Evaluation of Current Methods for the Measurement of Serum Anti–Double-Stranded DNA Antibodies

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ABSTRACT: Autoantibodies to double-stranded DNA (dsDNA) are, by definition, serological markers of systemic lupus erythematosus. However, the clinical value of anti-dsDNA antibodies largely depends on the assay principle and analytical variables of the methods used to quantitate and immunologically characterize them. In the present article, an overview of current methods for anti-dsDNA antibody detection is presented, together with a look at the future trends in technologies newly employed in this field.

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INTRODUCTION

The detection of autoantibodies to many different nuclear antigens in patient's serum is an important tool in the diagnostic algorithm of systemic autoimmune diseases. Among the antinuclear antibodies, those targeting double-stranded DNA (dsDNA) are so important for the diagnosis of systemic lupus erythematosus (SLE) that they are included in the established classification criteria.[1] They are considered specific markers for SLE,[2] being generally related to disease activity and tissue/organ damage, especially nephritis.[3] Their potential in predicting the disease, disease relapses, and consequently efficacy of established or new therapeutic approaches, has been assessed.[4–8] However, the clinical value of anti-dsDNA antibodies largely depends on the assay principle and analytical variables of the methods used to quantitate and immunologically characterize them.[9,10] In fact, the diversity and polyclonality of the autoimmune response to native DNA in individual patients may contribute to discrepant results among assays and makes it difficult to establish a unique laboratory strategy for the detection of these antibodies.

Many steps forward have been made since the first description of anti-DNA antibodies in lupus sera by means of highly specific but poorly sensitive techniques, such as agar gel double diffusion, complement fixation, or passive hemagglutination.[11] The increasing knowledge of the induction of T and B cell autoimmunity to DNA and the widespread diffusion of new sensitive assays have led to a revisitation of clinical and pathogenic relevance of anti-dsDNA antibodies.

In the present article, an overview of analytical aspects and clinical performance of most currently used methods for anti-dsDNA antibody detection is presented, together with a look at the future trends in technologies newly employed in this field.

INSIGHTS INTO THE IMMUNOCHEMICAL PROPERTIES OF DNA/ANTI-DNA IMMUNE RESPONSE

DNA and histones are the major autoantigens in both murine and human SLE. However, there is no experimental evidence of their immunopathogenic role in the disease. Naked DNA and free histones per se are weak immunogens[12]; in contrast, they acquire immunostimulating properties by linking with charged proteins or nucleic acids, respectively.[13] Recent evidence assigns a central role to nucleosomes (dsDNA-histones native complexes) in both the immunizing and effector phases of SLE pathogenesis, being most probably the antigen that initiates development of antibodies to DNA by epitope spreading.

TABLE 1. Sensitivity and specificity for the diagnosis of systemic lupus erythematosus of current anti–double-stranded DNA antibody assays

Assay	Sensitivity (%)	Specificity (%)
CLIFT	47–55	98–100
Farr	42–85	95–99
ELISA	56–67	91–96

CLIFT = indirect immunofluorescence on *Crithidia luciliae*; Farr = Farr radioimmunoassay; ELISA = enzyme-linked immunosorbent assay.

In addition, the clinical relevance of antinucleosome antibody testing in SLE diagnosis and monitoring has been demonstrated.[14,15]

Either dsDNA or single-stranded DNA (ssDNA) of all species may be recognized as antigen, but related antibodies largely differ in species specificity, avidity, and consequently pathogenic potential. The choice of DNA source and preparation in anti-DNA assays is crucial: human genomic DNA or pure dsDNA of adequate length (from 40 to hundreds of base pairs) have the best performance.[16] Autoantibody pathogenicity is strongly related to avidity for dsDNA, IgG class, complement fixing ability, and cross reactivity with glomerular basement membrane components. The clinical usefulness of anti-DNA assays depends on their ability to determine pathogenic autoantibody subtypes and to measure them by a standardized quantitative approach (TABLE 1).[17,18]

INDIRECT IMMUNOFLUORESCENCE ON *CRITHIDIA LUCILIAE*

The antigen source is the kinetoplast of the hemoflagellate *C. luciliae*, which contains naked circular dsDNA. The test detects medium–high avidity isotype-specific anti-dsDNA antibodies, thus coupling high disease specificity with good sensitivity. Antibody quantitation by serum titration is not adequate to measure changes in anti-dsDNA levels during the disease course.[3]

RADIOIMMUNOASSAYS

The assay principle consists in the separation of radiolabeled DNA/anti-DNA complexes from the free radioactive DNA by ammonium sulfate (Farr assay) or poly-ethylene-glycol (PEG assay) precipitation in solution. Iodinated plasmid or bacteriophage DNA are preferred as antigen source. RIA quantitatively measures levels of high avidity anti-dsDNA antibodies, and antibody fluctuations strictly correlate with disease relapses, especially nephritis.[3,19] However, histone or nucleosome-containing immune complexes may also be

responsible for anti-dsDNA reactivity in the Farr assay, mostly in sera from active SLE patients, thus causing apparent false-positive results.[20]

CLASSICAL AND NEW IMMUNOENZIMATIC ASSAYS

Solid-phase ELISA assays are very heterogeneous in technical equipment and procedures but they generally do not provide a good diagnostic accuracy, being mainly more sensitive but less specific than CLIFT and Farr assays. They all can measure both low- and high-avidity isotype-specific anti-DNA antibodies, in a easily performed and automatized manner; however, at present, changes in ELISA levels do not reliably reflect/predict changes in clinical disease activity.[19,21] Recently, a new ELISA assay (Farrzyme, The Binding Site, Birmingham, UK, distributed in Italy by Radim, Pomezia, Rome) suitable to detect primarily high-avidity anti-dsDNA antibodies has been developed, and its diagnostic performance is under evaluation.[22,23] The heterogeneity of DNA antigen source and coating on solid-phase greatly affects the assay reliability. Low molecular weight recombinant DNA is to be preferred to high molecular weight calf thymus DNA as a nucleic acid source, because the latter one may contain regions of ssDNA causing a false positive reactivity in patients with anti-ssDNA antibodies.[24] An efficient coating of dsDNA molecule to plastic is achieved by means of different precoating linkers or activating procedures that, however, more or less affect the test specificity.

More recently, a new era in ELISA testing has begun with the use of highly purified biotinylated synthetic oligonucleotides as an antigenic substrate coated on either classic microplate wells[25] or microparticles (The Liaison dsDNA assay), and the development of automated enzyme fluoroimmunoassays in which antibodies to plasmid dsDNA are detected fluorometrically by a fluorocrome-conjugated secondary antibody (Elia dsDNA).[26] These new approaches seem to be analytically promising but their performance on a clinical setting has to be extensively investigated.

MULTIPLEXED AND MICROARRAY TECHNOLOGIES

Multiplexed and microarray immunoassays are becoming the future techniques in many fields of laboratory research and diagnostics, including autoimmunity. The multiplexed assay combines the high-resolution power of flow cytometry to the widespread recombinant DNA technology and permits the quantitative detection of up to 10 autoantibodies simultaneously in each patient.[27,28] Protein arrays and genomic microarrays have the potential of becoming very important tools in molecularly defining the pathophysiologic role of autoantibody profiles.[29]

CONCLUSIONS

Whereas the panel of methods for laboratory detection of anti-dsDNA antibodies is continually increasing, assays traditionally employed in the routine laboratory work are still far from being standardized and widely accepted. The diagnostic accuracy and concordance rates are dependent on analytical variability and patient's population studied, and clinicians should be aware of this in interpreting test results.

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