

Commentary and update: Serological tests for diagnosis of systemic lupus erythematosus (SLE)¹

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Since the description of the lupus erythematosus (LE) cell phenomenon by Hargraves et al,¹ and with the realization by Haserick and Bortz² that this phenomenon could be induced in normal cells by a component of plasma from patients with systemic lupus erythematosus (SLE), the nature of this "LE cell factor" has been clarified. It is an IgG antibody that reacts with deoxyribonucleoprotein³ and as such belongs to an increasingly well-characterized group of antibodies that combine with various components of cell nuclei. Detection of certain of these antinuclear antibodies (ANA) has assumed increasing importance in the diagnosis and management of SLE and related diseases.

Among the serological tests for diagnosis of SLE, the LE cell phenomenon was followed by fluorescent assays for ANA with the use of various cellular substrates,^{4,5} tests for antibodies against double- or single-stranded DNA,⁶⁻⁸ and assays for antibodies against an acidic nuclear glycoprotein referred to as Sm antigen.⁹ Although these assays are in widespread use, and in many institutions combinations of them are often ordered as a "lupus battery," little quantitative information about the meaning and interpretation of positive or negative test results is available in the literature. We reviewed the laboratory and clinical records of The Cleveland Clinic Foundation, including Haserick's original work to up-

date and evaluate serological tests for diagnosis of SLE.

Methods

Antinuclear antibody (ANA) was assayed by indirect immunofluorescence⁵ with the use of rat kidney as substrate and a polyvalent, fluoresceinated rabbit anti-human immunoglobulin serum as indicator; a titer $\geq 1:40$ was considered positive. Anti-native DNA (anti-nDNA) was assayed by a modification of the Farr assay as previously described¹⁰; values $\geq 10\%$ binding were considered positive. Anti-Sm was assayed by double diffusion in agarose with the use of ribonuclease-treated rabbit thymus extract¹¹; positive sera formed a line of identity with a known positive standard (kindly supplied by Carol Peebles, National Jewish Hospital, Denver, Colorado). The LE cell test was performed as previously described by Haserick and Bortz²; positives were ranked 1+ or greater.

Sensitivities (prevalence of true-positives in the SLE population) were determined by performing each of the tests on sera from a group of 102 well-studied SLE patients previously reported.¹¹ Specificities (prevalence of true-negatives in the non-SLE population) were determined by reviewing clinical records of 573 patients selected for test positivity or negativity in approximately equal numbers for each test. These patients were classified as SLE or non-SLE; from this information together with the frequency of overall test positivity, specificities were calculated. In most cases data from more than one test were available

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on each patient. Sensitivities and specificities are shown in the *Table*.

Results

Predictive values¹² for positive and negative results of each of the four tests are shown in *Figures 1 and 2*, respectively. The predictive value of a positive test is the prevalence of SLE among all patients with a positive test result; the tests with the highest positive predictive values were anti-nDNA and anti-Sm. The predictive value of a negative test result is the prevalence of non-SLE among all patients with a negative test result; the test with the highest negative predictive value was ANA.

Test efficiency¹² is defined as the prevalence of true-positive and true-negative results among all the tests performed. It measures the correlation of test results with clinical situation (sum of SLE patients with positive test and non-SLE patients with negative test divided by total number

of tests done). Efficiency for tests examined is shown in *Figure 3*; the most efficient test was the LE cell preparation.

Quantitative correlations were drawn for ANA titer and degree of positivity of LE preparation with prevalence of SLE as well as with certain other non-SLE diagnoses (*Figs. 4 and 5*, respectively). In both tests the prevalence of SLE increased as degree of test positivity increased, although this relationship was clearer for LE cell preparation.

Discussion

We examined the results of four tests (LE cell preparation, ANA, anti-nDNA, and anti-Sm) in 675 patients, 102 of whom were selected because they were diagnosed on clinical grounds as having SLE; the remainder were selected from laboratory records of the four tests. The results permit calculation of sensitivity and specificity figures (*Table*), of predictive values of positive and negative results (*Figs. 1 and 2*), and of test efficiency (*Fig. 3*) for each test. These figures show how strongly a positive or negative test result standing alone, without other data, indicates a diagnosis of SLE. The specificity figures in the *Table* are probably conservative (underestimate specificity) since the results are biased by the fact that the test was ordered in the first place; non-SLE patients on whom ANA was or-

Table. Sensitivity and specificity of serological tests for SLE

Test	Sensitivity	Specificity
LE Prep	.704	.946
ANA	.990	.690
Anti-nDNA	.569	.993
Anti-Sm	.220	.999

ANA = antinuclear antibodies.

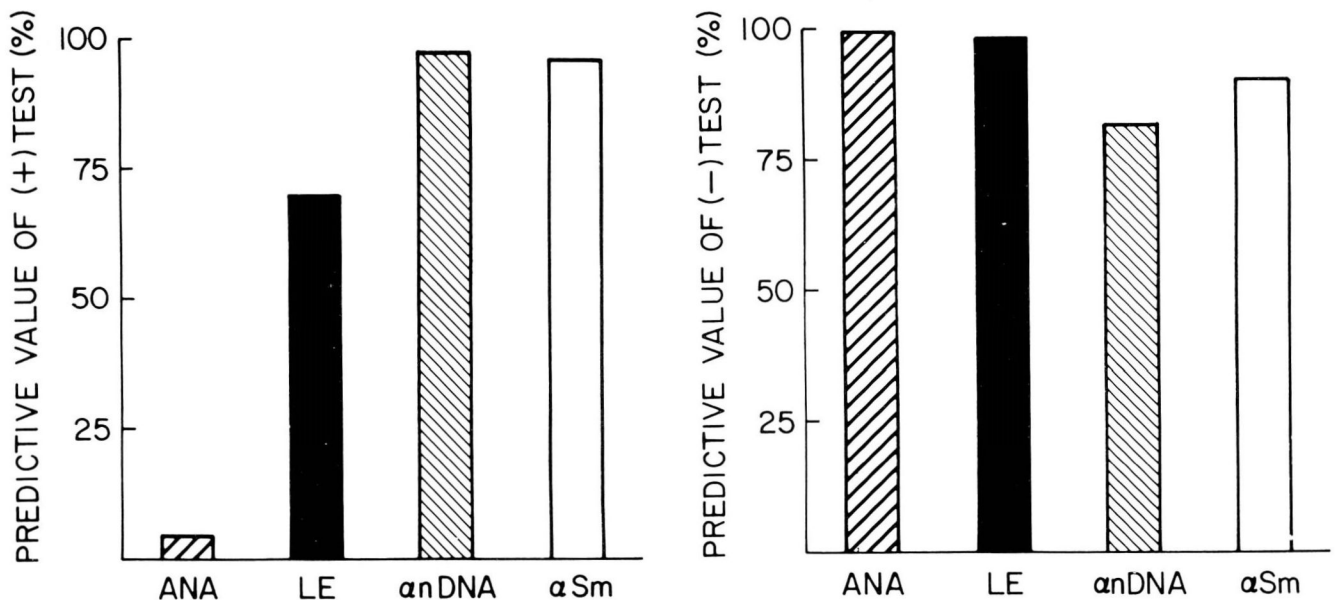


Figure 1. Predictive value of positive results for 4 serological tests used in the diagnosis of SLE; value is the percent of patients with a positive test who actually have SLE.

Figure 2. Predictive value of negative results for 4 serological tests used in the diagnosis of SLE; value is the percent of patients with a negative test who do not have SLE.

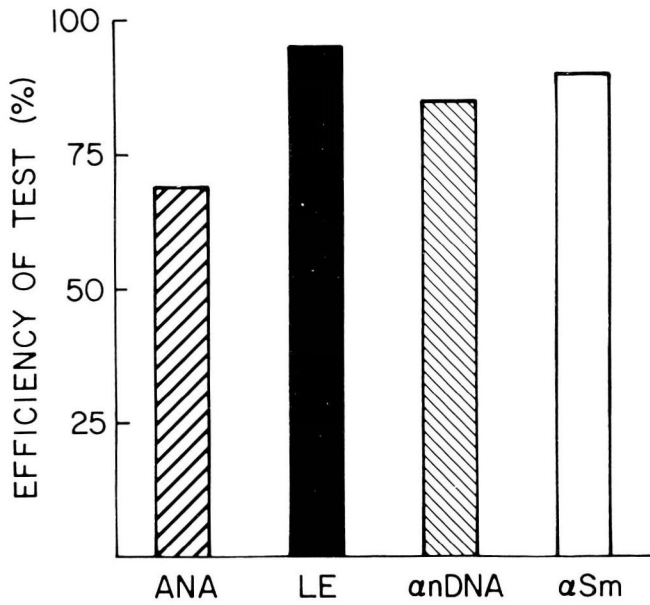


Figure 3. Efficiency of 4 serological tests used in diagnosis of SLE; value is the percent of patients in whom the outcome of the test agrees with the diagnosis.

dered, for example, may be more likely to have positive ANA than non-SLE patients in whom this was not even considered. However, the tests are valuable as discriminators only within the population on which they are performed, so this bias is probably not especially important.

The results illustrate the principle that for a highly specific test (such as anti-nDNA or anti-Sm) the predictive value of a positive result is high whereas for a highly sensitive test (such as ANA) the predictive value of a negative test is

high. The LE cell prep was intermediate in specificity and sensitivity, but as a single test had the greatest efficiency.

It is clear, however, that these test results are never considered in a vacuum. The tests are ordered because of suspicion based on clinical grounds that they may be positive, and the result obtained exerts either a positive or negative effect on the clinician's estimation of the likelihood that the patient has lupus. This clinical estimation of likelihood can be quantitated with a scoring system based on the American Rheumatism Association (ARA) criteria for the classification of SLE, according to the sensitivity and specificity of the criteria for diagnosis of SLE as we have previously reported.¹³ This allows establishment of pretest probability of SLE in a given patient. If this probability is low, a positive outcome of a test with a high positive predictive value (e.g., anti-nDNA, anti-Sm) would have the most profound effect on the diagnostic likelihood. On the other hand, for a patient with a high pretest probability of SLE, the greatest effect on probability would be exerted by a negative outcome of a test with a high negative predictive value (e.g., ANA). Because of its nonspecificity, a positive ANA exerts little effect on the likelihood of SLE, and, because of relatively low sensitivity, negative anti-DNA and anti-Sm results do not greatly influence the likelihood of SLE. Thus, as often happens in mild or inactive SLE, the combination of positive ANA, negative anti-nDNA, and negative anti-Sm is not especially helpful. If this

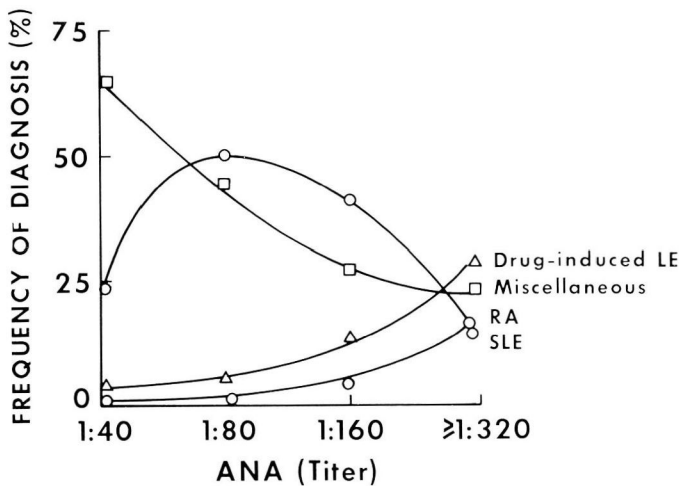


Figure 4. Predictive value of positive results of ANA at different titers for idiopathic SLE, drug-induced LE, rheumatoid arthritis (RA), and all other diagnoses (miscellaneous).

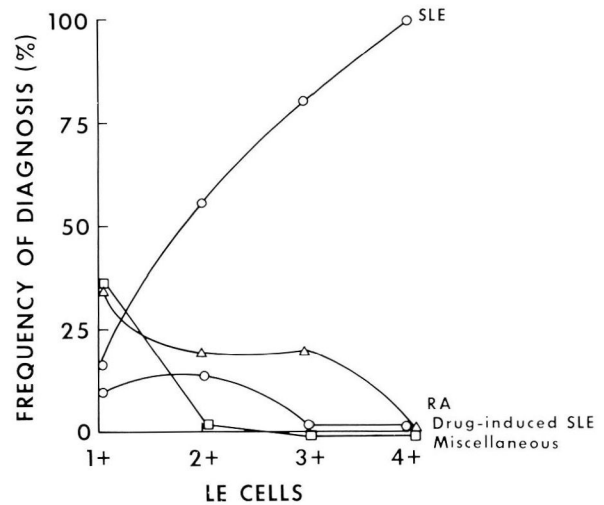


Figure 5. Predictive value of different levels of positivity of LE cell preparation for idiopathic SLE, drug-induced LE, rheumatoid arthritis (RA), and all other diagnoses (miscellaneous).

combination of results occurs in an individual for whom the pretest probability of SLE is low (e.g., a patient with fibrositis), the clinician is simply faced with the unpleasant task of explaining a positive ANA to someone in whom SLE was not strongly suspected initially. This realization might affect the decision to order ANA in this setting.

However, anti-nDNA and possibly anti-Sm have values in addition to their importance in diagnosis. Anti-nDNA levels correlate well with activity of SLE⁸ and, indeed, anti-nDNA is thought to be an important pathogenic antibody in SLE, particularly with glomerulonephritis.^{14,15} Furthermore, the combination of positive anti-nDNA and positive anti-Sm has in our experience been associated with a more severe form of lupus in which diffuse, proliferative glomerulonephritis is common (occurring in 73% of such patients) as opposed to patients in whom these antibodies are not found together, where severe renal disease is considerably less common (23%, $p < 0.001$).¹¹ Thus, adequate monitoring of disease activity requires serial determinations of anti-nDNA along with certain other tests (complement, creatinine, urinalysis, blood count), and determinations of anti-nDNA and anti-Sm may be helpful in estimating prognosis.

References

- Hargraves MM, Richmond H, Morton RJ. Presentation of two bone marrow elements: the "Tart" cell and "LE" cell. Proc Staff Meet Mayo Clin 1948; **23**:25-28.
- Haserick JR, Bortz DW. A new diagnostic test for acute disseminated lupus erythematosus. Cleve Clin Q 1949; **16**: 158-161.
- Holman HR, Deicher HRG, Kunkel HG. The L.E. cell and LE Serum factors. Bull NY Acad Med 1959; **35**:409-418.
- Holborow EJ, Weir DM, Johnson GD. A serum factor in lupus erythematosus with affinity for tissue nuclei. Br Med J 1957; **2**:732-34.
- Cleymaet JE, Nakamura RM. Indirect immunofluorescent antinuclear antibody tests: comparison of sensitivity and specificity of different substrates. Am J Clin Pathol 1972; **58**:388-393.
- Casals SP, Friou GJ, Myers LL. Significance of antibody to DNA in systemic lupus erythematosus. Arthritis Rheum 1964; **7**:379-390.
- Aarden LA, deGroot ER, Feltkamp TEW. Immunology of DNA. III. *Crithidia luciliae*, a simple substrate for the determination of anti-dsDNA with the immunofluorescence technique. Ann NY Acad Sci 1975; **254**:505-515.
- Clough JD. Measurement of DNA-binding immunoglobulins in systemic lupus erythematosus. J Immunol Methods 1977; **15**:383-394.
- Tan EM, Kunkel HG. Characteristics of a soluble nuclear antigen precipitating with sera of patients with systemic lupus erythematosus. J Immunol 1966; **96**:464-471.
- Krakauer RS, Clough JD, Alexander T, Sundeen J, Sauder DN. Suppressor cell defect in SLE: relationship to native DNA binding. Clin Exp Immunol 1980; **40**:72-76.
- Couri JM, Mowafi N, Clough JD. Association of anti-Sm with increased risk of diffuse lupus nephritis (abst). Clin Res 1981; **29**:781A.
- Galen RS, Gambino SR. Beyond Normality. The Predictive Value and Efficiency of Medical Diagnosis. New York: John Wiley and Sons, 1975.
- Clough JD, Elrazak M, Calabrese LH, Valenzuela R, Braun WE. Weighted criteria and test selection in the diagnosis of SLE (abst). Clin Res 1982; **30**:804A.
- Tan EM, Schur PH, Carr RI, Kunkel HG. Deoxyribonucleic acid (DNA) and antibodies to DNA in serum of patients with systemic lupus erythematosus. J Clin Invest 1966; **45**:1732-1740.
- Clough JD, Valenzuela R. Relationship of renal histopathology in SLE nephritis to immunoglobulin class of anti-DNA. Am J Med 1980; **68**:80-85.