Information on Diagnosis and Management of Systemic Lupus Erythematosus Derived from the Routine Measurement of 8 Nuclear Autoantibodies

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ABSTRACT. ***Objective.*** To determine the value of routine measurement of a panel of 8 nuclear autoantibodies (ANA/8) for the diagnosis and management of patients with systemic lupus erythematosus (SLE).

Methods. To estimate disease sensitivity of ANA/8, we studied 25 patients with new SLE and 114 with new and established SLE. To estimate disease specificity, 100 patients with other autoimmune rheumatic diseases were included. We used computerized statistical analysis of the level of 8 ANA in relation to clinical activity determined as Systemic Lupus Activity Measure disease activity scores (DAS). Data were collected retrospectively from the charts of 114 patients with 698 visits and evaluated by multiple and piece-wise linear regression analysis (PWLRA) and correlation and cluster analyses.

Results. The disease sensitivity of the 3 types of SLE profiles identified was 100% for new SLE patients (n = 25) and 87% for mixed SLE patients; the disease specificity was 98%. Autoantibody levels of anti-ssDNA, dsDNA, and Scl-70 were the best individual correlates of general and organ-specific DAS. Twenty-four percent (R^2) of the variability in the general DAS was explained by the multiple regression (R = 0.49), with significant contribution made by anti-Scl-70 (ß = 0.39), dsDNA (ß = 0.17), Sm (ß = 0.10), and SSA (ß = 0.08). PWLRA indicated that for 68% of the 698 clinical presentations (average 6/patient), the observed DAS and the predicted DAS from autoantibody levels were both low and clustered; they were partially discrepant for the remaining 32%, which was explained by the relatively high correlation of DAS with prior changes in autoantibody levels (R = 0.6). The changes in DAS and in anti-dsDNA levels were significantly predicted by the multiple regression at one prior visit, with anti-ssDNA as the main contributor.

Conclusion. The ANA/8 profile showed ~ 100% sensitivity and ~ 98% specificity for SLE and correlated with contemporary and subsequent changes in DAS and autoantibody levels. Among autoantibodies of this profile, anti-ssDNA (ssDNA) was the most sensitive indicator of SLE and the main contributor to prediction of subsequent changes in DAS. (J Rheumatol 2003;30:1761–9)

Key Indexing Terms:
SYSTEMIC LUPUS ERYTHEMATOSUS
ANTINUCLEAR ANTIBODIES
DISEASE ACTIVITY
ssDNA

Recent guidelines on the use of tests for antinuclear antibodies were issued by a committee of the College of American Pathologists and the American College of Rheumatology (ACR)[1,2]. According to these guidelines, (1) measurement of panels of autoantibodies has no clinical value for the diagnosis and management of patients with systemic lupus erythematosus (SLE); and (2) measurement of antibodies against denatured DNA (ssDNA) has no clinical value and should be used only for research purposes. However, no publication was cited to support these 2 guidelines.

Most studies on autoantibodies in patients with SLE have focused on those considered disease markers and included in the ACR diagnostic criteria for SLE[3,4]: anti-dsDNA and anti-Sm. However, other autoantibodies have been used in the diagnosis of SLE and related syndromes, including anti-ssDNA (denatured DNA, total DNA or ssDNA), histones, nRNP, SSA (Ro), SSB (La), ribosomal protein P, and Scl-70[5]. The presence of multiple autoantibodies in different assortments and concentrations reflects both the polyclonality and the diversity of the autoimmune response process in individual patients with SLE[6,7].

After the diagnosis of SLE is made, only anti-dsDNA autoantibody concentrations are routinely used as correlates or predictors of flare and disease activity[8-13]. The problem

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Supported in part by Campus Research Board, University of Illinois at Chicago.

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Submitted July 31, 2002; revision accepted January 3, 2003.

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with this approach is that only about 65% of patients with SLE have anti-dsDNA[5]. Although the individual autoantibodies mentioned above have been correlates of disease activity[5,14], there is no consensus on their clinical utility.

A metaanalysis of reports generated from a variety of methods (e.g., Farr assay, ELISA, immunofluorescence assay, double diffusion in agar gel, passive hemagglutination), mainly qualitative (positive/negative), was used to generate the above guidelines[1]. As a result they are difficult to extrapolate to the current practice of rheumatology, considering that quantitative and semiquantitative ELISA tests are used for > 90% of the reportable results. We investigated the clinical value of 8 autoantibodies measured against consistent standards in 114 patients with SLE on an open-ended scale ELISA over a period of 7 years. As controls we used 100 patients with other autoimmune collagen vascular diseases. The results were in strong support of the clinical utility of a panel of autoantibodies and of antibodies against ssDNA in the diagnosis and management of SLE. The results also suggest the feasibility of pattern recognition computer software to be used as an adjunct in diagnosis and management of SLE.

MATERIALS AND METHODS

Over a period of 7 years at the University of Illinois at Chicago, 114 patients with SLE who met ACR criteria[3,4] were tested for a panel of 8 autoantibodies (ANA/8) against ssDNA, dsDNA, Sm, nRNP/Sm, SSA, SSB, histones, and Scl-70. Nine additional patients with new onset SLE were added after the completion of the study. Random patients with other collagen vascular diseases were included as controls: 24 with rheumatoid arthritis, 52 with progressive systemic sclerosis (SSc) (patients with SSc/SLE overlap were excluded), 17 with primary Sjögren's syndrome, and 7 others. All control patients with an identified diagnosis met ACR or other accepted diagnostic criteria[15-17].

All tests were performed by ELISA using commercial kits (EL-ANA/8, TheraTest Laboratories, Lombard, IL, USA) according to the manufacturer's instructions. The clinical specificities of the tests, calculated by percentile ranking from levels obtained on multiple sets of 100 random blood donors, were as follows: ssDNA (denatured calf thymus DNA or total DNA) 95%, dsDNA (bacteriophage λ DNA) 99%, Sm 99%, nRNP/Sm complex 98%, SSA 98%, SSB 99%, histone 99%, and Scl-70 99%. All protein antigens were from bovine thymus or spleen. For these 114 patients, 695 ANA/8 tests were performed, or about 6.1 measurements/patient, with a range from one to 21. For the controls there was only one measurement at the time of diagnosis or admission to the clinic. All measurements were performed to the endpoint and data were expressed in units/ml as compared to a standard (calibrator).

The charts of all 114 patients and 100 controls were reviewed retrospectively for clinical information. For each visit of an SLE patient we recorded the general clinical Disease Activity Scores (DAS) as well as the DAS for each contributing organ or system: skin, lung, cardiovascular, neuromotor, joint, renal, and hematologic. For each organ or system, the DAS varied between 0 and 20, except for Raynaud's, where it was recorded as 0 or 1. This information was organized with a retrospective Systemic Lupus Activity Measure (SLAM)[18,19], reported to be well correlated (R = 0.67) with prospective SLAM[18]. Missing information in the charts for about 15% of the visits was given a score of 0. A total of 695 SLAM evaluations were performed and were matched on the same spreadsheet as contemporary to their autoantibody measurements. The treatment was not recorded.

We minimized bias in our study by having the investigators collecting the clinical data blinded to the laboratory results. Also, the guidelines for the 2 investigators who collected the data were consistent and, when collected from the same charts, there was very good correlation between their DAS findings (R = 0.92).

Of the 114 patients, 68% were black, 19% Hispanic, and 13% white; 95% were female. The large proportion of black subjects suggests that the frequency of anti-Sm and anti-nRNP, but not other nuclear autoantibodies, was significantly higher than expected for the general population of patients with SLE[20]. The mean disease duration was 8.6 years (range 1 month to 45 years).

Statistical analysis. We used the statistical software package Statistica for Windows, v 5.1 (StatSoft, Tulsa, OK, USA). Since the collected data exhibited a great variability over a very wide range and showed a tendency toward nonlinear behavior, we used both raw and transformed data in the form of logarithmic values and distribution-free percentile values. Ranking of values over time for the same patient was also used. The data were evaluated by multiple linear regression and piece-wise linear regression analysis with the breakpoint determined by computer. We also studied the data by means of correlation and cluster analysis. Correlation was expressed as R with a maximum possible of 1 and as R^2, which gives the percentage of correlation as explained by the autoantibody levels. The degree of contribution to R^2 was expressed as ß, i.e., the higher the absolute value of ß the higher the contribution, with a maximum possible of 1. For example, if R = 0.5 the R^2 was 0.25%; this 25% is equal to 1 for the calculation of the contribution as ß. All p values are shown only as low as < 0.001, although most were substantially lower. For multiple comparisons, application of Bonferroni or Sidak corrections did not change the posted p value.

RESULTS

Frequency of the 8 autoantibodies. There was a large variability in the ANA/8 profiles in individual patients (Table 1). The frequencies of the 8 autoantibodies in the cohort of 114 patients, based on the highest level recorded during multiple tests (average 6.1 measurements/patient) were: 87% anti-ssDNA, 52% anti-dsDNA, 40% anti-Sm, 58% anti-RNP/Sm, 48% anti-SSA, 33% anti-SSB, 53% anti-histone, and 35% anti-Scl-70 (Table 2) (for example, if a patient had 10 levels recorded over 4 years we chose the highest value to calculate the frequency). However, to obtain a more accurate view of the autoantibody profile at the time of diagnosis, we identified 25 patients tested prior to the start of any treatment: 16 from among the 114 patients and 9 added after the completion of the study. All patients had at least 2 autoantibodies at abnormal concentrations (Table 1) and individual autoantibodies were found with the following frequency: 100% anti-ssDNA, 64% anti-dsDNA, 44% Sm, 52% anti-RNP/Sm, 56% anti-SSA, 44% anti-SSB, 54% anti-histone, and 37% anti-Scl-70 (Table 2). For 95% confidence, there was no statistical difference between the frequency of each autoantibody in the new untreated patients and the cohort of 114 patients, when the results were selected as the highest recorded during their followup.

Three types of SLE profiles were identified (Table 1): type I had at least anti-ssDNA and anti-dsDNA (Patients 1–16, Table 1), type II had at least anti-ssDNA and anti-Sm (Patients 17–20), and type III had at least anti-ssDNA and anti-SSA (Patients 21–25) (or anti-nRNP/Sm based on data from the cohort of 114 patients). The 3 profiles combined

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Table 1. Levels of 8 autoantibodies for 25 new, untreated patients (normal values shown as —). Values are units per ml.

Main Profile Type	Patient	Upper Limit of Normal (N) and Percentile (%) Cutoff for 100 Ranked Normals ssDNA, u N = 99 95%	dsDNA, iu N = 40 99%	Sm, u N = 90 99%	RNP/Sm, u N = 83 98%	SSA, u N = 91 98%	SSB, u N = 73 99%	Histone, u N = 96 98%	Scl-70, u N = 32 99%
I	1	21,200	19,700	3490	10,300	—	96	192	1170
I	2	5167	220	3402	4574	—	—	ND	ND
I	3	613	97	—	—	—	—	139	—
I	4	408	711	—	—	—	—	—	—
I	5	1956	1944	151	339,000	148	285	361	216
I	6	697	141	—	—	12,000	870	—	—
I	7	9662	3855	—	—	—	181	1194	107
I	8	3277	3453	1801	15,977	212,000	417	355	64
I	9	2716	777	—	—	—	—	253	—
I	10	1170	282	110	89	283	—	710	—
I	11	1980	790	50,100	191,000	3270	167	920	167
I	12	30,800	594	1230	3210	—	—	1500	139
I	13	8100	6130	—	109	298	452	352	205
I	14	9990	11,010	—	86	3150	222	1210	1190
I	15	3990	200	—	—	—	—	—	152
I	16	469	151	—	—	—	376	—	—
II	17	21,500	—	176,000	368,000	302	—	217	—
II	18	133	—	226	185	—	—	—	—
II	19	490	—	384	178,000	—	—	—	—
II	20	156	—	329,000	950,000	211	246	100	—
III	21	112	—	—	—	256,000	141	—	—
III	22	3270	—	—	—	246	—	—	—
III	23	448	—	—	—	1887	—	—	—
III	24	11,080	—	—	—	635	—	—	—
III	25	681	—	—	—	105	—	—	—

ND: not done. * Some patients could be included in more than one profile type.

Table 2. Percentage of patients with antinuclear antibodies and SLE profiles in 4 populations: new (untreated) SLE, general SLE population, other collagen vascular diseases (CVD), and random blood donors.

Antibody	New SLE, n = 25	All SLE in Study*, n = 123	Other CVD, n = 100†	Blood Donors, n = 100
ssDNA	100	87	3	5
dsDNA	64	54	1	1
Sm	44	42	0	1
RNP/Sm	44	59	6	2
SSA	56	48	14	2
SSB	44	33	8	1
Histone	54	53	29	1
Scl-70	37	35	11	1
Type I SLE**	64	54	0	0
Type II SLE	44	39	0	0
Type III SLE	76	73	2	0
Type I+II+III	100	87	2	0

* Based on highest value recorded during the observation of each patient. ** Types of SLE profiles: Type I: at least anti-ssDNA and anti-dsDNA; type II: at least anti-ssDNA and anti-Sm; type III: at least anti-ssDNA and anti-SSA or anti-dsDNA and anti-nRNP/Sm. * Some patients belonged to more than one profile type. † Patients with other CVD included: 24 rheumatoid arthritis, 52 scleroderma, 17 Sjögren's syndrome, and 7 others.

had a disease sensitivity of 100% for new patients and 87% for all patients (including patients with relatively quiescent disease) (Table 2). The disease specificity, calculated based on random blood donors, was 100%, and based on disease controls, 98%. Thus, the SLE profiles had a disease sensitivity of 87–100% and specificity of 98–100%).

Correlation between individual autoantibody levels and DAS. The level of each autoantibody was correlated sepa-

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rately with a contemporary general and organ-specific DAS. Raw values as well as their log and percentile transforms (data not shown) were used. The levels of autoantibodies against ssDNA, dsDNA, and Scl-70 correlated significantly ($p < 0.001$) with general DAS and with several organ systems (lung, renal, and hematologic) (Table 3) for $\log_{10}$ and for the other 2 forms of input data. The best individual correlate of renal and hematologic DAS was anti-Scl-70. At the other extreme was anti-SSA, which did not correlate with the general DAS in any form of input data. Each of the remaining autoantibodies had significant correlations with general DAS of various organs, depending on the transform used (data not shown). For example, raw data for anti-Sm and anti-RNP/Sm were not significantly correlated with the general DAS, but the log or percentile transforms were. Thus, all individual components of the ANA/8 had some contemporary correlation with general and/or organ-specific DAS.

Correlation of DAS with multiple contemporary autoantibody levels. Using multiple regression analysis on 695 ANA/8 profiles (5560 individual autoantibody values), we investigated to what extent the DAS, both general and organ-specific, assessed at the time of each test, could be predicted from the autoantibody levels (Table 4). The regression analysis was performed on raw data as well as on $\log_{10}$ and percentile transforms. For the general and renal DAS the best results were obtained with $\log_{10}$ values. We found that 24% (R^2) of the variability in the general DAS was explained by multiple regression ($R = 0.49$, $p < 0.001$). Significant contribution to this correlation was made by anti-Scl-70 (ß = 0.39, $p < 0.001$) followed by anti-dsDNA (ß = 0.17, $p < 0.001$), anti-Sm (ß = 0.10, $p < 0.05$), and anti-SSA (ß = 0.08, $p < 0.025$) (Table 4).

For organ-specific DAS there was no single transform of the input variables that yielded the best correlation. Log values yielded the best correlation for renal (Table 4) and hematological activity, percentile ranking for joint, cardiovascular, and Raynaud's, and raw values for lung and neuromotor (data not shown). With this approach, there was a significant correlation between autoantibody levels and each of the organs and systems evaluated ($p < 0.001$, except < 0.01 for cardiovascular). However, the set of autoantibody specificities, which contributed significantly to the correlation, was different for each organ. Anti-Scl-70 was the best contributor for renal, lung, and hematological involvement, anti-Histone for neuromotor, anti-SSB for joint, and anti-Sm for skin. Anti-Sm was actually the strongest contributor of all autoantibodies to any organ involvement, with ß = 0.45

Table 3. Contemporary correlations (R) between the level of each autoantibody ($\log_{10}$ values) and the general and organ-specific DAS (n = 695; only R values significant at $p < 0.01$** and $p < 0.001$* are shown).

		DAS by Organ and System							
Autoantibody	General DAS	Skin	Lung	CV	NM	Renal	HEM	Joint	Raynaud's
Scl-70	0.45*	0.19*	0.17*		0.10**	0.36*	0.33*	0.14*	0.13*
dsDNA	0.35*	0.15*	0.20*	0.11**	0.09**	0.27*	0.21*	0.12*	
ssDNA	0.22*		0.09**			0.21*	0.21*		
Histone			0.10**			0.16*	0.19*		
Sm	0.17*	0.15*				0.13*	0.15*	0.11**	
RNP/Sm	0.12*	0.16*					0.13*	0.11*	
SSB							0.10**		
SSA									0.13*

DAS: disease activity score, CV: cardiovascular; NM: neuromuscular, HEM: hematologic.

Table 4. Correlation of multiple autoantibody levels ($\log_{10}$) with general and renal DAS based on multiple regression analysis.

		Significant Contributors*		
Dependent Variable: DAS	Correlation R, $p < 0.001$	Autoantibody Specificity	ß*	p
General	0.49	Scl-70	0.39	< 0.001
		dsDNA	0.17	< 0.001
		SM	0.10	< 0.05
		SSA	0.08	< 0.025
Renal	0.40	Scl-70	0.32	< 0.001
		dsDNA	0.10	< 0.02
		RNP/Sm	0.13	< 0.001
		Sm	0.12	< 0.02
		SSA	0.10	< 0.005

* The sum of all ß values is not 1, since the other factors did not have a statistically significant contribution.

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for skin manifestations. Anti-dsDNA was a significant contributor only for kidney disease; it was secondary, however, to Scl-70. Thus, the linear regression analysis showed that all autoantibodies taken together contributed to DAS, but for each organ the assortment of autoantibodies and the level of significance of contribution was different.

Piece-wise linear regression analysis of autoantibody levels and DAS. To correlate the autoantibody levels with DAS, the scores observed were plotted against the scores predicted from the contemporary autoantibody levels by piece-wise linear regression analysis (PWLRA). The computer selected the percentile-ranked values best for this analysis (Figure 1), although $\log_{10}$ and raw data yielded similar results (data not shown). PWLRA separated the predicted DAS into 2 major clusters: one well defined (Figure 1, lower left corner) and one scattered over a wide range. The predicted scores of the clinical presentations in the well defined cluster, which contained 62% of all presentations, were < 5.5, and those of the second cluster > 7.5, with a clear gap between the 2 sets of values. Thus, PWLRA indicated that 62% of clinical presentations were quiescent in terms of both autoantibody levels and clinical activity, i.e., an excellent correlation. The remaining 38% of presentations, predicted as having various DAS levels by autoantibody levels, did not cluster, but were scattered over a wide range. Some appeared as having relatively high predicted values by autoantibody but relatively low DAS, others had both high autoantibody activity and high DAS (Figure 1). Thus, for most presentations in the second cluster, a discrepancy was observed between the DAS indicated by multiple autoantibody levels and that recorded from clinical and other laboratory observations. Similar results were obtained when each organ was analyzed separately (data not shown).

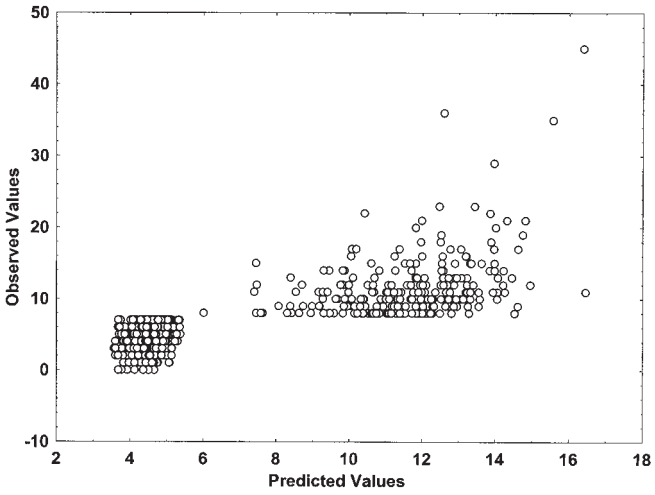

Figure 1. Relationship between general disease activity scores observed (y axis) and those predicted from contemporary autoantibody levels (x axis) analyzed by PWLRA (698 observations). For 68% of all visits, when the disease activity was relatively low, the actual and calculated (predicted) values were similar and formed a well defined cluster (lower left corner). The remaining 32% spread over a wide range, suggesting possible time lags between changes in autoantibody and disease activity (see Table 5).

Longitudinal relationships of autoantibody levels with DAS. An explanation was sought for the modest, although significant, correlation by PWLRA between autoantibody levels and DAS for the 38% of the presentations with predicted values > 7.5 (Figure 1) when the autoantibody levels and DAS data were contemporary. Thus, we considered the possibility that the correlation of DAS could be higher with autoantibody levels obtained at prior visits. To test this hypothesis, a subset of 10 patients with an average of 16 readings/patient (range 11–21) during the study period were analyzed separately. The average time between visits in which both ANA/8 and clinical data were available was 3.3 months (range 1–8 months, median 3.2). To suppress the undesirable effect of the great variability in autoantibody levels, e.g., from 0 to 125,000 for anti-ssDNA, we substituted the actual values by their rank obtained separately for each patient. Thus, 16 values obtained from a patient were replaced by their rank values of 1 to 16 (e.g., assume that among the values obtained from a given patient the 11th visit yielded the 7th highest rank for anti-ssDNA; our analysis established whether this 7th rank value could be predicted from the ranks of selected autoantibody levels obtained during previous visits). To investigate the time relationships among these ranked values by means of multivariate regression analysis, we generated time-lag versions of these variables for one to 5 visits. This approach in effect disqualified the first 5 visits as targets of prediction. The results showed contribution by each one of the autoantibodies. However, only the results obtained with the first-ranked contributor are shown. We performed 2 types of analyses: (1) prediction of general and organ-specific DAS from past autoantibody levels; and (2) prediction of autoantibody levels from past autoantibody levels.

Clinical DAS, both general and organ-specific, were significantly predicted by autoantibody levels (Table 5). Namely, 30% to 46% of the variability in organ-specific DAS and 35% of the general DAS were explained by prior autoantibody levels (R^2). Anti-ssDNA at one prior visit had the highest contribution (ß = 0.24) to the prediction of general DAS, with additional information provided by anti-ssDNA 5 visits prior (ß = 0.21; data not shown). This was also reflected in the finding that anti-ssDNA was the best predictor for lung, cardiovascular, renal, and hematologic DAS changes. Anti-histone was the best predictor for neuromotor and joint, anti-Scl-70 for skin. Thus, autoantibody levels, particularly anti-ssDNA, were significant predictors of subsequent change (increase or decrease) in disease activity.

The prediction of future change (increase or decrease) in autoantibody level by autoantibody was very strong, with overall R = 0.83 ($p < 0.001$), i.e., 69% (R^2) of variability was explained by the previous levels of autoantibodies (Table 6).

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Table 5. Prediction by multiple regression of change in DAS by autoantibody measured during the 5 prior visits.

Predicted Disease DAS	R, $p < 0.001$ Based on all 8	Best Predictor at 1 Prior Visit	ß*	p
General	0.60	ssDNA	0.24	< 0.05
Skin	0.60	Scl-70	0.28	< 0.005
Lung	0.56	ssDNA	0.56	< 0.001
Cardiovascular	0.63	ssDNA	0.36	< 0.01
Neuromotor	0.61	Histone	0.28	< 0.005
Renal	0.55	ssDNA	0.27	< 0.01
Hematologic	0.63	ssDNA	0.41	< 0.001
Joint	0.68	Histone	0.27	< 0.001

* The difference between the ß values and 1 is represented by the contribution of other factors, which did not reach statistical significance.

Table 6. Prediction of change in autoantibody levels by other autoantibodies measured during all 5 prior visits (overall prediction by multiple regression; $R = 0.83$; $p < 0.001$).

Predicted Antibody Against	R, $p < 0.001$ Based on all 8	Best Predictor at 1 Prior Visit	ß*	p
Sm	0.65	Sm	0.34	< 0.001
RNP/Sm	0.75	RNP/Sm	0.47	< 0.001
SSA	0.64	SSA	0.31	< 0.001
SSB	0.70	ssDNA	0.41	< 0.001
Histone	0.59	Histone	0.23	< 0.01
Scl-70	0.66	ssDNA	0.20	< 0.07, NS
ssDNA	0.83	ssDNA	0.60	< 0.001
dsDNA	0.78	ssDNA	0.48	< 0.001

* The difference between the ß values displayed and 1 is represented by the contribution of the same or other antibodies at other prior visits.

Anti-ssDNA was the best single predictor for 4 of the autoantibodies, dsDNA, Scl-70, SSB, and ssDNA, at one prior visit. The remaining Sm, RNP/Sm, SSA, and histone were the best predictors of themselves at one prior visit, i.e., on average the changes were predicted by 3.3 months.

Reliability of retrospective clinical data collection. To establish the consistency of the classification of DAS, the 2 investigators evaluated and recorded independently the data (overlap) from the same 80 clinical examinations. There was very good correlation between the DAS obtained by the 2 investigators ($R = 0.92$). Moreover, if the retrospective clinical data collection was adequate, it was expected that clinical involvement of some systems and organs would be closer to each other. For example, kidney involvement was expected to be closer to the general DAS than other organs. Therefore, DAS were used to calculate the Euclidean distance or linkage between any given organ and the general DAS based on their percentile ranking of values (Figure 2). This analysis showed 3 main groups of organ DAS in the following order of distance from the general DAS: (1) renal and hematological, (2) lung with cardiovascular and Raynaud's, and (3) neuromuscular with skin and joint. Renal involvement was clearly the closest to general DAS. Thus, the linkages support the adequacy of collection of clinical data.

DISCUSSION

This study showed that measurement of 8 autoantibodies offered a disease sensitivity of 87–100% and a specificity of 98–100%. It provided significant objective information regarding the contemporary and subsequent clinical status of patients with SLE. We found that the DAS, general or organ-specific, correlated significantly with autoantibody levels. For about 2/3 of clinical presentations a clear clustering was possible with low DAS and low contemporary autoantibody levels. For the remaining 1/3, the partial correlation between autoantibody levels and DAS was explained by the observation that changes in the 8 autoantibody levels were better early predictors than contemporary correlates of DAS. In this regard, the level of anti-ssDNA autoantibody (ssDNA), which was abnormal in all new untreated patients, was also the best contributor to early prediction of the levels of other autoantibodies and of DAS.

The disease sensitivity of the 3 SLE profiles of 100% for the 25 new patients and 87% for all patients (with active or inactive disease) was far superior to a sensitivity of < 50%

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for the widely used *Crithidia luciliae* test, and also displayed superior specificity, 98–100%[11,21]. The anti-ssDNA antibody test, recommended only as a research test[1,2], is essential to obtain this high level of sensitivity and specificity for patients seen by rheumatologists. The widely held misconception[1,2] that the test for anti-ssDNA has low disease specificity is largely based on the use of the test by itself and/or on poorly designed test systems.

As in previous prospective studies, our retrospective study confirmed the significant contemporary correlation between general DAS and anti-dsDNA levels[9,10]. Also, significant increases in the level of anti-dsDNA 8–10 weeks prior to lupus nephritis flares have been revealed both by the Farr assay and by ELISA[10,22]. However, anti-dsDNA is found only in a subset of about 65% of SLE patients, whereas anti-ssDNA was found in essentially all new SLE patients (Tables 1 and 2)[5], and both anti-dsDNA and anti-ssDNA have been shown to correlate with kidney histopathology[23,24] and with kidney DAS (Table 3). Of particular interest was the finding that anti-ssDNA autoantibody was the main contributor to early prediction of the level of several autoantibodies, including anti-dsDNA (Table 6), as well as DAS (Table 5). Although this pattern was described in case reports[25,26], it has never been statistically documented. Thus, the increase in the levels of autoantibodies is either the cause or is closely associated with the cause of the disease, in a manner similar to that observed in mice developing SLE[27,28]. The increase in anti-ssDNA before anti-dsDNA was also seen in the first reported case of antinuclear autoantibody produced de novo by a neonate[29]. Anti-ssDNA autoantibody measured by ELISA prepared with denatured eukaryotic DNA is actually a misnomer, since the denatured eukaryotic DNA contains all of the DNA epitopes identified by autoantibody in patients with SLE[7,14,30]. Therefore, anti-DNA is a better definition.

The presence of multiple autoantibody specificities is characteristic of SLE[7,31]. It was therefore not surprising to see that the correlations obtained with multiple autoantibodies were superior to those obtained with any of the individual autoantibodies taken separately (Tables 3 and 4). PWLRA showed a very good correlation by clustering 68% of all clinical presentations (visits) in the low activity group by the SLAM score and by autoantibody levels (Figure 1). However, in the second cluster of 32% of clinical presentation, a discrepancy was observed between the clinical activity and autoantibody level. This discrepancy could be explained by changes in autoantibody levels and disease activity at different times, as apparent from the time-lag analysis (Table 5), where a good correlation was observed between DAS and autoantibody levels from prior visits.

Anti-Scl-70 autoantibody, long considered a marker for scleroderma, was abnormal in about 35–37% of patients, either new or when the highest level was recorded for each patient (Table 2), and in about 25% of random SLE patients in previous reports[32,33]. This high proportion of abnormal values was in spite of the 99% specificity (based on blood donors) of the ELISA test. It has been associated with pulmonary hypertension and nephritis[32], an observation also seen in this cohort (Table 3).

Anti-histone autoantibody levels have been reported as correlated[34] or not correlated[35] with DAS. The confusion may be the result of measurement: most of the "anti-histone autoantibody" may in fact be circulating immune complexes or "anomalous IgG" that bind to histone on the solid phase and are measured as autoantibodies[36]. In our statistical analysis we did not differentiate between anti-histone autoantibody and circulating immune complexes. Nevertheless anti-histone autoantibody concentration was a significant contributor to the multiple regression for neuromotor and joint manifestations (Table 5).

As in our study, contemporary or time-lag correlations between DAS and antibody levels have been reported for individual autoantibodies: anti-Sm[37], anti-RNP[11,38-40], and anti-SSA[13,41-43]. However, as an individual factor, concentration of anti-SSA was not significantly correlated with contemporary general or organ/system-specific DAS (Table 3), although it was a small, but significant, contributor to the multiple regression (Table 4). In this study the most valuable contemporary or early predictor of disease activity was not any particular autoantibody but the change in concentration of multiple autoantibodies, although the contribution of each autoantibody to the variance was different in importance. Most previous work focused on a particular autoantibody, which is difficult to apply to the large diversity of autoantibodies in individual patients with SLE (Table 1).

The retrospective collection of clinical data represents the main limitation of our study. However, our study was statistical in design and a significant correlation was found between data collected by prospective and retrospective analyses of charts with the help of SLAM data ($R = 0.67$, $p < 0.0001$)[19]. The absence of data in about 15% of the visits is more likely to mean "0," as we recoded, than a value indicating active involvement of an organ or system. Nevertheless, the reliability of clinical data collection was reflected in the expected Euclidian distance between various organs and systems (Figure 2), and in the significant correlation between disease activity and anti-dsDNA autoantibodies, well established from prospective studies. For example, renal and hematological activities were closely linked to the general DAS, but at a relatively large distance from joint and skin activity. However, any correlation between the relatively hard values representing autoantibody levels and the "soft" routine clinical data is difficult. For example, nephritis is present in all SLE patients if the biopsy is used[44], but only in about 50% of patients if we rely on other clinical laboratory results.

One of the practical consequences of our study is that anti-ssDNA antibody appears to have ~ 100% sensitivity for

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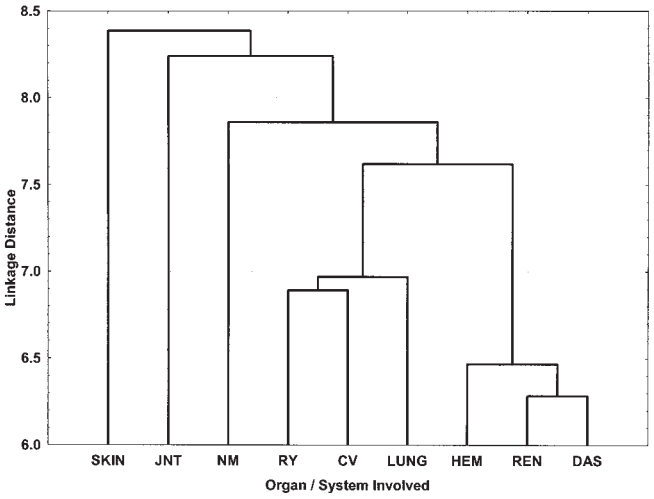

Figure 2. Linkage (Euclidian distance) between the general activity score (DAS) and the activity scores of different organs and systems (JNT: joint, NM: neuromotor, RY: Raynaud's phenomenon, CV: cardiovascular, HEM: hematological, REN: renal, DAS: general Disease Activity Score). As expected for a good collection of clinical data, the renal and hematologic manifestations were closely linked to DAS, but very distant from joint and skin manifestations.

SLE (i.e., it could rule out a new onset SLE) and, in combination with other autoantibodies, 98% specificity. It was the main contributor to the ability of the ANA/8 to predict changes in DAS. This ability may be useful in development of pattern recognition software for preemptive and effective early therapy[45]. However, additional large population studies are necessary. Another practical consequence is the possibility of using multiple autoantibody measurements, collected prospectively and processed by pattern recognition software, as guidance tools in the diagnosis and management of SLE.

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