Diagnostic value of combined evaluation of neopterin and anti-DNA antibody levels for assessment of disease activity in systemic lupus erythematosus

G. Nagy1,2, M. Brózik2, L. Tornóci3, P. Gergely2

13rd Department of Internal Medicine; 2Central Laboratory of Immunology; 3Institute of Pathophysiology, Semmelweis University, Budapest, Hungary

Abstract

Objective

The present study was designed to investigate whether the combined evaluation of laboratory tests could improve their diagnostic value. Laboratory parameters of systemic lupus erythematosus (SLE), such as anti-dsDNA, neopterin, soluble IL-2 receptor (sIL-2R), C3, C4, and complement haemolytic activity (CH50), and a logistic model that provides the probable clinical activity (PCA) of a given patient, calculated by stepwise multiple logistic regression analysis from the parameters mentioned above - were evaluated as SLE activation markers.

Methods

Serum samples from 101 patients were assayed by ELISA (neopterin, sIL-2R, anti-dsDNA), radial immunodiffusion (C3, C4) and haemolytic complement assay (CH50).

Results

Among the parameters investigated, PCA showed the greatest difference between the values representing active and inactive stages of the disease (p < 0.001), the highest correlation with SLEDAI (p < 0.001) and the highest sensitivity and specificity values (0.91, 0.88, respectively). Moreover, this value of PCA was the most useful in discriminating between active and inactive sample pairs.

Conclusion

We conclude that PCA is more useful in evaluating SLE activity than the single parameters studied.

Key words

Systemic lupus erythematosus, anti-DNA, neopterin.
Neopterin, anti-DNA and SLE activity / G. Nagy et al.

György Nagy, MD; Márta Brózik, PhD; László Tornóci, MD, PhD; and Peter Gergely, MD, PhD.

This work was supported by grants from the Ministry of Health No. 502, the Ministry of Education No. 0856, and the Hungarian National Science Foundation No. T026525.

Please address correspondence and reprint requests to: György Nagy, Central Laboratory of Immunology, Semmelweis University, Maria u 41, H-1085 Budapest, Hungary. E-mail: ngyorgy@kat.sote.hu

Received on February 28, 2000; accepted in revised form on September 8, 2000. © Copyright CLINICAL AND EXPERIMENTAL RHEUMATOLOGY 2000.

Introduction
One of the greatest challenges of systemic lupus erythematosus (SLE) is distinguishing patients with active disease from those with inactive disease. There are a number of laboratory tests used for monitoring disease activity in SLE, but we still lack an indicator which is both sensitive and specific (1-4).

The serum anti-dsDNA antibody level is one of the most frequently used laboratory tests. Numerous reports have found a correlation between the level of anti-dsDNA antibodies and disease activity (2,3,5,6). Laboratory tests measuring the activation of the complement system, such as complement haemolytic activity (CH50), C3 and C4 levels are also widely used to measure SLE activity (1-3, 6-8). In contrast with previous tests based on the activation of the humoral immune system, there are other tests reflecting a primarily cellular immune response. Neopterin and sIL-2R levels are prominent members of this latter group (9,10). Both neopterin and sIL-2R levels have been shown to be valuable in assessing SLE activity (11-25).

Thus different measures which at least partially reflect different parts of the activation of the immune system have been found to be valuable, suggesting that combinations of different tests could be more informative than the single parameters. The general problem with combinations is that the interpretation of the results is difficult, because the errors of the tests used are also present in the combinations. Mathematical models can be used to overcome this problem. With these it is possible to find the best combinations from the arithmetical possibilities of the model which was used to reach the lowest rate for both false negatives and false positives. However, very few attempts have been made to evaluate a combination of tests in order to obtain more information from laboratory data (26-30).

The aim of the present study was to assess the value of a logistic model that is calculated by stepwise multiple logistic regression analysis using CH50, C3, C4, anti-dsDNA, neopterin and sIL-2R, and to compare this model with the value of the single tests mentioned above.

Patients and methods
Patients and samples
101 (95 female, 6 male) Caucasian patients suffering from SLE, fulfilling 4 or more of the American Rheumatism Association (ARA) criteria for SLE (31), were included. Single samples were taken from 81 patients, and 2 samples from 20 patients. The samples of these latter patients were used to evaluate active and inactive sample pairs. In the present study 9 patients had renal involvement, with one or more of the following renal manifestations: hematuria, proteinuria, pyuria, or urinary casts. All patients, even those with renal involvement, had normal renal excretory function (serum creatinine < 120 mM/l). Informed consent was obtained from each participant.

Patients with the definitive diagnosis of a viral or bacterial infection or an inflammatory disorder other than SLE at the time of investigation were excluded from this study.

Routine laboratory tests
Blood and urine samples were taken at each visit. Venous blood samples were drawn into vacutainer tubes, centrifuged immediately and refrigerated. Routine laboratory tests included a complete blood cell count, serum creatinine, erythrocyte sedimentation rate, urinary sediment and total protein measurement.

Immunological tests
Serum anti-(native)dsDNA, serum neopterin (BRAMS Diagnostica GmbH, Berlin, Germany) and sIL-2R (Genzyme Diagnostics, Cambridge, MA, USA) levels were measured using ELISA methods. CH50 was measured in a haemolytic assay. C3 and C4 levels were measured using single radial immunodiffusion methods. The cut-off value for the anti-dsDNA level was 7.18 U/ml (mean + 2 SD), based on the data for the healthy control group (n = 100). The normal ranges were 48 - 105 U/ml for CH50, 0.7 - 1.8 g/l for C3 and 0.08 - 0.3 g/l for C4 based on the data of the healthy control group (n = 130). The cut-off values (mean+2 SD) for sIL-2R and neopterin were 2315 pg/ml and 9.2 nmol/l respectively based on the data for the healthy control group (n = 38); this was comparable with the manufacturers’ data.
Determination of disease activity
Patients were considered to have active lupus if they met both of the following criteria: (a) a score of at least 2 on the clinical activity scale (SLEDAI); and (b) the requirement for immediate treatment to bring symptoms under control symptoms (32). The physician’s global assessment was, in all cases, in accordance with the diagnosis of the activity mentioned above. Clinical assessment was made without knowledge of the laboratory data.

Statistical analysis
The SAS statistical package version 6.12 was used for all calculations. Stepwise logistic regression analysis was used to find the best variables to be incorporated in a logistic regression model and an estimation for the model parameters (A1, A2 and C, see below).

Differences between the parameters measured in the patient groups with active and inactive stages of SLE were tested using the Mann-Whitney’s test. Differences between gender in the patient groups with active and inactive stages of SLE were tested using Fisher’s exact test. The correlation of parameters with each other and with the SLEDAI score was calculated by the Spearman’s rank correlation test and by linear regression analysis. To avoid statistical bias from those patients who had two samples, only the active one was used for statistical analysis.

A modified version of the SLEDAI index (mSLEDAI) was used to evaluate the correlation between clinical activity and measured factors, and also to define active disease, as mentioned above, because the SLEDAI criteria (33) contain anti-dsDNA and complement. mSLEDAI was calculated by omitting anti-DNA and complement from the SLEDAI (34).

Specificity and sensitivity were calculated using the standard formula. Receiver operator characteristic (ROC) curves (35) were used to identify cutoff points for disease activity, measured by ‘PCA’, neopterin and anti-dsDNA, which provide the greatest sensitivity and specificity in determining lupus disease activity, based on the criteria mentioned above.

Results
Thirty-two (26.44%) of the samples were classified as being from patients with active SLE and 89 (73.55%) from patients with inactive disease. Ten (31.25%) of the 32 active samples were from patients with activity in more than one organ system and 22 (68.75%) were from patients with activity in one organ system [musculoskeletal (10), dermal (5), central nervous system (CNS) (4) and renal (3)]. The patients’ characteristics are presented in Table I. No significant differences were found in gender, age or disease duration between active and inactive patients. The levels of PCA, neopterin and anti-dsDNA in patients with different organ manifestations are shown in Figure 1. Neopterin and PCA levels did not differ significantly between active SLE patients with renal manifestation and active SLE patients without renal manifestation. In contrast levels of anti-dsDNA were significantly higher in active SLE patients with renal manifestation than in active SLE patients without renal manifestation (p = 0.025).

Logistic regression analysis
We have found that the logistic regression model using anti-DNA and neopterin best discriminates the active and inactive disease patients. Adding any other of the studied laboratory parameters (CH50, C3, C4, sIL-2R) did not significantly improve the model.

The model is described by the following formula:

\[ PCA = \frac{1}{1 + e^{-(C+A1X1+A2X2)}} \]

where \( C = -3.8412, A1 = 0.042, X1 = \) anti-dsDNA plasma concentration, \( A2 = 0.218, \) and \( X2 = \) neopterin plasma concentration.

The model provides the PCA value that helps to estimate disease activity in a given patient. The PCA value is between 0 and 1 (0 < PCA < 1). The higher it is, the more likely that the patient has active disease. This PCA value was tested with the other parameters as a potential SLE activation marker.

Discriminative power of PCA and the single tests between patients with active and inactive disease
The mean values and standard deviations in samples from patients with active and inactive disease, the differences in the PCA, anti-dsDNA, neopterin, CH50, C4, sIL-2R and C3 values between active and inactive disease patients are shown in Table II. The greatest difference be-

<table>
<thead>
<tr>
<th>Table I. Patients’ characteristics.</th>
<th>Active</th>
<th>Inactive</th>
<th>Inactive vs. active</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>32</td>
<td>69</td>
<td>n.s.</td>
</tr>
<tr>
<td>Male/female</td>
<td>1/31</td>
<td>5/64</td>
<td>n.s.</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>35.2 ± 10.3</td>
<td>37.1 ± 11.2</td>
<td>n.s.</td>
</tr>
<tr>
<td>Disease duration (yrs)</td>
<td>7.92 ± 6.47</td>
<td>11.1 ± 8.78</td>
<td>n.s.</td>
</tr>
<tr>
<td>SLEDAI</td>
<td>8.19 ± 2.67</td>
<td>1.42 ± 1.57</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>mSLEDAI</td>
<td>5.9 ± 2.1</td>
<td>0.4 ± 0.6</td>
<td>p &lt; 0.001</td>
</tr>
</tbody>
</table>

| Table II. Values of PCA, anti-dsDNA, neopterin, CH50, C4, sIL-2R and C3 (mean ± SD) in the active and inactive patient groups, Z values and the significance levels (p) of the differences. |
|-------------------------------------|--------|----------|---------------------|
| Test                                | Active n = 32 | Inactive n = 69 | Inactive vs. active |
| PCA                                 | 0.63 ± 0.31 | 0.16 ± 0.16 | Z = -6.35 | p < 0.001 |
| Anti-dsDNA [U/ml]                   | 32.96 ± 28.49 | 11.36 ± 23.98 | Z = -5.43 | p < 0.001 |
| Neopterin [nmol/l]                  | 18.67 ± 13.38 | 7.29 ± 4.21 | Z = -4.73 | p < 0.001 |
| CH50 [U/ml]                         | 56 ± 25 | 75 ± 29 | Z = -3.28 | p = 0.001 |
| C4 [g/l]                            | 0.12 ± 0.09 | 0.18 ± 0.1 | Z = -2.73 | p = 0.006 |
| sIL-2R [pg/ml]                      | 4016 ± 2269 | 2990 ± 1603 | Z = -1.98 | p = 0.047 |
| C3 [g/l]                            | 1.1 ± 0.6 | 1.3 ± 0.59 | Z = -1.98 | p = 0.047 |
Fig. 1. The mean levels and standard deviations of PCA, neopterin and anti-dsDNA in patients with active SLE. The organ manifestations and the number of patients with different manifestations are indicated.

Fig. 2. ROC curves of PCA, anti-dsDNA and neopterin. Arrows indicate the cutoff points in each case that provided the greatest sensitivity and specificity in determining lupus disease activity.

between patients in the active and inactive stages was found in the values of PCA (p < 0.001). Lower, but also highly significant differences were found in the anti-dsDNA, neopterin, CH50 and C4 levels. In the case of the sIL-2R and C3 values, the difference between the patients with active and inactive disease was less, but still significant.

Active and inactive sample pairs
Twenty patients had a flare during the study period. PCA followed the changes in the activity of the disease in 20/20, neopterin in 17/20, sIL-2R in 16/20, anti-dsDNA, CH50, C4 in 14/20 and C3 in 12/20 cases (data not shown).

Sensitivity and specificity of PCA and parameters measured
Receiver operator characteristic (ROC) curves were constructed for the three parameters showing the greatest difference between the active and inactive stages and the highest correlation with the mSLEDAI: PCA, neopterin and anti-dsDNA (Fig. 2). Based on the analysis of the ROC curves, the optimal sensitivity and specificity were found to be 0.91 and 0.88, respectively, at the cutoff point of 0.25 for PCA; 0.75 sensitivity and 0.76 specificity at the cutoff point of 8.9 nmol/l for neopterin; and 0.78 sensitivity, and 0.75 specificity at the cutpoint point of 13.9 U/ml for anti-dsDNA. The sensitivity and specificity of CH50, C4, sIL-2R, and C3 are shown in Table III.

Correlation of PCA and the single tests with mSLEDAI and with each other
Dot plots of the mSLEDAI and PCA, neopterin levels, anti-dsDNA levels of the patients studied are shown in Figure 3. mSLEDAI displayed the highest correlation with PCA (p<0.001, r=0.63). mSLEDAI was also highly correlated with anti-dsDNA (p<0.001, r=0.43) and neopterin (p<0.001, r=0.49).

A significant positive correlation was observed between mSLEDAI and sIL-2R values (p=0.025), while a significant negative correlation was observed between mSLEDAI and CH50 (p<0.001), C4 (p=0.004) and C3 (p=0.038) values. PCA was the most prominent correlating factor with mSLEDAI in the
Neopterin, anti-DNA and SLE activity / G. Nagy et al.

Discussion

Our findings confirm previous observations that tests for evaluating SLE activity partially but imperfectly measure the true disease activity; thus no single test is sufficiently sensitive and specific to assess SLE activity (1-4). Neopterin was reported to have 62% sensitivity and 72% specificity and sIL-2R was reported to have 64% sensitivity and 77% specificity (13, 23) for detecting SLE activity. Anti-dsDNA, C3, C4 and CH50 were reported to have 35-60% sensitivity and 50-70% specificity (3, 7, 32). Our results concerning the diagnostic value of the applied single tests, except sIL-2R, are similar, or even better than those reported in the literature. The previously reported combinations of more laboratory tests suggested that the clinical value of the combined evaluation may be higher than that of the single tests (26-30). Our present findings support and extend these results; we calculated a simple formula which may be applicable in the clinical laboratory.

Based on laboratory parameters evaluated in this study, stepwise logistic regression analysis revealed that neopterin and anti-dsDNA levels provide the best model for estimating disease activity. Using this model, PCA probability was obtained for every sample, and thus was thoroughly tested as a potential activation marker. PCA showed the highest difference between active and inactive stages of the disease. In addition, it showed the best correlation with mSLEDAl among the parameters studied in the whole patient population and in the populations of clinically active patients with or without renal manifestations. It also had the highest overall sensitivity and specificity. Moreover, PCA was the only parameter which followed the changes in the activity of the disease in all 20

**Table III.** Sensitivity, specificity and the cut-off values of PCA, anti-dsDNA, neopterin, CH50, C4, sIL-2R and C3

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Cut-off</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCA</td>
<td>91</td>
<td>88</td>
<td>0.25</td>
</tr>
<tr>
<td>Anti-dsDNA</td>
<td>78</td>
<td>75</td>
<td>13.9 [U/ml]</td>
</tr>
<tr>
<td>Neopterin</td>
<td>75</td>
<td>76</td>
<td>8.9 [nmol/l]</td>
</tr>
<tr>
<td>CH50</td>
<td>68</td>
<td>66</td>
<td>65 [U/ml]</td>
</tr>
<tr>
<td>C4</td>
<td>62.5</td>
<td>60</td>
<td>0.15 [g/l]</td>
</tr>
<tr>
<td>sIL-2R</td>
<td>56</td>
<td>60</td>
<td>3000 [pg/ml]</td>
</tr>
<tr>
<td>C3</td>
<td>56</td>
<td>57</td>
<td>1.1 [g/l]</td>
</tr>
</tbody>
</table>

Fig. 3. Dot plots of the mSLEDAl and PCA, neopterin levels, anti-dsDNA levels of the patients studied. Vertical lines separate the diagrams at the cut-off value in each case that provided the greatest sensitivity and specificity in determining lupus disease activity calculated by the analysis of the ROC curves. Horizontal lines separate the clinically active and inactive patients. Linear regression coefficients are shown in each case.
patients studied. Thus PCA was found to be a considerably better SLE activation marker than the other single tests measured and than most previously reported tests or test combinations. Anti-dsDNA, C3, C4 and CH50 levels reflect primarily humoral immunity. According to our results, they showed a highly significant correlation with each other. However, anti-dsDNA showed a higher difference between active and inactive stages of the disease and a better correlation with the mSLEDAI than C3, C4 or CH50. Serum neopterin and sIL-2R levels reflect cellular immunity (9, 10). Neopterin did not show a significant correlation with any of the studied parameters, but correlated well with mSLEDAI and showed significant differences between the active and inactive stages. Neopterin was also found to be useful in following changes in disease activity in 17/20 cases. The clinical value of neopterin was found to be higher than that of sIL-2R in accordance with others’ observations (13). Based on the above mentioned correlation studies, anti-dsDNA as a useful marker of humoral immune system and neopterin as a useful marker of cellular immune system can be selected, as both are valuable in evaluating SLE activity. However, they at least partially reflect the activity of different parts of the immune system, which is supported by the fact that they do not correlate with each other. This suggests that they can complement each other and may provide an explanation as to why the logistic regression model contains anti-dsDNA and neopterin.

The shape of the ROC curve of anti-dsDNA reflects that it is a sufficiently sensitive but not specific marker of SLE activity. In contrast, neopterin is a specific SLE activation marker, but its sensitivity is lower. Thus, the two ROC curves cross each other. This finding helps to explain why the anti-dsDNA level does not correlate with the neopterin level and supports the fact that information provided by anti-dsDNA and neopterin complement each other. However, the ROC curve of PCA reflects a higher clinical value than that of anti-dsDNA or neopterin alone. The observed significant difference in anti-dsDNA levels between active SLE patients with renal manifestation and active SLE patients without renal manifestation - which is in accordance with others’ observations (2,3,5) - also may help to explain the lack of correlation between neopterin and anti-dsDNA levels. However, neopterin and anti-dsDNA levels did not correlate significantly with each other in the population of active SLE patients with renal manifestation, or in the population of active SLE patients without renal manifestations. The lack of a significant correlation was previously reported between urine neopterin and anti-dsDNA (13).

In conclusion, a logistic regression model was found to be more valuable in evaluating SLE activity than any of the other studied parameters. Further longitudinal studies are needed with a larger sample group to establish the role of this marker in estimating SLE activity, and to assess its power in differentiating disease flares from other conditions, such as intercurrent infections.

Acknowledgements
We are indebted to Kirschfink Michael DVM, PhD, Professor of Immunology (Ruprecht-Karls Universität, Heidelberg, Germany) and to George Füst MD, PhD, Professor of Immunology (Semmelweis University, Budapest, Hungary) for their critical reading of the manuscript.

References

704


