Cryoglobulinemia in Sjögren Syndrome: A Disease Subset that Links Higher Systemic Disease Activity, Autoimmunity, and Local B Cell Proliferation in Mucosa-associated Lymphoid Tissue

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ABSTRACT. ***Objective.*** To compare systemic disease activity by validated tools, i.e., the European League Against Rheumatism Sjögren Syndrome Disease Activity Index (ESSDAI) and the Clinical ESSDAI (ClinESSDAI) scores, between primary Sjögren syndrome (pSS) with positive serum cryoglobulins and pSS without serum cryoglobulins.

Methods. There were 825 consecutive patients with pSS who were retrospectively evaluated.

Results. The ESSDAI and the ClinESSDAI scores were significantly higher in cryoglobulin-positive patients ($p < 0.0001$, for both scores). Cryoglobulinemia was significantly associated with these domains: constitutional ($p = 0.003$), lymphadenopathy ($p = 0.007$), glandular ($p = 0.0002$), cutaneous ($p < 0.0001$), peripheral nervous system ($p < 0.0001$), hematological ($p = 0.004$), and biological ($p < 0.0001$).

Conclusion. Cryoglobulin-positive patients show the highest systemic activity in pSS. (J Rheumatol First Release May 15 2017; doi:10.3899/jrheum.161465)

Key Indexing Terms:
PRIMARY SJÖGREN SYNDROME NON-HODGKIN LYMPHOMA
RHEUMATOID FACTOR CRYOGLOBULINEMIA VASCULITIS

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Accepted for publication April 4, 2017.

Primary Sjögren syndrome (pSS) is a chronic inflammatory autoimmune disease characterized by a chronic infiltration of exocrine glands, mainly salivary glands[1]. Disease spectrum is broad[2]. A subset of patients with extraglandular involvement, vasculitis, hypocomplementemia, and cryoglobulinemia may be at increased risk of lymphoma and mortality, thus requiring a close followup[3,4].

Symptomatic cryoglobulinemic vasculitis is observed in about 3%–4% of pSS and it is closely linked to lymphoma[5,6]. In particular, cryoglobulinemia appears to be linked to mucosa-associated lymphoid tissue (MALT) lymphoproliferation in pSS, which is different from hepatitis C virus (HCV)–related cryoglobulinemic vasculitis. However, some key pathogenetic events are shared, e.g., the expansion of rheumatoid factor (RF)–positive B cell clones preferentially using definite heavy and light chain genes[7].

Finally, cryoglobulins are often type II cryoglobulins and persistently positive in pSS with cryoglobulinemic vasculitis or lymphoma[8]. Overall, a heavier B cell commitment to cryoglobulin production appears to be linked to a higher risk of malignancy in pSS.

The objective of our study is to evaluate the clinical, histological, and biological differences between pSS with positive serum cryoglobulins and pSS without serum cryoglobulins. The new tools of European League Against Rheumatism

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Sjögren Syndrome Disease Activity Index (ESSDAI) and the Clinical ESSDAI (ClinESSDAI), recently introduced to standardize the evaluation of disease activity in pSS, have been herein applied.

MATERIALS AND METHODS

We retrospectively evaluated 825 consecutive patients with pSS from 5 centers, all fulfilling the American-European Consensus Group (AECG) criteria. Data were collected regarding sex, age at diagnosis, fulfillment of each item of the AECG criteria, antinuclear antibodies (ANA), RF, low complement factor 3 (C3), low C4, cryoglobulins, ESSDAI, and ClinESSDAI. Determination of laboratory variables was described elsewhere[5]. The patients were negative for anti-HCV antibody and were tested for serum cryoglobulins at least twice[9]. This group of patients corresponded to 83.1% of the whole population. In each center, once the diagnosis of pSS had been made, serum cryoglobulins were routinely tested and then in the followup according to clinical judgment to stratify the patient risk profile to lymphoma development. Cryoglobulin-positive patients with pSS were defined if they presented persistent positivity of serum cryoglobulins (i.e., if they showed 2 consecutive positive results). Disease activity at diagnosis was identified by the ESSDAI[10] and ClinESSDAI scores[11].

There were 786 women (95.3%), and the cohort had a mean age of 52 ± 14 years. The focus score, defined as the number of inflammatory infiltrates of at least 50 cells present in 4 mm^2 of gland surface unit[12], was available in 258 patients (31.3%) and was 2.4 ± 1.9. Median ESSDAI was 6 (range 0–63) and median ClinESSDAI was 6 (range 0–60).

Descriptions of the single ESSDAI domains involved are reported in Table 1.

Missing data accounted for < 1%. No differences regarding the number of laboratory tests or lip biopsies were observed between cryoglobulin-positive and cryoglobulin-negative patients (data not shown).

Comparisons between cryoglobulin-positive and cryoglobulin-negative groups were performed by applying parametric or nonparametric tests for independent samples after verifying the assumption.

Approval for data collection and sharing was obtained by informed consent in each center. To protect patient privacy, anonymizing was guaranteed by using only numeric symbols in the database to identify each patient. Approval by the local ethics committee was not required in accordance with the policies of our institutions.

RESULTS

Seventy-three patients were positive for serum cryoglobulins (8.8%). No differences were observed regarding age at diagnosis and sex (Table 1).

Global systemic disease activity. The ESSDAI score and the ClinESSDAI score were significantly higher in cryoglobulin-positive patients ($p < 0.0001$ for both scores by Mann-Whitney U test; Table 1 and Figure 1).

Single ESSDAI domain involvement. Cryoglobulin-positive patients showed a higher rate of involvement in the following ESSDAI domains: constitutional ($p = 0.003$ by Pearson chi-square), lymphadenopathy ($p = 0.007$ by Pearson chi-square), glandular ($p = 0.0002$ by Pearson chi-square), cutaneous ($p < 0.0001$ by Pearson chi-square), peripheral

Table 1. Clinical, histological, and serological features. Values are n (%) unless otherwise specified.

Variables	Overall, n = 825	Cryoglobulin-positive, n = 73	Cryoglobulin-negative, n = 752	p
Female	786 (95.3)	68 (93.2)	718 (95.5)	0.38
Age at diagnosis, yrs, mean ± SD	51.6 ± 13.9	49.9 ± 16.4	51.7 ± 13.7	0.36
Focus score, median (range)	2 (0–12)	2.85 (1–9)	1.97 (0–12)	0.02
ESSDAI score, median (range)	6 (0–63)	12 (0–50)	5 (0–42)	< 0.0001
ClinESSDAI score, median (range)	6 (0–60)	11.5 (0–48)	5 (0–41)	< 0.0001
ESSDAI domains				
Constitutional	119 (14.4)	19 (26)	100 (13.3)	0.003
Lymphadenopathy	228 (27.6)	30 (41.1)	198 (26.3)	0.007
Glandular	229 (27.8)	34 (46.6)	195 (25.9)	0.0002
Articular	507 (61.5)	45 (61.6)	462 (61.4)	0.97
Cutaneous	120 (14.5)	36 (49.3)	84 (11.2)	< 0.0001
Pulmonary	61 (7.4)	7 (9.6)	54 (7.2)	0.45
Renal	27 (3.3)	5 (6.8)	23 (3)	0.08
Muscular	9 (1.1)	0	9 (1.2)	ND
PNS	54 (6.5)	18 (24.7)	36 (4.8)	< 0.0001
CNS	14 (1.7)	0	14 (1.9)	ND
Hematological	232 (28.1)	31 (42.5)	201 (26.7)	0.004
Biological	447 (54.2)	71 (97.3)	376 (50)	< 0.0001
Serologic profile				
ANA, > 1:40	724 (87.8)	70 (98.6)	654 (87.3)	0.005
Rheumatoid factor	403 (48.8)	64 (87.7)	339 (45.5)	< 0.0001
Low C3	174 (21.1)	32 (43.8)	142 (19)	< 0.0001
Low C4	110 (13.3)	38 (52.8)	72 (9.6)	< 0.0001
Anti-SSA/Ro	580 (70.3)	60 (82.2)	520 (69.1)	0.02
Anti-SSB/La	295 (35.8)	44 (60.3)	251 (33.4)	< 0.0001

EULAR: European League Against Rheumatism; ESSDAI: EULAR Sjögren Syndrome Disease Activity Index; ClinESSDAI: Clinical ESSDAI; PNS: peripheral nervous system; CNS: central nervous system; ANA: antinuclear antibody; C3: complement factor 3; ND: not done because of insufficient data to perform adequate statistical comparisons.

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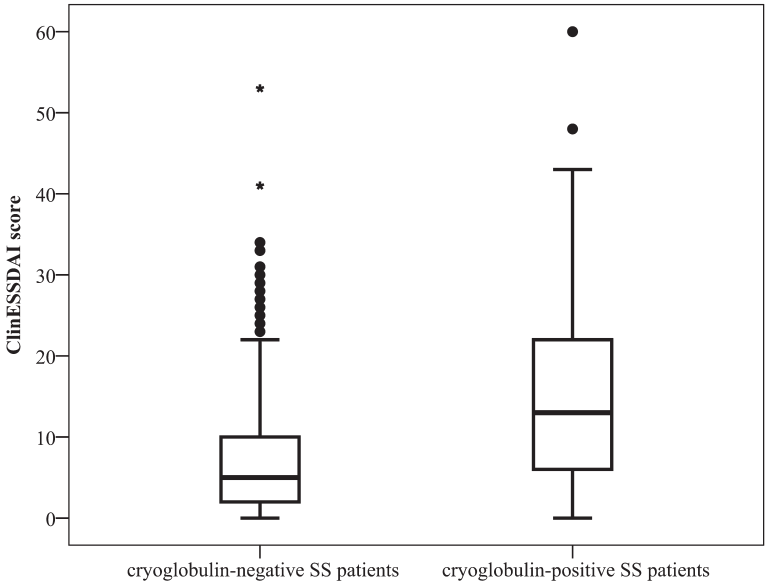

Figure 1. The ClinESSDAI score comparison between SS with or without cryoglobulinemia. EULAR: European League Against Rheumatism; ClinESSDAI: Clinical EULAR Sjögren Syndrome Disease Activity Index; SS: Sjögren syndrome.

nervous system ($p < 0.0001$ by Fisher's exact test), hematological ($p = 0.004$ by Pearson chi-square), and biological ($p < 0.0001$ by Pearson chi-square; Table 1).

Because the single domains of the ESSDAI score may not be independent of one another, an analytical multivariate analysis was performed by considering the presence of serum cryoglobulins as the independent variable, while the following clinical domains selected from the univariate analyses were the dependent variables: constitutional, lymphadenopathy, glandular, cutaneous, and peripheral nervous system. Notably, besides the cutaneous domain (as expected by the presence of purpura, a typical sign of cryoglobulinemia), the glandular domain was also selected (OR 7.4, 95% CI 4.3–12.5, $p < 0.0001$, and OR 2.4, 95% CI 1.4–4.0, $p = 0.002$, respectively).

Histologic infiltrate in the minor salivary glands. Focus score was significantly higher in cryoglobulin-positive patients ($p = 0.022$ by Mann-Whitney U test; Table 1, Figure 2A, and Figure 2B).

Serologic profile. Interestingly, cryoglobulin-positive patients showed a significantly higher rate of positivity of anti-SSB/La antibody ($p < 0.0001$ by Pearson chi-square), while positivity for anti-SSA/Ro was slightly higher ($p = 0.02$ by Pearson chi-square). Additionally, in cryoglobulin-positive patients, ANA positivity (> 1:40) was significantly higher ($p = 0.005$ by Pearson chi-square), as well as RF ($p < 0.0001$ by Pearson chi-square), low C3 ($p < 0.0001$ by Pearson chi-square), and low C4 ($p < 0.0001$ by Pearson chi-square), as largely expected for these last 3 variables given the presence of serum cryoglobulins (Table 1).

DISCUSSION

Primary SS is usually considered an indolent systemic autoimmune disease, affecting quality of life rather than mortality[4]. However, a subset of patients requires a closer followup owing to a higher risk of complications[5]. This subgroup of patients is clearly identified by some serological hallmarks, such as hypocomplementemia, lymphopenia, anti-SSB/La autoantibody, monoclonal gammopathy, increased titers of RF, and cryoglobulinemia[13,14]. In particular, cryoglobulinemia can help the clinician to identify patients with pSS at high risk of extraglandular manifestations and lymphoma[15,16]. Because many targeting therapies would be available for pSS, defining different subsets of disease is very important, and serological markers might help in the early identification of patients who are candidates to receive treatments to prevent the development of complications[14].

The aim of our study was to compare patients with pSS with or without serum cryoglobulins by using validated tools to assess disease activity in pSS (i.e., ESSDAI and ClinESSDAI).

Our data demonstrated that systemic disease activity was higher in pSS with serum cryoglobulins. Importantly, it was confirmed by also applying the ClinESSDAI score, which was derived from the ESSDAI score after excluding the biological domain, where cryoglobulinemia is present[11], thus avoiding redundancy. Notably, the ESSDAI domains significantly linked to the presence of cryoglobulinemia were not only those obviously related to the clinical appearance of cryoglobulinemic vasculitis (i.e., constitutional, cutaneous, peripheral nervous system), but also those domains that

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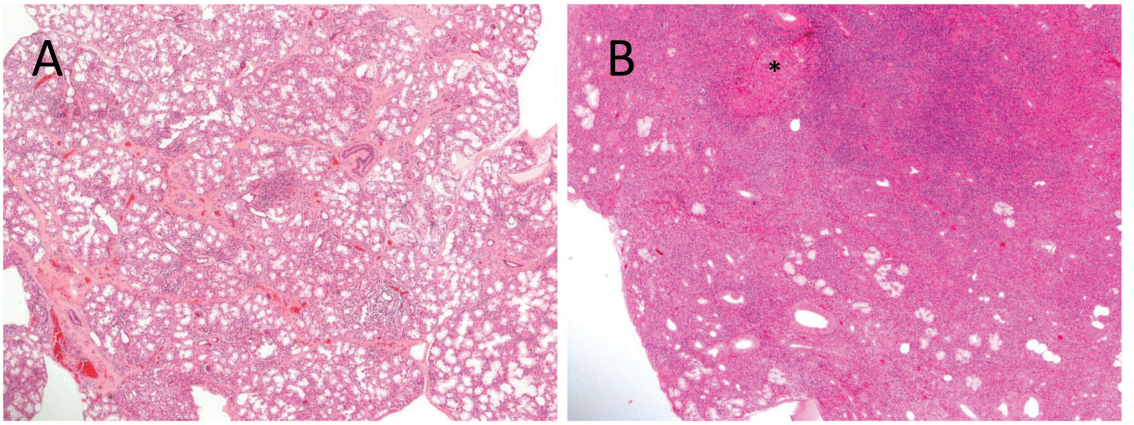

Figure 2. (A) Example of lip biopsy showing a low focus score in a patient with SS without cryoglobulinemia (H&E staining, 40× magnification). (B) Example of lip biopsy showing a high focus score in a patient with SS with cryoglobulinemia. * A myoepithelial island is highlighted (H&E staining, 40× magnification). SS: Sjögren syndrome.

represent local proliferation of MALT in pSS (i.e., glandular). This feature was even noted at the multivariate level of significance. Thus, a link between B cell expansion in MALT tissue, presence of serum cryoglobulins, and systemic disease activity may be highlighted, supporting the hypothesis that the biologic background of cryoglobulinemia in pSS is lymphoproliferation of MALT[17]. The herein reported observation of a higher focus score in cryoglobulin-positive patients with pSS can support this conclusion. This is not surprising, since ectopic germinal center-like structures in the minor salivary glands have been associated with lymphoma development in pSS[18] and the focus score appeared to be independently associated with lymphoma[19]. Finally, cryoglobulin-positive patients explained the highest level of systemic biologic activity. Thus the key prognostic value of cryoglobulins in pSS suggests that all patients should be tested for serum cryoglobulins at least at the time of pSS diagnosis, and the test should be repeated during the followup in all patients presenting a suspicion of vasculitis or lymphoma. Moreover, anti-SSB/La autoantibodies were significantly linked to cryoglobulinemia. Thus, the positivity of anti-SSA/Ro and anti-SSB/La antibodies may have a greater prognostic significance as a hallmark of a more aggressive pSS subset with more pronounced B cell proliferation[20], and physicians should be alert for possible cryoglobulinemia in pSS.

Practically, given that difficulties may arise in real-world settings with the measurement of serum cryoglobulins, patients with pSS could be removed from this analysis if they are at low risk of cryoglobulin positivity, i.e., RF-negative with normal levels of serum complement fractions, or RF-negative and anti-SSA/Ro and anti-SSB/La–negative.

Because our study was retrospective, a selection bias may have occurred. Patients with cryoglobulins would have had deeper and more extensive laboratory testing and clinical evaluation because of the known association of cryoglobulins with extraglandular manifestations and lymphoma in pSS. However, the frequency of serum cryoglobulins in the whole cohort was almost 9%, consistent with previous data[2], and the number of patients who had a lip biopsy, as well as an extensive laboratory evaluation, did not differ between cryoglobulin-positive and cryoglobulin-negative patients.

Cryoglobulinemia in pSS is a clear red flag for a possible progression of the disease from an indolent to an aggressive state. The test should be done for all patients at diagnosis and possibly repeated in the followup according to the clinical indication. Glandular swelling and lymphadenopathy are linked to cryoglobulinemia, thus the need is reinforced to target MALT to prevent lymphoma and extraglandular manifestations in pSS[14].

ACKNOWLEDGMENT

We thank our pathologist Cathryn Anne Scott, MD, for providing the histopathologic images, and Professor Miriam Isola for her support in the statistical analyses.

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