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Comparison of the Farr radioimmunoassay, 3 commercial enzyme immunoassays and *Crithidia luciliae* immunofluorescence test for diagnosis and activity assessment of systemic lupus erythematosus

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ABSTRACT

Backgrounds: Among anti-double-strand (ds)DNA antibody assays, Farr radioimmunoassay is decreasingly used because it requires radioactive material and is labor intensive. We evaluated the performance of Farr, three commercial enzyme immunoassays (EIAs) and the *Crithidia luciliae* immunofluorescence test (CLIFT) in systemic lupus erythematosus (SLE).
Methods: Anti-dsDNA antibodies were determined in 99 SLE patients, 101 healthy subjects, and 53 patients with autoimmune rheumatic diseases.
Results: Farr performed better than the 3 EIAs and CLIFT for the diagnosis of SLE at the manufacturer's cut off and at the cut off set to achieve a specificity of 95%. To achieve a similar level of specificity, some EIAs had a decrease in sensitivity which was dramatic for some tests. Farr was also the best at distinguishing patients with quiescent to mildly active disease from patients with more active disease at the cut off value of 93 IU/ml. Using manufacturer's cut off did not allow distinguishing between patients with quiescent and active SLE.
Conclusions: Farr was the best global test to assess the level of anti-dsDNA antibodies for both diagnosis and disease activity evaluation in SLE with adequately determined cut off values. Some EIA had low performances limiting their use in decision-making regarding diagnosis and/or treatment.

1. Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune disorder characterized by remissions and exacerbations [1]. Differentiating patients with SLE from those with other autoimmune rheumatic diseases can be difficult because they may share clinical and biological features [2]. Autoantibodies to double strand (ds) DNA are considered a key diagnostic test for SLE and are included in the American College of Rheumatology (ACR) criteria for the disease as a formal classification tool [3]. Some previous studies appeared to show that the level of anti-dsDNA antibodies was correlated with the activity of SLE whereas others failed to show any clear correlation [4,5]. Such discrepancies might be due to variations in techniques or interpretation in assessing the presence and the level of anti-dsDNA antibodies. Indeed, the sensitivity and specificity of anti-dsDNA antibodies for both the diagnosis of SLE and the assessment of disease activity depend closely on three parameters: the techniques used to assess the presence and measure the level of anti-dsDNA antibodies, the control group used (either healthy subjects or patients with other autoimmune rheumatic diseases) and finally the cut off value chosen to give a positive or a negative result. The gold standard for determining the presence of anti-dsDNA antibodies is usually Farr, a radioimmunoassay [6]. Enzyme immunoassays (EIAs) and the *Crithidia luciliae* immunofluorescence test (CLIFT) are also widely used [6,7], mainly because the Farr method requires radioactive material and is more labor intensive. Moreover, new generations of automated EIAs

Abbreviations: SLE, systemic lupus erythematosus; ds, double strand; ACR, American College of Rheumatology; EIA, enzyme immunoassay; CLIFT, *Crithidia luciliae* immunofluorescence test; SLEDAI-2K, Systemic Lupus Erythematosus Disease Activity Index 2000; AUC, area under the curve.

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are now available in medical laboratories and are presented as performing better in terms of specificity than previous EIAs on microplate [6]. However, these anti-dsDNA antibody assays could have different levels of performance for the diagnosis of SLE and disease activity assessment [8–10]. Indeed, some studies recently showed that Farr was superior to some EIAs for the diagnosis of SLE [2] or for the correlation with measures of global SLE activity [11].

The aim of our study was to compare the diagnostic performance for SLE of Farr, one microplate EIA, 2 automated EIAs and CLIFT. We included both a control group of healthy subjects and a control group of patients with other autoimmune rheumatic diseases. In addition to using the cut off values provided by the manufacturers, we also determined for each assay both cut off value and sensitivity when we set the specificity to 95% for SLE diagnosis and disease activity assessment.

2. Patients and methods

2.1. Patients

The study retrospectively included 99 consecutive unselected patients with SLE (92 females; median age, 32 years; range, 18–76 years), classified according to the 1997 revised ACR criteria [3], who attended the outpatient Department of Internal Medicine at Lille University Hospital. Renal involvement was biopsy proven. Seven teen patients had a history of renal involvement and 5 patients had active nephritis at the time of blood sample. For each patient, all items of the SLE Disease Activity Index 2000 (SLEDAI-2K) were recorded. The SLEDAI-2K was then calculated per standard protocol [12]. For the purpose of this study, the anti-dsDNA antibody component of the SLEDAI-2K was excluded to allow us to evaluate the performance of the anti-dsDNA antibody assays. Therefore, in this study, mSLEDAI-2K refers to the SLEDAI-2K instrument modified by excluding the anti-DNA antibody component. We classified SLE disease activity as quiescent (mSLEDAI-2K = 0), mild (0<mSLEDAI-2K≤4) or moderate to severe (mSLEDAI-2K>4).

Healthy controls comprised 101 blood donors (86 females; median age, 36 years; range, 19–66 years) matched for sex and age with SLE patients. The control group of patients with other autoimmune rheumatic diseases was constituted prospectively and included 53 patients (46 females; median age, 47 years; range, 18–74 years) comprising 16 patients with rheumatoid arthritis according to the ACR criteria [13], 18 patients with primary Sjögren's syndrome according to the revised American–European classification criteria [14], 12 patients with systemic sclerosis according to the Leroy and Medsger criteria [15], and 7 patients with polymyositis according to the Peter and Bohan criteria [16].

2.2. Laboratory measurements

Sera were prospectively collected and several aliquots were generated from each sample and were stored at −20 °C until they were evaluated within 12 months after blood sampling. In the first part of the study, we evaluated the diagnostic value for SLE of five anti-dsDNA kits. Of these 5 kits, 3 were based on EIA: one microplate method (Diastat®, Axis Shield, UK) and two automated assays (Liaison®, Diasorin, Antony, France and Elia®, Freiburg, Germany). Anti-dsDNA antibodies were also assayed using Farr (Trinity Biotech, Ireland) and CLIFT (Diasorin, Antony, France). Details of the 5 assays are given in Table 1. All the kits were evaluated in a “one run” time, according to the manufacturer's instructions and blinded to the clinical data.

In the second part of the study, we compared the performance of the 3 EIA tests, Farr and CLIFT in assessing SLE activity according to mSLEDAI.

2.3. Statistical analysis

Statistical analysis was performed using the SPSS 11 Statistical Software. For each test, we determined the sensitivity, specificity and likelihood ratio (LR) for the diagnosis of SLE and disease activity assessment using the cut off value provided by the manufacturers. Area under curve (AUC) was assessed by using the ROC curve method. Then, we set the specificity to 95% and determined the new cut off value to achieve this specificity, the new sensitivity and LR. For numerical parameters, the group comparisons were performed by means of analysis of variance and multiple comparisons were performed using Bonferroni correction. Significant relations between numerical variables were evaluated by Spearman's correlation coefficient.

3. Results

3.1. Diagnostic performance of the three EIAs, Farr, and CLIFT

3.1.1. Diagnostic performance for SLE of the three EIAs, Farr and CLIFT using healthy subjects as a control group (Table 2)

Using the cut off value provided by the manufacturer, we found that Farr had the highest sensitivity and specificity for SLE diagnosis when compared to the 3 EIAs. The LR for SLE diagnosis was higher with a positive Farr result than a positive EIA result (Table 2).

3.1.2. Diagnostic performance for SLE of the three EIAs, Farr, and CLIFT using patients with other autoimmune rheumatic diseases as a control group (Table 3)

When we used patients with other autoimmune rheumatic diseases as a control group, we observed a decrease in specificity for each test (except CLIFT) at the manufacturer's cut off (Table 3A). When we set the specificity to 95% for each test (except for CLIFT which had a specificity of 100%) (Table 3B), we observed a dramatic increase in cut off value for one EIA (Diastat®), a moderate increase for another EIA (Liaison®) whereas Farr and Elia® cut offs were quite similar to manufacturer's cut off. With a specificity set to 95%, Farr assay had the best sensitivity and positive as well as negative LR whereas Diastat® and Liaison® experienced a significant decrease in sensitivity (from 77.8% to 35.4% for Diastat® and from 78.6% to 67.3% for Liaison®). Sensitivity of Elia® did not change significantly but was lower than Farr sensitivity (62.1% vs 75.8%). Overall, for a specificity

Table 1
Characteristics of the six EIAs, Farr and the *Crithidia luciliae* immunofluorescence test.

Assay	Manufacturer	Method	Isotypes detection	dsDNA origins	Manufacturer cut off
Diastat® anti-dsDNA	Axis Shield, UK	Microplate/manual	IgG, IgM	Calf-thymus anti-dsDNA	30 IU/ml
Liaison® dsDNA	Diasorin, Antony, France	Automate, Liaison®	IgG	Human recombinant dsDNA	20 IU/ml
Elia® dsDNA	Phadia (Pharmacia), Freiburg, Germany	Automate, Unicap®	IgG	Circular recombinant plasmid dsDNA	15 IU/ml
Anti-dsDNA CL	Diasorin, Antony, France	CLIFT/manual	IgG	Kinetoplast DNA	Presence of a fluorescence of the kinetoplaste for a serum diluted at 1/10
Farr	Trinity Biotech, Ireland	RIA/manual	IgG, IgM	Recombinant plasmid dsDNA	7 IU/ml

EIA, enzyme immunoassay; CLIFT, *Crithidia luciliae* immunofluorescence test; RIA, radioimmunoassay.