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*Original Article*

# Anti-Telomere Antibodies in Lupus Glomerulonephritis

Daniela Monova[1], Simeon Monov[1] and Trenka Argirova[2]

[1]Department of Internal Diseases, Medical University – Sofia, [2]Department of Biochemistry - Sofia University, Sofia, Bulgaria

**Abstract**

**Background.** Differentiating Systemic lupus erythematosus (SLE) from other autoimmune rheumatic diseases and undifferentiated connective tissue diseases may be difficult. Diagnosing SLE early and more accurately is important for patient education and for the institution of appropriate treatment to reduce morbidity. The aim of this study is to investigate the prevalence and diagnostic significance of antibodies against telomeric DNA in 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 225 patients with SLE and 108 with other autoimmune rheumatic diseases (96 rheumatoid arthritis, 12 mixed connective tissue disease). Other assays used were two commercial ELISA assays for anti-dsDNA using calf thymus as antigen, Crithidia luciliae immunofluorescence and immunofluorescence using Hep-2 cells for ANA.

**Results**. The prevalence of anti-telomere in SLE was 58,67 %, versus 5,21 % in rheumatoid arthritis and 25 % in mixed connective tissue disease. Specificity of anti-telomere for SLE was 90,6 %, positive and negative predictive value were 94,2 % and 45,8 %, respectively. Other anti-dsDNA assays had low sensitivities. The association of anti-telomere with a history of nephritis in patients with SLE was stronger (P=0,005) than the other assay. The correlations between the different assays were good (P<0,001 for all comparisons).

**Conclusions.** We found that the ELISA assay for anti-telomeric DNA antibodies was a sensitive and highly specific test for SLE. The most sensitive test was ANA by immunofluorescence, followed by anti-dsDNA and anti-telomere. Anti-telomere was clearly more specific than the first two tests.

**Keywords:** antibodies, anti-telomere, glomerulonephritis, systemic lupus erythematosus

## Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by involvement with a broad spectrum of clinical manifestations and the existence of multiple species of autoantibodies. 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 immunofluo-rescence using calf thymus or Crithidia luciliae as target antigens [1]. 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 day can easily give false positive results with other autoimmune rheumatic diseases and in concomitant infections [2].

Telomeres are highly repetitive sequences of DNA (TTAGGG/CCCTAA) at the end of eukaryotic chromosomes. 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 [3,4].

## Patients and methods

225 patients (21 males, 204 females; mean age 30,02 ± 7,38 years) with SLE, who fulfilled the SLE criteria of the American College of Rheumatology [5] and 108 patients (20 males, 88 females; mean age 36,28 ± 9,47) with other autoimmune rheumatic diseases (96 rheumatoid arthritis, 12 mixed connective tissue disease) were enrolled in the study. The disease activities in SLE patients were scored according to SLE Disease Activity Index (SLEDAI) [6]. Lupus nephritis was verified by light microscopy, immunofluorescence, and electronic microscopy analysis.

Anti-telomere IgG antibodies were determined using a specific ELISA test and anti-dsDNA antibodies to calf thymus dsDNA by ELISA were determined by two assays according to the manufacturers' (Biohit plc, Finland; Shield Diagnostics, UK; Inova Diagnostics, USA) instructions. Anti-dsDNA and antinuclear antibodies (ANA) by indirect immunofluorescence technique were detected using Crithidia luciliae cells (Inova Diagnostics, USA) and Hep-2 cells (Inova Diagnostics, USA) as substances, respectively.

Anti-dsDNA was determined also by Farr radioimmunoassay.

The following statistical methods were applied: parametric Student's t-test, correlation analysis, and variation analysis. The sensitivities, specificities, and positive (PPV) and negative (NPV) predictive values were calculated.

## Results

Anti-telomere antibodies were found in 132 (58,67 %) of the patients with SLE, versus 5 (5,21 %) in rheumatoid arthritis and 3 (25 %) in mixed connective tissue disease. By the two other ELISA assays, 106 (47,11 %) or 84 (37,33 %) of the patients had anti-dsDNA antibodies. Anti-dsDNA

*Correspodence to:* Daniela Monova, Veliko Turnovo st., Sofia – 1504, Bulgaria;
Fax:++3599803649; E-mail: dmonova@abv.bg

by radioimmunoassay and immunofluorescence were present in 97 (43,11 %) and 65 (28,9 %) of patients with SLE, respectively. ANA, by immunofluorescence using Hep-2 cells as antigen, was present in 141 (62,67 %) of SLE patients. There isn't correlation between the presence of anti-telomere antibodies and histological type of lupus nephritis.

**Table 1.** Histological type of lupus nephritis

| Histological type of lupus nephritis | Number (%) of patients |
|---|---|
| Minimal mesangial lupus nephritis | 21 (11,93 %) |
| Mesangial proliferative lupus nephritis | 31 (17,61 %) |
| Focal lupus nephritis | 14 (7,95%) |
| Diffuse lupus nephritis | 68 (38,64 %) |
| Membranous lupus nephritis | 42 (23,86 %) |

Specificity of anti-telomere for SLE was 90,6 %, sensitivity was 58,4%, PPV and NPV were 94,2 % and 45,8 %, respectively. The most sensitive assays were anti-dsDNA by ELISA (Shield) – 66,2 % and ANA by immunofluorescence (73,1%), but their specificities were lower (63,5 % and 44,1 %), respectively. The combination of anti-telomere and anti-dsDNA had high sensitivity (81,2 %) and an acceptable specificity (66,2%). SLE patients with a history of nephritis, confirmed (176 patients) by renal biopsy (Table 1), had higher concentrations of anti-telomere antibodies than patients without nephritis (P=0,005). The associations of other tests were also statistically significant (Table 2). Anti-telomere antibodies also correlated with SLEDAI score (r=0,389, p=0,006). The correlation coefficients between the different assays are summarized in Table 3. In general, anti-telomere antibodies correlated well with the ELISA assays ($r_1$ = 0,8021 and $r_2$ = 0,6362) and less well with other tests. ANA and Crithidia immunofluorescence had the lowest correlations with other tests.

**Table 2.** Associations of the antibody assays with a history of lupus nephritis

| ANTIBODY ASSAY | P - VALUE |
|---|---|
| Anti-telomere | 0,005 |
| Anti-dsDNA (Shield Diagnostics) | < 0,010 |
| Anti-dsDNA (Inova Diagnostics) | < 0,010 |
| Radioimmunoassay | < 0,010 |
| Chrithidia luciliae | < 0,050 |
| Antinuclear antibodies | < 0,010 |

**Table 3.** Correlation coefficients of the tests among study patients

| ANTIBODY ASSAY | Anti-telomere | Anti-dsDNA (Shield) | Anti-dsDNA (Inova) | RIA | Chithidia luciliae | ANA |
|---|---|---|---|---|---|---|
| Anti-telomere | 1,000 | 0,8021 | 0,6362 | 0,7607 | 0,3084 | 0,4423 |
| Anti-dsDNA (Shield) | | 1,000 | 0,7853 | 0,4528 | 0,3206 | 0,4825 |
| Anti-dsDNA (Inova) | | | 1,000 | 0,4792 | 0,3101 | 0,4536 |
| RIA | | | | 1,000 | 0,4988 | 0,4675 |
| Chithidia luciliae | | | | | 1,000 | 0,2987 |
| ANA | | | | | | 1,000 |

## Discussion

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. We found that the ELISA assay for anti-telomeric DNA antibodies was a sensitive and highly specific test for SLE. The most sensitive test was ANA by immunofluorescence, followed by anti-dsDNA and anti-telomere. To counterbalance that D. J. Walace *et al*. ascertain that anti-telomere was more sensitive than anti-dsDNA by the Farr assay [4]. Anti-telomere was clearly more specific than the first two tests. The highest sensitivities were reached by ANA together with either anti-telomere or anti-dsDNA.

The prevalence of anti-telomere in SLE and other autoimmune rheumatic diseases in our study was similar to other published values [3,4,7]. D. J. Walace *et al*. reported that anti-telomere antibody was present in 49% of 220 patients with SLE and E. M. Salonen *et al*. – in 60% of 305 patients with SLE [4,7]. 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 D. J. Wallace *et al*. (35% of SLE patients with nephritis and 21% of patients without nephritis were anti-telomere positive) we found that anti-telomere antibodies positivity correlated with disease activity score as assessed by SLEDAI [4]. Further longitudinal studies with larger numbers of patients are needed to evaluate whether anti-telomere relates to the activity of SLE.

Why should the anti-telomere assay be better than other anti-native DNA antibody assays? Quantitative immunochemical studies in patients with active diseases 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.

## Conclusions

We found that the ELISA assay for anti-telomeric DNA antibodies was a sensitive and highly specific test for SLE. The most sensitive test was ANA by immunofluorescence, followed by anti-dsDNA and anti-telomere. Anti-telomere was clearly more specific than the first two tests.

*Conflict of interest statement.* None declared.

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