EXTENDED REPORT

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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Objective: 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 as antigen, *Crithidia 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%, v 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 as antigen, sensitivities were 69% and 29% and specificities 66% and 96%, respectively. Other anti-dsDNA assays had low sensitivities (RIA 43%, *Crithidia 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.¹ In a large retrospective European multicentre study including 1000 patients with SLE, the first symptoms preceded the clinical diagnosis by two years.² 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 *Crithidia luciliae* as target antigens.³ 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.^{4–5}

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 study⁶ 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.⁷ 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,^{6–7} and the comparisons with other relevant tests have been limited.⁸

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 *Crithidia luciliae* immunofluorescence assay using kinetoplast DNA as antigen, and with an antinuclear 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.⁹ 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, antinuclear antibody; anti-dsDNA, anti-double-stranded DNA; MCTD, mixed connective tissue disease; SLE, systemic lupus erythematosus

Table 1 Antibody positivity among study patients

Diagnosis	Anti-telomere (ELISA)	Anti-dsDNA (ELISA, Shield)	Anti-dsDNA (ELISA, Inova)	<i>Crithidia luciliae</i> (IF)	Farr assay (RIA)	ANA (IF)
SLE	164/275 (60%)	142/205 (69%)	82/279 (29%)	38/298 (13%)	126/291 (43%)	227/298 (76%)
RA	4/76 (5%)	17/78 (22%)	4/78 (5%)	1/77 (1%)	ND	28/78 (36%)
Primary SS	4/13 (30%)	20/32 (63%)	1/32 (3%)	0/32 (0%)	ND	27/32 (84%)
MCTD	1/8 (13%)	4/8 (50%)	0/8 (0%)	0/8 (0%)	ND	8/8 (100%)
Scleroderma	0/4 (0%)	0/4 (0%)	0/4 (0%)	0/4 (0%)	ND	3/4 (75%)
Others*	0/3 (0%)	0/3 (0%)	0/3 (0%)	0/3 (0%)	ND	3/3 (100%)

*One case of polymyositis, one of primary biliary cirrhosis, and one of fibrosing alveolitis, the two last both with secondary Sjögren's syndrome. ANA, antinuclear antibodies; ELISA, enzyme linked immunosorbent assay; IF, immunofluorescence; MCTD, mixed connective tissue disease; ND, not done; RA, rheumatoid arthritis; RIA, radioimmunoassay; SLE, systemic lupus erythematosus; SS, Sjögren's syndrome.

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.¹⁰ 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 eight with mixed connective tissue disease (MCTD),¹³ four with scleroderma,¹⁴ one with polymyositis,¹⁵ 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 76 years, and two of them were male (2%).

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 controls were stored at -20°C until tested. Not all antibody determinations were done in all patients. Missing data reflected small amounts of sera in unselected patients.

Laboratory methods

Anti-telomere antibody 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 measured with a BP800 microplate reader (catalogue No 740 030, Biohit plc). The absorbance values obtained were

converted to IU/ml according to the formula included in the test kit:

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

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^{TM} anti-dsDNA, Shield Diagnostics, Dundee, UK, and Quanta Lite^{TM} 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^{TM} dsDNA, Inova Diagnostics) and Hep-2 cells (Nova Lite^{TM} ANA, Inova Diagnostics) as substrates, respectively. A titre of at least 320 in the ANA assay and at least 10 in the *Crithidia* assay were considered positive,

Table 2 Spearman correlation coefficients of the tests among study patients

Antibody assay	Anti-telomere	Anti-dsDNA (Shield)	Anti-dsDNA (Inova)	RIA	<i>Crithidia luciliae</i>	ANA
Anti-telomere	1.000	0.720	0.650	0.604	0.273	0.360
Anti-dsDNA (Shield)		1.000	0.711	0.492	0.343	0.429
Anti-dsDNA (Inova)			1.000	0.568	0.284	0.373
RIA				1.000	0.401	0.395
<i>Crithidia luciliae</i>					1.000	0.213
ANA						1.000

All values were significant at the 0.01 level.

ANA, antinuclear antibodies; anti-dsDNA, anti-double-stranded DNA; RIA, radioimmunoassay.

Table 3 Autoantibodies in systemic lupus erythematosus and other autoimmune rheumatic diseases

Autoantibody (method)	Positive	SLE	Sensitivity	Specificity	PPV	NPV
Anti-telomere	176/379 (46%)	164/275	60%	91%	95%	46%
Anti-dsDNA (Shield)	185/330 (56%)	142/205	69%	66%	77%	57%
Anti-dsDNA (Inova)	87/404 (22%)	82/279	29%	96%	94%	38%
<i>Crithidia luciliae</i> (IF)	39/422 (9%)	38/298	13%	99%	97%	32%
ANA (IF)	296/423 (70%)	227/298	76%	45%	77%	44%
<i>Best combinations of two assays</i>						
Anti-telomere+anti-dsDNA (Shield)	174/287 (61%)	140/183	77%	67%	80%	62%
Anti-telomere+ANA	290/377 (77%)	235/273	86%	47%	81%	56%
Anti-dsDNA (Shield)+ANA	259/331 (78%)	181/206	88%	38%	70%	65%

ANA, antinuclear antibodies; anti-dsDNA, anti-double-stranded DNA; IF, immunofluorescence; NPV, negative predictive value; PPV, positive predictive value; SLE, systemic lupus erythematosus.

respectively. At these titres fewer than 4% and 1% of blood donors are positive (Miettinen A, unpublished results).

Statistical methods

The sensitivities, specificities, and positive and negative predictive values (PPV and NPV, respectively) were calculated as indicated in table 3. Spearman's rank correlation was applied in studying correlations between the different assays. The Mann–Whitney U test was used to compare the levels of the antibodies in patients with or without nephritis. All p values were two tailed, and differences at 0.05 were considered significant.

RESULTS

Prevalence of the antibodies

If the cut off was set at the 98% centile, anti-telomere antibodies were found in 60% of patients with SLE (table 1). In patients with rheumatoid arthritis and primary Sjögren's syndrome, the prevalences were 5% and 30%, respectively. In other autoimmune rheumatic diseases, anti-telomere antibodies were rare (7%, 1/15).

By the two other ELISA assays, 69% or 29% of the patients had anti-dsDNA antibodies (table 1). Anti-dsDNA by RIA and immunofluorescence were present in 43% and 13% of patients with SLE, respectively. ANA by immunofluorescence using Hep-2 cells as antigen was present in 76% of SLE patients.

Correlations between different assays

Correlation coefficients between the different assays are summarised in table 2. In general, anti-telomere antibodies correlated well with the ELISA assays ($r = 0.650$ to 0.720) and less well with other tests ($r = 0.273$ to 0.479). ANA and *Crithidia* immunofluorescence had the lowest correlations with other tests. All values were, however, significant at the 0.01 level.

Table 4 Associations of the antibody assays (titres) with a history of lupus nephritis

Antibody assay	p Value
Anti-telomere	0.005
Anti-dsDNA (Shield)	0.029
Anti-dsDNA (Inova)	0.024
RIA	0.006
<i>Crithidia luciliae</i>	0.049
ANA	0.999

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

Table 5 Anti-telomere and anti-dsDNA by enzyme linked immunosorbent assay in systemic lupus erythematosus and other autoimmune rheumatic diseases

	Cut off point	Positive	SLE	Sensitivity	Specificity	PPV	NPV
Anti-telomere	95th centile	204/379 (54%)	188/275	68%	85%	92%	50%
	98th centile	173/379 (46%)	164/275	60%	91%	95%	46%
	99th centile	93/379 (25%)	90/275	33%	97%	97%	35%
	Mean+2SD	199/379 (53%)	184/275	67%	86%	92%	49%
Anti-dsDNA (Shield)	95th centile	265/330 (80%)	184/205	90%	35%	69%	68%
	98th centile	185/330 (56%)	142/205	69%	66%	77%	57%
	99th centile	175/330 (53%)	136/205	66%	69%	78%	55%
	Mean+2SD	244/330 (74%)	173/205	84%	43%	71%	63%
Anti-dsDNA (Inova)	95th centile	141/404 (35%)	121/279	43%	84%	86%	40%
	98th centile	87/404 (22%)	82/279	29%	96%	94%	38%
	99th centile	79/404 (20%)	75/279	27%	97%	95%	37%
	Mean+2SD	151/404 (37%)	129/279	46%	82%	85%	41%

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.

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.⁶⁻⁸ In a recent study including substantial numbers of patients, Wallace *et al*⁸ 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% *v* 50%). We used the Farr assay in 291 SLE patients and, similarly, found that it was less sensitive than anti-telomere (43% *v* 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.¹⁶

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*⁸ 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.¹⁶ 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.

About 0.15% of the whole genome is composed of the repetitive telomere DNA sequence. Thus it is possible that this sequence plays a role as an immunogen for anti-dsDNA antibody production in SLE. However, at present there is no evidence for sequence specificity of the anti-DNA antibodies in this disease. Likewise there is no evidence for any biological role for such antibodies. Quantitative immunochemical studies are needed in which telomeric DNA is compared with ordinary dsDNA and with single stranded DNA in absorption and cross competition experiments with SLE sera. The results of such studies will eventually tell us if there is something unique in telomeric DNA.¹⁷

Why should the anti-telomere assay be better than other anti-native DNA antibody assays? Currently, most laboratories use indirect immunofluorescence, the Farr assay, or various ELISA techniques with different types of DNA as antigens. Most ELISA assays use DNA extracted from mammalian thymic tissues. In the *Crithidia luciliae* immunofluorescence assay, the antigen is eukaryotic kinetoplast DNA, and many Farr and ELISA assays use bacterial or plasmid DNA.

There is evidence for antigenic heterogeneity among the various DNAs. Unmethylated CpG motifs are frequent in bacterial DNA, and antibodies against bacterial DNA not binding to human DNA can be detected in normal human sera.¹⁸ The dsDNA antibodies of SLE recognise antigenic epitopes involving the sugar-phosphate backbone of DNA, and react with bacterial, viral, plant, and animal DNA.¹⁹ Thus the source of DNA may have only a minor effect on the binding of the SLE anti-DNA antibodies. The use of bacterial DNA as antigen—or DNA containing stretches of single stranded DNA—may increase the binding of antibodies of non-SLE patients or normal control subjects and decrease the specificity of the assay. The anti-telomere assay using synthetic mammalian dsDNA of known sequence as antigen is easy to standardise, eliminates binding of non-specific antibodies, and increases the specificity of the assay.

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