How immunological profile drives clinical phenotype of primary Sjögren syndrome at diagnosis: Analysis of 10,500 patients (Sjögren Big Data Project)

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SHORT TITLE: Immunological profile in the Sjögren Big Data Cohort

ABSTRACT

Objective. To evaluate the influence of the main immunological markers on the disease phenotype at diagnosis in a large international cohort of patients with primary SjS.

Methods. The Big Data Sjögren Project Consortium is an international, multicentre registry created in 2014. As a first step, baseline clinical information from leading centers on clinical research in SjS of the 5 continents was collected. The centers shared a harmonized data architecture and conducted cooperative online efforts in order to refine collected data under the coordination of a big data statistical team. Inclusion criteria were the fulfilment of the 2002 classification criteria. Immunological tests were carried out using standard commercial assays

Results. By January 2018, the participant centres had included 10500 valid patients from 23 countries. The cohort included 9806 (93%) women and 694 (7%) men, with a mean age at diagnosis of primary SjS of 53 years, mainly White (78%) and included from European countries (71%). The frequency of positive immunological markers at diagnosis was 79.3% for ANA, 73.2% for anti-Ro, 48.6% for RF, 45.1% for anti-La, 13.4% for low C3 levels, 14.5% for low C4 levels and 7.3% for cryoglobulins. Positive autoantibodies (ANA, Ro, La) correlated with a positive result in salivary gland biopsy, while hypocomplementemia and especially cryoglobulinemia correlated with systemic activity (mean ESSDAI score of 17.7 for cryoglobulins, 11,3 for low C3 and 9.2 for low C4, in comparison with 3.82 for negative markers). The immunological markers with a great number of statistically-significant associations ($p<0.001$) in the organ-by-organ ESSDAI evaluation were cryoglobulins (9 domains), low C3 (8 domains), anti-La (7 domains) and low C4 (6 domains).

Conclusion. We confirm the strong influence of immunological markers on the phenotype of primary SjS at diagnosis in the largest multi-ethnic international cohort ever analysed, with a greater influence for cryoglobulinemic-related markers in comparison with Ro/La autoantibodies and ANA. Immunological patterns play a central role in the phenotypic expression of the disease already at the time of diagnosis, and may guide physicians to design a specific personalized management during the follow-up of patients with primary SjS.

KEY WORDS: primary Sjögren syndrome, salivary gland biopsy, Ro/La autoantibodies, hypocomplementemia, cryoglobulinemia, ESSDAI

INTRODUCTION

Primary Sjögren syndrome (SjS) is a systemic autoimmune disease that mainly affects middle-aged women with a frequency in general population ranging between 0.01 and 0.72% [1]. Etiopathogenically, the disease targets the exocrine glands that are infiltrated by lymphocytes (focal sialadenitis)[2]. More than 95% of patients present with oral and/or ocular dryness[3], although they may also develop a wide number of systemic (extraglandular) manifestations, which may be the first clinical manifestation of the disease [4].

Patients with primary SjS produce a wide variety of circulating autoantibodies directed to antigens either nuclear or cytoplasmic; in some cases, the target antigen is present within specific tissues. B lymphocyte hyperactivation, the most typical immunopathogenic peripheral abnormality of primary SS, accounts for these autoantibodies[5,6]. Immunological markers play a central role not only in the diagnosis of the disease, but also in predicting their outcome as prognostic markers[7]. The key immunological markers are anti-Ro antibodies, as the most specific SjS-related autoantibody, and cryoglobulins and hypocomplementaemia, as the main prognostic markers[8]. Among the variety of immunological markers, rheumatoid factor (FR) and anti-La antibodies are found in nearly half the patients with primary SjS, and although are not included in the recent ACR/EULAR set of classification criteria[9], they should clinically considered as key immunological markers of the disease[10,11]. Previous studies in large multicentre national registries have analysed the association between immunological markers and the clinical disease phenotype[3,11–13], with heterogeneous results, although most identified patients carrying anti-Ro/La antibodies as the subset with the most clinically and immunologically “active” phenotype[14].

The objective of this study was to evaluate the influence of the main immunological markers on the disease phenotype at diagnosis in a large international cohort of patients with primary SjS.

METHODS

Patients

The Big Data Sjögren Project Consortium is an international, multicentre registry established in 2014 to take a "high-definition" picture of the main features of primary SjS following a worldwide data-sharing cooperative merging of pre-existing clinical SjS databases from leading centers on clinical research in SjS of the 5 continents (see reference 14 for additional methodological details[15]). The centers share a harmonized data infrastructure and conduct cooperative online efforts in order to refine already collected data in each center. Inclusion criteria were the fulfilment of the 2002 classification criteria[16]. Exclusion criteria for considering SjS as a primary disease were chronic HCV/HIV infections, previous lymphoproliferative processes, and associated systemic autoimmune diseases. Diagnostic tests for SjS (ocular tests, oral tests and salivary gland biopsy) were carried out according to the recommendations of the European Community Study Group[17]. The study was approved by the Ethics Committee of the Coordinating Centre (Hospital Clinic, Barcelona, Spain, registry HCB/2015/0869).

Definition of variables

Disease diagnosis was defined as the time when the attending physician confirmed fulfilment of the 2002 criteria. At this time, the main features of the disease were retrospectively collected and analysed. The following clinical variables were selected in order to be harmonized and further refined: age, gender, ethnicity, country of residence, fulfilment of the 2002 criteria items, antinuclear antibodies, rheumatoid factor, C3 and C4 levels, cryoglobulins, and organ-by-organ ESSDAI scores. By January 2018, the participant centres had included 10500 valid patients from 23 countries. Systemic involvement at diagnosis was retrospectively classified and scored according to the ESSDAI[18], which evaluates 12 domains or organ systems, and clinESSDAI[19], which evaluates the same domains but excluding the last (biological domain). Each domain is divided into 3-4 levels according to the degree of activity and scored as 0 (no activity), 1 (low activity), 2 (moderate activity) or 3 (high activity). Immunological tests were carried out using standard commercial assays (>95% of cases), using indirect immunofluorescence to detect ANA, ELISA to detect Ro/La antibodies, nephelometry for measuring RF and complement levels, and serum cryoglobulins by standard

measure as previously described[20]. We divided the results obtained according to the following two immunological subsets: patients with autoantibodies (ANA, Ro, La) and those presenting with cryoglobulin-related markers (RF, complement levels, cryoglobulins).

Statistical analysis

Descriptive data are presented as mean and standard deviation (SD) for continuous variables and numbers and percentages (%) for categorical variables. The Chi-square test was used to study the association between immunological markers with gender, diagnostic tests for SjS and systemic involvement. T-test was used to compare the mean age at diagnosis. All significance tests were two-tailed. P-values were adjusted for multiple comparisons using the false discovery rate (FDR) correction and values of $p < 0.001$ were considered significant to avoid false positive significant results. A heatmap was constructed to represent the association pattern between immunological markers and disease phenotype. All analyses were conducted using the R V.3.2.3 for Windows statistical software package (https://www.R-project.org/).

RESULTS

The baseline characteristics of the cohort are summarized in **Table 1**. The cohort included 9806 (93%) women and 694 (7%) men (female: male ratio, 14:1), with a mean age at diagnosis of primary SjS of 53.1 (SD 14.1) years, mainly White (78%) and included predominantly from European countries (71%). Dry mouth was reported by 9832 (94%) of patients, dry eyes by 9684 (92%), abnormal ocular tests in 8167/9745 (84%), abnormal oral tests in 6373/8115 (78%) and positive salivary gland biopsy in 6368/7777 (82%) patients.

a) Phenotype of patients carrying autoantibodies

ANA+ patients. ANA were tested in 9784 patients, and were positive in 7749 (79%). ANA-positive patients had a lower mean age at diagnosis (52 vs 56 yrs), a higher frequency of abnormal ocular tests (86% vs 82%), positive biopsy (84% vs 79%), mean ESSDAI score (6.7 vs 4.5) and a higher frequency of activity in the lymphadenopathy (10% vs 5%), articular (40% vs 35%), cutaneous (11% vs 4%), hematological (25% vs 11%) and biological (57% vs 31%) ESSDAI domains in comparison with ANA-negative patients (**Table 2**).

Ro+ patients. Ro autoantibodies were tested in 10417 patients and were positive in 7617 (73%). Ro-positive patients had a lower mean age at diagnosis (52 vs 57 yrs), had a lower frequency of dry mouth (91% vs 95%) and dry eyes (92% vs 97%), a lower frequency of positive biopsy (74% vs 96%), a higher mean ESSDAI score (6.7 vs 4.7) and a higher frequency of activity in the constitutional (10% vs 7%), cutaneous (11% vs 5%), renal (5% vs 2%), hematological (26% vs 13%) and biological (58% vs 31%) ESSDAI domains in comparison with Ro-negative patients (**Table 2**).

a3. La+ patients. La autoantibodies were tested in 10362 patients and were positive in 4662 (45%). La-positive patients had a lower mean age at diagnosis (51 vs 54 yrs), had a higher frequency of ocular (86% vs 82%) and oral (81% vs 76%) diagnostic tests, a lower frequency of positive biopsy (73% vs 87%), a higher mean ESSDAI score (7.2 vs 4.3) and a higher frequency of activity in the constitutional (11% vs 7%), lymphadenopathy (10% vs 8%), glandular (24% vs 19%), cutaneous (12% vs 7%), renal (6% vs 3%), muscular (3% vs 2%), hematological (28% vs 18%) and biological (65% vs 40%) ESSDAI domains in comparison with La-negative patients (**Table 2**).

Ro/La combination patterns. The 3 different combination patterns of anti-Ro/La antibodies (isolated Ro, isolated La and combined Ro and La) were associated with differentiated phenotypes (**Table 3**). Patients with isolated La+ had the highest frequency of dry eye (p=0.001) and active glandular and muscular domains (p<0.001), while patients carrying both autoantibodies showed the highest frequency of abnormal ocular and oral (p<0.001) diagnostic tests, and the highest frequencies of systemic activity in the constitutional, lymphadenopathy, cutaneous, renal, hematological and biological ESSDAI domains (p<0.001).

b) Phenotype of patients with cryoglobulin-related markers

RF+ patients. RF was tested in 8758 patients and was positive in 4245 (48.5%). RF-positive patients had a lower mean age at diagnosis (51 vs 54 yrs), had a higher frequency of abnormal ocular (88% vs 83%) and oral (82% vs 76%) tests, a higher mean ESSDAI score (7.3 vs 5.6) and a higher frequency of activity in the glandular (26% vs 19%), articular (44% vs 37%), cutaneous (12% vs 8%), hematological (29% vs 18%) and biological (66% vs 39%) ESSDAI domains in comparison with RF-negative patients (**Table 4**).

Cryoglobulinemic patients. Cryoglobulins were tested in 4732 patients, and were positive in 342 (7%). Cryoglobulinemic patients had a higher frequency of abnormal oral tests (87% vs 76%), a higher mean ESSDAI score (17.7 vs 7.2) and a higher frequency of activity in the constitutional (25% vs 11%), lymphadenopathy (23% vs 10%), glandular (39% vs 28%), cutaneous (38% vs 11%), renal (15% vs 5%), muscular (8% vs 3%), PNS (24% vs 7%), CNS (6% vs 2%), hematological (44% vs 25%) and biological (91% vs 50%) ESSDAI domains in comparison with non-cryoglobulinemic patients (**Table 4**).

C4 hypocomplementemic patients. C4 values were measured in 8556 patients and were low in 1234 (14%). C4-hypocomplementemic patients had a lower mean age at diagnosis (51 vs 53 yrs), had a lower frequency of positive biopsy (75% vs 81%), a higher mean ESSDAI score (9.2 vs 6.0) and a higher frequency of activity in the constitutional (13% vs 10%), lymphadenopathy (13% vs 8%), cutaneous (18% vs 9%), renal (7% vs 4%), PNS (12% vs 5%), hematological (37% vs 21%) and biological (85% vs 47%) ESSDAI domains in comparison with C4-normocomplementemic patients (**Table**

4).

C3 hypocomplementemic patients. C3 values were measured in 8573 patients and were low in 1146 (13%). C3-hypocomplementemic patients had a lower mean age at diagnosis (49 vs 53 yrs), had a lower frequency of dry mouth (89% vs 94%) and dry eyes (89% vs 92%), a higher mean ESSDAI score (11.3 vs 5.7) and a higher frequency of activity in the constitutional (17% vs 9%), lymphadenopathy (18% vs 8%), cutaneous (22% vs 8%), pulmonary (15% vs 10%), renal (11% vs 4%), PNS (14% vs 5%), CNS (3% vs 2%), hematological (43% vs 21%) and biological (86% vs 48%) ESSDAI domains in comparison with C3-normocomplementemic patients (**Table 4**).

DISCUSSION

In the three last internationally-accepted classification criteria for primary SjS[9,16,17], autoantibodies have always been one of the included criteria and always the only laboratory criterion. However, the number of autoantibodies accepted as criteria has been reduced progressively. The 1993 European Criteria included 4 antibodies (ANA, RF, Ro/SS-A, and/or La/SS-B), the 2002 Criteria 2 (anti-Ro/SS-A and anti-La/SS-B) and the 2016 ACR/EULAR, only one (Ro/SS-A)[9,16,17], in the search for a significant improvement of sensitivity and especially specificity. However, the figures for sensitivity/specificity obtained in the three sets of criteria are quite similar (0.93/0.94 for the 1993 criteria, 0.96/0.94 for the 2002 criteria, and 0.96/0.95 for the 2016 criteria). In contrast, other immunological markers (cryoglobulins, hypocomplementemia) that are strongly associated with disease prognosis and outcomes have been never included in the criteria. In this worldwide study, we have confirmed the close association of all these immunological markers with the phenotype of the disease at the time of diagnosis in the largest cohort of primary SjS patients ever studied.

We found ANA in 80% of patients with primary SjS, and as much the immunological marker most frequently detected. ANA+ patients had a specific phenotype (older age at diagnosis, higher frequency of abnormal diagnostic tests, higher mean ESSDAI and a higher frequency of activity in the lymphadenopathy, cutaneous and laboratory-related domains) (**Figure 1**). Some of these features may be related to a late diagnosis (age, enhanced frequency of diagnostic and laboratory tests) in comparison with patients with negative ANA, who are often diagnosed earlier on the basis of systemic features and positive anti-Ro[21] (nearly 10% of Ro+ patients may be ANA negative[22]). However, the figures for the main systemic features are quite similar to that found in patients with anti-Ro antibodies, suggesting that a positive ANA result does not add specific value to the phenotype observed in anti-Ro carriers. Probably, the key usefulness of testing ANA would be the early suspicion of the disease in non-specialized healthcare settings. Since ANA are the most frequent autoantibodies in primary SjS and their detection is overwhelmingly available in standard healthcare settings, a positive result in a patient presenting with sicca features could help primary care physicians and other specialists to suspect an autoimmune origin of sicca symptoms and therefore, to refer the patient to the autoimmune specialist to discard the disease.

We found anti-Ro antibodies in 73% of our patients, a figure very close to that found for ANA. This is a logical consequence of the strong weight of these autoantibodies in the classification criteria used (2002), as mandatory criteria together with salivary biopsy. Various studies have correlated the presence of anti-Ro with most of the SjS-related features, including parotidomegaly, lymphadenopathy, cutaneous vasculitis, neurologic disease and serologic hallmarks such as the presence of hypergammaglobulinemia, rheumatoid factor and cryoglobulins[10]. Our results confirm a specific phenotype consisting of patients diagnosed at younger age, with a lower frequency of sicca syndrome and positive salivary gland biopsy, and a higher frequency of activity in the constitutional, cutaneous and laboratory ESSDAI domains. A recent study by Quartuccio et al compared Ro/La+ and Ro/La- patients[23] and found a younger age at diagnosis and a higher frequency of glandular swelling, purpura, leukopenia, lymphoma, low C3, low C4, hypergammaglobulinemia, rheumatoid factor and serum cryoglobulins in Ro/La+ patients, while we have recently reported that anti-Ro/SS-A and anti-La/SS-B antibodies were also associated with global systemic activity, especially anti-Ro/SS-A, whose positivity at diagnosis also correlated with a higher activity score in the articular, cutaneous and renal domains in a Spanish multicentre study[3].

Anti-La antibodies were detected in the 45% of our patients and overwhelmingly associated with the presence of anti-Ro antibodies (95% of cases). Probably for this reason, the phenotype of La carriers was very similar to that reported for Ro carriers. However, when we analysed the phenotype of Ro/La patients according to the different antibody combinations, we found that the most striking phenotypic differences were found in patients carrying the two antibodies in comparison with those who carried only a single antibody, with a higher frequency of abnormal diagnostic tests, the highest mean ESSDAI score among the three groups, and the highest frequency of systemic activity in nearly all the ESSDAI domains (especially in the constitutional, lymphadenopathy, cutaneous, renal and hematological domains) (**Figure 1**). In a previous study, Locht et al[24] reported a higher frequency of internal organ involvement in patients carrying anti-La and anti-Ro in comparison with those carrying anti-Ro alone, and other studies also reported similar results[25,26]. In contrast, recent studies have reported a lower frequency of abnormal diagnostics tests (Schirmer test, UWSF and salivary gland biopsy) in isolated La carriers[27,28]. The influence of Ro/La on the

phenotypic expression of primary SjS at diagnosis could be driven by immunogenetic differences. The presence of these autoantibodies has been significantly linked with specific HLA-D epitopes (B1*03 and QB1*02, an association even more prominent and extended to QA1*0501 when patients were stratified according to the presence of La/SSB autoantibodies[29], suggesting a similar (but not identical) genetic susceptibility for Ro and La carriers.

Rheumatoid factor was detected in nearly half our patients, who also showed a specific phenotype consisting of a young age at diagnosis, a higher frequency of abnormal diagnostic tests, a high mean ESSDAI score, and a high frequency of systemic activity in the glandular, articular, cutaneous and hematological domains (**Figure 1**). Previous studies reported that RF has an independent association with the main clinical and immunological features of the disease[10], and we found recently that RF was associated with a higher ESSDAI score both at diagnosis and at the end of follow-up[30]. Thus, RF detection in primary SjS is clinically useful, especially for the diagnosis of some subsets of patients with primary SS, such as those with extraglandular manifestations or with circulating cryoglobulins.

Cryoglobulinemia had no influence on the glandular disease expression for both subjective and objective glandular features (except for an increased frequency of abnormal oral diagnostic tests), but play a key role in driving a multisystemic phenotype with statistically-significant higher frequencies in all ESSDAI domains but one (articular) (**Figure 1**). In fact, patients with cryoglobulinemia showed the highest mean ESSDAI among all the immunological subsets, being 4-fold higher than the mean score found in patients with no immunological markers and 3-fold higher than that found in ANA+ or Ro+ patients (**Figure 2**). This is closely related to the presence of a systemic vasculitic process, since although many patients with cryoglobulinemia remain asymptomatic, the percentage of patients with circulating cryoglobulins who develop vasculitic symptoms in primary SjS is 35%[20]. The presence of cryoglobulinemic vasculitis at the diagnosis of primary SS is independently associated with mortality, and is closely linked with a higher baseline ESSDAI score[31].

In previous studies in multicentre national cohorts, we found a significant association between low complement levels and the main systemic SS features, including both extraglandular disease (fever, articular involvement, cutaneous vasculitis, and

peripheral neuropathy) and immunological markers (cryoglobulinemia, rheumatoid factor)[7,32], and recently Shiboski et al[33] have reported that sicca patients with hypocomplementemia were 6 times more likely to progress to definite SjS. In addition, hypocomplementemia is also closely associated with the two main adverse outcomes of primary SS (lymphoma development and death)[34], although two studies[7,35] reported a predominant role for low C4. This study is the first to analyse separately the phenotype associated with either low C4 or low C3 values, and we found significant differences. Patients with C4-hypocomplementemia were older and had an enhanced frequency of positive salivary biopsy, while those with C3-hypocomplementemia were younger and had a lower frequency of sicca symptoms. Both subsets of patients showed higher mean ESSDAI scores (**Figure 2**) and a close association with systemic activity in the ESSDAI domains, although systemic activity was more pronounced in C3-hypocomplementemic patients (**Figure 1**). This is a new finding, in contrast with previous studies carried out in more geographically-homogeneous populations that showed a predominant role for low C4 levels. Probably, the different degree of association between hypocomplementemia and cryoglobulinemia (cryoglobulinemia is more frequently associated with consumption of C4 factor) could explain these differences with previous studies, since the frequency of cryoglobulinemia is strongly influenced by geographical and ethnicity determinants[15].

The results of this study, however, should be interpreted with caution, and some limitations should be pointed out. Studies including clinical big data may detect some differences which, although statistically significant, may not be relevant clinically, and further studies are necessary to confirm their clinical relevance in smaller, but more homogeneous, populations. This was the reason why we considered statistically-significant p-values less than 0.001 after adjusting for multiple comparisons using the false discovery rate. The predominant presence of European patients could also limit the generalization of the results in other ethnic subpopulations less frequently reported. Other sources of heterogeneity may include the variable amount of missing data for some variables and the immunological assays used by the different centers, although all are commercial tests and more than 80% used the same technique (ELISA) to test for Ro/La autoantibodies and ANA were overwhelmingly tested for by indirect immunofluorescence.

In summary, we confirm a strong influence of immunological markers on the phenotype of primary SjS at diagnosis in the largest multi-ethnic international cohort ever analysed, with a greater influence for cryoglobulinemic-related markers in comparison with Ro/La autoantibodies and ANA. Immunological patterns play a central role in the phenotypic expression of the disease already at the time of diagnosis, and may guide physicians to design a specific personalized management during the follow-up of patients with primary SjS.

APPENDIX 1

Members of the EULAR-SS Task Force Big Data Consortium:

a) Members of the EULAR-SS Task Force

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FIGURE LEGENDS

Figure 1. Heat map of the main statistically-significant associations (adjusted P-values < 0.001) between immunological markers and disease phenotype.

Figure 2. Mean ESSDAI score (2a) and clinESSDAI score (2b) according to each immunological marker.