 0 as 2 standard deviations of the mean was <1 IU/mL. The manufacturer claims that no significant interference has been observed with sera containing haemoglobin, lipids, or bilirubin. In the current study, IgG and IgM at high concentrations (1865 mg/dL and 3915 mg/dL, respectively) did not interfere with the Cobas Core Anti-dsDNA results.

Comparison of Methods

Using both of the anti-dsDNA methods, we analyzed 229 samples sent to our laboratory for routine ANA testing. The concordance of the qualitative results is shown in Table 2. We used two cutoff values for the Gull method, but we used only one for the Cobas Core Anti-dsDNA because in the latter method there is no difference in sample classification between both cutoffs (25 and 35 IU/mL). Good concordance was seen between the qualitative results obtained with the Roche and Gull methods, with a cutoff of 50 IU/mL for Gull ($\kappa=0.84$), and a very good concordance was seen with a cutoff of 35 IU/mL for Gull ($\kappa=0.91$). The results were

97.8% coincident for the 30 IU/mL cutoff, and 96.5% coincident for the 50 IU/mL cutoff. The quantitative results obtained by both methods are shown in Fig. 1. There was a linear relation between the Gull and Cobas Core Anti-dsDNA tests with a Spearman correlation coefficient of 0.854; $P<0.001$ ($n=229$). The relation between the low concentrations of anti-dsDNA is shown in the inset of Fig. 1.

Clinical Studies

The results obtained from the groups of SLE patients, other autoimmune diseases, and blood donors, using the cutoffs recommended by the manufacturers as clearly positive (35 and 50 IU/mL for the Roche and Gull methods, respectively), are shown in Table 3. Of 48 serum samples from patients with SLE, 20 were positive according to the Roche method (42%), and 13 were positive with the Gull method (27%). In the group of other autoimmune diseases, two of 47 serum samples were positive according to both methods. One sample was from a patient with antiphospholipid syndrome, and the other was from a patient with an undefined systemic autoimmune disease. In the blood donor group, no positive results were obtained.

The diagnostic sensitivity and specificity, positive and negative predictive values, and likelihood ratios for SLE obtained with both methods are shown in Table 4. A prevalence of 18.6% was used, and the group of other autoimmune diseases was used as the contrast group. The same specificity was obtained with both methods (96%), but the Roche technique provided a greater

TABLE 1. Cobas Core anti-dsDNA intra-assay and inter-assay precision for three samples with low, medium, and high anti-dsDNA concentrations

	Intra-assay			Inter-assay		
	Mean (IU/ml)	SD (IU/ml)	CV (%)	Mean (IU/ml)	SD (IU/ml)	CV (%)
Low	21.0	1.0	4.9	20.5	1.9	9.3
Medium	44.8	1.1	2.5	39.7	3.3	8.3
High	97.6	3.9	4.0	87.0	5.0	5.8

TABLE 2. Comparison of qualitative results at different cut-offs

Roche	Gull, 30 IU/mL			Gull, 50 IU/mL		
	Negative	Positive	Total	Negative	Positive	Total
Negative	195	5	200	197	3	200
Positive	0	29	29	5	24	29
Total	195	34	229	202	27	229

Kappa indexes: 0.91 (cut-off = 30 UI/ml) and 0.84 (cut-off = 50 UI/ml).

Fig. 1. Comparison of the quantitative results obtained by the two methods (n = 229 samples). Linear regression equation $y = -0.776 + 0.484x$, $S_{yx} = 17.8$; Spearman correlation coefficient $r = 0.854$. Inset: Increased resolution of low anti-dsDNA.

TABLE 3. Serum anti-dsDNA antibodies detected in patients with SLE and other autoimmune diseases by the Roche and Gull assays

Patients	Roche anti-dsDNA EIA		Gull anti-dsDNA EIA	
	Positive	Negative	Positive	Negative
SLE (n = 48)	20 (42%)	28 (58%)	13 (27%)	35 (73%)
Other AD (n = 47)	2 (4%)	45 (96%)	2 (4%)	45 (96%)
Blood donors (n = 74)	0	74	0	74

TABLE 4. Sensitivity, specificity, predictive values, and likelihood ratios of anti-dsDNA antibodies measured by EIA applied to patients with SLE and other autoimmune diseases

	Roche anti-dsDNA EIA	Gull anti-dsDNA EIA
Sensitivity (%)	41.7	27.1
Specificity (%)	95.7	95.9
PPV (%)	71.1	61.3
NPV (%)	28.0	23.6
LR ⁺	9.7	6.8
LR ⁻	0.6	0.8

TABLE 5. SLE activity and anti-dsDNA antibodies detected by the Roche and Gull assays

SLE	Roche anti-dsDNA EIA			Gull anti-dsDNA EIA		
	Negative	Positive	Total	Negative	Positive	Total
Active disease	2	9	11	5	6	11
Non-active disease	9	3	12	10	2	12

Gull anti dsDNA Ab EIA: χ^2 with continuity correction and Fisher F tests non-significant. Roche anti dsDNA Ab EIA: χ^2 with continuity correction ($p=0.032$) and Fisher F tests ($p=0.030$) statistically significant.

sensitivity (42%) than the Gull technique (27%). Likewise, the Roche method provided better positive and negative predictive values than the Gull technique (Table 4).

Relationship Between Anti-dsDNA and Disease Activity in SLE

Table 5 shows the results obtained when disease activity in SLE patients was taken into account. No significant association was observed between anti-dsDNA and disease activity with the Gull method, whereas a significant association ($P=0.03$) between disease activity and anti-dsDNA was found with the Roche method.

DISCUSSION

There is no definitive method for the measurement of anti-dsDNA antibodies. Comparative studies between different methods have provided conflicting results (6). While some studies (10,11) obtained a low correlation (<0.5) between the different methods assayed, others reported a better agreement (12,13). In the current study, we obtained a very good agreement in the qualitative results with a high percentage of coincidence. The quantitative data provided a moderate linear relationship with a significant correlation coefficient (0.854).

In terms of reproducibility, the Roche kit showed a relatively high precision. This is in agreement with a previous analysis (14) of other EIA kits for ANAs of defined specificities.

The clinical usefulness of a test is reflected by the diagnostic sensitivity and specificity achieved, and high values of both characteristics are desirable. However, as the decision level of the test is changed, specificity and sensitivity move in opposite directions. For diagnosis, the most important aspect is specificity, whereas for monitoring the most useful one is sensitivity. Since anti-dsDNA antibodies are used for both diagnosis and follow-up, both qualities are important.

The method evaluated in the present study (the Roche Cobas Core Anti-dsDNA) has a high specificity (96%), which makes it an appropriate first-line method for screening. However, it did not have a very high sensitivity (42%) in this study. This may be because most of the SLE patients studied here were undergoing treatment. In a significant number of patients, the disease was inactive or in remission. The Roche method uses plasmid dsDNA instead of calf thymus as the source of antigen, which may explain the better accuracy obtained. Using the dsDNA AF-CDC1 standard, Tan et al. (14) evaluated different anti-dsDNA antibody EIAs. They obtained better accuracy when plasmid dsDNA was used as the antigen. They also considered the use of dsDNA plasmid to be a factor in the increased accuracy of the anti-dsDNA antibody measurement (14).

Anti-dsDNA antibodies correlate with disease activity. However, the correlation is not as strong as that seen for diagnosis, and the methodology used may affect this (15). When only patients with active and non-active SLE were considered, a significant association with activity was found only when the anti-dsDNA antibodies were measured according to the Roche method.

As far as predictive values are concerned, it should be emphasized that these apply only to the defined population used to obtain the estimation of prevalence. In the current study, this population consisted of only those patients with SLE. To avoid the influence of prevalence, we considered the likelihood ratio. This index is independent of prevalence. A positive likelihood ratio close to or higher than 10 notably increases post-test SLE probability, while a close to 0.1 negative ratio decreases post-test SLE risk (16,17). In our study, better likelihood ratios were obtained with the Roche anti-dsDNA. The positive likelihood ratio was 9.7, and the negative likelihood ratio was 0.6.

In summary, a good agreement was obtained between the two EIA methods studied, but the best accuracy, predictive values, likelihood ratios, and correlation with active disease were obtained with the Roche anti-dsDNA assay, which uses plasmid dsDNA as the antigen.

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