Diagnostic and Prognostic Significance of Measuring Antibodies to α-Fodrin Compared to Anti-Ro-52, Anti-Ro-60, and Anti-La in Primary Sjögren's Syndrome

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ABSTRACT. ***Objective.*** To compare sensitivity and specificity of autoantibodies to α-fodrin with conventional anti-Ro and anti-La antibodies in patients with primary Sjögren's syndrome (pSS). Data on internal organ manifestations were correlated with presence of autoantibodies.

Methods. We collected clinical and laboratory data from 321 patients with pSS (Copenhagen criteria), of which 205 fulfilled the new American-European 2002 consensus criteria. Sera were tested for autoantibodies against α-fodrin and recombinant Ro-52, Ro-60, and La proteins.

Results. Antibodies to α-fodrin were not diagnostically superior to conventional anti-Ro/La testing. IgG anti-La had the highest specificity (97%). A highly significant association was found between presence of anti-La and internal organ manifestations (OR 6, 95% CI 2.99–12.03) or hematological abnormalities. The pattern of autoantibodies was relatively independent of disease duration, indicating that these antibodies appeared early in pSS, probably even years before the first symptoms were manifest.

Conclusion. We could not confirm that antibodies to α-fodrin had higher specificity or sensitivity than anti-Ro/La. Anti-La antibodies were strongly correlated to organ involvement and cytopenias, and thus could serve as a prognostic marker in pSS. (First Release Mar 15 2008; J Rheumatol 2008;35:845–9)

Key Indexing Terms:
PRIMARY SJÖGREN'S SYNDROME ANTI-RO-52 ANTI-RO-60 ANTI-LA ANTI-α-FODRIN

Primary Sjögren's syndrome (pSS) is a chronic autoimmune condition commonly affecting middle-aged women (female/male ratio 9:1). The clinical hallmark is extreme tiredness combined with irritation of the eyes and persistent dryness of the mouth due to chronic inflammation of the salivary and lachrymal glands. The main histopathological feature is focal lymphocytic infiltrations of the exocrine glands, leading to acinar cell death probably through apoptosis[1]. A considerable proportion of patients with pSS develop non-exocrine manifestations involving internal organs (lungs, liver, kidneys, pancreas) as well as skin, blood, lymphatic systems, muscles, and joints[2].

pSS is characterized by the presence of a variety of autoantibodies, both organ-specific and non-organ-specific. Autoantibodies directed to the cytoplasmic/nuclear ribonucleoprotein particles (RNP) containing small uridine-rich fragment of RNA[3,4] have attracted special attention owing to their widespread occurrence in patients with pSS. U1-RNP antibodies against the Ro/SSA-60, Ro/SSA-52, and La/SSB proteins are claimed to be present in 50%–90% of patients with pSS and to a lesser degree in patients with systemic lupus erythematosus (SLE)[5].

In 1997 Haneji, *et al*[6] found that serum samples from patients with SS reacted with a 120 kiloDalton organ-specific antigen purified from the salivary glands of NSF/sld mice (a mouse model of human SS). The amino-terminal residues were identical to the ubiquitously expressed human cytoskeletal protein α-fodrin. Forty-one of 43 subjects with pSS reacted with the purified and recombinant human α-fodrin protein, whereas none of the control patients with SLE or rheumatoid arthritis (RA) or healthy blood donor controls were positive. In addition, 5 of 8 sera from patients with secondary SS also demonstrated reactivity to this antigen. Recently, a number of studies found antibodies to α-fodrin to be a reliable and specific marker for pSS, with a sensitivity of around 60% and specificity in relation to SLE of over 90%[7-9]. Others, however, failed to show similar results[10,11].

We assessed the diagnostic utility of measuring anti-α-fodrin antibodies of the IgG and IgA isotype compared to the classical IgG anti-Ro/La antibodies in a large cohort of patients with pSS. We evaluated whether a positive test for any of these autoantibodies could be correlated to clinical manifestations in terms of internal organ involvement or hematological abnormalities.

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MATERIALS AND METHODS

Patients. A prospective collection of clinical and serological data on patients with pSS has been undertaken at the Sjögren's Syndrome Research Centre in Malmö since 1984. All data were registered according to a standardized research protocol and stored in a database. Patients were initially included if they fulfilled the 1986 Copenhagen classification criteria for pSS[12], according to which it is mandatory to have both keratoconjunctivitis sicca and xerostomia verified by at least 2 objective test results from the eyes and mouth, respectively, i.e., a minimum of 4 abnormal test results from the organs affected.

The patient cohort comprised 321 individuals satisfying the Copenhagen classification criteria. A new set of classification criteria for SS from the American-European Consensus Group appeared in 2002, in which at least 4 of 6 items should be positive. Among the positive items, a positive labial gland biopsy with a focus score > 1.0 and/or the presence of anti-Ro/La is an absolute requirement[13]. Following these criteria it was actually possible to be diagnosed with pSS with sicca symptoms from one exocrine organ only, i.e., xerostomia or keratoconjunctivitis, thus departing from the original conception of SS, which required both. Of the 321 patients, 205 fulfilled the 2002 Consensus criteria.

The distribution of anti-α-fodrin and anti-Ro/La autoantibodies was calculated and the sensitivities compared according to each set of criteria. Clinical manifestations were correlated to the presence of the various autoantibodies.

Specificity was determined from control groups consisting of healthy blood donors (n = 76), SLE patients (n = 108), and patients with RA (n = 95).

Permission to collect clinical data and serum samples was obtained and approved by the regional Ethical Committee for Malmö/Lund, Sweden.

Clinical data. The medical history for the individual patients was retrieved from the database. Internal organ injury was defined for lungs (bronchial/bronchiolar damage on radiograph, computed tomographic scans, decreased diffusion capacity for carbon monoxide, or a pathological pulmonary function test), kidneys (elevated creatinine, decreased clearance, signs of renal tubular acidosis, or persistent proteinuria), liver, and pancreas (pathological liver/pancreas enzyme levels). Biochemical or hematological abnormal results were required to have occurred on at least 2 occasions.

Laboratory tests. Routine laboratory measures, i.e., hemoglobin, leukocyte counts, and thrombocyte concentrations, were collected from the database.

Frozen sera from all patients were tested for antibodies of the IgG and IgA isotype to α-fodrin by use of a commercial kit (Aesku.lab Diagnostika, Wendelsheim, Germany) according to the protocol from the manufacturer; the cutoff was 15 U/ml.

IgG antibodies to Ro-60, Ro-52, and La were determined by in-house ELISA at the Department of Autoimmunology, Statens Serum Institut, Copenhagen. The cutoff was defined as the mean of 100 donors + 3 standard deviations (SD) and was adjusted to 10 arbitrary units/ml for all assays.

The Ro and La antigens were recombinant proteins produced (at Department of Biochemistry, Statens Serum Institut) in E. coli, using the expression system developed by Studier and Moffatt[14] (Ro-52 and Ro-60), or in insect cells using the Baculovirus expression system introduced by Miller[15] (La). The recombinant proteins were provided with N-terminal oligo-His extensions, and purified by affinity chromatography on a HiTrap chelating column (Amersham Pharmacia Biotech, Uppsala, Sweden).

Statistics. Categorical variables were analyzed with the chi-square or Fisher's exact test. Logistic regression was performed to elucidate the correlation between the clinical manifestations and the presence of various autoantibodies.

RESULTS

Specificity and sensitivity of autoantibodies. The distribution of individual antibodies according to the Copenhagen and EU/US criteria is shown in Table 1. IgA and IgG anti-α-fodrin was found in 32% and 31%, respectively, of subjects with pSS according to the Copenhagen criteria set, whereas IgG anti-Ro-52, anti-Ro-60, and anti-La were present in 38%, 26%, and 20%. In the subcohort of individuals satisfying the EU/US criteria the prevalence figures for IgA and IgG anti-α-fodrin were 35% and 37%, respectively, and for anti-Ro-52, anti-Ro-60, and anti-La 60%, 42%, and 32%. The specificity was calculated for the combined control group of blood donors and patients with SLE and RA (total n = 279). It was 88% and 94% for IgA and IgG anti-α-fodrin antibodies, respectively, and 92%, 93%, and 97% for each of anti-Ro-52, anti-Ro-60, and anti-La autoantibodies. In the cohort of SLE patients, 25 of 108 (23%) had either anti-Ro-52/Ro-60 or anti-La; in the RA group one of 95 (1%) was positive; and none of the 76 control donors was positive for these autoantibodies. IgA or IgG anti-α-fodrin antibody was found in 24 (23%) SLE patients, 14 (15%) RA patients, and 4 (5%) control donors.

The overall specificity for IgG or IgA anti-α-fodrin was 85% and when sera were positive for one of IgG anti-Ro-52 or anti-Ro-60 or anti-La, it was 91%.

Disease duration and presence of autoantibodies. The prevalence of anti-Ro/La and anti-α-fodrin antibodies in relation to disease duration of pSS is shown in Figure 1 for the 202 patients constituting the subcohort satisfying the EU/US criteria (data on disease duration were missing for 3 subjects).

Extraglandular complications and autoantibodies. The numbers of patients with at least one affected organ (lung, kidney, liver, pancreas) were for the Copenhagen group 156 (49%) and for the EU/US group 108 (53%). Individual figures for the Copenhagen versus the EU/US cohort were lungs 59 (18%)/38 (18%), kidneys 104 (32%)/78 (38%), liver 30 (9%)/21 (10%), and pancreas 8 (2.5%)/5 (2.4%). The fractions with cytopenia (one or more of anemia, leuko-

Table 1. Sensitivity and specificity of the 5 individual assays when patients were grouped according to the Copenhagen or the subgroup fulfilling the European/American consensus (EU/US) criteria. Specificity was calculated for the entire control group of blood donors and patients with SLE and RA. Data are percentages.

Sensitivity	Copenhagen Criteria, n = 321	EU/US Criteria, n = 205	Specificity
IgA anti-α-fodrin	32	35	88
IgG anti-α-fodrin	31	37	94
IgG anti-Ro-52	38	60	92
IgG anti-Ro-60	26	42	93
IgG anti-La	20	32	97
IgA or IgG anti-α-fodrin	45	50	85
IgA and IgG anti-α-fodrin	18	22	97
IgG anti-Ro-52 or Ro-60 or La	43	69	91
IgG anti-Ro-52 and Ro-60 and La	12	19	98

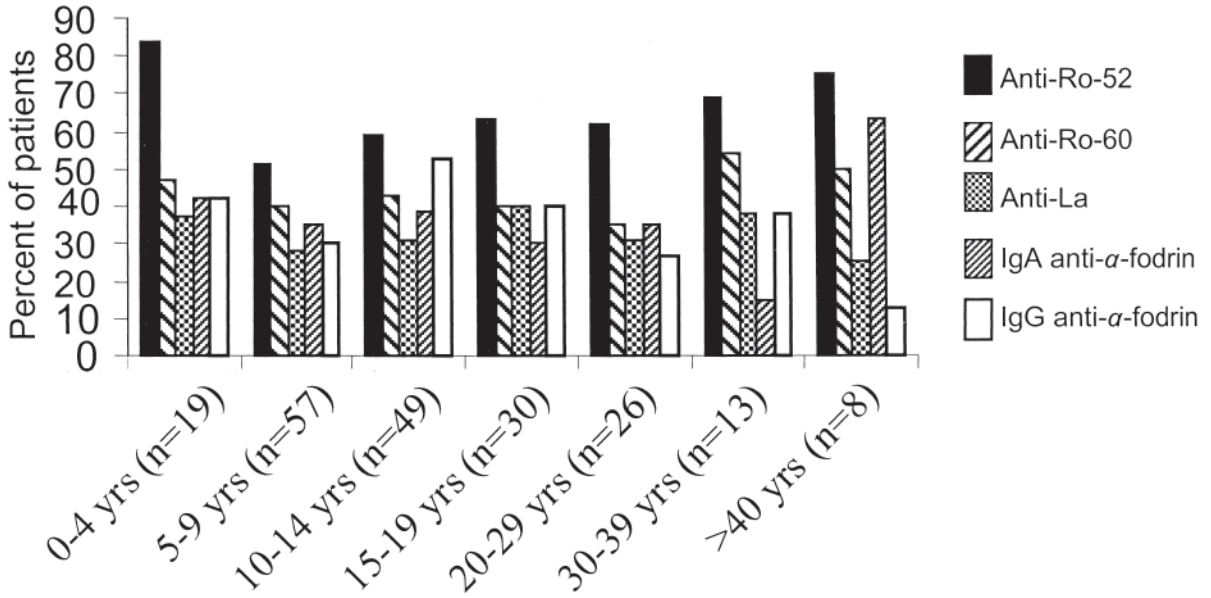

Figure 1. Prevalence of IgG anti-Ro-52, anti-Ro-60, anti-La, and IgG/IgA anti-α-fodrin autoantibodies in 202 patients with pSS, classified according to the EU/US consensus criteria. The pattern of antibodies is plotted against disease duration in years.

cytopenia, thrombocytopenia) correspondingly were 91 (28%)/76 (37%). Apart from cytopenia, which occurred slightly more frequently in the Consensus criteria group, there were virtually no differences between the 2 cohorts. The subsequent calculations are therefore restricted to the 205 subjects from the EU/US consensus group.

There were 125 patients positive for anti-Ro-52, of which 70 (56%) had at least one organ manifestation. Eighty-five subjects were positive for anti-Ro-60 and 47 (55%) had organ manifestation, and 65 were positive for anti-La and 52 (80%) had organ damage. For the patients with IgA and IgG anti-α-fodrin antibodies the figures were 45 out of 72 (63%) with organ manifestation, and 38 out of 76 (50%) were seropositive. A highly significant association was found for the combination of at least one organ affected and a positive test for anti-La (OR 6.0, 95% CI 2.99–12.03). A weak association was shown between presence of IgA anti-α-fodrin and organ complication (OR 1.85, 95% CI 1.03–3.33); however, the presence of IgG anti-α-fodrin pointed slightly (although not significantly) in the opposite direction by indicating a protective effect against internal organ manifestations (OR 0.84, 95% CI 0.48–1.49) (Table 2). A logistic regression model of the above variables confirmed that anti-La was a significant predictor of organ damage.

There was a very significant correlation between presence of anti-La and occurrence of cytopenia (anemia, leukopenia, or thrombocytopenia) (OR 4.62, 95% CI 2.47–8.66, $p < 0.0001$). Cytopenia was also correlated with the number of affected organs ($p = 0.0015$; data not shown).

DISCUSSION

In this large, well characterized cohort of patients with pSS we observed a sensitivity of IgG anti-Ro/La and IgA/IgG anti-α-fodrin in the range of 20%–38%. The highest sensitivity was found for anti-Ro-60 and the lowest for anti-La when patients were classified according to the Copenhagen classification criteria. These figures may deviate somewhat from other reports, which claim that the prevalence of anti-

Table 2. Distribution of individual autoantibodies between the groups with minimum one organ affected (lung, kidney, liver, or pancreas). Odds ratio denotes odds for having at least one organ manifestation given a positive test result.

Status	Affected Organs, n = 108 (%)	No Affected Organs, n = 97 (%)	OR	95% CI	p
Anti-La-positive	52 (48)	13 (13)	6.00	2.99–12.03	< 0.0001
Anti-Ro-52-positive	70 (65)	55 (57)	1.41	0.80–2.47	0.25
Anti-Ro-60-positive	47 (44)	38 (39)	1.20	0.68–2.09	0.57
IgA anti-α-fodrin-positive	45 (42)	27 (28)	1.85	1.03–3.33	0.04
IgG anti-α-fodrin-positive	38 (35)	38 (39)	0.84	0.48–1.49	0.56

Ro/La is in the order of 50%–90%[2,16-18]. Obviously these figures are highly dependent on several factors such as the use of different classification criteria or recruitment from referral centers with varying degrees of specialization, or due to the large variety of diverse assay techniques currently in use. The double-immune-diffusion method is still preferred by some as well as counterimmune electrophoresis, although many investigators now favor the ELISA platform because of its low cost and high throughput rate. To compare frequencies of anti-Ro/La between pSS patients fulfilling the Copenhagen versus the EU/US consensus criteria makes virtually no sense as determination of these autoantibodies is one of the items included in the Consensus set of criteria. Thus it is not surprising that the sensitivity of anti-Ro/La was considerably greater when the consensus standards were applied. In accord with others' results[19,20], we found that the occurrence of anti-Ro/La was notably lower in the control population of patients with SLE. Twenty-five of 108 (23%) SLE controls had either anti-Ro-52/Ro-60 or anti-La. The sensitivity for anti-Ro-60 may also be influenced by the use of recombinant Ro-60 protein in the ELISA. There are indications that anti-Ro-60 antibodies are directed to conformational rather than linear epitopes, resulting in a lower number of positives than would have been obtained by use of native antigens[21,22].

The fraction of patients positive for anti-Ro/La or anti-α-fodrin was relatively constant and was unaffected by duration of the illness (Figure 1). Autoantibodies are probably generated very early in the disease process or even before clinical symptoms emerge. This may imply that once present these antibodies do not change much during the course of illness in the individual patient. This is probably equivalent to the situation in RA, where autoantibodies such as rheumatoid factor and anti-cyclic citrullinated peptides appear years before the diagnosis is established[23,24].

A number of encouraging reports have claimed that measurement of antibodies to α-fodrin provided better diagnostic sensitivity and specificity in pSS than anti-Ro/La[7,9,25]. We were unable to confirm this. The sensitivity for IgA and IgG anti-α-fodrin was comparable to anti-Ro-52 and anti-Ro-60. Indeed, the highest sensitivity was obtained for anti-Ro-52 (38%), without significant loss of specificity. IgA anti-α-fodrin has also been found to be superior to the IgG isotype. In this study the performance of IgA and IgG anti-α-fodrin antibodies was equally sensitive. IgG even had the highest specificity. Interestingly, the proportion of sera positive for α-fodrin increased only slightly when the EU/US consensus criteria were applied. From these combined data there is nothing to substantiate that measurements of anti-α-fodrin antibodies should replace the traditional use of anti-Ro/La in the diagnosis of pSS.

It is well known that pSS can be accompanied by symptoms in virtually every organ. Nonetheless, the overall mortality rate seems to match that of the background population[26]. One exception is a significant increased incidence of malignant lymphoma, reported to be in the order of 15 to 20 times that in the average population. Prognostic markers of future development of lymphoma have been shown to be hypergammaglobulinemic purpura, low complement factor C3 and C4, or low counts of CD4-positive T lymphocytes[26]. Other organ manifestations, e.g., bronchial epithelitis of the lungs, might be disturbing for the individual patient but do not seem to influence the mortality rate[26,27]. The same observation probably applies to kidney impairment, where endstage renal failure in pSS is a most uncommon feature. It is uncertain whether pathological liver/pancreas enzyme concentration could be taken as an indicator of organ damage, although it may indicate some degree of organ malfunction. In contrast to treatment of RA or SLE, drugs with severe side effects are not commonly used in pSS. Therefore the liver/pancreas enzyme abnormalities observed in pSS probably indicate effects on the various organ systems of the disease itself and are not caused by iatrogenic factors.

We found no indication that increased diagnostic sensitivity or specificity for pSS could be obtained by the use of IgG or IgA anti-α-fodrin antibodies; conversely, the traditional anti-Ro/anti-La autoantibodies were slightly superior. Internal organ damage and hematological abnormalities were closely associated with the presence of anti-La antibodies. As autoantibodies appear early, or before the disease manifestations become clinically apparent, determination of anti-La may prove to be a reliable prognostic factor in pSS.

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