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Clinical significance of autoantibodies recognizing Sjögren's syndrome A (SSA), SSB, calpastatin and alpha-fodrin in primary Sjögren's syndrome

V. Goëb,* V. Salle,† P. Duhaut,† F. Jouen,‡ A. Smail,† J.-P. Ducroix,† F. Tron,‡ X. Le Loët* and O. Vittecoq*
*Department of Rheumatology, Rouen University Hospital & Inserm U519 (IFRMP23), Institute for Biomedical Research, University of Rouen, Rouen, France, †Department of Internal Medicine, Amiens University Hospital, Amiens, France, ‡Immunology Laboratory, Rouen University Hospital & Inserm U519 (IFRMP23), Institute for Biomedical Research, University of Rouen, Rouen, France

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Correspondence: Vincent Goëb, Rouen University Hospital – Rheumatology, CHU-Hôpitaux de Rouen, Rouen 76031, France.
E-mail: goebvince@yahoo.fr

Summary

The aim of our study was (i) to compare the clinical and biological characteristics of 148 (137 women, 11 men) primary Sjögren's syndrome (pSS) patients at diagnosis as a function of their sex and (ii) to assess the prognostic value of anti-calpastatin and anti-alpha-fodrin autoantibodies. In addition, the presence of anti-nuclear antibodies (ANA), anti-52- and 60-kDa Sjögren's syndrome A (SSA), anti-Sjögren's syndrome B (SSB), anti-cyclic citrullinated peptide (CCP) antibodies and rheumatoid factors (RF) of IgA, IgG and IgM isotypes was sought in sera collected at pSS onset. Raynaud's syndrome, significantly more frequent in women, was the only systemic manifestation of pSS whose frequency differed significantly as a function of the patient's sex ($P = 0{\cdot}02$). ANA ($P = 0{\cdot}001$) and anti-60-kDa SSA autoantibodies ($P = 0{\cdot}03$) were significantly more common in women, while men never synthesized detectable levels of anti-SSB, anti-calpastatin or IgG anti-alpha-fodrin autoantibodies. In addition, anti-CCP autoantibodies were found in low percentages of pSS patients (4% F/18% M). The absence of autoantibodies does not exclude the diagnosis of pSS in men that will be based mainly on the anatomopathological findings of a minor salivary gland biopsy. Positivity of anti-60-kDa SSA, anti-SSB, anti-calpastatin, IgA and IgG anti-alpha-fodrin antibodies is not associated with pSS clinical and biological severity.

Keywords: autoantibodies, calpastatin, fodrin, Sjögren's syndrome

Introduction

Primary Sjögren's syndrome (pSS) is a common autoimmune disease (prevalence: 0·1–0·2% of the general adult population) that affects primarily women (F/M sex ratio: 9) [1]. Responsible for a sicca syndrome, predominantly buccal and ocular, caused by inflammatory involvement of the salivary and lacrimal glands, pSS can also induce extraglandular or systemic disorders that can be life-threatening (lymphoma [2,3], pulmonary fibrosis [4], renal [5] or neurological involvement [6,7]). A diagnosis of pSS is considered when it occurs alone, independently of another autoimmune disease such as rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE).

pSS is diagnosed based on the detection of circulating anti-nuclear autoantibodies, such as anti-Sjögren's syndrome A (anti-SSA/Ro) or B (anti-SSB/La), and/or the presence of Chisholm grades 3 or 4 [8] nodular inflammatory lymphocyte infiltrates in a minor salivary gland biopsy. These two objective criteria are consistent with the buccal and/or ocular dryness described by the patients. Indeed, they are included in the 'revised' American and European criteria for the diagnosis of pSS [9], and ensure a good compromise between sensitivity (90%) and specificity (95%). In routine practice, the diagnosis of pSS is often difficult for several reasons: its onset is often insidious; a sicca syndrome can be the consequence of diverse causes (smoking, medications); initial non-specific extraglandular manifestations (myalgias, arthralgias) can precede the appearance of sicca manifestations by several years; and the absence of a biological (especially immunological) marker specific to the disease.

Several autoantibodies populations are associated with pSS: rheumatoid factors (RF), anti-SSA, anti-SSB, anti-alpha-fodrin and anti-type M3 muscarinic receptors [10–12]. RF are detected in approximately 60% of pSS patients and their frequency is higher in men [13]. Antinuclear antibodies (ANA) are found in more than two-thirds of pSS patients, but they are not specific to this disease [1]. The most characteristic autoantibodies of pSS are those directed against soluble nuclear antigens [e.g. anti-extractable nuclear antigen (ENA)], anti-SSA and anti-SSB. Anti-SSA autoantibodies are found in 30–60% of pSS

patients and are not considered totally specific to the disease because they are seen in 30% of SLE patients [1,11]. They seem to be associated with pSS forms with vasculitis and/or leucopenia [14]. The anti-SSA autoantibodies can bind to several antigenic epitopes expressed by two proteins of molecular masses of 52 and 60 kDa associated with RNA [15,16]. Only the anti-SSA autoantibodies recognizing the 60-kDa protein are sought routinely. Notably, it seems that the reactivity profile can differ for patients afflicted with pSS (isolated anti-52-kDa SSA reactivity) or SLE (anti-60-kDa reactivity) [15,16]. Anti-SSB autoantibodies are also characteristic of pSS. They are detected in 5–50% of the patients and co-exist with anti-SSA autoantibodies in more than 50% of the patients; their co-existence is highly suggestive of pSS [11]. Autoantibodies to alpha-fodrin, a ubiquitous cytoskeletal protein described as the target of isotype IgG antibody in pSS [12,17], are accorded much attention because their appearance precedes those of anti-SSA and -SSB autoantibodies, and might therefore contribute to obtaining an earlier diagnosis of pSS. Anti-alpha-fodrin autoantibodies could be a sign of systemic involvement of pSS [18] and, pertinently, were found at higher titres (but not significantly so) in patients with neurological manifestations [19]. Autoantibodies to calpastatin, the endogenous inhibitor of calpains which are intracellular calcium-activated cysteine proteases, have been identified in systemic rheumatic diseases [20]. They were also shown to be associated with secondary Sjögren's syndrome in SLE [21]. Finally, autoantibodies to cyclic citrullinated peptide (anti-CCP), known to be highly specific to RA, are detected in 3–7% of pSS patients [22].

Thus, diagnosing pSS is made difficult by the diversity of initial manifestations and the heterogeneity of the autoimmune markers described during the course of the disease. More importantly, no factors able to predict the course of pSS are available. Therefore, the aim of this study was (i) to describe retrospectively the clinical and biological (predominantly autoimmune) characteristics of pSS patients, hypothesizing that those of men and women differed, and (ii) to assess the prognostic value of the anti-calpastatin and anti-alpha-fodrin autoantibodies for pSS and particularly to evaluate if their positivity is linked to the most severe pSS clinical and/or biological manifestations.

Patients and methods

Patients

The sera from 148 pSS patients recruited retrospectively from the Rouen University Hospital Department of Rheumatology and the Amiens University Hospital Department of Internal Medicine were analysed. Only patients with at least four of the diagnostic criteria of the American–European classification [3] and presenting at least anti-SSA/Ro and/or anti-SSB/La autoantibodies and/or Chisholm grade ≥ 3 [2] nodular lymphocytic sialadenitis as determined by minor salivary gland biopsy were included. Patients affected with another autoimmune disease, e.g. RA, or with viral hepatitis or human immunodeficiency virus (HIV)-positive, were not included. Finally, 77 patients from Rouen and 71 from Amiens were recruited for this study. In accordance with French law, all patients had given their written informed consent that their sera will be collected and stored for future analyses.

Clinical and biological manifestations

The patients' medical files were consulted to collect the clinical manifestations occurring during the disease: parotiditis, keratitis, arthralgias, polyarthritis, myositis, lung involvment (non-productive tracheobronchitis, pulmonary fibrosis), central or peripheral nervous system involvement, psychiatric disorder (depressive syndrome), pulmonary artery hypertension, purpura or other vasculitides (determined by histological examination of biopsies), Raynaud's syndrome and previous episodes of autoimmune thyroiditis.

The following biological parameters at diagnosis were collected when available: abnormal white blood count (leucopenia and/or lymphopenia), gammaglobulinaemia, presence of a monoclonal gammapathy, cryoglobulinaemia, erythrocyte sedimentation rate 1st h (ESR) and C-reactive protein (CRP) concentration.

Because of the lack of assessed clinical or biological criteria of pSS severity in the literature, we have defined (i) clinical severity as the presence of at least one of the following manifestations: parotiditis, pulmonary fibrosis, lymphoma, neurological involvment and/or vasculitis; and (ii) biological severity as the presence of at least one of the following abnormalities: lymphopenia, monoclonal gammapathy, hypogammaglobulinaemia and/or cryoglobulinaemia.

Detection of autoimmune markers

Enzyme-linked immunosorbent assays (ELISA) were run with the sera stored at −80°C in each hospital's serum library and Biobanque de Picardie to detect: IgG, IgM and IgA RF isotypes and anti-52- and 60-kDa SSA, anti-SSB, IgA and IgG anti-alpha-fodrin, anti-CCP and IgG anti-calpastatin autoantibodies.

ELISA determination of IgM, IgG and IgA RF isotypes were home-made in the Immunology Laboratory of Rouen. Flat-bottomed 96-well microtitre plates (Virion, Roche, Rosny-sous-Bois, France) were coated with a 10-μg/ml solution of purified rabbit IgG (Jackson Immunoresearch Laboratories, Westgrove, PA USA) in 0·1 M carbonate-bicarbonate, pH 9·6, at 4°C overnight. After coating, the plates were incubated for 2 h at 37°C with test serum samples and with a serial dilution of a standard serum for each isotype. Bound RF were detected with $F(ab)_2$ goat anti-human immunoglobulins (IgM, IgA or IgG) conjugated to

alkaline-phosphatase (Sigma Immunochemicals, Munich, Germany). Finally, a 1-mg/ml solution of p-nitrophenol phosphate (Sigma, St Louis, MO, USA) was added to the wells and the optical density (OD) generated by the enzymatic reaction was read at 405 nm in a microplate reader. The upper limit of normal antibody values was considered to be 6 standard deviations (s.d.) above the mean level in serum samples from 100 healthy blood donors. These thresholds of positivity were determined previously by using receiver operating characteristic (ROC) curves to discriminate RA from other rheumatic diseases, as described by Visser *et al*. [23]. The RF titres at which the accuracy of these tests was greatest in terms of sensitivity and specificity were chosen as optimal cut-off titres.

The calculation of the titres and cut-offs of the autoantibodies to 52- and 60-kDa SSA, SSB, IgA and IgG alpha-fodrin (Aeskulab® kits; GMBH, Wendelsheim, Germany) and anti-CCP (EuroImmun, GMBH, Groß Grönau, Germany) were those recommended by the manufacturers.

An ELISA in which the antigen was a synthetic peptide corresponding to the 27 C-terminal amino acids of calpastatin (SSKAPKNGGKAKDSAKTTEETSKPKDD) was set up according to the procedure described by Schlosser *et al*. [24], with some modifications [25]. Half the wells of 96-well plastic microtitre plates were coated overnight at room temperature with the synthetic peptide (Neosystem, Strasbourg, France) dissolved in phosphate-buffered saline (PBS) at 0·1 μg/ml. The purity of this peptide was > 75%; the preparation may have contained smaller fragments of the peptide sequence but no exogenous contaminant. The remaining wells received 100 μl of PBS. After four washes with PBS containing 0·1% Tween 20 (PBST), the wells were saturated for 2 h at room temperature with 200 μl of PBST solution containing 50 g/l of sucrose and 3% milk powder. After removal of excess buffer, test sera diluted 1 : 200 in 1% bovine serum albumin–PBST were incubated for 30 min at room temperature. After washing with PBST, $F(ab')_2$ fragments of goat anti-human IgG conjugated to alkaline phosphatase, diluted 1 : 2000 in PBST, were added and the preparations were incubated for 30 min at room temperature. Plates were then washed four times and 100 μl of a solution containing 1 mg/ml of *p*-nitrophenol phosphate dissolved in 0·1 M Tris–HCl (pH 9·8) and 1·35 M NaCl was added. After 15 min at room temperature, the OD of the different wells were determined. The OD were calculated by subtracting the OD measured in uncoated wells from that measured in coated wells. A calibration curve was generated with an ACAST-C27-positive reference serum at dilutions ranging from 1 : 200 to 1 : 4800. This reference serum, which was obtained from one RA patient followed in the Department of Rheumatology, had a high OD at a dilution of 1 : 200. Serum autoantibodies titres were expressed in arbitrary units (AU) relative to the reference serum, whose OD at a dilution of 1 : 200 was equal to 240 AU. The threshold of positivity was 2 s.d. above the mean value calculated for the sera of 70 healthy blood donors whose OD values were distributed normally, i.e. 20 AU/ml.

All positive autoantibody detections in these sera led systematically to repeat titreing of the same serum sample. In the case of discordant findings, a third and ultimate evaluation was made. ANA positivity was assessed on Hep2 cells (Immunoconcept, Sacramento, CA, USA).

Statistical analyses

A χ^2 test, Fisher's exact test (when fewer than five samples were compared) and Wilcoxon's test were applied to evaluate the significance of the differences between frequencies of each clinical and biological factor according to the patients' sex and to the clinical or biological severity of the disease. Significance was defined as $P < 0{\cdot}05$.

Results

Demographic, clinical and classical biological data, and diagnostic criteria

Among the 148 pSS patients recruited retrospectively but followed actively in our two hospitals, 137 (93%) were women and 11 (7%) were men. Their median age at the time of pSS diagnosis was 54·3 (women) and 58 (men) years. The main demographic and clinical data for women and men and their comparisons are given in Table 1. No significant differences were found for the distributions of the clinical manifestations as a function of patients' sex, except for vascular involvement (Raynaud's syndrome, purpura, hypertension and/or vasculitis) and particularly Raynaud's syndrome, which was significantly more frequent in women ($P = 0{\cdot}03$ and 0·02, respectively; Fisher's exact test). Because renal involvement was well documented for only one patient, it was not evaluated in this study.

At pSS onset, four or five of the international diagnostic criteria were satisfied, respectively, by eight men and 89 women, or three men and 48 women. All 11 men and 126 women had Chisholm grades 3/4 lymphocytic infiltrates in their minor salivary gland biopsies, and 5% of the women had grade 2 inflammation.

None of the classical biological parameters evaluated [ESR (100 F/6 M), CRP (92 F/6 M), leucopenia or lymphopenia (133 F/11 M), gammaglobulinaemia (77 F/8 M) (50% ≤ 12 g/l and 75% ≤ 16 g/l, overall range: 2–28 g/l), monoclonal gammapathy] differed between men and women.

Autoantibodies and diagnosis of pSS

Among the various autoantibodies populations studied in the sera of these pSS patients (Table 2), only ANA and anti-60-kDa SSA differed significantly between men and women ($P = 0{\cdot}001$ and 0·03, respectively; Fisher's exact test), and

Table 1. Clinical manifestations of the 148 primary Sjögren's syndrome (pSS) patients according to sex.

Parameter	Men ($n = 11$)	Women ($n = 137$)	*P*-value
Diagnostic criteria			
International consensus criteria [3]			0·75
4 satisfied	8 (73)	89 (65)	
5 satisfied	3 (27)	48 (35)	
MSGB grade 4	3 (27)	45 (33)	1·0
Demographic and clinical data			
Age (years), median (range)	58 (27–69)	54·3 (20–96)	
Parotiditis	1 (9)	16 (12)	1·0
Keratitis	2 (18)	22 (16)	1·0
Musculoskeletal system	6 (55)	88 (64)	0·5
Arthritis	2 (18)	32 (23)	1·0
Myositis	0	4 (3)	1·0
Pulmonary involvement	3 (27)	16 (12)	0·2
Tracheobronchitis	2 (18)	10 (7)	0·2
Fibrosis	1 (9)	6 (4)	0·4
Vascular manifestations	1 (9)	59 (43)	0·03
Raynaud's syndrome	0	51 (37)	0·02
Pulmonary artery hypertension	1 (9)	2 (1)	0·2
Purpura	0	7 (5)	1·0
Vasculitis	0	12 (9)	0·6
Autoimmune thyroiditis	1 (9)	26 (19)	0·7
Neurological involvement	2 (18)	21 (15)	0·7
Central nervous system	2 (18)	9 (7)	0·2
Peripheral nervous system	0	12 (9)	0·6
Depressive syndrome	1 (9)	13 (9)	1·0
Lymphoma	1 (9)	4 (3)	0·3

Values are *n* (%) unless indicated otherwise. MSGB: minor salivary gland biopsy.

Table 2. Rates of autoantibodies expression in primary Sjögren's syndrome (pSS) patients according to sex.

Autoantibody	Men	Women	*P*-value
ANA ($n = 135$)	1 (10)	77 (62)	0·001
Anti-52-kDa SSA ($n = 134$)	2 (18)	20 (16)	0·7
Anti-60-kDa SSA ($n = 138$)	0	41 (32)	0·03
Anti-SSB ($n = 140$)	0	24 (19)	0·2
Anti-CCP ($n = 147$)	2 (18)	6 (4)	0·1
RF ($n = 148$)			
IgA	1 (9)	25 (18)	0·7
IgG	1 (9)	29 (21)	0·5
IgM	3 (27)	48 (35)	0·7
IgA anti-alpha-fodrin ($n = 147$)	2 (18)	35 (26)	0·7
IgG anti-alpha-fodrin ($n = 148$)	0	23 (17)	0·2
Anti-calpastatin ($n = 148$)	0	19 (14)	0·3

Values are *n* (%) unless indicated otherwise. ANA: anti-nuclear antibodies; SSA: Sjögren's syndrome A; SSB: Sjögren's syndrome B; CCP: cyclic citrullinated peptide;

were more frequent in women. Notably, our male and female pSS patients had only low percentages of anti-CCP autoantibodies, highly specific for RA (18% and 4%, respectively). Pertinently, anti-60-kDa SSA, anti-SSB, anti-calpastatin and IgG anti-alpha-fodrin were never detected in sera from male patients.

Anti-calpastatin, anti-alpha-fodrin and pSS clinical and biological severity

The positivity and titres of the anti-calpastatin, IgG and IgA anti-alpha-fodrin autoantibodies according to the clinical and biological severity of pSS patients are summarized in Tables 3 and 4, respectively. There was no significant relationship between the presence/titres of these autoantibodies populations, considered alone or in combination, and the criteria of severity. Furthermore, there was no significant association between IgA ($P = 0·602$) and IgG ($P = 0·531$) anti-alpha-fodrin autoantibodies and pSS neurological manifestations.

Discussion

In this retrospective study, we compared a variety of clinical and biological parameters between 148 men and women with pSS followed actively in two university hospitals. We also assessed the interest of anti-calpastatin and anti-alpha-fodrin autoantibodies in pSS. Our analysis of differences associated with the patients' sex showed that Raynaud's syndrome occurred significantly more frequently in women, and that anti-60-kDa SSA, anti-SSB and IgG anti-alpha-fodrin autoantibodies were not associated with pSS in men.

Table 3. Positivity/titres of anti-Sjögren's syndrome A (SSA), Sjögren's syndrome B (SSB), calpastatin and alpha-fodrin autoantibodies according to primary Sjögren's syndrome (pSS) clinical severity.

	Clinical severity			
Autoantibodies presence	Presence ($n = 87$)	Absence ($n = 61$)	P	Odds ratio IC (95%)
Anti-52 kDa SSA	12 (9%)	10 (7·5%)	0·703	0·838
Titres*	48 (16–110)	49 (9–111)		(0·333–2·098)
Anti-60 kDa SSA	25 (18%)	16 (11·6%)	0·642	1·193
Titres*	64 (22–151)	63 (25–146)		(0·566–2·513)
Anti-SSB	16 (11·5%)	8 (5·7%)	0·376	1·515
Titres*	19 (1–91)	20 (13–67)		(0·601–3·82)
Anti-calpastatin	13 (9%)	6 (4%)	0·361	1·610
Titres*	9 (9–190)	9 (9–498)	0·717	(0·576–4·503)
IgG antialpha-fodrin	10 (7%)	13 (9%)	0·105	0·480
Titres*	8 (2–88)	7 (1–122)	0·690	(0·195–1·179)
IgA antialpha-fodrin	23 (16%)	14 (10%)	0·670	1·181
Titres*	9 (1–132)	8·5 (0–59)	0·898	(0·550–2·537)
Anti-calpastatin and/or IgG anti-alpha-fodrin	20 (14%)	17 (11%)	0·565	0·773 (0·365–1·636)
Anti-calpastatin and IgG anti-alpha-fodrin	3 (2%)	2 (1%)	1·000	1·054 (0·171–6·502)

*Titres (median and extremes), expressed in international units (IU).

Our data show that anti-calpastatin and anti-alpha-fodrin autoantibodies are not associated with the most severe pSS forms.

pSS is known to be uncommon in men, with an F/M ratio of 9 : 1 [1] and a ratio of 13 : 1 in our study. The clinical and biological data from male and female pSS patients were compared previously and their significant differences are summarized briefly in Table 5. In particular, extraglandular involvement, namely, thyroid disease and carpal tunnel syndrome [13] or arthritides and Raynaud's syndrome [26], were significantly more frequent in women in those populations that had respective F/M ratios of 521/28 and 69/36. In contrast, Molina *et al*. [27] and Anaya *et al*. [28] did not identify any significant clinical differences between men and women with pSS, but their study populations were much smaller. None of those studies demonstrated higher frequencies in women than men of other clinical signs of pSS severity (lymphoma, pulmonary fibrosis, vasculitis or central nervous system involvement, for example). Our analysis confirmed the significantly higher frequency of Raynaud's syndromes in women found by Drosos *et al*. [26], but not the arthritides, thyroid or neurological involvement described by others. In addition, the women in our cohort did not develop more clinical manifestations of pSS severity than the men. Thus, a potential triggering role of sex hormones in the pathophysiology of pSS was not supported by more severe disease. Moreover, close monitoring and/or more intensive therapy cannot be justified by the patient's sex.

Table 4. Primary Sjögren's syndrome (pSS) biological severity and positivity of anti-Sjögren's syndrome A (SSA), Sjögren's syndrome (SSB), calpastatin and alpha-fodrin autoantibodies.

	Biological severity			
Autoantibodies presence	Presence ($n = 63$)	Absence ($n = 15$)	P	Odds ratio IC (95%)
Anti-52 kDa SSA	11 (15%)	2 (2·7%)	1	1·375
Titres*	50 (21–111)	(40–85)		(0·268–7·046)
Anti-60 kDa SSA	20 (27·4%)	4 (5·48%)	0·703	1·282
Titres*	58 (22–151)	42 (35–110)		(0·357–4·605)
Anti-SSB	13 (17·3%)	1 (1·3%)	0·276	3·872
Titres*	31 (5–77)	7		(0·465–32·25)
Anti-calpastatin	6 (8%)	2 (3%)	0·646	0·684
Titres*	9 (9–190)	9 (9–55)	0·453	(0·124–3·783)
IgG antialpha-fodrin	8 (10%)	5 (6%)	0·115	0·291
Titres*	8 (1–70)	10 (3–101)	0·192	(0·079–1·073)
IgA antialpha-fodrin	19 (24%)	3 (4%)	0·536	1·727
Titres*	10 (1–64)	7 (2–68)	0·227	(0·437–6·830)
Anti-calpastatin and/or IgG anti-alpha fodrin	13 (17%)	5 (6%)	0·318	0·520 (0·151–1·788)
Anti-calpastatin and IgG anti-alpha-fodrin	1 (1%)	2 (3%)	0·092	0·105 (0·009–1·244)

*Titres (median and extremes) are expressed in international units (IU).

Table 5. Previously reported significant clinical and/or biological differences between men and women with pSS.

Reference	Female/male ratio	Significant difference	
		Clinical	BiologicalM: RF, ANA
Diaz-Lopez *et al.* [13]	521/28	F: thyroid involvement, carpal tunnel syndrome	F: ANA, anti-60-kDa SSA
Drosos *et al.* [26]	30/12	F: arthritides, Raynaud's syndrome	F: RF, anti-60-kDa SSA
Molina *et al.* [27]	69/36	None	None
Anaya *et al.* [28]	25/13	None	F: ANA, anti-60-kDa SSA
This study	137/11	F: Raynaud's syndrome	

F: female, RF: rheumatoid factor, ANA: anti-nuclear antibodies.

Our most intriguing finding was the total undetectability of ANA and anti-60-kDa SSA autoantibodies in sera from men with pSS. The significantly higher rates of ANA and anti-60-kDa SSA autoantibodies in women confirm the results of Drosos *et al.* [26] (ANA and anti-60-kDa SSA) and Molina *et al.* [27] (anti-60-kDa SSA). Our results are also consistent with those of Garcia-Carrasco *et al.* [29] that described a lower prevalence of immunological features (notably a lower frequency of anti-SSA autoantibodies) in male patients. In light of the more common development of autoimmunity in women, these findings are not surprising. The possible influence of intercurrent factors (chronic viral infections, medications, etc.) on the higher frequency of ANA in women seems unlikely, but was not examined in this study. In addition, neither hormone-replacement therapy nor oral contraceptive use which might induce a bias is known to induce the synthesis of ANA. Moreover, although the difference did not reach significance, no anti-SSB autoantibodies were detected in the men studied, as opposed to 19% of our female patients. Furthermore, the RF frequencies were always lower for our male pSS patients.

Because of the low frequency or negativity of the autoantibodies usually associated with pSS in our male patients, we wondered whether the search for other autoantibody populations would be informative. Therefore, we looked for anti-52-kDa SSA, anti-calpastatin and IgG anti-alpha-fodrin autoantibodies. The former were found in the sera of 20% of our male pSS patients compared to 16% of the women's sera, while all the men's sera were negative for these latter in contrast to, respectively, 14% and 17% positivity for female pSS patients.

The aim of our study was also to assess the prognostic value of anti-calpastatin and anti-alpha-fodrin autoantibodies for pSS. Because of their role in apoptosis and the relative frequency of evolution of pSS towards lymphoma in which apoptosis dysregulation plays a main role, we had posited the hypothesis that anti-calpastatin and anti-alpha-fodrin autoantibodies would also be associated with the most severe forms of pSS. In fact, our results show that there is no association between these autoantibodies and the clinical and biological pSS severity as we defined it. Moreover, there was no significant association between IgA and IgG anti-alpha-fodrin autoantibodies and pSS neurological involvement, contrary to the results of de Sèze *et al.* [19]. Anti-calpastatin autoantibodies had never been assessed previously for their prognostic value in pSS. Salle *et al.* [21] have shown that they were associated with SS secondary to SLE. In our study, the prevalence of anti-calpastatin autoantibodies is not related to patient's gender, whereas they were never detected in sera from male patients. Furthermore, anti-calpastatin are not correlated with pSS severity. As it is the first study describing anti-calpastatin in pSS, independent studies are needed to conclude definitively upon their prognostic value in pSS.

Some authors [22,30] reported the presence of anti-CCP autoantibodies in the sera of pSS patients. Only eight (5%) of our patients' sera were positive for these autoantibodies, with no difference in the rates for men and women. Anti-CCP autoantibodies are highly specific for RA (found in approximately 97% of the patients) and, when combined with RF positivity, are almost definitively diagnostic for RA. Rather than raise doubt concerning the primary nature of SS diagnosed in this setting, the positivity of anti-CCP autoantibodies should alert the physician to the possible later appearance of true RA.

This study demonstrates that female pSS patients suffer more frequently from Raynaud's syndrome than their male counterparts. Otherwise, no major clinical differences at the time of pSS diagnosis were found between men and women and, notably, the women did not have clinical manifestations of more severe disease. Although our male pSS patients satisfied the American–European consensus diagnostic criteria, none of their sera contained detectable anti-60-kDa SSA, anti-SSB, anti-calpastatin or IgG anti-alpha-fodrin autoantibodies at the time of diagnosis. Thus, in our study, the autoantibodies associated classically with pSS do not contribute to making this diagnosis in men. Therefore, in routine practice, obtaining a minor salivary gland biopsy appears to be essential. In addition, the search for anti-52-kDa SSA autoantibodies could prove useful when pSS is suspected in a man with a negative immunological profile. Most importantly, the failure to detect the classical autoantibodies associated with pSS should not exclude this diagnosis in a man who has not undergone minor salivary gland biopsy. Finally, autoantibodies usually associated with pSS, anti-alpha-fodrin and anti-calpastatin autoantibodies are not associated with the most severe forms of the disease.

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