

Performance of Antinuclear Antibody Connective Tissue Disease Screen

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ABSTRACT: Antinuclear antibodies (ANAs) have become routine laboratory parameters in clinical hospitals. However, ANA testing by indirect immunofluorescence (IIF) assays is not an automated laboratory test. Efforts are being made to develop easy and semi- or automated methods to screen for ANAs. We evaluated the clinical performance of a new ELISA developed to screen for connective tissue disease related ANAs. The presence of serum ANA was studied with a commercial ELISA (Varelista ANA CTD Screen) in 472 patients (202 SLE, 41 Sjögren syndrome, 11 CREST, 59 rheumatoid arthritis, 30 seronegative spondyloarthropathies, 77 inflammatory bowel disease, 13 reactive arthritis, 11 giant cell arteritis, 28 ankylosing spondylitis). A hundred and five sera from healthy subjects were used as controls. Receiver operator characteristics (ROC) analysis was carried out in order to optimize the cutoff. At target specificities of 80/90%, sensitivities of 80.8/ 73.9% were achieved. At the manufacturer's cutoff (ratio ≥ 1.0) sensitivity/specificity of 71.4/91.2% was found. At that cutoff, a positive likelihood ratio of 8.11 was found. For helping in the diagnosis of connective tissue diseases a test employing a subset of the most prevalent specificities reveals a good compromise as indicated by a high-positive likelihood ratio. However, the presence of ANAs in pathologies other than connective tissue diseases, such as SLE or Sjögren syndrome, may be of clinical significance as well. In these cases an IIF assay test is still mandatory, especially in autoimmune laboratories.

KEYWORDS: antinuclear antibodies; connective tissue disease; screen; ELISA; indirect immunofluorescence

Systemic connective tissue diseases (CTD), such as systemic lupus erythematosus (SLE), mixed connective tissue disease (MCTD), Sjögren's syndrome (SS), scleroderma, and polymyositis, are characterized by the production of a diverse array of autoantibodies. Among them, the detection of antinuclear

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antibodies (ANAs) in the serum of patients with autoimmune symptoms provides evidence in the screening, diagnosis and monitoring of CTD.¹ Evidence-based guidelines for ANA testing clearly establish that indirect immunofluorescence (IIF) on Hep-2 cells slides is the standard method.² This is in part due to the large number of autoantigens present on Hep-2 cells that are not coated in the alternative methods that are being developed by the manufacturers, since ANA specificities described reach over 80. As a consequence, measurement of ANAs by IIF has a high-positive predictive value for diagnosing CTD.

Screening for CTDs is a current clinical practice in every patient with systemic manifestations. This has a great impact on laboratory work since the volume of analysis has exponentially grown and the performance must be faster but keeping specificity and sensitivity. As stated above, IIF on Hep-2 cells is the gold standard but it is very time-consuming and requires experienced staff that clinical laboratories, especially small ones, do not usually have. Although it is a subject of debate, many manufacturers try to develop a screening method to lighten the laboratory work in the diagnosis of systemic CTD.³

The aim of the present study was to analyze the clinical performance of a new developed ELISA for the screening of systemic CTD.

PATIENTS AND METHODS

From a cohort of 472 patients, where material is stored in the serum bank, one aliquot from each subject was randomly selected. Our serum bank is made up by samples received for routine tests at our laboratory and has been approved by the local ethical committee. The distribution of patients with systemic CTD in the cohort was: 202 SLE, 41 Sjögren syndrome, and 11 CREST. As control diseases we selected 59 rheumatoid arthritis (RA), 28 ankylosing spondylitis (AS), 77 inflammatory bowel disease (57 Crohn's disease [CD], 20 ulcerative colitis [UC]), 11 giant cell arteritis (GCA), 13 reactive arthritis, and 30 seronegative spondyloarthropathies. Finally, 105 sera from healthy subjects under 35 years old were used as controls. Demographic data are shown in TABLE 1.

All samples were tested with a commercial ELISA (Varelista® ANA CTD Screen, hereafter called CTD Screen, Phadia GmbH, Freiburg, Germany). The CTD Screen is an indirect noncompetitive enzyme immunoassay for the qualitative determination of 11 ANAs (purified dsDNA, Sm, and histones H1-H3, and recombinant U1-RNP, SSa, SSb, Scl-70, Jo-1, Pm-Scl, CENP-A,B,C, and ribosomal P) in serum or plasma. The wells of a microplate are coated with human recombinant and native purified nuclear antigens and dsDNA. Antibodies specific for the nuclear antigens present in a patient sample bind to these nuclear antigens. In a second step the enzyme labeled second antibody (conjugate) binds to the antigen-antibody complex which leads to the

TABLE 1. Demographic characteristics of the population included in the study

Group	<i>N</i>	Age	Male/female
Systemic lupus erythematosus	202	20–85	10/192
Sjögren syndrome	41	31–89	4/37
CREST	11	47–85	1/10
Rheumatoid arthritis	59	20–87	10/49
Spondyloarthropaties	58	18–79	22/36
Reactive arthritis	13	13–86	5/8
Inflammatory bowel disease	77	16–54	37/47
Giant cell arteritis	11	61–87	3/8
Healthy donors	105	21–34	46/59

formation of an enzyme labeled conjugate–antibody–antigen complex. The enzyme labeled antigen–antibody complex converts the added substrate to form a colored solution. The rate of color formation from the chromogen is a function of the amount of conjugate complexed with the bound antibody and thus is proportional to the initial concentration of the respective antibodies in the patient sample.

Data were summarized as frequencies of positive results for each cutoff in the CTD Screen. Fisher's exact test was used for comparison of frequency of positive result rate. Receiver operating characteristics (ROC) curves analysis was performed and experimental cutoff indexes were determined. Clinical performance of the assay was done by the Taylor method.

RESULTS

By using the cutoff recommended by the manufacturer (CTD ratio of 1.0), among the patients included in the cohort of CTD, 75.4%, 73.17%, and 100% of SLE, SS, and CREST patients were positive for CTD assay, respectively. The frequencies of sera positive from the disease control groups were always lower than 10% except for GCA that reached almost 20%. 10.48% of healthy blood donors had a positive CTD test (FIG. 1). All sera positive within the healthy subjects showed a ratio between 1.0 and 1.4 and none of them have developed any manifestation of systemic CTD after a mean follow-up of 3.45 ± 4.72 years.

The experimental cutoff ratios for the three CTD diseases considered together (SLE, SS, CREST) were calculated by the ROC curves and represented the most accurate point in which minimal false-positive and false-negative results were obtained (FIG. 2). The area under curve (AUC) was 0.866 (95% CI: 0.834–0.897). At target specificities of 80/85/90/95%, sensitivities achieved were 80.5/76.3/73.9/62.4% for CTD Screen ratios of 0.63 / 0.7 / 0.86 / 1.52. These data are reasonable comparing the cutoff ratio recommended by the manufacturer (i.e., 1.0 as recommended cutoff ratio) as shown in TABLE 2.

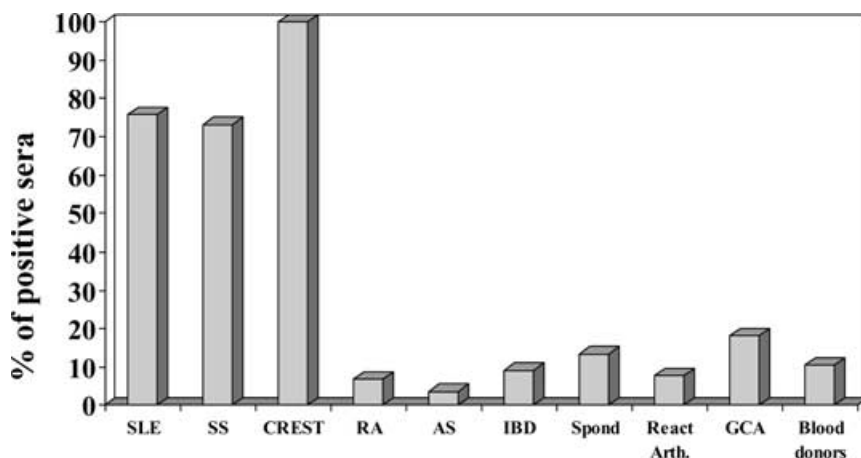


FIGURE 1. Frequency of positive results for the CTD Screen assay at the recommended cutoff ratio 1.0 in the cohort of patients included in the study: systemic lupus erythematosus (SLE, $n = 202$), Sjögren's syndrome (SS, $n = 41$), CREST ($n = 11$), rheumatoid arthritis (RA, $n = 59$), ankylosing spondylitis (AS, $n = 28$), inflammatory bowel disease (IBD, $n = 77$), seronegative spondyloarthropathy (Spond, $n = 30$), reactive arthritis (React Arth, $n = 13$), and giant cell arteritis (GCA, $n = 11$). As healthy donors, sera from 105 blood donors were used.

The positive likelihood ratios calculated for cutoff ratios 1.0 and 1.4 indicated, that the results obtained gave valuable information with respect to the clinical diagnosis of CTD.

DISCUSSION

The present study was designed to evaluate the clinical performance of the CTD Screen assay in the diagnosis of CTD, mainly SLE, SS, and CREST, which are the most prevalent systemic CTD at our center. The aim was not to compare it with IIF on Hep-2 cells since both assays are not comparable to each other. The CTD Screen includes a mixture of the most representative autoantigens recognized by autoantibodies present in CTD, while Hep-2 cells per se offer the whole nucleus with more than 30 known specificities.

Results demonstrated a reasonable good performance of the test, especially in terms of specificity. Only GCA patients showed a high percentage of positive sera but it is probably due to the old age of these patients. Although sensitivity did not reach that of IIF on Hep-2 cells, which in our hands was 87%, it is still good enough to be considered as screening method. It is possible that the 75.4% of sensitivity in SLE patients could be due to the inactivity of the disease since 27% of inactive SLE showed a ratio <1 . Thus CTD Screen can detect anti-dsDNA antibodies that are clearly associated with lupus flares.⁴ In case of

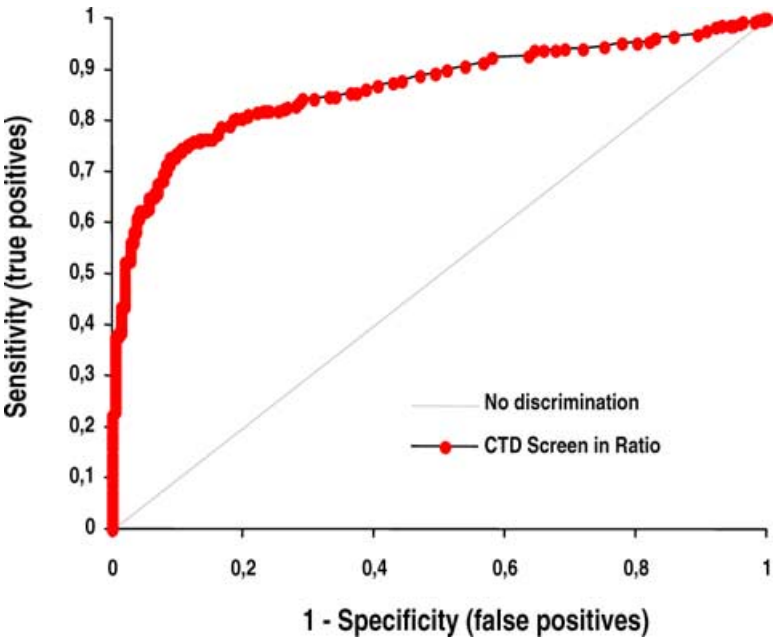


FIGURE 2. ROC curve of the CTD Screen assay. SLE, SS, and CREST patients were considered together as the CTD to diagnose. The remaining diseases considered in the study, but not healthy donors, were used as disease control groups for the analysis. (In color in Annals online.)

a positive CTD Screen result, further testing for ANAs by IIF on Hep-2 cells and determination of specificities with further tests should follow. We believe that the use of CTD Screen as first step in the suspicion of CTD could be useful in reducing the laboratory work, especially in autoimmune laboratories that study samples referred from diverse origins (i.e., from primary care to university hospitals), especially when the clinical suspicion of CTD is very low. Thus it would increase the efficiency of the routine testing with respect to costs. ANA testing is a very time-consuming task despite the introduction

TABLE 2. Diagnostic characteristics of the CTD Screen assay for the diagnosis of systemic CTD

	Cutoff ratio ≥ 1.0
Sensitivity (95% CI)	76.4 (71.2–81.6)
Specificity (95% CI)	90.7 (87.5–93.9)
Positive likelihood ratio (95% CI)	8.2 (5.8–11.6)**
Negative likelihood ratio (95% CI)	0.27 (0.21–0.33)*

* Little value for diagnostic decision.
** Moderate value for diagnostic decision.

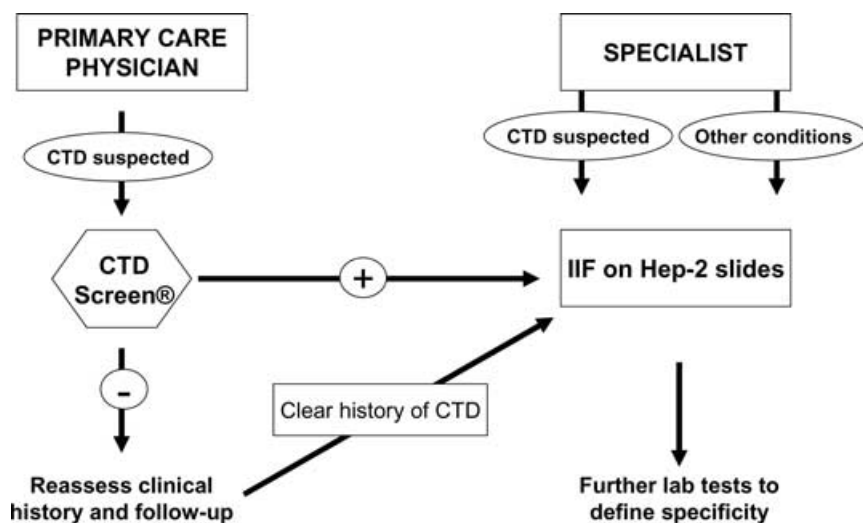


FIGURE 3. Algorithm proposed for the use of the CTD Screen in the diagnosis of systemic CTD in the autoimmunity laboratories.

of automated equipment for the preparation of the serum dilutions and slides.⁵ The vast majority (74%) of the whole ANA tests performed routinely at our laboratory is found negative. This is especially the case for those samples referred from general practitioners. Indeed such a situation is not uncommon and for a modern laboratory underlines the needs to utilize methods such as the CTD Screen in order to reduce the workload apparent in CTD diagnostic procedures.

Obviously, CTD Screen can not substitute ANA testing in the diagnosis of these diseases since it still remains as the “gold standard” method and, for instance, the presence of ANA is a classification criterion of the ACR for SLE.⁶ After diagnosis, determination of ANA in the follow-up of CTD should be done by IIF on Hep-2, since changes in the titers as well as in the staining patterns have been described.⁷ Furthermore, determination of ANAs in sera from other autoimmune or inflammatory diseases may be of help in their management because their presence may either be diagnostically helpful (i.e., some forms of juvenile chronic arthritis), may indicate an ongoing inflammatory response or a more aggressive progression of the disease.⁸

A limitation of the present study is that sera studied were from patients with well established diagnosis. The utility of the test analyzed remains to be elucidated in the early diagnosis of CTD. It was recently demonstrated that ANAs detected by IIF are present in the sera of patients that develop SLE years before the diagnosis.⁹ Thus, ANAs on Hep-2 have been proven to be useful in the early diagnosis of CTD. In the same work it was shown that anti dsDNA,

anti-Ro and anti-La were the specificities that earliest appeared and these are included in the CTD Screen.⁹

Further evidence for the use of CTD Screen in patients from primary care in order to reduce clinically false-positive results comes from the article by Narain *et al.*¹⁰ Here the authors showed that a high percentage of the patients referred to a specialist for suspicion of CTD was not diagnosed of CTD despite the ANA positive result. Thus, ANA positivity in absence of clinical signs or symptoms is of limited diagnostic utility.

In summary, our results demonstrate that the CTD Screen has a very good clinical performance in the diagnosis of established systemic CTD. Since the request of ANA from primary care patients yields a very low frequency of positivity, we see the possibility of implementation of the CTD Screen in a possible algorithm in an autoimmunity laboratory (Fig. 3).

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