Usefulness of IgA Anti-α-fodrin Antibodies in Combination with Rheumatoid Factor and/or Antinuclear Antibodies as Substitute Immunological Criterion in Sjögren Syndrome with Negative Anti-SSA/SSB Antibodies

Gabriela Hernández-Molina, Carlos Nuñez-Alvarez, Carmen Avila-Casado, Luis Llorente, Carlos Hernández-Hernández, María Luisa Calderillo, Verónica Marroquín, Claudia Recillas-Gispert, Juanita Romero-Díaz, and Jorge Sánchez-Guerrero

ABSTRACT. ***Objective.*** We aimed to evaluate the usefulness of anti-α-fodrin antibodies (AFA) in combination with rheumatoid factor (RF) and/or antinuclear antibodies (ANA) as an alternative immunological criterion for Sjögren syndrome (SS) among patients with negative anti-Ro/La serology.

Methods. The study included 350 patients (100 with rheumatoid arthritis, systemic lupus erythematosus, and systemic sclerosis, and 50 with primary SS) randomly selected and assessed for SS. All patients were tested for ANA, RF, anti-SSA/SSB, and AFA antibodies. SS diagnosis was made on a clinical basis by 2 rheumatologists based on the 6-item screening questionnaire, Schirmer-I test, nonstimulated whole salivary flow rate, fluorescein staining test, autoantibodies, lip biopsy, and medical chart review. Non-SS was defined as lack of clinical diagnosis and not fulfilling the American-European Consensus Group classification criteria and the American College of Rheumatology (ACR) criteria. The ACR criteria were applied substituting the immunological criteria as follows: (1) RF plus ANA > 1:320, (2) RF plus AFA, (3) ANA > 1:320 plus AFA, (4) RF alone, and (5) 2 positive tests out of RF, ANA > 1:320, or AFA. We estimated the sensitivity, specificity, positive predictive value, negative predictive value, and likelihood ratio positivity with 95% CI for each criterion.

Results. There were 236 patients (67%) who tested negative for anti-SSA/SSB antibodies, of whom 65 (27.5%) were clinically diagnosed as SS, and 149 (63%) with non-SS. RF + AFA and ANA + AFA performed similarly to RF + ANA > 1:320. The model 2 out of 3 of RF, ANA, or AFA improved the sensitivity from 56.9% to 70.7%, although the specificity decreased.

Conclusion. The combination AFA + RF, AFA + ANA > 1:320, or at least 2 out of 3, performed well as a proxy immunological test for patients with SS and negative Ro/La serology. (J Rheumatol First Release August 1 2016; doi:10.3899/jrheum.151315)

Key Indexing Terms:
PRIMARY SJÖGREN SYNDROME ANTI-α-FODRIN ANTIBODIES

Sjögren syndrome (SS) is an autoimmune disease affecting mainly the exocrine glands, whose serologic hallmark is the presence of anti-Ro/SSA and/or anti-La/SSB antibodies. Indeed, these antibodies are independent items of both the American-European Consensus Group classification criteria (AECG)[1] and the American College of Rheumatology (ACR)

From the Department of Immunology and Rheumatology, the Dental Service, and the Ophthalmology Service, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, Mexico; Department of Pathology, University Health Network, Toronto General Hospital, and Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada.

Supported by a grant from the Mexican Consejo Nacional de Ciencia y Tecnología, grant SEP/CONACYT.

G. Hernández-Molina, MS, Department of Immunology and Rheumatology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán; C. Nuñez-Alvarez, PhD, Department of Immunology and Rheumatology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán; C. Avila-Casado, PhD, Department of Pathology, University Health Network, Toronto General Hospital, University of Toronto; L. Llorente, MD, Department of Immunology and Rheumatology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán; C. Hernández-Hernández, MD, Dental Service, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán; M.L. Calderillo, MD, Dental Service, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán; V. Marroquín, MD, Dental Service, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán; C. Recillas-Gispert, MD, Ophthalmology Service, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán; J. Romero-Díaz, MS, Department of Immunology and Rheumatology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán; J. Sánchez-Guerrero, MS, Mount Sinai Hospital and University Health Network, University of Toronto.

Address correspondence to J. Sánchez-Guerrero, Mount Sinai Hospital and University Health Network, University of Toronto, 60 Murray St., 2-005, Toronto, Ontario M5T 3L9, Canada. E-mail: JSanchez-Guerrero@mtsinai.on.ca

Accepted for publication June 20, 2016.

SS classification criteria[2], and their detection strongly supports the diagnosis. Depending on the method applied for their identification, anti-Ro/SSA and anti-La/SSB antibodies are detected in about 50% to 70% of patients with SS[3].

In the ACR criteria, the association of antinuclear antibodies (ANA) ≥ 1:320 and rheumatoid factor (RF) was considered equivalent to anti-SSA/-SSB positivity, as a way to identify patients who have negative anti-SSA and/or anti-SSB serology[2]. Nevertheless, this criterion did not confirm its clinical benefit because the majority of patients with SS who display high titers of ANA and RF also had anti-SSA/-SSB antibodies[2].

One subject of research in SS has been the presence of autoantibodies against α-fodrin (AFA), an intracellular cytoskeletal protein binding the actin filament in secretory cells and associated with membrane ion channels and pumps in several epithelial cells. There have been conflicting results regarding AFA prevalence, diagnostic accuracy, and clinical meaning[4-10,11-15].

In SS, the prevalence of the AFA-IgA isotype ranges between 32.5% and 88%, and for the AFA-IgG isotype between 21.3% and 95%[14]. Hu, *et al*, in a metaanalysis that included 23 studies, reported a pooled sensitivity (SN) of 39.3% and specificity (SP) of 83% for these antibodies in the diagnosis of SS. Both subtypes IgG and IgA had SP above 80%[15].

Thus the overall utility of AFA antibodies has been arguable and possibly does not add much to the diagnosis of SS. We hypothesized that AFA are useful as a substitute immunological criterion for patients with SS and negative anti-SSA/SSB antibodies.

We undertook the present study to determine whether AFA in combination with RF and/or ANA could be used as a substitute immunological criterion in this subset of patients with SS.

MATERIALS AND METHODS

We conducted a posthoc analysis within a population of patients with rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), systemic sclerosis (SSc), and primary SS (pSS) that had a standardized assessment for SS for research purposes[16].

Patients. The study was conducted in a tertiary-care center where the rheumatology clinic provides regular care to 5942 patients, of whom 4813 (81%) have systemic autoimmune diseases. One hundred out of 2527 patients with RA, 100 of the 1860 with SLE, and 100 of the 136 with SSc according to the classification criteria, and 50 out of 81 patients with a clinical diagnosis of pSS were selected from our patient registry using random numbers and assessed for SS using a structured approach[16].

Patients were excluded if they had taken any medication that would reduce salivary flow (e.g., antihistamines, sedatives, β-blockers, diuretics) within 48 h before the study, had a history of hepatitis C or human immunodeficiency virus infection, sarcoidosis, IgG4-related disease, lymphoma, graft versus host disease, or history of neck/head radiotherapy.

The study was approved by the Institutional Biomedical Research Board of the Instituto Nacional de Ciencias Médicas y Nutrición SZ (Reference 1423), and all patients gave signed informed consent to participate according to the Declaration of Helsinki.

Assessment of SS. Participating patients had a standardized evaluation designed in 3 phases: screening, confirmatory, and lip biopsy. In addition, we reviewed the medical charts including the clinical notes of rheumatology, ophthalmology, and dental clinics.

Participants were asked to refrain from eating, drinking, smoking, chewing, or oral hygiene procedures for at least 1 h prior to the evaluation, and were seen during the morning in a closed room with no air conditioning or heating.

Screening phase. All patients had a face-to-face interview with a single rheumatologist using a standardized form that included questions about demographic data and use of medications. In addition, a validated 6-item screening questionnaire for oral and ocular sicca symptoms was applied, and the Schirmer-I and the wafer test were carried out[17,18].

The Schirmer-I test was done using 2 standardized sterile tear measurement strips (Tear Flo; Rose Stone Enterprises), and the wafer test was done as described[17,18].

A blood sample was drawn for autoantibody testing, including anti-Ro/La (IgG isotype) determined by ELISA (Orgentec Diagnostika), RF (IgG isotype), and ANA. RF was measured by commercially available ELISA method (Orgentec Diagnostika) and ANA-IgG by indirect immunofluorescence using HEp-2 cells (INOVA Diagnostics) in an ASP 1200 (HTZ Ltd.). The ANA titration was obtained with the software AutoCyte Image Titer.

Patients with an affirmative response to at least 1 of the screening questionnaires, Schirmer-I test ≤ 5 mm in 5 min, or wafer test > 4 min were considered to have a positive screening.

Confirmatory phase. Patients with positive screening underwent this phase, consisting of fluorescein staining test and nonstimulated whole salivary flow rate (NSWSF). The fluorescein staining test was performed by 2 ophthalmologists blinded to the patients' diagnoses. The test was considered positive with a score ≥ 4 according to the van Bijsterveld scale in at least 1 eye[1]. For purposes of the current analysis, the ACR criteria ocular staining score ≥ 3 was substituted by van Bijsterveld score ≥ 3[19]. NSWSF collection was performed as reported[20]. Saliva was collected during 5 min and volume expressed as ml/5 min.

Lip biopsy. Lip biopsy was proposed for all patients who had > 2 of the following results: at least 1 affirmative answer to the oral component of the screening questionnaire, wafer test > 4 min, presence of keratitis by fluorescein staining test, NSWSF < 1.5 ml/5 min, and positive anti-Ro/La antibodies.

An expert pathologist evaluated all biopsies while blinded to clinical data. Light microscopy examination was carried out on H&E staining at 4× magnification using a scale grid. Histological evaluation focused on the presence of lymphoid infiltrate. Focal lymphocytic sialadenitis was diagnosed based on a focal score of 1 or more lymphocytic foci (> 50 lymphocytes per 4 mm^2)[1].

Diagnosis of SS. Diagnosis of SS was made on a clinical basis by 2 rheumatologists. For this purpose, each of them independently evaluated every patient, considering the results of the 6-item screening questionnaire, history of parotid enlargement, Schirmer-I test, wafer test, NSWSF rate, fluorescein staining test, autoantibodies, lip biopsy, and medical chart review. Each patient was diagnosed as SS or non-SS. We considered a diagnosis of SS when both rheumatologists agreed independently on whether the patient fulfilled the AECG or ACR classification criteria.

Non-SS diagnosis was defined as a lack of clinical diagnosis and not fulfilling AECG and/or ACR criteria.

The agreement from the independent evaluation by the 2 rheumatologists was 79.1%. Patients in whom discrepancy existed underwent further review and discussion between both rheumatologists, and a final diagnosis was reached.

Determination of AFA. We tested AFA (IgA and IgG isotypes) by quantitative commercially available ELISA method (Orgentec Diagnostika) according to the manufacturer's instructions. Briefly, highly purified antigen was coated to microplate. The serum sample was diluted 1:100. All wash steps

were done with TBS-Tween. Results were expressed as U/ml derived off a standard curve (0–100). We used kits with the same lot number for testing the sera of all patients and controls. Cutoff points for AFA were considered positive according to reference values of the immunology laboratory of our institution. These correspond to values above the 95th percentile of 79 normal controls (IgA 5.4 U/ml and IgG 5.9 U/ml). All ELISA were processed in a DSX System (DYNEX Technologies). The intraassay and interassay coefficients of variation of the method were IgG 2.1%, IgA 5.9%, and IgG 6.0%, IgA 4.0%, respectively.

Statistical analysis. The target population was patients testing negative for anti-SSA and/or anti-SSB antibodies. Using the clinical diagnosis of SS as gold standard, we applied the ACR SS criteria to each study participant, substituting the immunological criteria as follows: (1) RF plus ANA > 1:320, (2) RF plus AFA, (3) ANA > 1:320 plus AFA, (4) RF alone, and (5) 2 positive tests out of RF, ANA > 1:320, or AFA. We estimated the SN, SP, positive predictive value, negative predictive value, and positive likelihood ratio with 95% CI, for each set of criteria.

Categorical variables were compared by chi-square or Fisher's exact test when appropriate; continuous variables were compared using Student t test or Mann-Whitney U test. Two-tailed $p < 0.05$ was considered significant. All analyses were performed using SPSS for Windows 20.0 (IMB SPSS Inc.).

RESULTS

From the original 350 patients, 236 (67%) tested negative for both anti-Ro/SSA and anti-La/SSB antibodies (Figure 1). These seronegative patients were the target of the present study. Mean age of patients was 48.4 ± 14.9 years, and 222 (94%) were women.

Of the 236 patients, 192 had a positive screening and all of them were subjected to the confirmatory phase. We identified 150 patients with a positive confirmatory phase to whom a lip biopsy was proposed; 65 patients had a lip biopsy, 69 declined it, 14 were under anticoagulation, and 2 patients had severe thrombocytopenia (Figure 1).

Diagnosis of SS. Sixty-five patients were diagnosed as SS and 149 as non-SS; the remaining 22 patients were not clinically diagnosed as SS but fulfilled 1 set of SS criteria (AECG n = 11, ACR n = 11, both = none), so they were excluded from the analysis.

The clinical and serological features among patients with and without SS are shown in Table 1. Patients with SS more often had sicca symptoms, and had positive oral and ocular tests; however, the prevalence of RF, ANA > 1:320, and anti-α-fodrin IgA or IgG isotypes did not differ.

In an SN analysis, comparing patients with clinical diagnosis of SS and fulfilling either AECG and/or ACR (n = 45) versus the 149 patients without SS, the SS group had more frequently RF (51.1% vs 32.9%, p = 0.02), RF plus AFA-IgA isotype (46.7% vs 23.5%, p = 0.003), and the combination 2 of 3 of RF, ANA > 1:320, or AFA-IgA isotype (26.7 vs 10.1%, p = 0.01).

Based on these results, we decided to analyze the data using AFA-IgA isotype only.

Performance of ACR criteria with substitute immunological criterion. The performance of ACR criteria using diverse substitute immunological criteria is shown in Table 2. The combination RF + AFA-IgA and ANA > 1:320 + AFA-IgA performed similarly to RF + ANA > 1:320 as a substitute immunological criterion. Although RF + ANA > 1:320 performed well, incorporating AFA-IgA improved the number of patients with SS classified by the ACR criteria. Particularly, the model 2 out of 3 (RF, ANA, or AFA-IgA) improved the SN of ACR criteria to identify patients with SS who had negative anti-SSA/SSB serology from 37 to 46 out of 65 patients, although the SP decreased.

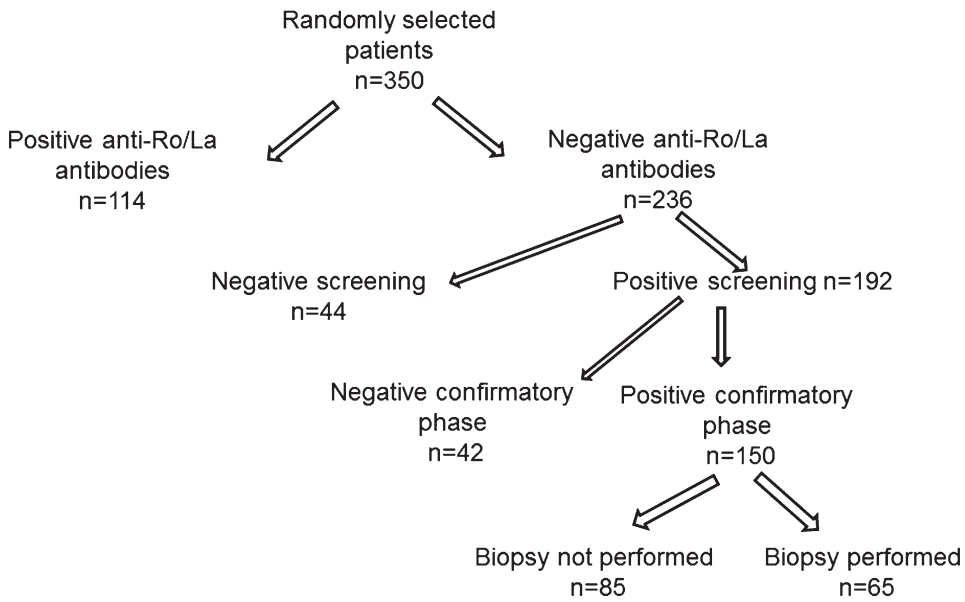

Figure 1. Flow of patients through study phases.

Table 1. Clinical and immunological features among patients with and without Sjögren syndrome (SS). Data are n (%) unless otherwise specified.

Variables	SS, n = 65	No SS, n = 149	p
Age, yrs, mean ± SD	51.8 ± 13.8	46.8 ± 15.1	0.02
Women	65 (100)	135 (90.6)	0.01
Oral symptoms	37 (56.9)	26 (17.4)	< 0.0001
Ocular symptoms	44 (67.7)	35 (23.5)	< 0.0001
Parotid enlargement	8 (12.3)	2 (1.3)	0.001
Schirmer-I test	43 (66.2)	49 (32.9)	< 0.0001
Wafer test	60 (92.3)	79 (53)	< 0.0001
Keratoconjuctivitis sicca	51/64 (79.6)	43/94 (45.7)	< 0.0001
NSWSF < 0.1 ml/min	61 (93.8)	57/122 (46.7)	< 0.0001
Focal sialadenitis	31/42 (73.8)	0/17 (0)	< 0.0001
RF	28 (43.1)	49 (32.9)	0.15
ANA ≥ 1:320	32 (49.2)	85 (57)	0.29
ANA ≥ 320 and RF	13 (20.3)	21 (11.1)	0.30
AFA-IgA	44 (67.7)	94 (63.1)	0.51
AFA-IgG	20 (30.8)	48 (32.2)	0.83
Median AFA-IgA, U/ml (range)	5.8 (4.3–48.5)	5.7 (4–48)	0.50
Median AFA-IgG, U/ml (range)	5.4 (4.3–10.9)	5.5 (4.3–17.9)	0.25
RF and AFA-IgA	22 (33.8)	35 (23.5)	0.11
ANA ≥ 1:320 plus AFA-IgA	25 (38.5)	54 (36.2)	0.75

NWSFS: nonstimulated whole salivary flow rate; RF: rheumatoid factor; ANA: antinuclear antibodies; AFA: anti-α-fodrin antibodies.

DISCUSSION

The presence of AFA has been described in murine SS models (NFS/sld and NOD)[21,22] and in patients with SS[4,11,23,24,25,26]. In childhood pSS, the presence of these antibodies conferred high SP and SN[5,27,28]. AFA have been suggested as markers for early diagnosis[5,10]. Conversely, other authors do not agree that they provide additional value for the SS diagnosis[7,9,13]. Discrepancies in the prevalence and diagnostic performance in former studies may be explained by the use of different methodology (ELISA, Western blotting, immunoprecipitation), isotype evaluated, and different study population[23]. More recently, a meta-analysis concluded that AFA had moderate accuracy for the diagnosis of SS (low SN and high SP) and that to decrease the misdiagnosis, a combination of the anti-SSA/SSB antibody and AFA may be necessary[15].

The presence of anti-Ro/SSA and anti-La/SSB as well as focal sialadenitis are the main features for the diagnosis of SS; however, lip biopsy is not always available and a third of the patients are seronegative for anti-Ro/SSA or anti-La/SSB antibodies, hampering the establishment of the diagnosis. Also, it has been reported that these patients have a lower prevalence of lymphoma and clinical manifestations[29].

According to Sordet, *et al*, the diagnostic value of AFA in seronegative pSS is limited, given the overlap of both antibodies. However, in their study only 4 of 107 patients were positive for IgA or AFA-IgG and negative for anti-Ro/La[7].

Our study included a large population with negative anti-Ro/SSA and anti-La/SSB serology and explored diverse combinations of immunological criteria, substituting the original serological item of the ACR classification criteria, including AFA, ANA > 1:320, and RF.

We found that among patients with negative anti-Ro/La serology, RF plus AFA-IgA, ANA ≥ 1:320 plus AFA-IgA, or 2 out of these 3 elements were useful markers of SS, and performed similarly to the proposed RF plus ANA > 1:320 criterion[2]. We focused on the ACR criteria because it is the only set that includes a validated serological equivalent of anti-Ro/SSA and anti-La/SSB positivity; however, we do not aim to propose our results as part of new classification criteria for negative anti-Ro/SSA and anti-La/SSB patients.

We could not reproduce our results when we measured the IgG isotype. Witte and co-workers also showed that IgA antibodies against AFA provided a higher SN than the IgG isotype[24]. The reason for this is not clear; it cannot be explained by a general increase in serum IgA levels, because IgA antibodies are produced in the salivary glands of patients with SS. Therefore, production of AFA-IgA antibodies may indicate a specific inflammation at the site of tissue injury.

On the other hand, the clinical and prognostic implications of AFA are uncertain. A positive correlation with the degree of lymphocytic infiltration in salivary glands has been reported[25], as well as an association with neurologic manifestations[30], recurrent parotid swelling[10], and Hashimoto thyroiditis[31].

We acknowledge some limitations of our study. First,

Table 2. American College of Rheumatology (ACR) and modified immunological criterion ACR performance. Data in parentheses are 95% CI.

Immunological Criterion	SN	SP	PPV	NPV	LR+	LR–
RF + ANA ≥ 1:320	56.9 (44–69.1)	93.9 (88.8–97.2)	80.4 (66–90.6)	83.3 (76.8–88.6)	9.4 (4.8–18.3)	0.46 (0.35–0.61)
RF + AFA-IgA	61.5 (48.6–73.3)	88.5 (82.3–93.2)	70.1 (56.6–81.5)	84.0 (77.4–89.4)	5.3 (3.3–8.7)	0.43 (0.32–0.59)
AFA-IgA + ANA ≥ 1:320	64.6 (51.7–76.8)	85.9 (79.2–91.0)	66.6 (53.6–78.0)	84.7 (78.0–90.0)	4.5 (2.9–7.0)	0.41 (0.29–0.56)
RF	66.15 (53.3–77.4)	87.2 (80.8–92.1)	69.2 (56.3–80.4)	85.53 (78.9–80.4)	5.19 (3.29–8.17)	0.39 (0.27–0.55)
Two out of three of RF, ANA ≥ 1:320, or AFA-IgA	70.7 (58.1–81.4)	78.5 (71.0–84.8)	58.9 (47.2–69.9)	86.0 (78.0–91.3)	3.3 (2.3–4.6)	0.37 (0.25–0.55)

SN: sensitivity; SP: specificity; PPV: positive predictive value; NPV: negative PV; LR: likelihood ratio; RF: rheumatoid factor; ANA: antinuclear antibodies; AFA: anti-α-fodrin antibodies.

immunosuppressive therapy can downregulate the production of AFA antibodies[32]; we evaluated patients with diverse connective tissue diseases (CTD) and many of them were receiving these treatments. Second, because of the transversal design, we were not able to evaluate temporality of the antibody presentation. Third, there is some degree of ascertainment bias because not all the patients had lip biopsy; nevertheless, we were able to determine SS status in most of them. A strength of our study was the inclusion of a large number of seronegative anti-Ro/La patients, randomly selected from a large registry of patients with CTD, who encompassed both primary and secondary SS varieties.

RF plus AFA-IgA, and ANA > 1:320 plus AFA-IgA, performed as well as RF plus ANA > 1:320 as a proxy immunological test for patients with SS and negative Ro/La serology, an important yet forgotten subgroup of patients in clinical studies of SS.

Since the submission of this manuscript for publication, a new set of SS criteria were presented at the 2015 annual meeting of the ACR. These criteria have not been published yet and are still under review by the ACR and the European League Against Rheumatism. They focus on pSS and have not been validated in patients with other autoimmune diseases (secondary SS). Also, they are not different in SN and SP from the ACR and AECG criteria. Therefore they should not invalidate our results and conclusions.

REFERENCES

1. Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, et al. Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Ann Rheum Dis 2002;61:554-8.
2. Shiboski SC, Shiboski CH, Criswell L, Baer A, Challacombe S, Lanfranchi H, et al. American College of Rheumatology Classification Criteria for Sjögren's syndrome: a data-driven, expert consensus approach in the Sjögren's International Collaborative Clinical Alliance Cohort. Arthritis Care Res 2012;64:475-87.
3. Tzioufas AG, Tatouli IP, Moutsopoulos HM. Autoantibodies in Sjögren's syndrome: clinical presentation and regulatory mechanisms. Presse Med 2012;41:e451-60.
4. Hanejil N, Nakamura T, Takio K, Yanagi K, Higashiyama H, Saito I, et al. Identification of alpha-fodrin as a candidate autoantigen in primary Sjögren's syndrome. Science 1997;276:604-7.
5. Kobayashi I, Kawamura N, Okano M, Shikano T, Mizumoto M, Hayashi Y, et al. Anti-alpha-fodrin autoantibody is an early diagnostic marker for childhood primary Sjögren's syndrome. J Rheumatol 2001;28:363-5.
6. Ruffatti A, Ostuni P, Grypiotis P, Botsios C, Tonello M, Grava C, et al. Sensitivity and specificity for primary Sjögren's syndrome of IgA and IgG anti-alpha-fodrin antibodies detected by ELISA. J Rheumatol 2004;31:504-7.
7. Sordet C, Gottenberg J, Goetz J, Bengoufa D, Humbel RL, Mariette X, et al. Anti-α-fodrin autoantibodies are not useful diagnostic markers of primary Sjögren's syndrome. Ann Rheum Dis 2005;64:1244-5.
8. Goëb V, Salle V, Duhaut P, Jouen F, Smail A, Ducroix JP, et al. Clinical significance of autoantibodies recognizing Sjögren's syndrome A (SSA), SSB, calpastatin and alpha-fodrin in primary Sjögren's syndrome. Clin Exp Immunol 2007;148:281-7.
9. Ruiz T, Lopez-Longo F, Santamaría B, Urrea R, Carreño L, Estecha A, et al. Prevalence of IgG anti- α-fodrin antibodies in Sjögren's syndrome. Ann N Y Acad Sci 2005;1050:210-6.
10. Willeke P, Gautbitz M, Schotte H, Becker H, Mickholz E, Domschke W, et al. Clinical and immunological characteristics of patients with Sjögren's syndrome in relation to α-fodrin antibodies. Rheumatology 2007;46:479-83.
11. 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.
12. Zanbelt M, Vogelzangs J, van de Puttee L, Van Venrooij WJ, Van Den Hoogen FH. Anti-α-fodrin antibodies do not add much to the diagnosis of Sjögren's syndrome. Arthritis Res Ther 2004;6:R33-8.
13. Tuckcapar N, Olmez U, Turkak H, Duman M. The importance of alpha-fodrin antibodies in the diagnosis of Sjögren's syndrome. Rheumatol Int 2006;26:354-9.
14. Witte T. Antifodrin antibodies in Sjögren's syndrome. Ann N Y Acad Sci 2005;1051:235-9.
15. Hu Q, Wang D, Chen W. The accuracy of the anti-alpha-fodrin antibody test for diagnosis of Sjögren's syndrome: a meta-analysis. Clin Biochemistry 2013;46:1272-6.
16. Hernández-Molina G, Avila-Casado C, Nuñez-Alvarez C, Cárdenas-Velázquez F, Hernández-Hernández C, Luisa Calderillo M, et al. Utility of the American-European Consensus Group and American College of Rheumatology classification criteria for Sjögren's syndrome in patients with systemic autoimmune diseases in the clinical setting. Rheumatology 2015;54:441-8.
17. Vitali C, Bombardieri S, Moutsopoulos HM, Balestrieri G, Bencivelli W, Bernstein RM, 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.
18. Sánchez-Guerrero J, Aguirre E, Pérez M, Kraus A, Cardiel MH, Soto-Rojas AE. The wafer test: a semi-quantitative test to screen for xerostomia. Rheumatology 2002;41:381-9.
19. Whitcher JP, Shiboski CH, Shiboski SC, Heidenreich AM, Kitagawa K, Zhang S, et al. A simplified quantitative method for assessing keratoconjunctivitis sicca from the Sjögren's Syndrome International Registry. Am J Ophthalmol 2009;149:405-15.
20. Navazesh M. Methods of collecting saliva. Ann N Y Acad Sci 1993;694:72-7.
21. Saegusa K, Ishimaru N, Yanagi K, Mishima K, Arakaki R, Suda T, et al. Prevention and induction of autoimmune exocrinopathy is dependent on pathogenic autoantigen cleavage in murine Sjögren's syndrome. J Immunol 2002;169:1050-7.
22. Yanagi K, Ishimaru H, Haneji N, Saegusa K, Saito I, Hayashi Y. Anti-120-kDa alpha-fodrin immune response with Th1 cytokine profile in the NOD mouse model of Sjögren's syndrome. Eur J Immunol 1998;28:336-45.
23. Maruyama T, Saito I, Hayashi Y, Kompfner E, Fox RI, Burton DR, et al. Molecular analysis of the human autoantibody response to α-fodrin in Sjögren's syndrome reveals novel apoptosis-induced specificity. Am J Pathol 2004;165:53-61.
24. Witte T, Matthias T, Arnett F, Peter HH, Hartung K, Sachse C, et al. IgA and IgG autoantibodies against alpha-fodrin as markers for Sjögren's syndrome. Systemic lupus erythematosus. J Rheumatol 2000;27:2617-20.
25. Witte T, Matthias T, Oppermann M, Helmke K, Peter HH, Schmidt RE, et al. Prevalence of antibodies against alpha-fodrin in Sjögren's syndrome: comparison of 2 sets of classification criteria. J Rheumatol 2003;30:2157-9.
26. Qin Q, Wang H, Wang AZ, Huang YL, Li H, Zhang WW, et al. Diagnostic accuracy of anti-alpha-fodrin antibodies for primary Sjögren's syndrome. Mod Rheumatol 2014;24:793-7.
27. Takahashi K, Tatsuzawa O, Yanagi K, Hayashi Y, Takahashi H.

Alpha-fodrin autoantibody in Sjögren's syndrome and other autoimmune diseases in childhood. Eur J Pediatr 2001;160:520-1.
28. Maeno N, Takei S, Imanaka H, Oda H, Yanagi K, Hayashi Y, et al. Anti-α-fodrin in Sjögren's syndrome in children. J Rheumatol 2001;28:860-4.
29. Quartuccio L, Baldini C, Bartolini E, Priori R, Carubbi F, Corazza L, et al. Anti-SSA/SSB negative Sjögren's syndrome shows a lower prevalence of lymphoproliferative manifestations, and lower risk of lymphoma evolution. Autoimmun Rev 2015;14:1019-22.
30. de Seze J, Dubucquoi S, Fauchais A, Matthias T, Devos D, Castelnovo G, et al. Alpha-fodrin autoantibodies in the differential diagnosis of MS and Sjögren syndrome. Neurology 2003;61:268-9.
31. Szanto A, Csipo I, Horvath I, Biro E, Szodoray P, Zeher M. Autoantibodies to alfa-fodrin in patients with Hashimoto thyroiditis and Sjögren's syndrome: possible markers for a common secretory disorder. Rheumatol Int 2008;11:1169-72.
32. Ulbricht KU, Schmidt RE, Witte T. Antibodies against alpha-fodrin in Sjögren's syndrome. Autoimmun Rev 2003;2:109-13.