Anti-dsDNA Antibody Testing by Farr and ELISA Techniques Is Not Equivalent

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ABSTRACT. ***Objective.*** To determine the degree of correlation between Farr and ELISA methods of detecting anti-dsDNA antibodies in patients with systemic lupus erythematosus (SLE), and their association with measures of disease activity.

Methods. Anti-dsDNA antibodies were assayed using the Farr and ELISA methods in patients followed between January 1, 2000, and December 31, 2002. Statistical correlations between Farr and ELISA were determined. Relationships between the 2 assays and measures of disease activity [SLE Disease Activity Index 2000 (SLEDAI-2K-DNA), renal, central nervous system (CNS), and vasculitis] were determined for the same clinic visit.

Results. 550 patients with 2940 clinic visits met the inclusion criteria. Correlation between Farr and ELISA levels was 0.46 using the first visit for each patient. When the Farr was abnormal, the ELISA was equally likely to be normal or abnormal. Abnormal Farr results were associated with higher SLEDAI-2K scores than normal Farr results (6.2 vs 4.3, respectively; $p < 0.0001$). There was less of a distinction with ELISA results (5.9 vs 4.8; $p = 0.04$). Farr levels were significantly associated with the presence of renal disease and vasculitis, while ELISA levels were not. Neither Farr nor ELISA results correlated with the presence of active CNS involvement.

Conclusion. Farr and ELISA techniques for the detection of anti-dsDNA antibodies in patients with SLE are poorly correlated. The Farr is superior to the ELISA in correlating with measures of global disease activity, as well as renal and vasculitis involvement. The Farr technique should continue to be used in clinical practice. The ELISA adds no additional information. (First Release Aug 15 2006; J Rheumatology 2006;33:1785–8)

Key Indexing Terms:
SYSTEMIC LUPUS ERYTHEMATOSUS FARR ASSAY
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Anti-double-stranded DNA (anti-dsDNA) antibodies are useful as a diagnostic test for systemic lupus erythematosus (SLE), reportedly about 97% specific for the diagnosis[1]. They are also used as a prognostic test for overall disease activity, as well as certain organ-specific involvement. Classically, anti-dsDNA antibodies are associated with renal disease activity, but not central nervous system (CNS) involvement[2-4]. Despite such associations, some patients with SLE are known to have serologic and clinical discordance, being either serologically active and clinically quiescent (SACQ) or clinically active and serologically quiescent (CASQ)[5,6]. It is unclear whether such discordance is a reflection of the true native disease states, or rather an indication of deficiencies in laboratory detection methods.

A number of factors may contribute to discrepant anti-dsDNA antibody detection abilities of laboratory assays. First, variation exists in the types of anti-dsDNA antibodies that may be detected. Anti-dsDNA antibodies may differ according to properties that influence their pathogenicity, including isotype (IgG, IgM), charge, complement-fixing ability, and avidity. For example, high avidity antibodies are felt to be more closely correlated with renal disease than low avidity antibodies[7]. Second, variation exists in the specific techniques used to measure anti-dsDNA antibodies. Such methodologic differences can affect the type, and therefore the importance and relevance, of antibody detected. For example, assays differ in the isotype or avidity of the antibodies detected, and some demonstrate false-positive results due to single-stranded DNA contamination or antibodies binding to other proteins.

The 2 most common methods in current use for detection of anti-dsDNA antibodies are the Farr and enzyme-linked immunosorbent assay (ELISA) techniques. The Farr is a radioimmunoassay, while the ELISA method uses a colorimetric reaction to detect antibodies bound to the antigen. Studies have revealed varying degrees of correlation between the 2 tests and their ability to mirror disease activity in

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patients with SLE, and have argued that either technique can be used in practice[8]. The Farr method has become less and less available, possibly due to the use of radioactive material and the fact that it is more labor-intensive. As a result, there is increasing reliance on the ELISA for anti-dsDNA antibody measurements. Because of the probable need to rely solely on the ELISA in the near future, we sought to determine how well the detection of anti-dsDNA antibody by Farr and ELISA techniques correlate with one another, with disease activity, and with complement levels in a large cohort of patients with SLE who have had anti-dsDNA antibody measured prospectively by both methods over a number of years.

MATERIALS AND METHODS

Patients. Patients followed in the University of Toronto Lupus Clinic between January 1, 2000, and December 31, 2002, who had anti-dsDNA antibody measurement by both Farr and ELISA techniques were identified. Patients followed at this clinic since 1970 are registered in a computer database with relevant clinical and laboratory information recorded. As of December 31, 2002, there were 1106 patients in the database. A total of 579 patients were seen between January 1, 2000, and December 31, 2002. A standard protocol is used and stored in the database for each of the clinic visits, which are scheduled every 3–4 months, regardless of clinical status. The protocol includes demographic data, organ-specific disease-related symptoms, physical findings, current medications, and recent laboratory values. In addition, clinical notes are recorded in patient charts at each visit. The longterm observational cohort study was approved by the Institutional Ethics Board. All clinic patients provided written informed consent agreeing to the use of the information for scientific study.

Laboratory measurements. Anti-dsDNA antibodies were assayed using the Farr (Amerlex anti-dsDNA radioimmunoassay kit; Trinity Biotech, Bray, Ireland) and ELISA (dsDNA ELISA test system; Zeus Scientific, Raritan, NJ, USA), which are components of the usual clinic protocol. Farr values > 7 U/ml and ELISA values > 180 IU/ml were defined as abnormal. Complement concentrations, which are also part of the usual clinic protocol, were measured by nephelometry (Dade Behring BN11). Complement levels were defined as abnormal if C3 was < 0.75 g/l and/or C4 was < 0.12 g/l.

Measures of disease activity. The SLE Disease Activity Index 2000 (SLEDAI-2K) was assessed per standard protocol and served as the measure of global disease activity[9]. For the purposes of this study, the DNA component of the SLEDAI-2K score was excluded in order to assess the performance of the 2 anti-dsDNA antibody tests against this measure of global disease activity. Here, SLEDAI-2K refers to the SLEDAI-2K instrument excluding the DNA component. Organ-specific disease activity for renal, CNS, and vasculitis involvement was determined per standard definitions. Active renal disease was defined as the presence of at least one of: positive urine protein, 24-hour urine protein > 500 mg, positive urine white blood cell (WBC), urine WBC count > 5 per high-powered field (HPF), positive urine red blood cell (RBC), urine RBC > 5 per HPF, or urinary casts. Active vasculitis was defined as the presence of at least one of: nailfold infarcts, splinter hemorrhages, vasculitic skin lesions, ulceration, gangrene, or skin or muscle biopsy demonstrating arteritis. Active CNS disease was defined as the presence of at least one of: seizures (focal, generalized, or both), chorea, benign intracranial hypertension, acute stroke syndrome (hemorrhagic or nonhemorrhagic), transient ischemic attack, subarachnoid hemorrhage, headache, aseptic meningitis, acute transverse myelitis, acute cranial neuropathy, acute peripheral neuropathy, organic brain syndrome, or psychosis.

Statistical analysis. Descriptive statistics were calculated for the characteristics of cohort patients. Statistical correlations between dsDNA levels by Farr and ELISA techniques were determined by Pearson's correlation coefficient. This was done using all the clinic visits and reanalyzed using a single visit per patient, which was defined as the first clinic visit during the study period. Correlation and regression models were used to evaluate the relationship of SLEDAI-2K and the Farr and ELISA tests, respectively, and between SLEDAI-2K and complement levels. A multiple linear regression model was used to analyze the association of both Farr and ELISA tests with SLEDAI-2K. T tests and Wilcoxon rank-sum tests were used to compare mean or median (as appropriate) SLEDAI-2K values among those with normal or abnormal anti-dsDNA test values, and to compare mean or median anti-dsDNA test values among those with or without organ-specific activity or low complement levels. Tests for independence were performed using the chi-square test to determine if the proportion of those with abnormal anti-dsDNA test or complement values differed among those with and without organ-specific involvement. These measures of global and organ-specific disease activity were evaluated in relation to Farr and ELISA at the same visit, the previous visit, and the next visit.

RESULTS

Patient characteristics. Five hundred fifty patients with 2940 clinic visits were identified. All but 6 patients satisfied the American College of Rheumatology criteria for the classification of SLE at the time of inclusion into the study[10,11]. Patient characteristics are shown in Table 1. The mean age of this cohort was 42.3 years, the majority being female. The mean disease duration was 12.5 years, with a mean SLEDAI-2K of 5.1. Using data for one visit per patient, 42.5% had an abnormal Farr result, while 29.1% had an abnormal ELISA result. Similarly, the prevalence of abnormal complement levels was 32.9%.

Correlation between assays. The correlation between the Farr and ELISA assays was 0.46 (confidence interval 0.39, 0.52; $p < 0.0001$). The correlations were no better when either test was abnormal or when both were normal. When the Farr was abnormal, the ELISA was equally likely to be normal or abnormal. For example, in evaluating the frequency distribution of normal and abnormal anti-dsDNA antibody test results, we found the ELISA results equally likely to be normal (116 patients) or abnormal (118 patients) when the Farr results were abnormal (234 patients). When the Farr results were normal (316 patients), the majority of ELISA results were also normal (274 normal, 42 abnormal). Similarly, when the ELISA was abnormal (160 patients), the majority of Farr results were also abnormal (118 abnormal, 42 normal).

Correlation of assays with SLEDAI-2K. In a regression model in which either the Farr or ELISA as independent predictor was abnormal (n = 276 subjects), increasing anti-dsDNA antibody titers by Farr correlated with increasing SLEDAI-2K values (beta parameter estimate = 0.067 ± 0.011; $p < 0.0001$), while no such association with ELISA was found (beta parameter estimate = –0.0006 ± 0.0013; $p = 0.7$). SLEDAI-2K values were lower with normal anti-dsDNA antibody results and were higher with abnormal anti-dsDNA antibody results. For example, with a normal Farr value, the mean SLEDAI-2K value was 4.29 ± 4.77 SD, while it was 6.16 ± 6.10 SD when the Farr was abnormal ($p = 0.0001$). The ELISA had less discriminatory power, with a SLEDAI-2K of 4.75 ± 5.11 when the ELISA was normal, and 5.90 ± 6.16 when the ELISA was

Table 1. Patient characteristics: 550 patients, 2940 clinic visits. All values are for first visit in interval.

Characteristic	Result
Mean age, yrs (SD), range, median	42.3 (14), 17.6–81.8, 41.6
Sex, % female	87.8
Mean disease duration, yrs (SD), range, median	12.5 (9.9), 10–46.7, 10.1
SLEDAI-2K, mean (SD), range, median	5.9 (5.7), 0–32, 4.0
Excluding DNA component	5.1 (5.5), 0–30, 4.0
Prevalence of	One visit per patient
Abnormal Farr (> 7 U/ml)	42.5% (n = 234)
Abnormal ELISA (> 180 IU/ml)	29.1% (n = 160)
Prevalence of abnormal complement levels	One visit per patient
C3	104 (19.1%)
C4	142 (26.1%)
Both	179 (32.8%)

abnormal (p = 0.04). Nonparametric analyses yielded similar results.

Correlation of assays with organ-specific disease activity and complement levels. Active renal involvement was present in 121 patients (22%). Farr values were statistically significantly higher in those with renal involvement than in those without, while the ELISA values were no different among those with and without renal involvement (Table 2). Further, more patients with renal involvement had an abnormal Farr value compared to those without renal involvement (50% vs 40%; p = 0.05), but roughly equal proportions of patients with and without active renal disease had abnormal ELISA values (30% vs 29%; p = 0.9). Similar findings were demonstrated in the correlation of the Farr assay with vasculitis compared to the ELISA (Table 2). Neither the Farr nor the ELISA values correlate with CNS disease (Table 2). These analyses were repeated using nonparametric techniques and yielded similar results.

Both Farr and ELISA values were significantly higher in those with low complement concentrations. When complement levels were normal (n = 366), the mean Farr level was 11 (SD 18.1), while with low complement levels (n = 179), the mean Farr level was 28.6 (SD 33.4) (p < 0.0001). Similarly, the mean ELISA level was 127 (SD 167) in the presence of normal complement levels, while with low complement levels, the ELISA had a mean value of 240 (SD 294) (p < 0.0001). Of those with low complement levels, 63.1% had an abnormal Farr result, compared to 32% in those with normal complement levels (p < 0.0001). Of those with low complement levels, 43% had an abnormal ELISA, while 22.1% of those with normal complement levels have an abnormal ELISA (p < 0.0001). However, complement levels could not distinguish between those with and those without organ-specific involvement (Table 2), with the exception of a borderline association with renal involvement. Similar results were obtained using nonparametric methods.

DISCUSSION

Using a large cohort with clinical and laboratory data collected prospectively, we evaluated the utility of 2 assays commonly used for the measurement of anti-dsDNA antibodies. Our results demonstrate a low level of agreement between the Farr and ELISA assays that, although statistically significant, is not clinically significant. Further, when the Farr assay is abnormal, the ELISA is equally likely to be normal or abnormal. The ELISA does not correlate with measures of global disease activity and lacks sufficient discriminatory power in comparing those with and without organ-specific disease activity. The Farr assay correlates with measures of disease activity, including global disease activity and renal activity.

Table 2. Farr, ELISA, and complement values in those with and without organ-specific involvement.

	Renal Involvement		Vasculitis		CNS Involvement	
	Yes, n = 121 mean ± SD	No, n = 429 mean ± SD	Yes, n = 17 mean ± SD	No, n = 533 mean ± SD	Yes, n = 67 mean ± SD	No, n = 483 mean ± SD
Farr, U/ml	22.5 ± 31.4	15.2 ± 23.4	29.5 ± 36.2	16.4 ± 25.1	22.6 ± 31.6	16.0 ± 24.5
	p = 0.02		p = 0.03		p = 0.1	
ELISA, IU/ml	166 ± 236	164 ± 219	210 ± 177	163 ± 224	182 ± 190	162 ± 227
	p = 0.95		p = 0.2		p = 0.5	
C3, g/l	0.95 ± 0.29	0.99 ± 0.28	0.98 ± 0.58	0.98 ± 0.21	0.96 ± 0.27	0.98 ± 0.29
	p = 0.2		p = 0.996		p = 0.5	
C4, g/l	0.18 ± 0.09	0.17 ± 0.08	0.16 ± 0.09	0.17 ± 0.08	0.17 ± 0.08	0.17 ± 0.08
	p = 0.054		p = 0.5		p = 0.5	

Despite the small number of patients with vasculitis, a significant difference was found in the Farr value among those with vasculitis compared to those without vasculitis, and there was a trend toward more patients in the vasculitis group having an abnormal Farr result compared to those without vasculitis. Consistent with previous studies, neither the Farr nor the ELISA values correlated with CNS activity. Complement levels appeared to correlate well with both Farr and ELISA values; however, complement levels could not distinguish between those with and those without organ-specific activity, with the exception of a borderline significant association with renal disease, which is not unexpected.

Riboldi, *et al*[8] recently reported that the Farr assay had the best diagnostic contribution in SLE. Our study confirms that the Farr technique is superior to the ELISA in correlating with measures of global and organ-specific disease activity and should therefore be used to follow anti-dsDNA antibody levels in patients with SLE.

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