Evolution of clinical, histological and serological features in a Primary Sjögren's Syndrome cohort and the limitations of the current classification criteria

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ABSTRACT

Objective. The classification and/or diagnosis of Primary Sjögren's Syndrome (PSS) requires a multidimensional approach. Although age and the duration of sicca symptoms can affect the clinical, serological and histological features found at initial evaluation, these are not considered when using classification criteria as a guide for PSS diagnosis. Our study aimed to explore if there is any relationship between the duration of symptoms and clinical, histopathological and serological findings.

Methods. An observational, retrospective study was performed. All the evaluated subjects were part of the "sicca cohort". Patients' clinical, serological and histological characteristics were assessed according to the duration of symptoms.

A Receiving Operator Characteristic (ROC) curve was performed to establish the duration of symptoms (months) that predicted a PSS diagnosis. Binary regression models and odds ratios were used to evaluate the association between the duration of symptoms and the clinical, serological, and histopathological profiles.

Results. One hundred and sixteen patients were included; 97(83.62%) fulfilled PSS criteria. Of the 116 patients, thirty-six (31.03%) had < 15 months presenting with sicca symptoms when receiving a diagnostic approach.

A duration of symptoms >15 months was associated with an altered Schirmer test (OR 2.76; 95% CI 1.15-6.61, *P*=0.02), low salivary flow rate (OR 3.5; 95% CI 1.34-9.13, *P*=0.01), ≥1 foci score (OR 1.21; 95% CI 1-1.45, *P*=0.04), ocular (OR 7.8; 95% CI 1.49-40.81, *P*=0.02) and severe oral symptoms (OR 2.61; 95% CI 1.16-5.87, *P*=0.02).

Conclusion. The time of evolution of symptoms plays a fundamental role in the clinical, histological and serological profiles in PSS.

Keywords: Primary Sjögren's Syndrome criteria; Sicca; Diagnosis; Duration of symptoms.

INTRODUCTION

Primary Sjögren's Syndrome (PSS) is a chronic systemic autoimmune disease affecting primarily exocrine glands which lead to dryness of the main mucosal surfaces, but may also be associated to other organ-specific manifestations[1,2]. The classification and/or diagnosis of PSS requires a multidimensional evaluation, considering clinical, histopathological, and serological features[3]. In recent years, several classification criteria have been proposed[4–6]. In 2002, the American-European Consensus Group (AECG) developed a set of subjective and objective criteria[4]; in 2012, the American College of Rheumatology (ACR) criteria focused on objective parameters; finally in 2016, according to the ACR and the European League Against Rheumatism (EULAR) criteria, evidence of a positive minor salivary gland biopsy (MSGB) and/or positive serology for anti-SSA/Ro antibody was required to classify a patient with PSS[5,6].

Duration of at least three months with symptomatology has been an arbitrary time range used in the classification of different chronic pathologies, such as PSS[7]. In the derivation cohorts used to develop PSS classification criteria, the sicca population had a duration of symptoms that ranged from 3 months to up to 8 years since clinical onset[8]. Although the duration of symptoms and age can affect the clinical, serological and

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histopathological findings during the initial diagnostic evaluation, these are not considered by the clinicians who use the classification criteria as a guide for PSS diagnosis[9–11]. This is the reason why the duration of sicca symptoms (therefore, the time of evolution) could have an impact in the utility, implementation and outcomes during the application of these criteria. Our study aimed to explore if there is any relationship between the duration of sicca symptoms and clinical, histological, and serological findings.

MATERIALS AND METHODS

DESIGN

An observational, retrospective, cross-sectional study was performed. All the subjects evaluated were part of the "sicca patient cohort" from the University Hospital "Dr. José Eleuterio González" in Monterrey, Mexico, during the period of September 2015 to September 2019. The local ethics and research committee approved the research protocol (RE20-00003), which was performed according to the Declaration of Helsinki.

PATIENTS

Patients who were part of the cohort could be enrolled in three different ways: 1) Patients referred by their ophthalmologist due to ocular dryness symptoms; 2) patients referred from the Dentistry department, secondary to oral dryness symptoms; and 3) patients initially evaluated in the Rheumatology clinic with suspicion of PSS. All the patients enrolled had received (if any) only symptomatic treatment before their inclusion in the cohort.

For their inclusion, patients should be ≥ 18 years-old, have sicca symptoms (dry eye/mouth) for more than 3 months and should be classified as PSS according to the 2002 AECG criteria, with a complete evaluation: clinical (medical record, Schirmer test, salivary flow-rate test), serological (antinuclear antibodies [ANA], rheumatoid factor [RF], anti-SSA/Ro and anti-SSB/La antibodies), and histopathological (MSGB) evaluation[12]. Those with any missing data were excluded.

ASSESSMENTS

For the entire sicca cohort, we recorded gender, age, sicca symptoms (with subjective and objective measures), and extra-glandular manifestations[13]. A clinical questionnaire was used to evaluate the time of onset and the duration of sicca symptoms and extra-glandular manifestations. Sixteen manifestations (glandular and extra-glandular) were evaluated (as presence or absence), and a score that ranged from 0-16 was created (the clinical manifestations score).

ClinESSDAI (Clinical European League against Rheumatism Sj gren's Syndrome Disease Activity Index) score was also documented[14].

OBJECTIVE MEASURES

The Schirmer test was performed as established in the literature. The use of artificial tears was avoided at least 1 hour before the test. Patients dried the eyes with a tissue and afterward the tear strips were placed in the middle and lateral areas of the lower eyelid for 5 minutes.

A positive result was considered when the tear strip wetted 5 mm or less in at least one eye during the 5 minutes.

An unstimulated whole saliva flow rate test was performed, patients were not allowed to eat or drink liquids or solids at least one hour before the test. After swallowing saliva, produced saliva was collected for 15 minutes by passive spitting in measured containers. The flow rate was expressed in mL/min. The test was classified as positive when there was a salivary flow rate of <1.5 mL/15 minutes.

Serology for ANA, RF, anti-SSA/Ro and anti-SSB/La antibodies were performed. The ANA titer was determined by the immunofluorescence staining method with HEp2 cells. The RF (IgA, IgG, and IgM) levels were determined by ELISA (EUROIMMUN). The anti-SSA/Ro and anti-SSB/La antibodies were determined using a fully automated Luminex based pre-coated multi-bead assay.

All the patients had a MSGB performed by a trained and certified physician (BRVF). The MSGB process and interpretation were performed according to recently published recommendations[15] by certified pathologists (LACF, GLL). The description of MSGB included reporting the number of foci scores (50 cells [mononuclear and lymphocytic aggregates, plasmatic cells, and histiocytes] in a 4 mm^2 field), lobules, and the presence of germinal center- like structures (GCS), atrophy, adipose tissue infiltration, and ductal dilatation[16].

MSGB PROCESS

Biopsy specimens were placed in formalin and sectioned through the mid-belly of the sphere in sections 4 microns thick and placed on glass slides to stain with

hematoxylin and eosin (H&E); afterwards, they were evaluated microscopically graduating qualitatively the presence or absence of the following findings, taking as a comparison the histology of a normal minor salivary gland biopsy. 1) Foci score: 50 cells (mononuclear and lymphocytic aggregates, plasmatic cells, and histiocytes) in a 4 mm^2 field. 2) Fibrosis: collagenous fibrosis lining the ducts or forming pathways that dissociate the lobes and cover the acini. 3) Ductal dilation: enlargement of the duct lumen with ablation of the epithelium. 4) Acinar depletion: dedifferentiation of the acinar epithelium and/or loss of the glandular parenchyma with replacement by fibrocollagenized connective tissue and in later phases with adipocyte infiltration. 5) Adipose infiltration: replacement of normal parenchyma by adipocytes.6) GCS: lighter areas within the lymphoid infiltrate composed of lymphoid and non-lymphoid cells.

Sialadenitis was defined as an inflammatory lymphocytic infiltrate with a peri-acinar and periductal distribution without forming a focus score. Sialadenitis was graded in percentages according to a microscopic evaluation: <30% was considered low-degree, 30-60% medium, and >60% high-degree sialadenitis.

DURATION OF SYMPTOMS

Patients were separated according to the duration of sicca symptoms in less than 1 year, less than 2 years, less than 3 years, and more than 3 years. Clinical, serological, and histopathological features were assessed at each established time.

STATISTICAL ANALYSIS

Descriptive statistics were used to summarize demographic, clinical, serological, and histopathological characteristics. Normality tests were performed using the Kolmogorov-Smirnov test. Normally distributed variables were compared using Student's *t*-test for continuous variables and the Chi-square test for categorical variables. The non-parametric variables were compared using the Mann-Whitney U test. A Receiving Operator Characteristic (ROC) curve was performed to establish the duration of symptoms (months) that predicted a PSS diagnosis. Positive and negative predictive values were calculated. Binary regression models and odds ratios were used to evaluate the association between the duration of symptoms (median) and the clinical, serological, and histopathology profiles. A *P*-value <0.05 was considered statistically significant. Data were analyzed using SPSS version 20.

RESULTS

A total of 174 patients were enrolled in the sicca cohort from September 2015 to September 2019. One hundred and sixteen sicca patients (67%) had a complete evaluation (see Table I) and 97 (84%) fulfilled 2002 AECG PSS criteria. Eighty-two (71%) patients with an ocular staining score were able to classify as PSS using the 2016 ACR-EULAR criteria.

Of the entire cohort, 26 (22%) patients fulfilled the 2002 AECG PSS criteria with a duration of sicca symptoms ≤12 months, 39 (34%) with ≤ 24 months, 51 (44%) with ≤ 36 months, and 97 (84%) with more than 36 months (Figure 1). Classification criteria and other clinical findings through time are shown in Supplementary Table I.

A ROC curve was performed to analyze the median duration of symptoms to classify as PSS, resulting in an area under the curve of 0.63 (95% CI 0.52-0.75, *P*=0.02). Fifteen months had a sensitivity of 73% and a specificity of 53%, a positive predictive value of 89%, and a negative predictive value of 28%. Patients with less than 15 months of symptom duration before the diagnosis were 36 (31%) (CI 22.4-40.5) (Figure 2).

CLINICAL FINDINGS

No difference was found in age (*P*=0.22) and sex (*P*=0.4) when comparing both groups (<15 months vs >15 months). A subanalysis was performed for age and its association with an altered Schirmer test (*P* <0.05), low salivary flow rate (*P*=0.14), foci score (*P*=0.54), atrophy (*P*=0.21), adipose tissue infiltration (*P* <0.05), and ductal dilatation (*P*=0.81). The median time of sicca symptoms in the < 15-month group was 12 (IQR 6-12) months compared to 60 (IQR 36-108) months in the > 15-month group.

An altered Schirmer test (*P* <0.05) and a low salivary flow rate (<1.5/15 minutes) (*P* <0.05) were found predominantly in the >15 months duration of symptoms group. Also in this group, more severe oral symptoms were found, such as cracker cookie (*P* <0.05), ocular symptoms (*P* <0.05), and renal involvement (*P* <0.05). The median score of clinical manifestations was of 9 (IQR 6.25-10) (Table I).

The mean clinical manifestations score (0-16) for the whole cohort was of 7.93 (SD 2.76) with a median of 8 (IQR 6-10). A score of 8 or more was associated with ≥ 15 months of sicca symptomatology (*P* =0.04) and an altered Schirmer test (*P*=0.03), but not with a low salivary flow rate (*P*=0.06) or positivity in serolo-

TABLE I. CLINICAL AND SEROLOGICAL FEATURES OF SICCA PATIENTS. CHARACTERISTICS OF SICCA PATIENTS STRATIFIED AS <15 MONTHS AND >15 MONTHS OF SYMPTOMATOLOGY

	Patients (n=116)	SICCA <15 months (n=36)	SICCA >15 months (n=80)	P
Demographics				
Female	112 (96.6%)	34 (94.4%)	78 (97.5%)	0.4
Years of age at diagnosis, mean(SD)†	53.33 (12.43)	51.22 (13.72)	54.28 (11.78)	0.22
Years of age at symptom onset , mean(SD)†	48.4 (13.11)	50.75 (13.74)	47.34 (12.76)	0.2
Months from symptom onset to diagnosis, median (IQR)	36 (12-84)	12 (6-12)	60 (36-108)	<0.05
Objective measurements				
Schirmer Right Eye	0 (0-6)	5 (0-10)	0 (0-4)	
Schirmer Left Eye	5 (2-13.75)	8 (3.25-75)	4 (1-12)	
Altered Schirmer test ¶	87 (75%)	22 (61.1%)	65 (81.3%)	<0.05
Salivary flow rate ml/15 minutes	0.8 (0.43-1.3)	1 (0.5-1.75)	0.73 (0.33-1.28)	
Altered Salivary flow rate test	94 (81%)	24 (66.7%)	70 (87.5%)	<0.05
Clinical manifestations				
Oral symptoms	103 (88.8%)	32 (88.9%)	71 (88.8)	0.98
Ocular symptoms	108 (93.1%)	30 (83.3%)	78 (97.5%)	<0.05
Cracker cookie	73 (62.9%)	17 (47.2%)	56 (70%)	<0.05
Choking	60 (51.7%)	14 (38.9%)	46 (57.5%)	0.06
Parotidomegaly	26 (22.4%)	9 (25%)	17 (21.3%)	0.65
Arthralgia	79 (68.1%)	22 (61.1%)	57 (71.3%)	0.28
Arthritis	32 (27.6%)	9 (25%)	23 (28.7%)	0.68
Fatigue	87 (75%)	28 (77.8%)	59 (73.8%)	0.64
Myalgia	65 (56%)	18 (50%)	47 (58.8%)	0.38
Raynaud	13 (11.2%)	6 (16.7%)	7 (8.8%)	0.21
Skin involvement	46 (39.7%)	10 (27.8%)	36 (45%)	0.08
Craurosis	34 (29.3%)	10 (27.8%)	24 (30%)	0.81
Lung involvement	14 (12.1%)	2 (5.6%)	12 (15%)	0.15
Nervous system involvement	51 (44%)	12 (33.3%)	39 (48.8%)	0.12
Hematologic involvement	5 (4.3%)	3 (3.8%)	2 (2.5%)	0.15
Renal involvement	37 (31.9%)	5 (13.9%)	32 (40%)	<0.05
Median Score of Clinical Manifestations (IQR)	8 (6-10)	6.5 (5-9.75)	9 (6.25-10)	0.3
Extraglandular manifestations	109 (94%)	33 (91.67%)	76 (95%)	0.49
Median score of ClinESSDAI (IQR)				
Serology	23.5 (13-32)	17 (9-27.7)	25 (17-33)	0.16
AntiRo titers	10.96 (2.05-86.68)	4.66 (1.39-57.39)	23.38 (2.3-87.98)	
Positive AntiRo	55 (47.4%)	15 (41.7%)	40 (50%)	0.41
AntiLa titers	3.39 (1.92-7.83)	3 (1.58-5.82)	3.6 (1.99-9.72)	
Positive AntiLa	19 (16.4%)	4 (11.1%)	15 (18.8%)	<0.05
Positive ANAs (>1:320)	68 (60.2%)	17 (47.2%)	51 (64.6%)	0.15
RF IgG titers	6.1 (2.67-11.31)	5.54 (0-10.35)	6.53 (2.99-11.74)	
RF IgM titers	18.32 (8.57-90.5)	27.31 (13.85-174.83)	13.85 (4.72-61.84)	
RF IgA titers	8.8 (3.27-26.9)	0	8.92 (3.27-33.5)	
Positive RF	54 (46.6%)	20 (55.6%)	34 (42.5%)	0.19

P value corresponds to the comparison of the two strata with Student's t, Mann Whitney-U test or Chi-squared tests. IQR =interquartil range, RF: Rheumatoid factor

†Normal variables: Age at diagnosis, Age at the time of symptom onset; ¶ Altered Schirmer Test <5mm/5 minutes; Altered Salivary flow rate test <1.5 ml/15 minutes; Extraglandular manifestations at the time of diagnostic approach

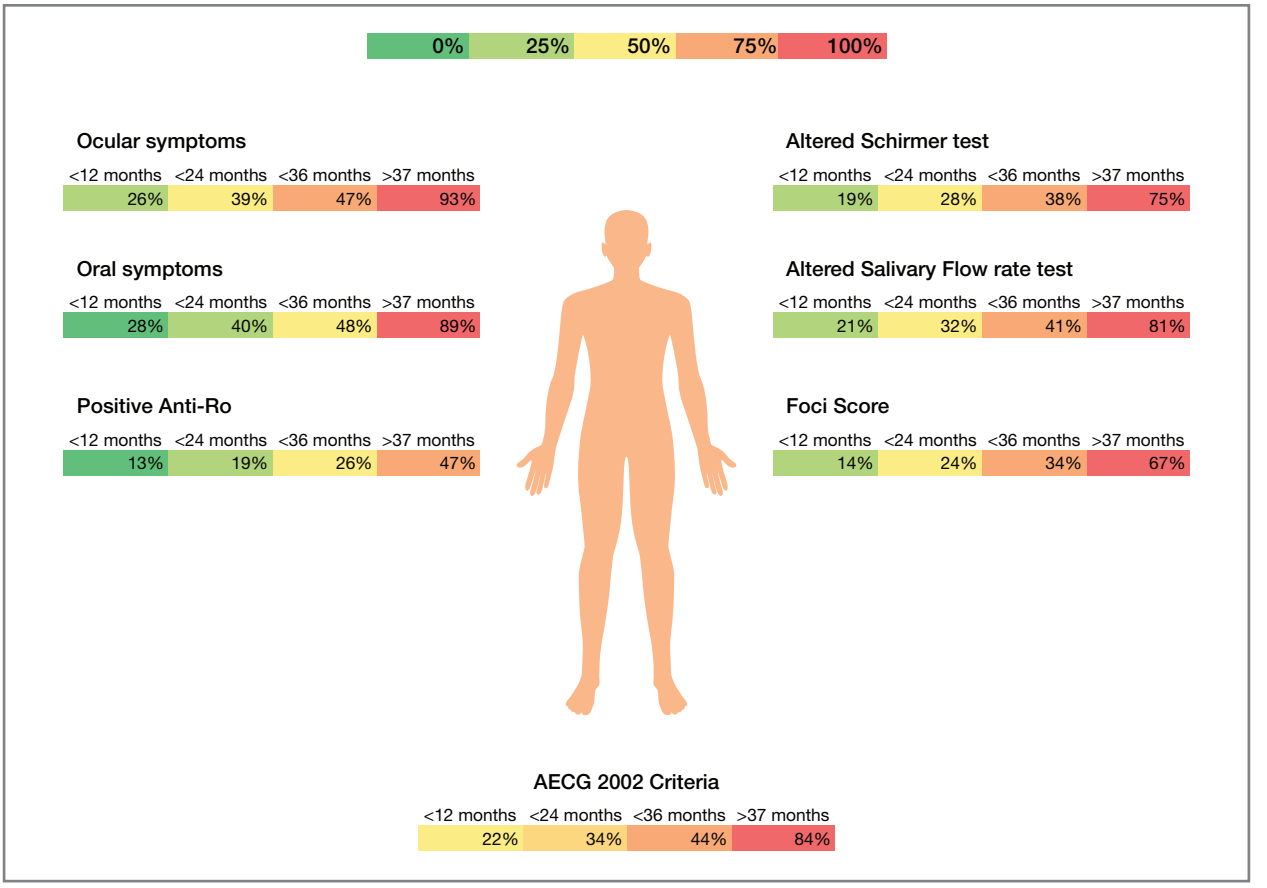

FIGURE 1.

gy: anti-SSA/Ro (*P*=0.07), anti-SSA/La (*P*=0.38), ANA (*P*=0.5), RF (*P*= 0.38). The histopathological features were neither associated: lobules (*P*=0.39), Foci score (*P*=0.06), atrophy (*P*=0.75), fatty infiltration (*P*=0.66), and ductal dilatation (*P*=0.67).

ClinESSDAI score was documented with a mean of 22.9 (SD 13.13) and a median of 25.5 (IQR 13-32). Patients with <15 months had a mean of 18.75 (SD 23.28) and a median of 17 (IQR 9-27.75); in the >15 months of sicca symptoms group ClinESSDAI score had a mean of 24.78 (SD 12.71) and a median of 25 (IQR 17-33) (Table I).

A ClinESSDAI score of ≥ 20 was associated with >15 months of sicca symptoms duration (*P*<0.05) and a low salivary flow rate (*P*=0.02),

No association was found in abnormal Schirmer test *(P=0.23),* positivity in serology: anti-SSA/Ro (*P*=0.22), anti-SSA/La (*P*=0.24), ANA (*P*=0.73), RF (*P*= 0.76) and histopathological features: lobules (*P*=0.85), Foci score (*P*=0.59), atrophy (*P*=0.53), fatty infiltration (*P*=0.75), and ductal dilatation (*P*=0.15).

SEROLOGIC FINDINGS

No difference was found in ANAs, anti-SSA/Ro or RF positivity when comparing these groups. Anti-SSB/La positivity was found predominantly in the group with a duration of symptoms of >15 months (*P*<0.05) (Table I).

HISTOPATHOLOGICAL FINDINGS

When analyzing MSGB, the <15 months group presented more lobules (*P*<0.05). Patients with more than 15 months of symptoms had higher foci scores (*P*<0.05). Atrophy and germinal center-like structures were predominantly found in the >15 months duration of symptoms group but this was not statistically significant (Table II).

A duration of symptoms >15 months was associated with more alterations in the Schirmer test (OR 2.76; 95% CI 1.15-6.61, *P*=0.02), salivary flow rate (OR 3.5; 95% CI 1.34-9.13, *P*=0.01), foci score (OR 1.21; 95%

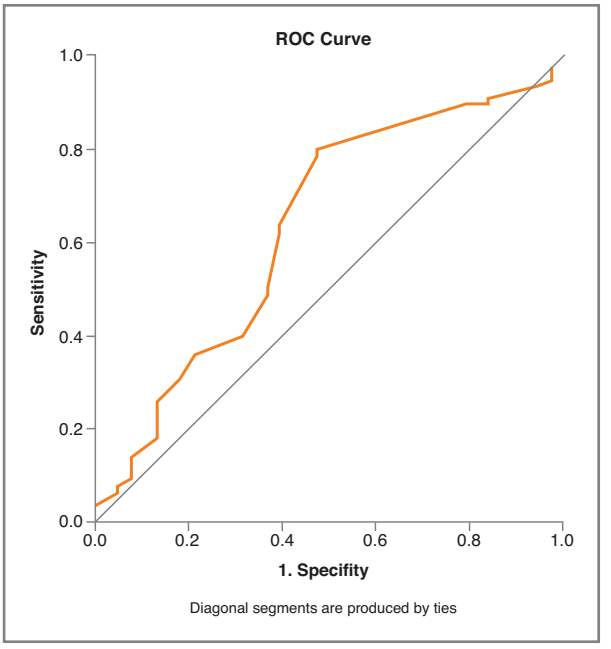

FIGURE 2.

CI 1-1.45, *P*=0.04), ocular (OR 7.8; 95% CI 1.49-40.81, *P*=0.02) and severe oral symptoms (OR 2.61; 95% CI 1.16-5.87, *P*=0.02).

When performing a multivariate analysis, ocular symptoms, a low salivary flow rate, and a lower number of lobules persisted as associated features with the classification of PSS. The foci score showed a positive association but this was not statistically significant (Table III).

DISCUSSION

There is a relation between the duration of sicca symptoms and clinical, histopathological, and serological findings. In our study, these relationships were more significant and frequent after 15 months of sicca symptoms onset. The application of classification or diagnostic criteria of PSS should consider the patient's age and the duration of symptoms[1]. Several classification and diagnostic criteria have been proposed. We used the 2002 AECG criteria to classify our patients because active enrollment started in 2015 and not all patients had an ocular staining score performed required for the implementation of ACR-EULAR 2016 criteria[4–6,17]. Our cohort included patients who were enrolled at an early course of symptom onset; the median duration of symptoms before the diagnosis was made was of 36 months (3 years) (IQR 12-84 months) and a mean of 48.52 months (4.04 years). The median duration of symptoms reported in our cohort was lower than that documented in other sicca cohorts, including those used to elaborate classification criteria (median 6-8 years). We consider this is due to an active enrollment of patients and early referral to the Rheumatology Clinic[8,12,18]. Other authors have reported a duration of symptoms that ranges from 4 to 312 months with a

TABLE II. HISTOPATHOLOGIC MINOR SALIVARY GLAND BIOPSY FEATURES OF SICCA PATIENTS

Feature	Patients, n=116	SICCA <15 months (n=36)	SICCA >15 months (n=80)	P-value
Positive Foci Score	78 (67.24)	16 (44.4)	62 (77.5)	<0.05
Atrophy	32 (27.6)	8 (22.2)	24 (30)	0.39
Adipose tissue infiltration	29 (25)	9 (25)	20 (25)	1
Ductal dilatation	24 (20.7)	11 (30.6)	13 (16.3)	0.08
Central germinal-like structures	2 (1.7)	0 (0)	2 (1.7%)	0.34
Sialadenitis	108 (93.1)	31 (86.2)	77 (96.25)	<0.05
Low	58 (50)	19 (52.8)	39 (48.7)	
Moderate	34 (29.3)	10 (27.8)	24 (30)	
Severe	16 (13.8)	2 (5.6)	14 (17.5)	
No sialadenitis	8 (6.9)	5 (13.8)	3 (3.8)	

Characteristics of SICCA patients are stratified as <15 months and >15 months of symptomatology.
Data are n (%). P value corresponds to the comparison of the two strata with the Mann Whitney-U test or Chi-squared tests.
Foci scores: 50 cells (mononuclear and lymphocytic aggregates, plasmatic cells, and histiocytes) in a 4-mm2 field
Sialadenitis: Low degree <30%, Moderate degree 30-60%, Severe degree >60%

TABLE III. ASSOCIATION BETWEEN CLINICAL, SEROLOGICAL, AND HISTOPATHOLOGICAL FEATURES IN SICCA PATIENTS AND >15 MONTHS OF SICCA SYMPTOMS DURATION

Feature	Univariate OR (95% CI)	P value	Multivariate OR (95% CI)	P value
Shirmer test <5mm/5 minutes	2.76 (1.15, 6.61) *	0.023	1.59 (0.45, 5.57)	0.47
Salivary flow rate <1.5/15 minutes	3.5 (1.34, 9.13)*	0.01	4.88 (1.31, 18.15)*	0.02
Positive AntiRo/SSA	1.4 (0.63, 3.1)	0.41	0.6 (0.18, 1.99)	0.4
Positive RF	0.59 (0.27, 1.31)	0.19	0.46 (0.16, 1.38)	0.17
Number of lobules	0.92 (0.87, 0.97)*	0.003	0.91 (0.85, 0.98)*	0.02
Atrophy	1.5 (0.6, 3.76)	0.39	2.69 (0.62, 11.62)	0.18
Adipose tissue infiltration	1 (0.4, 2.48)	1	0.38 (0.09, 1.58)	0.19
Ductal dilatation	0.44 (0.18, 1.11)	0.08	0.53 (0.15, 1.89)	0.33
Foci score	1.21 (1, 1.45)*	0.04	1.27 (0.97, 1.66)	0.08
Oral symptoms	0.99 (0.28, 3.44)	0.98	0.5 (0.09, 2.83)	0.43
Ocular symptoms	7.8 (1.49, 40.81)*	0.02	10.91 (1.11, 107.4)*	0.04
Cracker cookie	2.61 (1.16, 5.87)*	0.02	1.98 (0.56, 7.05)	0.29
Choking	2.12 (0.95, 4.75)	0.07	1.12 (0.31, 4.05)	0.87
Parotidomegaly	0.81 (0.32, 2.04)	0.65	0.47 (0.13, 1.68)	0.24

RF: Rheumatoid factor; OR: Odds ratio; CI: Confidence interval; p <0.05 *
Foci score: 50 cells (mononuclear and lymphocytic aggregates, plasmatic cells, and histiocytes) in a 4-mm^2 field

mean of 116 months before this led to PSS investigation; and from 4 to 312 months, with a mean of 91 months to biopsy performance[19].

Recent studies published in the last three years, such as a Canadian research in Toronto, documented a median delay of 4 years (1-28 years) between the onset of symptoms and diagnosis. Another small analysis in 81 PSS patients reported a median disease duration of 24 months (IQR 0–570 months) before diagnosis[19–22]. An earlier diagnosis in these recent reports could be due to the effort of clinicians in improving the detection and diagnostic approach of sicca patients.

It is essential to consider the duration of sicca symptoms when analyzing the clinical profiles and characteristics of these patients. Some studies evaluating patients with sicca symptoms at an early stage of the disease have found no histopathologic alterations, yet when repeating the MSGB later on, critical pathogenic findings have been documented[11].

Age has been found to influence sicca symptoms and histopathology findings reporting fatty infiltration related to advanced aging, similar to what we observed in our cohort[10].

In a study of 1,927 patients referring sicca symptoms, 886 were classified as PSS according to the AECG 2002 classification criteria. In this group, patients presented a mean duration of dry mouth and eye symptoms before diagnosis of 6.6 and 7.2 years, respectively. Patients who did not fulfill 2002 AECG PSS criteria but had at least one objective criteria altered, were classified as intermediate PSS; in this group duration of sicca symptoms before being classified as intermediate PSS was 5.9 years for xerostomia and 6.7 years for xerophthalmia. Sicca patients who did not fulfill objective criteria were classified as controls and had a lower time of evolution of oral and ocular symptoms with 4.5 and 5.3 years, respectively[12]. This cohort showed, as in our study, a more severe clinical and serological profile with longer duration of sicca symptomatology, mainly in patients with a positive biopsy. The mentioned study reported that symptom duration >10 years was associated with a low salivary flow rate; in our patients, this finding was observed with more than 15 months of sicca symptoms duration[12].

Other studies documented that several factors, such as time of evolution of symptoms, hypocomplementemia, and hypergammaglobulinemia predispose sicca patients to develop PSS. Even though we did not evaluate hypocomplementemia and hypergammaglobulinemia, we found that a duration longer than 15 months of sicca symptoms before diagnosis was associated with more clinical and histological findings and an increased probability of fulfilling PSS classification criteria[11].

In other cohorts, symptoms such as fever, Raynaud's phenomenon, rash, and myalgia have been found ear-

ly in the disease course of SS patients and C-reactive protein >6 mg/dL or ANA titer > 1:320 in PSS. In our study, these results were not found[20]. A multicenter study from the Big Data Sjögren Project Consortium registry, with 10,500 patients from 22 different countries showed that a positive serology was found for ANA in 79.3%, for anti-SSA/Ro in 73.2%, for anti-SSB/La in 45.1%, and for RF in 45.1%. Similar results were found in our cohort with a positive serology at diagnosis for ANA 60.2%, anti-SSA/Ro 47.4%, and RF 46.6% establishing that positive serology is frequently found at the time of performing a diagnostic approach and may precede symptom onset such as reported in Malmo University Hospital at Sweden, analyzing serology 7 (SD 5.5) years before symptom onset[19,23,24]. In our study, anti-SSB/La positivity was found only in 16.4% of the cohort and was associated with longer symptom duration. Recent interest in characterizing patients carrying isolated anti-SSB/La autoantibodies has emerged after the exclusion of this subset of patients from the 2016 ACR-EULAR criteria due to a lower altered ocular staining and salivary focus scores in the isolated anti-SSB/La positive when compared to anti-SSA/Ro positive carriers. Nonetheless, isolated anti-SSB/La positive carriers have shown a higher frequency of sicca symptoms, altered Schirmer test, and altered salivary flow rate when compared to the seronegative group[25–27].

Histopathologic alterations such as lymphocytic infiltration have been considered one of the most critical time-dependent criteria to be found in sicca patients[28,29]. A positive biopsy has been associated with more chronic and severe oral symptoms, as well as high titers for anti-SSA/Ro, similar to what our results portrayed; nonetheless, in a study performed at the State University of Rio de Janeiro, salivary flow rate and autoantibodies profiles were independent of biopsy status contrary to our findings. This probably could be due to the small number of patients evaluated in the referred study[21,25].

Valuable information on diagnosis and prognosis has been achieved from studying MSGB, helping clinicians to detect patients at risk of presenting critical clinical profiles and an increased likelihood of developing lymphoproliferative disorders by evaluating foci scores, germinal center like structures, and other ectopic lymphoid structures (30). In our group the presence of > 1 foci score and GCS was associated with more severe profiles and extraglandular manifestations[31].

Foci score analysis has been homogenized during the last years but the evaluation of GCS varies widely among pathologists, some using only H & E stains and others biomarkers such as Bcl-6,CD21,CD3/CD20. In our study only H&E stains were used and GCS were found in 2 cases with > 15 months of sicca symptomatology and higher foci scores, similar to what other reports have found[32].

Some of the studies that have evaluated different techniques have reported a good reliance with the use of H&E stains, yet these are operator dependent; when using biomarkers such as CD21 and Bcl-6 the concordance increased, being a more dependable method[33].

When evaluating the behavior of an established PSS diagnosis over time, an increased risk of extra-glandular manifestations and lymphoproliferative disorders have been documented in advanced and untreated stages[34]. In a study performed in the Participants of the Sjögren's International Collaborative Clinical Alliance Registry, they have reported a stable disease course after being classified as PSS, yet the follow-up performed in the mentioned study had a mean duration of 24 months, assuming this was a short follow-up period[11].

The aim of the SSF (Sjogren's Syndrome Foundation) is to reduce the time taken to diagnose SS below 3 years and we consider that the results of our study are crucial in the working progress of achieving and interpreting these results[22,35]. Awareness and education of physicians, patients, and dentists in the existence of this pathology may increase early referral and treatment.

The development of new diagnostic strategies such as salivary gland ultrasonography (SGU) has become an important assessment tool for diagnostic and prognostic evaluation in patients at an early stage of the disease, demonstrating a diagnostic sensitivity and specificity of 66% and 98%, respectively. This could be another method to considerate when evaluating diagnostic methods in patients who do not fulfill classification criteria at an early stages[36].

To date, no immunomodulator or immunosuppressive therapy treatment has demonstrated an efficient therapeutic response in patients with sicca symptoms. We deduce with our results that patients that finally classify as PSS are in a late phase of the disease and only symptomatic treatment is being offered, the contrary to modifying disease behavior if treatment was received in earlier stages[12].

An early diagnosis can contribute to the development of new treatment strategies, diminishing complications and extra-glandular manifestations, as well as preventing lymphoproliferative disorders; yet achieving

these goals becomes difficult when expecting patients to fulfill classification criteria that are time-dependent.

CONCLUSIONS

The duration of symptoms plays a fundamental role in the wide spectrum of clinical, serological, and histopathological features of PSS patients. Duration of sicca symptoms should be considered when interpreting and applying diagnostic and classification criteria. A duration of sicca symptoms >15 months at diagnosis was associated with more alterations. The presence of a foci score ≥1 in MSGB continues to be the best histopathologic diagnostic and prognostic marker of PSS, associated with more severe clinical profiles.

LIMITATIONS

Limitations include the retrospective design of the study evaluating patients referring sicca symptoms at a specific time by a clinical questionnaire that could be memory biased (time of onset of symptoms), lacking clinical, serological, and histopathological follow-up after the first diagnostic approach was performed. We did not register symptomatic treatment administered to patients before their inclusion in the sicca cohort and the differences found between patients who had received symptomatic treatment versus untreated evaluated patients. ClinESSDAI was used because the biologic domain was not available in our entire cohort. Biomarkers for the analysis of B and T cell distribution in MSGB were not performed.

ETHICS APPROVAL

The protocol was approved by our local ethics and research committee (RE20-00003) and conducted according to the Declaration of Helsinki.

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SUPPLEMENTARY TABLE

Characteristic	<12 months	<24 months	<36 months	>37 months
Oral symptoms	28%	40%	48%	89%
Ocular symptoms	26%	39%	47%	93%
Altered Salivary flow rate test	21%	32%	41%	81%
Altered Schirmer test	19%	28%	38%	75%
Positive Anti Ro	13%	19%	26%	47%
Positive Anti La	3%	6%	10%	16%
≥1 focus score	14%	24%	34%	67%
Criteria 2002 AECG	22%	34%	44%	84%
Cracker cookie	15%	22%	31%	63%
Choking	12%	18%	25%	52%
Parotidomegaly	8%	12%	16%	22%
Artritis	8%	14%	14%	28%
Renal involvement	4%	8%	12%	32%
Positive ANA	15%	23%	32%	59%
Positive RF	17%	24%	29%	47%
Atrophy	7%	9%	12%	28%
Adipose tissue infiltration	8%	9%	12%	25%
Ductal Dilatation	9%	11%	14%	21%