

Sensitivity and Specificity for Primary Sjögren's Syndrome of IgA and IgG Anti- α -Fodrin Antibodies Detected by ELISA

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ABSTRACT. Objective. To investigate the sensitivity and specificity of anti- α -fodrin antibodies in patients with primary Sjögren's syndrome (pSS).

Methods. IgA and IgG anti- α -fodrin antibodies were measured in the sera of 80 patients with pSS, 60 blood donors matched for age and sex, 50 patients with systemic lupus erythematosus (SLE), 30 with rheumatoid arthritis (RA), 20 with systemic sclerosis (SSc), and 10 with polymyositis or dermatomyositis (PM/DM) by an ELISA method employing recombinant human α -fodrin as antigen.

Results. The sensitivity of IgA and IgG anti- α -fodrin antibodies for pSS was 32.50% and 21.25%, respectively. When the prevalence of these antibodies in patients with SLE, RA, SSc, and PM/DM was evaluated, we observed specificity of these antibodies of 68.18% and 79.09%, respectively. The sensitivity and specificity for pSS of the combined determination of IgA and IgG anti- α -fodrin antibodies were 40% and 58.18%, respectively.

Conclusion. The prevalences of IgA and IgG anti- α -fodrin antibodies in our patients with pSS and other chronic autoimmune diseases have induced us to doubt their use as diagnostic markers of pSS. (J Rheumatol 2004;31:504-7)

Key Indexing Terms:

α -FODRIN AUTOANTIBODIES SENSITIVITY SPECIFICITY
PRIMARY SJÖGREN'S SYNDROME

Primary Sjögren's syndrome (pSS) is a systemic rheumatic disease characterized by a progressive lymphocytic and plasma cell infiltration of the salivary and lachrymal glands and the presence of several autoantibodies in the blood. In particular, serum antinuclear antibodies (ANA) are found in the majority of patients with pSS, anti-Ro/SSA antibodies in 50 to 80%, anti-La/SSB antibodies in 30 to 60%, and rheumatoid factor (RF) in 60 to 80%¹. However, all these antibodies are also observed in systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and other chronic autoimmune diseases. In 1997, Haneji, *et al*² reported that sera from patients with pSS reacted with purified antigen and recombinant 120 kDa human α -fodrin protein whereas those from patients with SLE or RA did not. An important role was thus attributed to anti- α -fodrin antibodies in the diagnosis of pSS. The presence of anti- α -fodrin antibodies

in the blood of adults and children with SS was subsequently described in a number of reports³⁻⁸, with a prevalence varying between 100% and 55.2% in pSS and between 100% and 40.9% in secondary SS. These antibodies, however, were also found in the sera of all asymptomatic mothers of offspring with neonatal lupus erythematosus³, in up to 41.6% of adults with RA⁵, in up to 55.5% of children with RA⁶, and in up to 83.3% of children with SLE⁶.

We investigated the sensitivity and specificity of anti- α -fodrin antibodies for pSS by testing the sera of patients with pSS and comparing the results with those obtained from the sera of patients with other chronic autoimmune rheumatic diseases with no sign or symptom of associated SS. We also evaluated whether the presence of anti- α -fodrin antibodies is correlated with the most frequent pSS antibodies.

MATERIALS AND METHODS

Patients. Eighty patients, 77 women and 3 men (mean age 49.58 years \pm 14.09, range 24-82), with pSS were examined. All patients fulfilled the European Community Study Group criteria for the classification of pSS⁹ and had a mean disease duration of 118.90 months \pm 67.75, range 1-444. Those showing signs or symptoms of other associated disease were excluded.

Controls. Sera from 60 blood donors matched for age and sex with the pSS patients were used.

Disease control group. A total of 110 patients with chronic rheumatic autoimmune diseases were enrolled: 50 with SLE according to the American College of Rheumatology (ACR) criteria^{10,11}, 30 with RA

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Submitted October 4, 2002; revision accepted August 11, 2003.

according to the ACR criteria¹², 20 with systemic sclerosis (SSc) according to the ACR criteria¹³, and 10 with polymyositis or dermatomyositis (PM/DM) according to Bohan and Peter's criteria¹⁴. All patients were without any signs or symptoms of SS; in particular none reported dry mouth or dry eyes, or had anti-Ro/SSA and anti-La/SSB antibodies.

Patients and controls were all Italian and all resided in Italy.

Assay for anti- α -fodrin antibodies. Antibodies binding to α -fodrin of immunoglobulin (Ig) classes A and G were measured using a commercial ELISA kit, kindly provided by Italiana Laboratori Bouty, Italy, and used according to the manufacturer's protocol (Aesku Lab Diagnostika, Germany). Antigen coated to microplates was recombinant human α -fodrin obtained using the standard procedure⁵. The sera were diluted 1:101. Results were expressed as arbitrary units (U) by reading off a standard curve, and the normal range for each test was established as a mean plus 2.5 standard deviation (SD) calculated from 100 blood donors, 50 men and 50 women with a mean age of 42.84 yrs \pm 10.21, range 21–64. The cutoff values of IgA and IgG anti- α -fodrin antibodies were 17 and 20 U, respectively. Kits with the same lot number (02200) were employed for testing the sera of all patients and controls. In order to evaluate the reliability of ELISA results, the pSS, disease control group, and the healthy control sera were retested for IgA and IgG anti- α -fodrin antibodies using kits with a different lot number (02330). The possibility of false-positive results due to nonspecific Ig background-binding was investigated by measuring and comparing the mean serum concentrations of IgA and IgG in IgA and IgG positive and negative sera, respectively.

Detection of other antibodies. Sera were screened for ANA by indirect immunofluorescence (IIF) on HEp-2000 cells (Immunoconcepts, Sacramento, CA, USA). Precipitating antibodies to Ro/SSA, La/SSB, and other extractable nuclear antigens (ENA) such as U1-RNP, Sm, topoisomerase I, PM1, Jo1, PCNA, Ku, and SL were investigated by an in-house counterimmunoelectrophoresis method. Anticentromere antibody was detected by a centromere fluorescence pattern on HEp-2000 cells. Anti-dsDNA antibodies were assayed by IIF using *Crithidia luciliae* (Immunoconcepts) as substrate. RF was detected by automated rate nephelometry.

Statistical analysis. McNemar's chi-square test for comparison of results from different lot number kits, Fisher's exact test for comparison of antibody prevalences, and Mann-Whitney U test for comparison of mean values of antibody levels were used. A p value of < 0.05 was considered statistically significant.

RESULTS

The results obtained from kits with different lot numbers showed no significant differences. As a result, only those from lot number 02200 are reported. IgA anti- α -fodrin antibodies (Figure 1) were detected in the sera of 26 patients

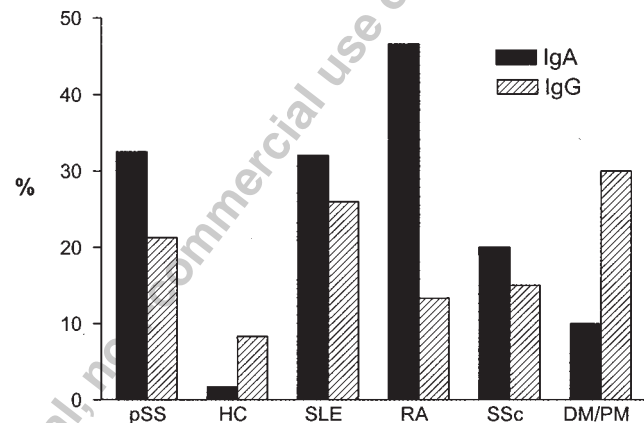


Figure 1. Prevalence of IgA and IgG anti- α -fodrin antibodies in patients with pSS, healthy controls (HC), and the disease control group.

(32.5%) with pSS, in 16 (32.0%) with SLE, in 14 (46.7%) with RA, in 4 (20.0%) with SSc, in 1 (10.0%) with PM, and in 1 control (1.7%). IgG anti- α -fodrin antibodies (Figure 1) were positive in the sera of 17 patients (21.3%) with pSS, in 13 (26.0%) with SLE, in 4 (13.3%) with RA, in 3 (15.0%) with SSc, in 3 (30.0%) with DM/PM, and in 5 (8.3%) controls. IgA and/or IgG anti- α -fodrin antibodies were observed in the sera of 32 patients (40.0%) with pSS, 21 (42.0%) with SLE, 17 (56.7%) with RA, 5 (25.0%) with SSc, 3 (30.0%) with DM/PM, and 6 (10.0%) controls. Thus the sensitivity of IgA and IgG antibodies against α -fodrin for SS was 32.5% and 21.3%, respectively, while the specificity was 68.2% and 79.1%, respectively. The sensitivity and specificity of the combined determination of IgA and IgG anti- α -fodrin antibodies for pSS were found to be 40.0% and 58.2%, respectively. When prevalences of both antibodies in pSS were compared with those with other diseases and healthy controls, only IgA anti- α -fodrin antibodies proved significant in pSS as compared to healthy controls ($p < 0.0001$).

The possibility of false positive results due to Ig background binding was excluded by the lack of a significant difference between mean serum levels of IgA and IgG in IgA and IgG anti- α -fodrin antibody positive and negative sera. Levels of IgA and IgG anti- α -fodrin antibodies are shown in Figure 2 and their mean values in Figure 3. Significantly higher mean levels in pSS of IgA or IgG anti- α -fodrin antibodies were not found when statistical analysis compared mean antibody levels in pSS and the other disease or control groups. Among the patients with pSS, ANA were detected in 68 of 78 examined sera (87.2%), anti-Ro/SSA in 63 of 79 (79.7%), anti-La/SSB in 42 of 79 (53.2%), and RF in 40 of 66 (60.6%). Anti-U1-RNP was positive in 1 of 79 sera (1.3%), anticentromere in 1 of 78 (1.3%), and anti-dsDNA in 6 of 79 (7.6%) at a titer of ≤ 40 . No significant association between IgA or IgG anti- α -fodrin antibodies and ANA, RF, anti-Ro/SSA, or anti-La/SSB antibodies was revealed by statistical analysis.

DISCUSSION

Our study found a low sensitivity of both IgA and IgG anti- α -fodrin antibodies for pSS. Moreover, a moderate specificity of both antibodies for pSS was observed when their prevalences in patients with SLE, RA, SSc, and PM/DM were evaluated. A slightly higher sensitivity and a slightly lower specificity for pSS were obtained when the combined determination of IgA and IgG anti- α -fodrin antibodies was employed. The statistical comparison of the prevalence of antibodies in pSS with that in other chronic autoimmune rheumatic diseases found no significant difference for IgA or IgG anti- α -fodrin antibodies. Moreover, mean antibody levels in pSS patients did not significantly differ from those in patients with SLE, RA, SSc, and PM/DM.

It should be emphasized that the detection of anti- α -

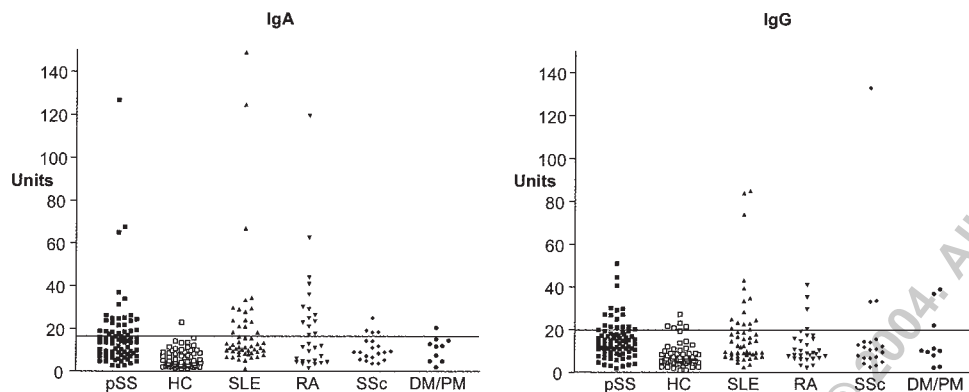


Figure 2. Levels of IgA and IgG anti- α -fodrin antibodies in patients with pSS, healthy controls (HC), and the disease control group.

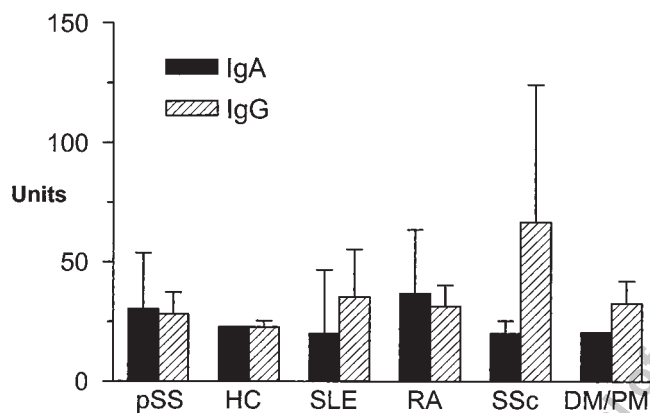


Figure 3. Mean levels and SD of IgA and IgG anti- α -fodrin antibodies in patients with pSS, healthy controls (HC), and the disease control group.

fodrin antibodies was performed with a human recombinant α -fodrin as antigen in accord with numerous studies³⁻⁸. Moreover, Witte, *et al*⁵ recently reported the same analytic sensitivity and specificity using both ELISA and immunoblotting methods for IgA anti- α -fodrin antibodies.

To evaluate the sensitivity of anti- α -fodrin antibodies for SS, only patients with primary SS were enrolled in this study. The diagnosis of pSS in these patients was based on the most commonly applied classification criteria⁹ and the prevalence in their sera of ANA, RF, anti-Ro/SSA, and La/SSB antibodies was in keeping with that reported in literature¹. In order to investigate the specificity for pSS of anti- α -fodrin antibodies, patients without secondary SS criteria⁹ and in particular without dry mouth, dry eyes, anti-Ro/SSA, and anti-La/SSB antibodies were included in the disease control group. As shown in Table 1, in previous studies the prevalence of anti- α -fodrin antibodies in adult patients varied by about 100% to 55% in pSS, by about 62% to 40% in secondary SS, and by about 0% to 41% in RA. The find-

ings in childhood patients (Table 2) are controversial, as they reveal the same prevalence only in pSS. Compared to other studies (Table 1), we found the lowest prevalence of anti- α -fodrin antibodies in pSS and the highest prevalence in adult SLE and RA. Moreover, for the first time we describe the presence of anti- α -fodrin antibodies in adult patients with SSc and PM/DM, at a frequency not significantly different from that of patients with pSS. These important differences in the sensitivity and specificity for pSS of anti- α -fodrin antibodies could be due to variations in the number of sera examined or in the racial composition of patient groups. In addition, it should be specified that according to Witte, *et al*⁵, the recombinant α -fodrin protein used in the ELISA kit was a 93 kDa protein coded by 103–1823 base pairs of α -fodrin cDNA. Other authors^{2,4,6-8} instead used a 120 kDa recombinant α -fodrin protein coded by 1–1784 base pairs of α -fodrin cDNA. This slight difference along with the possibility that the structure of the recombinant α -fodrin bound to the ELISA plate might be different from that in the immunoblot could explain the diversity between the sensitivity and specificity for SS of anti- α -fodrin antibodies detected by ELISA and those of anti- α -fodrin antibodies detected by immunoblot.

Our results indicate that these antibodies cannot be considered a diagnostic marker of pSS. This observation concurs with the negative or irrelevant correlation between anti- α -fodrin antibodies and anti-Ro/SSA or La/SSB antibodies reported by us and others⁵⁻⁷.

The presence of these antibodies in 100% of children with pSS according to all pediatric reports^{6,8}, and the experimental data on the role of α -fodrin as an autoantigen in the pathogenesis of SS^{15,16} indicate that these should be considered early, rather than diagnostic, markers of the disease. This suggestion is in keeping with a study by Kobayashi, *et al*⁸ that detected anti- α -fodrin antibodies in the sera of childhood patients with primary or secondary SS before anti-Ro/SSA and anti-La/SSB antibodies became positive.

Table 1. The prevalence of anti- α -fodrin antibodies in adult patients and healthy controls.

Antibody Isotype	Haneji ² , Not Specified (%)	Miyagawa ³ , IgG (%)	Watanabe ⁴ , Not Specified (%)	Witte ⁵ ,		Current Report,	
				IgA (%)	IgG (%)	IgA (%)	IgG (%)
Primary SS	41/43 (95.3)	11/11 (100)	7/9 (77.7)	54/85 (64)	47/85 (55.3)	26/80 (32.5)	17/80 (21.2)
Secondary SS	5/8 (62.5)	—	9/15 (60)	13/22 (59)	9/22 (40.9)	—	—
SLE	0/21 (0)	—	3/44 (6.8)	1/50 (2)	1/50 (2)	16/50 (32)	13/50 (26)
RA	0/14 (0)	—	—	2/12 (16.6)	5/12 (41.6)	14/30 (46.6)	4/30 (13.3)
SSc	—	—	—	—	—	4/20 (20)	3/20 (15)
DM/PM	—	—	—	—	—	1/10 (10)	3/10 (30)
Mothers of infants with NLE	—	5/5 (100)	—	—	—	—	—
Healthy controls	0/15 (0)	0/10 (0)	—	1/160 (0.6)	3/160 (1.8)	1/60 (1.6)	5/60 (8.3)

NLE: neonatal lupus erythematosus.

Table 2. The prevalence of anti- α -fodrin antibodies in childhood patients and healthy controls.

Antibody Isotype	Takahashi ⁶ , Total Ig (%)	Maeno ⁷ , IgG (%)	Kobayashi ⁸ , IgG (%)
	Primary SS	3/3 (100)	11/11 (100)
Secondary SS	—	4/4 (100)	2/4 (50)
SLE	5/6 (83.3)	2/16 (12.5)	1/7 (14.3)
RA	5/9 (55.5)	—	0/7 (0)
DM	—	—	0/7 (0)
Healthy controls	0/7 (0)	—	—

Clinical followup studies in patients with early signs or symptoms of the disease could verify whether anti- α -fodrin antibodies should be considered a predictive marker of SS and perhaps of other chronic autoimmune rheumatic diseases.

ACKNOWLEDGMENT

The authors thank L. Inverso for her assistance in preparing this manuscript.

REFERENCES

- Anaya JM, Talal N. Sjögren's syndrome and connective tissue diseases associated with other immunologic disorders. In: Koopman WJ, editor. Arthritis and allied conditions. Baltimore: Williams & Wilkins; 1997:1561-80.
- Haneji N, Nakamura T, Takio K, et al. Identification of α -fodrin as a candidate autoantigen in primary Sjögren's syndrome. *Science* 1997;276:604-7.
- Miyagawa S, Yanagi K, Yoshioka A, Kidoguchi K, Shirai T, Hayashi Y. Neonatal lupus erythematosus: maternal IgG antibodies bind to a recombinant NH₂-terminal fusion protein encoded by human α -fodrin cDNA. *J Invest Dermatol* 1998;111:1189-92.

- Watanabe T, Tsuchida T, Kanda N, Mori K, Hayashi Y, Tamaki K. Anti- α -fodrin antibodies in Sjögren's syndrome and lupus erythematosus. *Arch Dermatol* 1999;135:535-9.
- Witte T, Matthias T, Arnett FC, et al. IgA and IgG autoantibodies against α -fodrin as markers for Sjögren's syndrome. *J Rheumatol* 2000;27:2617-20.
- Takahashi K, Tatsuzawa O, Yanagi K, Hayashi Y, Takahashi H. Alpha-fodrin auto-antibody in Sjögren's syndrome and other autoimmune diseases in childhood. *Eur J Pediatr* 2001;160:520-1.
- Maeno N, Takei S, Imanaka H, et al. Anti- α -fodrin antibodies in Sjögren's syndrome in children. *J Rheumatol* 2001;28:860-4.
- Kobayashi I, Kawamura N, Okano M, et al. Anti- α -fodrin autoantibody is an early diagnostic marker for childhood primary Sjögren's syndrome. *J Rheumatol* 2001;28:363-5.
- Vitali C, Bombardieri VC, Moutsopoulos HM, et al. Preliminary criteria for the classification of Sjögren's syndrome. Results of a prospective concerted action supported by the European Community. *Arthritis Rheum* 1993;36:340-7.
- Tan EM, Cohen AS, Fries JF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982;25:1271-7.
- Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus [letter]. *Arthritis Rheum* 1997;40:1745.
- Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;3:315-24.
- Subcommittee for Scleroderma Criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Preliminary criteria for the classification of systemic sclerosis (scleroderma). *Arthritis Rheum* 1980;23:581-90.
- Bohan A, Peter JB. Polymyositis and dermatomyositis. *N Engl J Med* 1975;292:344-7, 403-7.
- Inoue H, Tsubota K, Ono M, et al. Possible involvement of EBV-mediated α -fodrin cleavage for organ-specific autoantigen in Sjögren's syndrome. *J Immunol* 2001;166:5801-9.
- Toda I. Autoantigens and Sjögren's syndrome. *Cornea* 2002;21:S13-6.