

Anti-SS-A/Ro autoantibodies are not part of the originally defined anti-SS-A/Ro reactivity

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ABSTRACT

Purpose: To determine how the techniques to measure anti-SSA-52 (Ro-52) and anti-SSA-60 (Ro-60) autoantibodies affect the sensitivity of detecting them. Determine the clinical sensitivities and specificities of these autoantibodies in autoimmune connective tissue diseases and control samples.

Methods: Whole cell extracts for double immunodiffusion (Ouchterlony) were prepared from Wil-2 cells or calf thymus. ELISA plates were made with native bovine SSA-60, recombinant human SSA-60, or recombinant human SSA-52 antigens. Sera from 204 control, 161 SLE, 72 Sjögren's syndrome, 122 scleroderma and 169 polymyositis/dermatomyositis (PM/DM) patients were tested on SSA-52 and SSA-60 ELISAs.

Results: Anti-SSA-52 autoantibodies are non-precipitating in Ouchterlony immunodiffusion with both extracts. Approximately 25% of samples that are positive for native SSA-50 in ELISA are negative on recombinant SSA-60. The specificities of anti-SSA-52 and anti-native SSA-60 autoantibodies are the same in normal blood donors (1% positive) and other control sera (3% positive). Though some patients are positive for only one or the other of these autoantibodies, their sensitivities are very similar in patients with SLE (35%), ANA positive Sjögren's syndrome (80%), and to a lesser extent, scleroderma (25% for anti-SSA-52 to 10% for anti-SSA-60) and PM/DM without synthetase syndrome (also 25% to 10%). However, there is a dramatic difference between the prevalence of these two autoantibodies in PM/DM patients with synthetase syndrome. Anti-SSA-52 reactivity is common (85%), while anti-SSA-60 reactivity is not (12%).

In our group of 92 PM/DM patients with synthetase syndrome, anti-SSA-52 autoantibodies were the most common autoantibody, found in 78 of these patients, compared with finding anti-Jo-1 autoantibodies in 61 of them.

Conclusions: Measuring both anti-SSA-52 and SSA-60 autoantibodies together adds useful sensitivity in detecting patients with SLE and Sjögren's syndrome. Because anti-SSA-52 autoantibodies are non-precipitating in Ouchterlony immunodiffusion, and have a subtly different clinical sensitivity compared to anti-SSA-60 autoantibodies, these two antibody reactivities should be considered separate antibodies. Besides SLE and Sjögren's syndrome, anti-SSA-52 autoantibodies may also be useful to help diagnose synthetase syndrome in PM/DM patients. Thus, depending on the patient group, it may be beneficial to measure anti-SSA-52 and SSA-60 together or separately.

INTRODUCTION

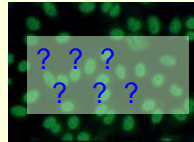
It is important for disease diagnosis and treatment to identify patients that have anti-SSA-52 autoantibodies. SSA-52 autoantibodies appear independently of SSA-60, and SS-B autoantibodies^{1,3}. Native SSA-60 is a better antigen than cloned SSA-60, while cloned SSA-52 is better than native SSA-52^{4,6}. The sensitivity of tests for SLE and Sjögren's Syndrome is increased when both SSA-60 and SSA-52 autoantibodies are assayed^{1,3}. Further, it has been found that patients with Sjögren's Syndrome sometimes have anti-SSA-52 autoantibodies but not anti-SSA-60 autoantibodies. Conversely some patients with SLE exhibit SSA-60, but not SSA-52 activity⁷. In our hands, patients with SLE and SS are sometimes positive for both antigens, but about 5% of the time they are positive for just one or the other. Polymyositis and Dermatomyositis (PM/DM) patients have anti-SSA-52, but not anti-SSA-60 activity^{8,9}. The group of PM/DM Patients that exhibit synthetase syndrome have a large increase in anti-SSA-52 autoantibody reactivity when compared to those without synthetase syndrome⁸. The recognition of synthetase syndrome is important because the treatment required differs from that for other typical autoimmune diseases.

METHODOLOGY

QUANTA Lite™ SS-A 52 ELISA

Purified SSA-52 antigen is bound to the wells of a polystyrene microwell plate under conditions that will preserve the antigen in its antigenic state. It has been reported that autoantibodies to both SSA-60 and SSA-52 are diagnostically important¹. Pre-diluted controls and diluted patient sera are added to separate wells, allowing any SSA-52 antibodies present to bind to the immobilized antigen. Unbound sample is washed away and an enzyme labeled anti-human IgG conjugate is added to each well. A second incubation allows the enzyme labeled anti-human IgG to bind to any patient antibodies, which have become attached to the microwells. After washing away any unbound enzyme labeled anti-human IgG, the remaining enzyme activity is measured by adding a chromogenic substrate and measuring the intensity of the color that develops. The assay can be evaluated spectrophotometrically by measuring and comparing the color intensity that develops in the patient wells with the color in the control wells.

IFA and SS-A 52



Conclusion: No discernible IFA pattern was seen with nine SSA-52 positive samples on HEp-2 cells, or salivary gland, spleen or liver slides.

Ouchterlony and Immunodiffusion

Positive by Ouchterlony Immunodiffusion on Wil-2 Extract
Retrospective study

N = 191	SSA-52 ELISA		
	SSA-60 ELISA	Pos	Neg
P os	184	6	
N eg	0	1	

High Titer Monospecific anti-SSA-52 Samples
Prospective study on Calf Thymus Extract

N=7	Ouchterlony	
	Pos	Neg
	0	7

Conclusion: Anti-SSA-52 antibodies do not form a precipitin line in Ouchterlony immunodiffusion

Sensitivity and Specificity

Disease	# Samples	SSA-52 +	SSA-60 +	Either +	Only 52
Norm B. D.	120	1%	1%	2%	1%
Other controls	84	7%	2%	8%	6%*
SLE	161	35%	37%	45%	7%
ANA+ Sjo Syn	72	86%	76%	89%	12%
Scleroderma	122	26%	9%	28%	19%

*These samples were all from the Hepatitis C control group

Conclusion: In the above diseases anti-SSA-52 and SSA-60 have similar sensitivities and specificities. The sensitivity for detecting both SLE and SS patients is increased if both types of activities are measured.

Polymyositis and Dermatomyositis

Disease / Autoantibody	# Samp.	SSA-52 +	SSA-60 +	52 + /60-
PM/DM/IBM (all)*	169	60%	14%	47%
Not Synthetase Synd*	77	31%	14%	17%
Synthetase Synd*	92 (66% are Jo-1+)	85%	13%	73%
Jo-1 Positive**	105	87%	17%	72%

*These samples were all from the University of Pittsburgh

**61 from the previous line in the table, plus 44 others.

Conclusion: Anti-SSA-52 is the most common autoantibody in polymyositis patients with synthetase syndrome.

Sensitivity of Native SS-A 60

	SSA-60 Reactivity in ELISA				
	Number	Native + Total	Recom. + Total	Native + Only	Recom. + Only
Blood Donors	65	1	1	1	1
Speckled ANA	97	71	52	20	1

Conclusion: More than 25% (20/71) of samples that are positive by ELISA with native SSA-60 antigen are negative using recombinant SSA-60 antigen.

CONCLUSIONS

- Early studies that used Western blot and ELISA with recombinant antigen to detect anti-SSA autoantibodies mistakenly thought that many samples that were positive for anti-SSA by Ouchterlony were mono-specifically reactive with SSA-52, thus incorrectly naming the new reactivity as if it were part of the originally defined SS-A/Ro.
- Anti-SSA-52 reactivity is not detected at all by Ouchterlony immunodiffusion¹¹ or immunofluorescence¹². It is detected with high sensitivity by Western blot and ELISA with recombinant antigen^{12,7}.
- Anti-SSA-60 reactivity is only detected 75% of the time by Western blot or by ELISA with recombinant antigen^{12,8}. It is detected with much greater sensitivity by Ouchterlony immunodiffusion, immunofluorescence and ELISA with native antigen^{12,7}.
- For most normal and disease controls, and for most autoimmune diseases, the sensitivities and specificities of anti-SSA-52 and SSA-60 autoantibodies are similar^{11,7}.
- However, anti-SSA-52 is the most common reactivity in patients with polymyositis/dermatomyositis who have synthetase syndrome, while anti-SSA-60 reactivity is not common in this group^{8,9}.
- Detecting anti-SSA-52 and anti-SSA-60 together adds sensitivity in SLE and SS patients, while anti-SSA-52 by itself may aid in diagnosing synthetase syndrome in patients with PM/DM.

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