Autoantibodies Against α-Fodrin in Sjögren's Syndrome with Neurological Manifestations

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ABSTRACT. ***Objective.*** To investigate the diagnostic value of autoantibodies against α-fodrin in patients with Sjögren's syndrome (SS) with neurological manifestations compared to SS patients without neurological manifestations, a control group, and patients with other neurological autoimmune diseases including systemic lupus erythematosus (SLE) with neurological manifestations and multiple sclerosis (MS).

Methods. We evaluated α-fodrin autoantibodies in 31 patients with SS with neurological manifestations, 53 SS patients without neurological symptoms, 38 patients with SLE, 60 with MS, and 160 controls.

Results. Twenty of the 31 SS patients with neurological manifestations (64.5%) had an increased concentration of IgA and/or IgG anti-α-fodrin. This was not statistically different from that of SS patients without neurological symptoms (73.6%), but was higher than the number with SSA/SSB antibodies, which were found in 15 (48%) of our SS patients without neurological manifestations. When the results of the 2 tests were combined, 28 of the 31 (90.3%) patients had positive autoantibodies (α-fodrin and/or SSA/SSB). α-fodrin antibodies were increased in 8 (13.3%) of the 60 patients with MS, in 6 (15.7%) of 38 patients with SLE, and in 10 (6.3%) of 160 controls.

Conclusion. Our results confirm that α-fodrin antibodies are an additional diagnostic tool for SS. This test is of particular interest for patients with SS with neurological manifestations, in whom anti SSA/SSB antibodies are less frequently found. (J Rheumatol 2004;31:500–3)

Key Indexing Terms:
SJÖGREN'S SYNDROME ALPHA-FODRIN MULTIPLE SCLEROSIS NEUROLOGICAL

Sjögren's syndrome (SS) is a frequent autoimmune disorder, affecting between 0.29 and 4.8% of the population, depending on the diagnostic criteria used[1]. Establishing the diagnosis of SS may be difficult since different criteria have been used for its classification and symptoms. Further, dryness of the eyes and mouth are common complaints in the elderly[2]. Recently, new criteria for SS have been proposed and validated by an American-European committee. They provide a relatively high level of sensitivity (90%) and specificity (95%)[2]. However, the diagnosis of SS with neurological manifestations has been a major problem as frequently only the nervous system is affected, systemic inflammation is rarely found, and anti-Ro (SSA) and anti-La (SSB) antibodies are found in fewer than 50% of cases[3,4].

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It was recently suggested that antibodies against α-fodrin, an intracellular protein associated with the cytoskeleton, are both a sensitive and a specific marker for SS[5,6]. Another study confirmed these first data, showing that IgA or IgG autoantibodies against α-fodrin are found in almost 70% of patients with primary SS and in fewer than 5% of blood donors[7]. Fodrin is a heterodimer composed of α and ß subunits and is an abundant protein in the eukaryotic cell membrane skeleton[8,9]. The 120 kDa amino terminal α-fodrin fragment was detected in tissue homogenates of lip biopsies from patients with primary SS, but not in those from controls[5]. Based on these findings, the 120 kDa amino terminal α-fodrin fragment was considered to be an important, organ-specific autoantigen in an animal model of SS as well as in patients with SS[5,7,10].

Apart from case reports, there have been only a few studies on the neurological manifestations of SS[3,4,11-14]. The main clinical features are peripheral neuropathies and central nervous system (CNS) involvement. Several groups have suggested that the main differential diagnosis of SS with CNS involvement is multiple sclerosis (MS)[11-14]. MS is a CNS-restricted disease without a specific antigenic target. However, there are similar findings in MS and SS with CNS involvement, both clinically (predominance of the disease in women, dissemination of the symptoms in time and space, and frequent spinal cord and optic nerve involvement) and

in terms of laboratory data (hyperintensities on magnetic resonance imaging, and oligoclonal bands in cerebrospinal fluid)[4,11,13].

We investigated the diagnostic value of autoantibodies against α-fodrin in SS patients with neurological manifestations compared to patients with SS without neurological manifestations, patients with MS or systemic lupus erythematosus (SLE) with CNS manifestations, and healthy control blood donors.

MATERIALS AND METHODS

Patients. We investigated 31 patients with neurological manifestations of SS. Ten had peripheral manifestations (axonal sensitive neuropathy) and 21 had CNS manifestations (spinal cord involvement in 17 cases and brain-restricted involvement in 4 cases). All these patients had definite SS according to the revised European criteria for SS, with 4 or more criteria with at least one histological or immunological criterion[2]. We also investigated 53 SS patients without neurological manifestations, 38 SLE patients with neurological manifestations (convulsions, n = 13; depression, n = 23; and psychosis, n = 2) following the American College of Rheumatology criteria[15], and 160 controls. No patient was receiving corticosteroid and/or immunological treatment at the time of blood sampling. As MS is the major differential diagnosis for SS with neurological manifestations, and especially for SS with CNS involvement, we also tested a control group of 60 patients with MS. Twenty patients had a relapsing-remitting form of MS and the remaining 40 had a progressive form of the disease (secondary progressive in 20 and primary progressive in 20). Demographic data are shown in Table 1. None of the patients with MS and SLE met the criteria for SS[2]. The Declaration of Helsinki principles were followed.

Laboratory methods. Sera were frozen at –20°C. All results were analyzed blind to the clinical data for both α-fodrin ELISA and anti SSA/SSB tests.

ELISA for α-fodrin. This method has been described[7]. Briefly, the sera were diluted 1:100 in a dilution buffer containing phosphate buffered saline (PBS; pH 7.4, 75 mM/l NaCl, 0.1% Tween 20); 100 µl aliquots of the diluted sera were incubated for 30 min on the ELISA plates. After 3 washes with dilution buffer using an automatic ELISA washer (SLT-Labinstruments, Grödig, Austria), horseradish peroxidase-labeled goat antiserum recognizing only IgA or IgG (Dianova, Hamburg, Germany) was added for 15 min. After 3 additional washes, tetramethylbenzidine (TMB) was added as substrate for 15 min. The reaction was stopped by addition of 100 µl 1 M HCl and the optical density at 450 nm was determined using an ELISA reader (Rainbow Reader, SLT-Labinstruments). Positive values for both IgG and IgA ELISA were determined using the control group (mean value + 3 standard deviations, SD).

Identification of SSA and SSB antibodies. As proposed by Morozzi, *et al*[16], we assessed 3 different tests for anti-SSA/anti-SSB detection. We assessed indirect immunofluorescence (Bioadvance, Emerainville, France), double immunodiffusion (Biomedical Diagnostics, Marne-la-Vallée, France), and Western blotting (standard in-house procedure). The fine specificity of the 52 kDa and 60 kDa anti-Ro/SSA response was determined by immunoblotting using the human HEp2 cell line (ATCC CCL 23) as substrate. Immunoblotting was performed on a home-made 10–20% acrylamide gel in order to optimize the separation of proteins with a molecular mass ranging from 40 to 70 kDa. For each patient, the patterns were blindly read and compared with SSB 48, SSA/Ro 52, and 60 positive sera, respectively. These controls were clinically and biologically defined as SS.

Statistical analysis. We performed chi-square tests for qualitative comparisons and Mann-Whitney U tests for quantitative comparisons. Values with $p < 0.05$ were considered significant.

RESULTS

The results for α-fodrin antibodies are summarized in Figure 1. Sera were considered positive if they had values ≥ 15 U/ml for both IgG and IgA ELISA. With these cutoff values, only 10 (6.3%) of the 160 healthy controls had a positive anti-α-fodrin test.

Twenty (65%) of the 31 SS patients with neurological manifestations had increased IgA and/or IgG anti-α-fodrin concentrations. This result was higher than for SSA/SSB, which was found in 15 (48%). Further, only 7 patients were positive for both SSA/SSB and α-fodrin. Consequently, if we considered the 2 tests (α-fodrin and/or SSA/SSB), 28 of the 31 (90.3%) patients had positive autoantibodies. The α-fodrin antibody level was not correlated with age, sex, duration of disease, or clinical symptoms (peripheral nervous system or CNS involvement). The results were not statistically different between SS patients with (64.5%) and those without (73.6%) neurological symptoms. However, in the latter subgroup, SSA/SSB antibodies were found in 91.1% of cases.

Antibodies to α-fodrin were found in 8 (13.3%) of the 60 MS patients, with no predominance in one of the 3 subgroups, and in 6 (15.8%) of the 38 patients with SLE.

DISCUSSION

Our study confirms that the α-fodrin antibody test is an interesting additional diagnostic tool for SS, and particularly for SS with neurological manifestations where anti SSA/SSB antibodies are less frequently found[3,4]. This test associated with SSA/SSB antibodies showed a sensitivity of 90.3%. However, other unpublished studies found a lower sensitivity of α-fodrin antibody detection with the same ELISA test. Further studies will be necessary to confirm these conflicting results. The variability of the results may

Table 1. Demographic data and percentage of patients with positive IgG or IgA.

	SS with Neurological Manifestations, n = 31	SS without Neurological Manifestations, n = 53	Multiple Sclerosis, n = 60	SLE, n = 38	Controls, n = 160
Mean age, yrs (range)	58.9 ± 6.5	53.5 ± 8	46.9 ± 8.5	48 ± 12	35 ± 9
Sex, F/M	19/12	53/0	34/26	38/0	80/80
IgA or IgG α-fodrin (%)	20/31 (64.5)	39/53 (73.6)	8/60 (13.3)	6/38 (15.8)	10/160 (6.3)

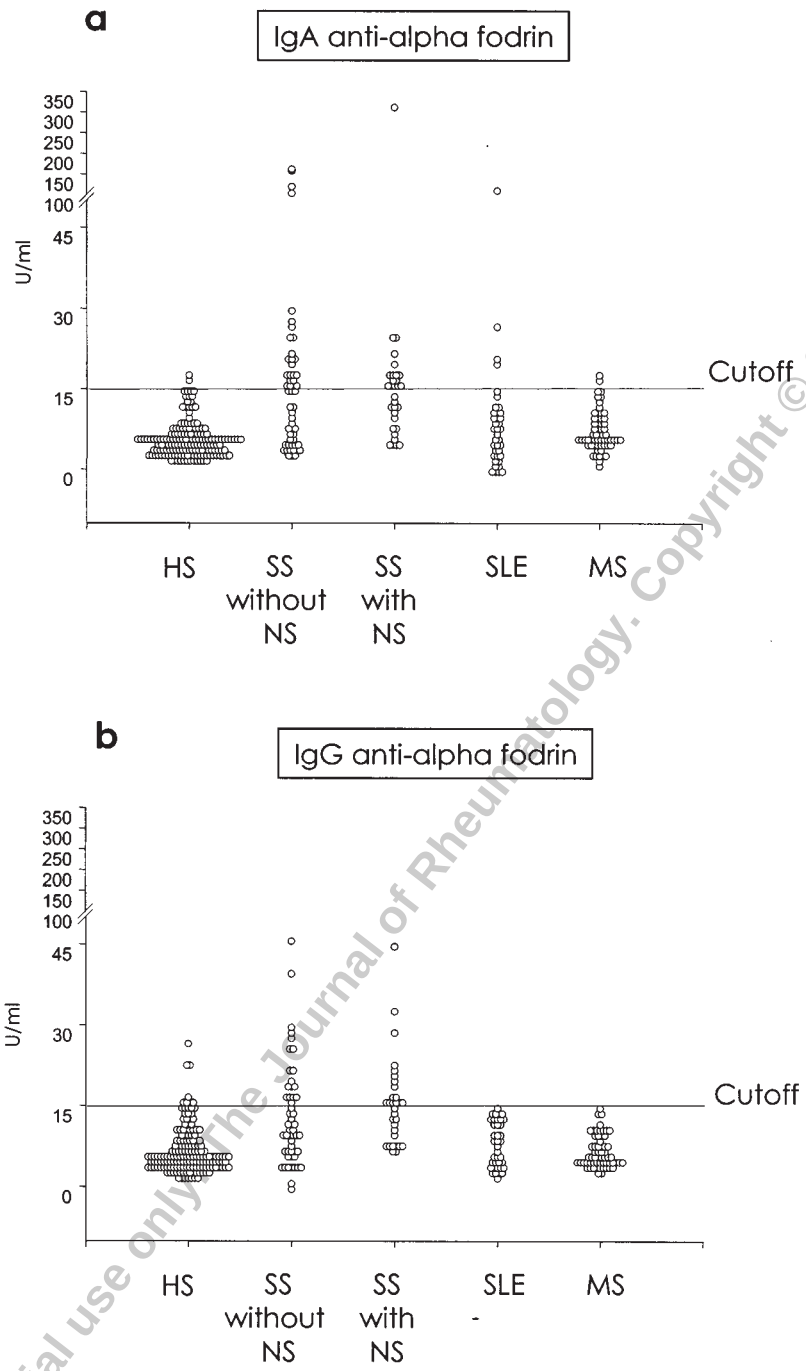

Figure 1. IgA (panel A) and IgG (B) anti-α-fodrin values of both groups of SS patients and controls. Sera were considered positive for values ≥ 15 UI/ml (cutoff value) for both IgG and IgA ELISA. Only 10 (6.3%) of 160 controls had a positive anti-α-fodrin test. α-fodrin antibodies were found in 13.3% (8/60) of MS patients and in 15.8% (6/38) of SLE patients. These antibodies were observed in SS patients with (64.5%) and without (73.6%) neurological symptoms. HS: healthy subjects; NS: neurological symptoms; SLE: systemic lupus erythematosus; SS: Sjögren's syndrome; MS: multiple sclerosis.

be due to selection criteria used for SS, or immunosuppressive drugs taken at the time of blood sampling.

SS can be very difficult to diagnose when the presenting symptoms are purely neurological and there can be a considerable delay[4]. There is a need for new diagnostic tests in order to increase the sensitivity of the detection of SS cases, especially those with neurological manifestations. The combination of the 2 tests used in our study gave very

good results in terms of sensitivity. In a previous study, we showed that the α-fodrin antibody test is specific when comparing patients with SS and healthy controls[7]. In this study we confirmed this result, as we found antibodies in only 6% of controls. This good specificity was also found when we compared SS patients with patients with other autoimmune diseases with neurological manifestations, such as MS and SLE, where we found α-fodrin antibodies in about 15% of cases.

In the ELISA used for detection of antibodies against α-fodrin, a recombinant protein produced in *E. coli* is coated to the plates. The protein core of the deglycosylated recombinant α-fodrin is identical to one described as the target structure for autoantibodies[5]. Due to the lack of glycosylation, the recombinant protein has a molecular weight of only 93 kDa compared to 120 kDa for the glycosylated apoptotic cleavage product of α-fodrin. Since the prevalence of antibodies against α-fodrin does not significantly differ in sera of Japanese patients with SS evaluated in immunoblot assays and our patients evaluated by ELISA, the glycosylation of α-fodrin does not appear to have a major influence on binding of the autoantibodies.

The main interest of α-fodrin is the lack of cross-reactivity with SSA/SSB antibodies, making the 2 tests complementary. α-fodrin is an abundant protein of the eukaryotic cell membrane skeleton, whereas SSA and SSB are nuclear proteins[8,9]. However, the specific role of these different proteins has yet to be determined. A previous study found that α-fodrin may be an organ-specific autoantigen in SS[5,7,10] but in this study we have shown that α-fodrin is also found in various forms of SS with neurological manifestations. It would be interesting to evaluate the *in situ* α-fodrin reactivity against both peripheral nerve and brain samples. However, α-fodrin and SSA/SSB are probably not the only target and may be an immunological marker of a more complex dysfunction.

It is not yet known whether α-fodrin can be considered as a marker of disease activity. It would be of interest to perform a sequential followup of the level of α-fodrin antibodies in order to evaluate whether it is correlated with the clinical course or therapeutic response.

Although the pathophysiological role of α-fodrin has yet to be determined, this new antigenic target appears to be implicated in SS, including those patients with neurological manifestations. Further studies are needed to determine whether this new antibody plays a specific role in SS and, if so, whether it is a marker of disease activity.

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