ANTI-TELOMERE ANTIBodies IN SYSTEMIC LUPUS ERYTHEMATOSUS (SLE): A COMPARISON WITH FIVE ANTINUCLEAR ANTIBODY ASSAYS IN 430 PATIENTS WITH SLE AND OTHER RHEUMATIC DISEASES

E M Salonen, A Miettinen, T K Walle, S Koskenmies, J Kere, H Julkunen

Objectives: To investigate the prevalence and diagnostic significance of antibodies against telomeric DNA in systemic lupus erythematosus (SLE) and other autoimmune rheumatic diseases, and to make comparisons with five conventional anti-DNA or anti-nuclear antibody (ANA) assays.

Methods: Antibodies to telomeres, which are highly repetitive sequences of DNA (TTAGGG/CCCTAA) at the end of eukaryotic chromosomes, were measured by an enzyme linked immunosorbent assay (ELISA) in 305 patients with SLE and 125 patients with other autoimmune rheumatic diseases (78 rheumatoid arthritis, 32 primary Sjögren’s syndrome, eight mixed connective tissue disease, seven miscellaneous rheumatic diseases). Other assays used were two commercial ELISA assays for anti-dsDNA using calf thymus DNA as antigen, C. luciliae immunofluorescence, and radioimmunoassay (RIA) for anti-dsDNA and immunofluorescence using Hep-2 cells for ANA.

Results: The prevalence of anti-telomere in SLE was 60%, vs 5% in rheumatoid arthritis and 18% in other autoimmune rheumatic diseases. Specificity of anti-telomere for SLE was 91%; positive and negative predictive values were 95% and 46%, respectively. For anti-dsDNA by two ELISA assays using calf thymus DNA as antigen, sensitivities were 69% and 29% and specificities 66% and 96%, respectively. Other anti-dsDNA assays had low sensitivities (RIA 43%, C. luciliae immunofluorescence 13%). The association of anti-telomere with a history of nephritis in patients with SLE was stronger (p = 0.005) than by any other assay (p = 0.006–0.999). The correlations between the different assays were good (p < 0.001 for all comparisons).

Conclusions: The new ELISA for anti-telomere antibodies using standardised human dsDNA as antigen is a sensitive and highly specific test for SLE.

Differentiating systemic lupus erythematosus (SLE) from other autoimmune rheumatic diseases and undifferentiated connective tissue diseases may be difficult. In a large retrospective European multicentre study including 1000 patients with SLE, the first symptoms preceded the clinical diagnosis by two years. This delay is mainly caused by the highly variable clinical picture of SLE, but it may also reflect the lack of a sensitive and specific laboratory test to diagnose the disease. Diagnosing SLE early and more accurately is important for patient education and for the institution of appropriate treatment to reduce morbidity.

The key diagnostic test for SLE is anti-double-stranded DNA (anti-dsDNA), which is conventionally determined by Farr assays, enzyme linked immunosorbent assays (ELISA), or indirect immunofluorescence using calf thymus or C. luciliae as target antigens. Farr assays and immunofluorescence are reasonably specific but not very sensitive for SLE. ELISA assays are more sensitive in detecting low affinity anti-dsDNA, but they can easily give false positive results with other autoimmune rheumatic diseases and in concomitant infections.

Telomeres are highly repetitive sequences of DNA (TTAGGG/CCCTAA) at the end of eukaryotic chromosomes. It is possible that human antibodies against DNA could be more specific towards this particular and common repeat than towards conventionally used DNA antigens of non-human origin in commercially available assays. A previous study showed that, of 52 patients with SLE, 96% had IgG class, 56% IgM class, and 85% IgA class anti-telomere antibodies by ELISA. In another study, anti-telomere antibodies were documented in 65% of SLE patients and in 75% of patients with active disease. In other autoimmune rheumatic diseases (primary Sjögren’s syndrome and scleroderma) and in controls, the frequencies of these antibodies were about 7%. These results suggest that the anti-telomere antibody assay may be a very sensitive and specific test for SLE, and further, that it may well correlate with the clinical activity of the disease. Previous studies on anti-telomere antibodies have been quite preliminary and done in small numbers of patients, and the comparisons with other relevant tests have been limited.

In this study, we employed a novel ELISA assay using human telomere dsDNA as antigen to test for anti-DNA antibodies. The results were compared with those obtained with two commercially available ELISA assays using calf thymus DNA as antigen, with a Farr assay using bacterial DNA as antigen, with a C. luciliae immunofluorescence assay using kinetoplast DNA as antigen, and with an anti-nuclear antibody assay using human Hep-2 cells as antigen.

METHODS

Patients and controls

The study included 305 patients with SLE as classified according to the ACR criteria. These patients attended the outpatient departments of the University Central Hospitals of Helsinki and Kuopio and 21 other major hospitals in Finland.

Abbreviations: ACR, American College of Rheumatology; ANA, anti-nuclear antibody; anti-dsDNA, anti-double-stranded DNA; MCTD, mixed connective tissue disease; SLE, systemic lupus erythematosus
and were included in a nationwide project, the aim of which was to identify all multiplex SLE families in Finland. The recruitment process, data collection, and clinical manifestations of the patients have been described previously. The mean (SD) age of the 305 SLE patients was 46.2 (13.5) years, range 19 to 84, and the mean number of SLE criteria fulfilled was 5.5 (1.4), range 4 to 10. The majority of the patients were female (95%).

Patients, with autoimmune rheumatic diseases other than SLE attended the outpatient departments of the Helsinki University Central Hospital and included 78 patients with rheumatoid arthritis,11 32 with primary Sjögren’s syndrome,12 14 with scleroderma,14 one with polymyositis,13 and one each with primary biliary cirrhosis and fibrosing alveolitis, both of these having secondary Sjögren’s syndrome. The mean age of these 125 control patients was 49.0 (12.4) years, range 18 to 84, and the mean number of SLE criteria fulfilled was 5.5 (1.4), range 4 to 10. The majority of the patients were female (95%).

Healthy controls consisted of 50 female and 35 male blood donors, 80 persons from the staff of the Helsinki University Central Hospital, and 150 persons from the staff of the Biohit laboratory. The sera from 430 patients and from 314 healthy donors, 80 persons from the staff of the Helsinki University Central Hospital and included 78 patients with rheumatoid arthritis,11 32 with primary Sjögren’s syndrome,12 eight with mixed connective tissue disease (MCTD),13 four with scleroderma,14 one with polymyositis,13 and one each with primary biliary cirrhosis and fibrosing alveolitis, both of these having secondary Sjögren’s syndrome.

Laboratory methods

Anti-telomere IgG antibodies assay

Anti-telomere IgG antibodies were determined using a specific ELISA test (anti-dsDNA test kit, catalogue No AI 003 010, Biohit plc, Helsinki, Finland). In the test the antigen, a 60 base pair long (10 repeats of human telomere sequence) dsDNA, biotinylated at the 5’-end of one strand, is attached to streptavidin coated microplate wells. Duplicate serum samples were tested at 1:100 dilution according to the manufacturer’s instructions. Between the reaction steps the plates were washed using a BW50 microplate strip washer (catalogue No 740 050, Biohit plc). The absorbances were 0.030, Biohit plc). The absorbance values obtained were converted to IU/ml according to the formula included in the test kit:

\[
\text{xA(patient sample)} - \text{xA(blank)} \\
\times \text{standard (IU/ml)} \\
\text{xA(standard)} - \text{xA(blank)}
\]

The upper reference value, 64 IU, for the test was set at the 98th centile for 150 healthy persons from the staff of the Biohit laboratory. This value corresponded to the 0.445 absorbance units (AU). Two samples of the 150 controls were above this value (0.514 and 0.960 AU, corresponding to 78 and 152 IU, respectively).

Other antibody assays

Anti-dsDNA antibodies to calf thymus dsDNA by ELISA were determined by two assays according to the manufacturers’ instructions (Diastat™ anti-dsDNA, Shield Diagnostics, Dundee, UK, and Quanta Lite™ dsDNA, Inova Diagnostics Inc, San Diego, California, USA). The upper reference value for the former test was set at the 98th centile for 165 healthy controls (85 blood donors and 80 persons from the staff of the Helsinki University Hospital) and corresponded to 0.201 OD (optical density) units. Two samples of 165 were above this value (0.209 and 0.235 OD units, respectively). The upper reference value for the latter test was set at the 98th centile for 165 healthy controls and corresponded to 0.526 AU. Three samples of 165 were above this value (0.538, 0.558, and 0.631 AU, respectively).

Iodinated bacterial DNA was used as antigen in the Farr assay (Anti-dsDNA test, Medix Biochemica, Kauniainen, Finland). Values of at least 7 mg/l were considered positive.

Anti-dsDNA antibodies and ANA by the indirect immunofluorescence technique were detected using Crithidia luciliae cells (NOVA Lite™ dsDNA, Inova Diagnostics) and Hep-2 cells (Nova Lite™ ANA, Inova Diagnostics) as substrates, respectively. A titre of at least 320 in the ANA assay and at least 10 in the C. luciliae assay were considered positive.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Antibody positivity among study patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosis</td>
<td>Anti-telomere (ELISA)</td>
</tr>
<tr>
<td>SLE</td>
<td>164/275 (60%)</td>
</tr>
<tr>
<td>RA</td>
<td>4/75 (5%)</td>
</tr>
<tr>
<td>Primary SS</td>
<td>4/13 (30%)</td>
</tr>
<tr>
<td>MCTD</td>
<td>1/8 (13%)</td>
</tr>
<tr>
<td>Scleroderma</td>
<td>0/4 (0%)</td>
</tr>
<tr>
<td>Others*</td>
<td>0/3 (0%)</td>
</tr>
</tbody>
</table>

*One case of polymyositis, one of primary biliary cirrhosis, and one of fibrosing alveolitis, the two last both with secondary Sjögren’s syndrome.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Spearman correlation coefficients of the tests among study patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody assay</td>
<td>Anti-telomere (ELISA)</td>
</tr>
<tr>
<td>Anti-telomere</td>
<td>1.000</td>
</tr>
<tr>
<td>Anti-dsDNA (Shield)</td>
<td>0.720</td>
</tr>
<tr>
<td>Anti-dsDNA (Inova)</td>
<td>0.650</td>
</tr>
<tr>
<td>RIA</td>
<td>0.604</td>
</tr>
<tr>
<td>Crithidia luciliae</td>
<td>0.273</td>
</tr>
<tr>
<td>ANA</td>
<td>0.360</td>
</tr>
</tbody>
</table>

All values were significant at the 0.01 level.

ANA, antinuclear antibodies; anti-dsDNA, anti-double-stranded DNA; RIA, radioimmunoassay.
Association of the tests with SLE
Table 3 shows that the anti-telomere antibody assay was a sensitive (60%) and specific (91%) test for SLE, with a high positive predictive value (95%). The most sensitive assays were anti-dsDNA by ELISA (Shield) (69%) and ANA by immunofluorescence (76%), but their specificities were lower (66% and 45%, respectively). Anti-dsDNA (Inova) and Crithidia immunofluorescence had poor sensitivities, but their specificities were high.

Table 3 shows the best combinations of two assays in the differential diagnosis of SLE. The highest sensitivities were reached by combining ANA with anti-telomere (86%) or anti-dsDNA by ELISA (Shield) (88%) but their specificities were poor (47% and 38%, respectively). The combination of anti-telomere and anti-dsDNA (Shield) had high sensitivity (77%) and an acceptable specificity (67%).

Association of the tests with nephritis
SLE patients with a history of nephritis had higher concentrations of anti-telomere antibodies than patients without nephritis (p = 0.005) (table 4). The associations of other tests, except ANA, were also statistically significant (p = 0.006 to 0.049).

DISCUSSION
We found that the ELISA assay for anti-telomeric DNA antibodies was a sensitive and highly specific test for SLE. The most sensitive test (best screening test) was ANA by immunofluorescence, followed by anti-dsDNA (Shield) and anti-telomere. Anti-telomere was clearly more specific than the first two tests. In our study, anti-dsDNA (Inova) and Crithidia immunofluorescence were too insensitive to be clinically useful in the differential diagnosis of SLE, although they were very specific.

An ideal diagnostic test would be sensitive, specific, and have a high PPV and NPV. Increases in sensitivity lead to decreases in specificity and vice versa, and the percentages depend on the cut off level set for positivity among the controls. We present the results for anti-telomere and anti-dsDNA by the two ELISA assays, choosing values higher than the 98th centile for healthy controls as positive. When the 95th and 99th centile and mean±2 SD were chosen as the cut off point, the conclusions were similar. Table 5 shows that the balance between sensitivity and specificity by anti-telomere was comparable with other ELISA assays. Using mean±2 SD or 95th centile as the cut off point would have increased the sensitivity of anti-telomere to 67–68% without a major loss of specificity.

By combining two assays, the highest sensitivities were reached by ANA together with either anti-telomere (88%) or anti-dsDNA (Shield) (86%). The specificities of these two combinations were, however, quite low and their overall
performance was not better than with individual tests. The combination of anti-telomere with anti-dsDNA (Shield) was as sensitive as but more specific than ANA. This combination was also more sensitive than the corresponding individual assays and had an acceptable specificity. It can thus be recommended as the combination of choice in differentiating SLE from other autoimmune rheumatic diseases.

The prevalence of anti-telomere in SLE and other autoimmune rheumatic diseases in our study was similar to other published values. In a recent study including substantial numbers of patients, Wallace et al. reported that anti-telomere antibody was present in 49% of 220 patients with SLE, in 11% of 18 patients with primary Sjögren’s syndrome, and in 8% of 79 patients with rheumatoid arthritis.

Wallace et al. also reported that, in a cohort of 48 SLE patients, anti-telomere was more sensitive than anti-dsDNA by the Farr assay (71% vs 50%). We used the Farr assay in 291 SLE patients and, similarly, found that it was less sensitive than anti-telomere (43% vs 60%). Data on the specificity of the Farr assay were not available, because the test was not carried out in controls with other rheumatic diseases. In general, the Farr assay is considered to be a specific and reasonably sensitive test for diagnosing SLE.

We found a strong correlation of anti-telomere with nephritis, suggesting that the test could be used as an aid for assessing the activity of SLE. In contrast to these findings, Wallace et al. reported that anti-telomere antibody positivity did not correlate with any specific symptom, sign, laboratory finding, or disease activity score as assessed by SLEDAI (SLE disease activity index). In their study, however, 35% of SLE patients with nephritis were anti-telomere positive compared with 21% of patients without nephritis. Statistical significance was not found, but this may have reflected the relatively small number of patients (48) in the comparison. Obviously, further (longitudinal) studies with larger numbers of patients are needed to evaluate whether anti-telomere relates to the activity of SLE.

Our study design does not fully mirror daily clinical practice in diagnosing SLE. We took serum samples from SLE patients being followed up routinely and from patients with other clearly defined autoimmune rheumatic diseases. A more ideal setting would be to use sera from symptomatic patients referred to the laboratory for determination of anti-dsDNA for diagnostic purposes. To use sera from patients with established diagnoses does not, however, influence the relative performance of the different tests. It is also probable that if more samples from patients with active disease had been available, the sensitivities of the tests for SLE would have been greater.

Table 5

<table>
<thead>
<tr>
<th>Cut off point</th>
<th>Positive SLE</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-telomere 95th centile</td>
<td>204/379 (54%)</td>
<td>188/275</td>
<td>68%</td>
<td>85%</td>
<td>92%</td>
</tr>
<tr>
<td>98th centile</td>
<td>173/379 (46%)</td>
<td>164/275</td>
<td>60%</td>
<td>91%</td>
<td>95%</td>
</tr>
<tr>
<td>99th centile</td>
<td>93/379 (25%)</td>
<td>90/275</td>
<td>33%</td>
<td>97%</td>
<td>97%</td>
</tr>
<tr>
<td>Mean-2SD</td>
<td>199/379 (53%)</td>
<td>184/275</td>
<td>67%</td>
<td>86%</td>
<td>92%</td>
</tr>
<tr>
<td>Anti-dsDNA (Shield) 95th centile</td>
<td>265/330 (80%)</td>
<td>184/205</td>
<td>90%</td>
<td>35%</td>
<td>69%</td>
</tr>
<tr>
<td>98th centile</td>
<td>185/330 (56%)</td>
<td>142/205</td>
<td>69%</td>
<td>66%</td>
<td>77%</td>
</tr>
<tr>
<td>99th centile</td>
<td>175/330 (53%)</td>
<td>136/205</td>
<td>66%</td>
<td>69%</td>
<td>78%</td>
</tr>
<tr>
<td>Mean-2SD</td>
<td>244/330 (74%)</td>
<td>173/205</td>
<td>84%</td>
<td>43%</td>
<td>71%</td>
</tr>
<tr>
<td>Anti-dsDNA 95th centile (Inova)</td>
<td>141/404 (35%)</td>
<td>121/279</td>
<td>43%</td>
<td>84%</td>
<td>86%</td>
</tr>
<tr>
<td>98th centile</td>
<td>87/404 (22%)</td>
<td>82/279</td>
<td>29%</td>
<td>96%</td>
<td>94%</td>
</tr>
<tr>
<td>99th centile</td>
<td>79/404 (20%)</td>
<td>75/279</td>
<td>27%</td>
<td>97%</td>
<td>95%</td>
</tr>
<tr>
<td>Mean-2SD</td>
<td>151/404 (37%)</td>
<td>129/279</td>
<td>46%</td>
<td>82%</td>
<td>85%</td>
</tr>
</tbody>
</table>

The effect of different cut off points for positivity in healthy controls is shown.

Anti-dsDNA, anti-double-stranded DNA; NPV, negative predictive value; PPV, positive predictive value; SLE, systemic lupus erythematosus.
REFERENCES

Anti-telomere antibodies in systemic lupus erythematosus (SLE): a comparison with five antinuclear antibody assays in 430 patients with SLE and other rheumatic diseases

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