Comparison of different methods for the detection of anti-Ro/SSA antibodies in connective tissue diseases


Institute of Rheumatology, University of Siena, Italy.

G. Morozzi, PhD; F. Bellisai, MD; A. Simpatico, Lab technician; G. Pucci, Lab technician; M.R. Bacarelli, Lab technician; V. Campanella, MD; R. Marcolongo, MD; M. Galeazzi, MD.

Please address correspondence and reprint requests to: Dr.ssa Gabriella Morozzi, Istituto di Reumatologia, Università di Siena, Policlinico “Le Scotte”, 53100 Siena, Italy. E-mail: morozzi@unisi.it

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ABSTRACT

Objective

To compare the performance characteristics of various tests commonly used to detect anti-SSA/Ro autoantibodies in the sera of patients affected by connective tissue diseases (CTD).

Methods

Indirect immunofluorescence (IIF) with HEp-2000 as substrate (ImmunoConcepts, USA), Ouchterlony’s double immunodiffusion (ID) (home made), commercial Varelisa ReCombi anti-Ro kit (Pharmacia & Upjohn, Germany), research kits (60 kDa and 52 kDa) with human recombinant antigens (Pharmacia & Upjohn, Germany) and a commercial western blot (WB) kit (MarDx, USA) were evaluated in our study. Sixty-four sera from patients affected by CTD were tested: 15 had primary Sjögren’s syndrome (SS), 34 only had sicca syndrome, and 15 had systemic lupus erythematosus (SLE). Thirty sera from healthy subjects were selected as controls.

Results

54 sera were positive by at least one method. The specificity of all tests was good. The prevalence of anti-SSA antibodies on 54 positive sera was 76% (ID), 89% (IIF), 89% (Varelisa), 89% (ELISA Ro-60 kDa), 67% (ELISA Ro-52 kDa) and 85% (WB). Some differences were found between WB and ELISA in the detection of anti-60kDa SSA and anti-52kDa SSA; in 3 SS sera only anti-52 kDa protein was found by WB.

Conclusion

Our data confirm that, although IIF HEp 2000 (Immuno Concepts) and Varelisa anti-Ro (Pharmacia & Upjohn) both performed well, a combination of 2 or more methods must still be recommended for anti-SSA antibody detection.

Introduction

Anti-Ro/SSA antibodies are antinuclear antibodies closely associated with Sjögren’s syndrome (SS), systemic lupus erythematosus (SLE) and neonatal lupus. The structure of the Ro particle remains a focus of debate.

Using non-denaturing methods, Boire et al. (1-3) found three different types of particles, Ro 60-kDa, La 48-kDa and Ro hY5. The 52-kDa Ro peptide, identified in 1988 by WB, is unable to bind hY-RNA. This observation led some investigators to hypothesize that the Ro 52 kDa protein does not form part of the Ro complex (3-6). On the other hand, others have suggested that it may participate in the complex by binding to the 60 kDa Ro peptide via a protein-protein interaction (7, 8), and, in particular, via calreticulin (9).

More recently the fine specificity of the autoimmune response to Ro/SSA ribonucleoproteins has been reported (10, 11). Numerous studies have addressed the specificity of anti-Ro antibodies for the 60 or 52 kDa component in autoimmune diseases, but the results have been conflicting. The discrepancies in the reports are probably due to differences in the methods used for the detection of these autoantibodies, particularly regarding those directed to the 52 kDa component.

The diagnostic and therapeutic implications of the anti-SS-A/Ro antibodies, especially in certain clinical situations such as congenital heart block or neonatal lupus, are so important that a highly sensitive and specific assay for their detection is definitely needed. The performance characteristics of commercially available procedures for detecting anti-SS-A/Ro (12-15) have been evaluated in several studies, but these reached different conclusions.

The aim of our study was to compare different assays: indirect immunofluorescence (IIF) using HEp-2000 as substrate (ImmunoConcepts, USA), Ouchterlony’s double immunodiffusion (ID) (home made), a commercial Varelisa ReCombi anti-Ro kit (Pharmacia & Upjohn, Germany), research kits (60 kDa and 52 kDa) based on human recombinant antigens (Pharmacia & Upjohn, Germany), and a commercial western blot (WB) kit (MarDx, USA).

Patients and methods

Patients

Sera from 64 patients affected by connective tissue diseases were selected on the basis of the anti-Ro positivity/negativity by ID and were tested using each method. For this study 41 positive sera (ID) from 15 patients with primary Sjögren’s syndrome (SS), 21 patients with sicca syndrome and undifferentiated con-
nective tissue disease, and 5 patients with SLE were examined. Of the remaining 23 negative sera (ID), 13 were from patients with sicca syndrome and had been sent to our laboratory for anti-SS-A/Ro antibody assay and 10 were from SLE patients. The SLE sera were selected as the autoimmune control, being negative for both anti-SS-A/Ro and anti-SS-B/La by ID, but positive for other autoantibodies (Sm, RNP, nDNA). Thirty sera from healthy subjects were tested as normal controls.

The diagnosis of SS was based on the presence of sicca symptoms, a salivary gland biopsy with a focus score ≥ 1, and the absence of findings that met the diagnostic criteria for another autoimmune disease.

Methods

Indirect immunofluorescence. A commercial ANA substrate HEP-2000 Fluorescent ANA-Ro test (Immuno Concepts, Sacramento CA, U.S.A) was used. This kit employs a full-length cDNA encoding a human 60 kDa SS-A/Ro protein that was transfected and overexpressed in the HEp-2 cell line. The IIF protocol outlined by the manufacturer was followed, using a goat fluorescein-conjugated anti-human immunoglobulin (HEp-2 Marblot, MarDx, USA) with anti-human IgG alkaline phosphatase conjugate was used. The performance characteristics of each test were evaluated in terms of its sensitivity and specificity.

Results

All sera were tested by each method. Sera from 54 out of 64 patients affected by connective tissue diseases were positive for anti-SS-A/Ro by at least one method. The specificity of all the tests was good and is shown in Table I. The sensitivity of the tests was calculated in comparison to 41 anti-SS-A/Ro positive sera in which the test was performed by Ouchterlony’s ID technique. Sensitivity was 100% for ID, 100% for IIF, 100% for VARELISA SS-A, 100% for ELISA Ro 60kDa, 73% for ELISA Ro 52kDa, and 83% for WB. The prevalence of anti-SS-A/Ro antibody in 54 positive sera from connective tissue diseases by each method is shown in Table II. The concordance between methods is shown in Table III. Interestingly, 3 sera from SS patients only showed anti-SS-A/Ro 52kDa by WB (MarDx). Furthermore the same sera were negative for anti-Ro when tested by other methods (data not shown).

Discussion

Many methods have been proposed and evaluated for anti-SS-A/Ro antibody detection (12-15). As there is no agreement on the best approach for their detection, in our study we have compared different techniques in order to establish their sensitivity and specificity; the results have also been evaluated in relation to

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<th>Table I. Specificity of the tests.</th>
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<td>Healthy subjects</td>
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<td>Autoimmune controls</td>
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The specificity was calculated on control sera; among them 30 were from healthy subjects and 10 were from SLE patients. All SLE sera were anti-SS-A/Ro negative by ID, but positive for anti-RNP and/or anti-Sm; 4/10 were positive for anti-nDNA.

<table>
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<tr>
<th>Table II. Prevalence of anti-SS-A/Ro antibodies on 54 connective tissue diseases sera, detected by different methods, expressed as.</th>
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<td>Positive/Total</td>
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<th>Table III. Concordance between methods.</th>
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<tr>
<td>ID (n=41)</td>
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<td>IIF (n=48)</td>
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<td>VARELISA (n=48)</td>
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<td>WB 60 kD and/or 52kD (n=46)</td>
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Certain clinical manifestations. Our results show that the specificity of all of these assays is good, as no reactivity was observed among the 30 blood donor sera, and only a few low/equivocal positive values by ELISA on autoimmune controls were obtained. We found an identical sensitivity for the IIF HEp 2000 (Immuno Concepts) and Varelisa anti-Ro (Pharmacia & Upjohn) tests. Less sensitivity was shown by WB (Mar Dx), ID and Varelisa Ro 52 kDa (research kit from Pharmacia & Upjohn).

Our data also show that 3 primary SS sera contained anti-Ro 52 kDa autoantibodies in the absence of any detectable anti-Ro 60 kDa autoantibodies. The possibility of finding isolated anti-Ro 52 kDa antibodies, without detectable anti-Ro 60 kDa, is still a matter of discussion. In fact some authors suggest that isolated anti-Ro 52 kDa antibodies are not true anti-Ro (3, 6). Further studies are needed to verify whether in these sera the 52-kDa peptide represents a true SSA; nevertheless, it is important to point out that our 3 anti-Ro 52 kDa positive patients had primary SS, diagnosed according to clinical and histologic criteria.

In conclusion, our results show that the use of different methods for the detection of anti-Ro may yield different results, especially in terms of the test’s sensitivity. The discrepancies may be due, at least in part, to the different molecular approaches used by companies to prepare the antigen. Depending upon the method utilised it is possible to detect autoantibodies directed to different epitopes of the same complex antigen showing either a linear or tridimensional conformation. ID and WB still show a low sensitivity, while IIF HEp 2000 (Immuno Concepts) and Varelisa anti-Ro (Pharmacia & Upjohn) are sensitive tests, but not sensitive enough to detect anti-Ro 52 kDa autoantibodies. On the other hand, as far as the detection of anti-Ro 52 kDa is concerned, our results show that WB is more sensitive than Varelisa. For these reasons we believe that a combination of two or more methods is still to be recommended for anti-Ro antibody detection and that the clinical characteristics of the patient always represent the central point for the evaluation of the diagnostic relevance of the test.

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