Evaluation of a Semiautomated Pretstandardized Immunofluorescence Test System for Detection of Anti-Native Deoxyribonucleic Acid Antibodies

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An immunofluorescence-native deoxyribonucleic acid (nDNA) antibody test system (Zeus Scientific, Inc.) was compared with a radioimmunoassay procedure (FARR assay) for detecting anti-nDNA antibodies in human serum. Double-blind studies of split samples obtained from 236 patients showed an 80% correlation between the immunofluorescence-nDNA antibody test system and the radioimmunoassay procedure. Studies of sera from patients with known diagnoses showed positive nDNA antibody findings in biopsy-proven systemic lupus erythematosus only. The immunofluorescence-nDNA antibody test system provides a reliable, simple, and economically feasible alternative method for detecting nDNA antibodies that can be employed in any laboratory equipped with a fluorescence microscope.

The clinical significance of double-stranded or native deoxyribonucleic acid (nDNA) antibodies in the pathogenesis and diagnosis of systemic lupus erythematosus (SLE) is well documented (3, 11-13). A number of reports (5, 10, 15) have concluded that monitoring nDNA antibodies in lupus patients is of value because these antibodies often correlate with the clinical course of the disease. A recent report by Steinman et al. (18) showed good correlation between the presence of circulating nDNA antibodies and the amount and location of glomerular electron dense deposits in renal biopsies obtained from lupus patients. The radioimmunoassay (RIA) or FARR assay has long been the method most widely employed to measure nDNA antibodies in patient sera. Recently, however, an indirect immunofluorescent (IF) method employing *Crithidia luciliae* was described by Aarden et al. (1). The *C. luciliae* IF-nDNA antibody procedure was subsequently reported to be a useful clinical test (4, 5, 15, 19), primarily because of its specificity for nDNA antibodies and their predominance in patients with SLE.

Recent studies by several investigators using the RIA method reported the presence of nDNA antibodies in patients with diseases other than SLE (7, 8, 14) and in normal subjects (9). It has been suggested that these reports are ambiguous because of contamination of the nDNA preparations used with single-stranded DNA (17). This is believed to be an inherent problem with RIA techniques, even though special procedures for removal of the single-stranded DNA may be employed. In addition, Aarden et al. (2) have shown that the fraction of DNA bound by a constant amount of SLE serum is linearly dependent on the molecular weight of the DNA employed. The use of different sources of DNA preparations could account for wide variations in the results of RIA assays.

This study was undertaken to ascertain whether the IF-nDNA antibody test system (Zeus Scientific, Inc.) provided a simplified and useful alternative method for anti-nDNA antibody testing. This study was performed in two segments. The first segment was designed to compare the relative sensitivity of the IF-nDNA antibody test system with the FARR technique. The second segment was designed to study antinuclear antibody (ANA)-positive sera from patients with known diseases including systemic lupus erythematosus (SLE) to determine the specificity of the IF-nDNA antibody test system for nDNA antibodies and patients with SLE. The FARR assay was not employed in this segment of the study.

**MATERIALS AND METHODS**

**Serum specimens.** In the first segment of this study, a double-blind evaluation of split serum specimens submitted to Roche Clinical Laboratories for quantitative nDNA antibody determinations was made. A total of 236 serum samples was tested in two different laboratories. The IF-nDNA antibody test system was employed at Saint Barnabas Medical Cen-
ter, and the FARR technique was employed at Roche Clinical Laboratories. The histories and diagnoses of the patients from whom these serum specimens were obtained were not included because of the logistical problem of obtaining this information from the numerous sources for these specimens. Consequently, no clinical correlations could be ascertained in this segment of the study.

**Disease association.** Serum samples from 63 patients with various diseases (including SLE) with positive ANA reactions were tested to determine the specificity of the IF-nDNA antibody test system for nDNA antibodies and patients with active biopsy-proven SLE. These 63 patients included 11 biopsy-proven SLE patients, 8 patients with chronic end-stage renal disease with hypertension and uremia, 3 patients with scleroderma, 15 patients with rheumatoid arthritis, 1 patient with Sjogren’s syndrome, 5 post-open-heart-surgery (bypass) patients with drug-induced ANAs, and 20 patients with positive ANAs without a specific diagnosis. Sera from 60 healthy hospital employees were also studied. The above diagnoses were ascertained from each patient’s discharge summary as stated by the respective attending physician.

**Immunofluorescence studies.** The IF-nDNA antibody test system (Zeus Scientific, Inc.) was employed. This system utilized the established C. luciliae substrate first described by Aarden et al. (1). All test procedures were performed according to the manufacturer’s directional insert. Any serum specimen that produced kinetoplast staining at a 1/10 or greater titer was considered positive for nDNA antibodies. Staining of the kinetoplast and nucleus simultaneously was interpreted as a positive test. Staining of the nucleus only was considered negative for nDNA antibodies.

**RIA studies.** RIA studies for nDNA antibody binding were performed according to the methods described by Pincus et al. (13). The [125I]DNA employed in these studies was obtained from Electronucleonics, Inc. This is an nDNA preparation harvested from human KB cells that were iodinated in culture with an iododeoxyuridine precursor.

**RESULTS**

The Zeus Scientific IF-nDNA antibody test system produced positive results in 80% of those specimens that produced significant (greater than 20%) DNA binding by the RIA method (Table 1). Of the 15 discrepancies, 12 occurred in the 20 to 30% DNA binding range, 2 occurred in the 31 to 40% range, and 1 occurred in the 41 to 50% range. Three serum samples were positive with the IF procedure only. Two of the serum samples produced nuclear staining of the C. luciliae substrate in the absence of kinetoplast staining. These two samples were also negative with the RIA-nDNA antibody procedure but did show high ANA reactivity. Serum samples with 51% or greater nDNA binding activity showed 4+ kinetoplast staining (Fig. 1) and produced nDNA antibody titers ranging from 1/160 to 1/2,560. In general, the greater the DNA binding, the higher the IF-nDNA antibody titer (Table 1).

Of 11 patients with biopsy-proven lupus nephritis, 9 produced positive kinetoplast staining (Table 2; Fig 2). Two of the 11 lupus sera that were negative for nDNA antibodies were from patients with high titer antibody activity directed against the SM extractable nuclear antigen. None of the patients with hypertension and uremia, scleroderma, rheumatoid arthritis, and Sjogren’s syndrome contained detectable nDNA in the sera.

**FIG. 1. Positive IF-nDNA test showing 4+ C. luciliae kinetoplast and nuclear staining. Magnification, x750.**

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**Table 1. Results of parallel IF-nDNA and RIA-nDNA antibody tests**

<table>
<thead>
<tr>
<th>Bound DNA (%)</th>
<th>No. of specimens</th>
<th>No. of RIAs</th>
<th>Positive IF-nDNA</th>
<th>Titer range</th>
<th>Discrepancies</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>163</td>
<td>16</td>
<td>4</td>
<td>1/10/1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12</td>
</tr>
<tr>
<td>20-30</td>
<td>16</td>
<td>9</td>
<td>7</td>
<td>1/160-1/640</td>
<td>2</td>
</tr>
<tr>
<td>31-40</td>
<td>9</td>
<td>4</td>
<td>3</td>
<td>1/160-1/1,280</td>
<td>1</td>
</tr>
<tr>
<td>41-50</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>1/160-1/2,560</td>
<td>0</td>
</tr>
<tr>
<td>51-100</td>
<td>44</td>
<td>44</td>
<td>44</td>
<td>1/160-1/2,560</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> RIA percent binding results greater than 20 were interpreted as positive.

<sup>b</sup> There was 80% agreement between the RIA and IF-nDNA methods.
antibodies. In addition, 5 post-open-heart-surgery (bypass) patients with drug-induced ANA titers (greater than 1/40) and 20 ANA-positive patients with unknown diagnoses were negative for nDNA antibodies.

**DISCUSSION**

To make a more definitive diagnosis of SLE, the patient’s history, clinical symptoms and the use of judiciously selected laboratory tests must be considered. The incorporation of the nDNA antibody test as part of the laboratory workup of a possible SLE patient has been advocated by a number of investigators (1, 3, 5–7, 11, 13). Until recently, the RIA test for nDNA antibodies was considered the method of choice and was usually available only at large research centers or commercial laboratories. Recent studies have cast doubt on the specificity of certain RIA procedures because of the inherent problem of single-stranded DNA contamination (15, 16) and because of the presence of nDNA antibodies in patients with diseases other than SLE (7, 8, 14) and in normal subjects (9). This study shows that the Zeus Scientific IF-nDNA antibody test system is a simple and reliable procedure for monitoring nDNA antibodies in SLE patients’ serum. Most of the discrepancies noted in Table 1 occurred in the low DNA-binding level (20 to 30%). It is conceivable that these discrepancies were due to antibodies present in the patient sera that reacted with small amounts of contaminant single-stranded DNA in the RIA method. This explanation has been proposed in recent reports (15, 16). It should be noted that low titer or negative nDNA antibody titers may be obtained in patients with active lupus nephritis. Davis et al. (5) have shown that deoxyribonuclease treatment of such sera may result in an increased DNA antibody titer upon retesting. This phenomenon may help explain the discrepancies between the IF-nDNA antibody test system and the FARR technique. These studies were not done in this evaluation because of the difficulty in tracing patients whose specimens are processed through the high-volume reference laboratory. Based on this study, the IF-nDNA antibody test system was found to be a simple, reliable, and economically attractive alternative method to the RIA procedure for anti-nDNA antibody testing. This test system can be employed in any laboratory equipped with a fluorescence microscope. The specificity of the Zeus Scientific IF test system for DNA antibodies and SLE is shown in Table 2. These results concur with those reported in a number of recent reports (1, 4, 5, 16, 19) designed to evaluate the specificity of the IF kinetoplast C. luciliae procedure for nDNA antibody testing and its value as a routine laboratory procedure. The nuclear C. luciliae staining produced by two patients’ sera in the absence of kinetoplast staining is not clearly understood. Both patients had high ANA titers and did not have a diagnosis of SLE. Similar findings have been noted in other reports.

**TABLE 2. Disease correlation of positive IF-nDNA antibody test results**

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Diagnosis</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ANA nDNA antibody</td>
</tr>
<tr>
<td>11**</td>
<td>Systemic lupus erythematosus</td>
<td>11 9</td>
</tr>
<tr>
<td>8</td>
<td>Hypertension and uremia</td>
<td>8 0</td>
</tr>
<tr>
<td>3</td>
<td>Scleroderma</td>
<td>3 0</td>
</tr>
<tr>
<td>15</td>
<td>Rheumatoid arthritis</td>
<td>5 0</td>
</tr>
<tr>
<td>1</td>
<td>Sjogren’s syndrome</td>
<td>1 0</td>
</tr>
<tr>
<td>5</td>
<td>Post open heart surgery</td>
<td>5 0</td>
</tr>
<tr>
<td>20</td>
<td>Other diseases</td>
<td>20 0</td>
</tr>
<tr>
<td>60</td>
<td>Normal</td>
<td>2 0</td>
</tr>
</tbody>
</table>

*With biopsy-proven lupus nephritis.*

**FIG. 2. Renal biopsy obtained from a patient with lupus nephritis and 4+ IF-nDNA kinetoplast staining showing dense immunoglobulin G immune complexes within the glomerular basement membrane and mesangium. Magnification, x300.
(1, 4, 16, 19). Only sera with positive ANA reactions produced positive nDNA antibody tests. The three sera that produced positive nDNA antibody reactions with the *C. luciliae* test alone constitute an interesting observation for which no immediate explanation is available. Further studies are in progress to evaluate this phenomenon.

**LITERATURE CITED**


