

# The Clinical Significance of Multiplex Analysis of Antinuclear Antibodies in Systemic Lupus Erythematosus

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**Abstract:** Background: The term antinuclear antibodies (ANA) refers to a large family of antibodies that react with nuclear constituents of cells, such as the nuclear membrane, nucleoplasm, nucleoli, and nuclear organelles. The measurement of ANA is a useful for the diagnosis of systemic lupus erythematosus (SLE).

Objective: To evaluate the clinical significance of multiplex bead-based immunoassay for the detection of ANA profile and evaluation of activity and severity of SLE.

Methods: 94 sera from SLE patients (pts) (SLICC, 2012 classification criteria) (80 F, age 35.9 [16.0-65.0] years, disease duration 113.5 [2.0-576.0] months), 50 sera from patients with other systemic autoimmune rheumatic diseases, 20 pts with non-autoimmune rheumatic diseases, and 30 sera from healthy controls were collected. Disease activity was assessed by SLEDAI-2K – 9,7 (0-40), and chronicity was assessed by SLICC damage index (SDI) - 1,6 (0-18). Serum samples were analyzed for SLE-associated ANA (anti-dsDNA, anti-Sm, anti-Chromatin, anti-SS-A/Ro 52 kDa and 60 kDa, anti-SS-B/La, anti-RNP-70, anti-Rib P) using automated multiplex bead-based immunoassay system BioPlex® 2200 (ANA Screen; Bio-Rad Laboratories Inc., USA). The following ANA values were considered positive: anti-dsDNA $\geq$ 10.0 IU/mL, other antigen-specific ANA $\geq$ 1.0 AI (Antibody Index).

Results: The detection of anti-dsDNA, anti-Sm, anti-Rib P antibodies by BioPlex assay demonstrated the highest overall specificity (0.95, 0.97, 0.99, respectively), and positive likelihood ratio (LR) (10.40, 9.67, 15.0, respectively) for diagnosis of SLE. BioPlex assay for anti-RNP-70, anti-SS-A/Ro, anti-Chromatin antibodies was a useful diagnostic test in SLE (positive LR 2.80, 2.31, 4.15, respectively). Anti-SS-B/La antibodies testing had no value in SLE diagnosis (positive LR 1.20). The frequency of 1 ANA in SLE pts was 79.8%, 2 ANA – 28.7%, 3 ANA – 15.6%, 4 ANA – 10.6%, 5 ANA – 5.3%, 6 ANA – 1.1%. Simultaneous detection of  $\geq$ 3 ANA increased the specificity of multiplex immunoassay up to 0.98-1.00, and positive LR – to a maximum value. SLEDAI-2K was positively correlated with serum concentration of anti-dsDNA ( $r=0.55$ ;  $p<0.05$ ), anti-Chromatin ( $r=0.65$ ;  $p<0.05$ ), anti-Rib P ( $r=0.32$ ;  $p<0.05$ ), anti-Sm ( $r=0.36$ ;  $p<0.05$ ) antibodies. Not found significant association between production of ANA and SDI.

Conclusions: The detection of ANA profile by multiplex bead-based immunoassay has a high specificity and positive likelihood ratio for diagnosis of SLE. Levels of anti-dsDNA, anti-Chromatin, anti-Rib P and anti-Sm in the serum were positively correlated with disease activity.

**Key words:** antinuclear antibodies, multiplex immunoassay, systemic lupus erythematosus