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


1 Autoimmune Diseases Unit, Department of Medicine, Hospital CIMA·Sanitas, Barcelona, Spain.
2 Sjögren Syndrome Research Group (AGAUR), Laboratory of Autoimmune Diseases Josep Font, IDIBAPS-CELLEX, Department of Autoimmune Diseases, ICMID, University of Barcelona, Hospital Clinic, Barcelona, Spain.
3 Department of Statistics, Faculty of Science and Letters, Mimar Sinan Fine Arts University, Istanbul, Turkey.
4 Institute of Cellular Medicine, Newcastle University, Newcastle Upon Tyne, UK.
5 Division of Clinical Immunology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary.
6 Arthritis and Clinical Immunology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK, USA.
7 Department of Rheumatology, Skane University Hospital Malmö, Lund University, Malmö, Sweden.
8 Center for Immunology of Viral Infections and Autoimmune Diseases, Assistance Publique – Hôpitaux de Paris, Hôpitaux Universitaires Paris-Sud, Le Kremlin-Bicêtre, Université Paris Sud, INSERM, Paris, France.
9 Department of Rheumatology and Immunology, Anhui Provincial Hospital, Hefei, China.
10 Rheumatology Unit, University of Pisa, Pisa, Italy.
11 Department of Rheumatology, Strasbourg University Hospital, Université de Strasbourg, CNRS, Strasbourg, France.
12 Department of Clinical Immunology & Rheumatology, Christian Medical College & Hospital, Vellore, India.
13 Clinic of Rheumatology, Department of Medical Area (DAME), University Hospital "Santa Maria della Misericordia", Udine, Italy.
14 Department of Internal Medicine and Medical Specialties, Rheumatology Clinic, Sapienza University of Rome, Rome, Italy.
15 Immunology and Rheumatology Department, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán. México City, Mexico.
16 Department of Internal Medicine, Hacettepe University, Faculty of Medicine, Ankara, Turkey.

* SJOGREN BIG DATA CONSORTIUM: http://www.sjogrenbigdata.org
17 Department of Rheumatology and Clinical Immunology, University Medical Center Utrecht, Utrecht, The Netherlands.
18 Seoul St. Mary's Hospital, The Catholic University of Korea, Seoul, South Korea.
19 Department of Medicine, Solna, Unit of Rheumatology, Karolinska Institutet, and Karolinska University Hospital, Stockholm, Sweden.
20 Department of Rheumatology, University Medical Centre, Ljubljana, Slovenia.
21 Department of Internal Medicine, Lariboisière Hospital, Assistance Publique-Hôpitaux de Paris, Paris Diderot University, Paris, France.
22 Rheumatology Unit, Department of Medicine, University of Perugia, Perugia, Italy.
23 Department of Internal Medicine, Hospital Vall d’Hebron, Barcelona, Spain.
24 Department of Rheumatology, School of Medicine, The University of Western Australia, Crawley, Australia.
25 Division of Rheumatology, Kanazawa University Hospital, Kanazawa, Ishikawa, Japan.
26 Centre for Rheumatology, Division of Medicine, University College London, London, UK.
27 Department of Medicine, Federal University of Espirito Santo and University Hospital HUCAM/EBSERH, Vitória, Brazil.
28 Department of Rheumatology and Internal Medicine, Wroclaw Medical Hospital, Wroclaw, Poland
29 Rheumatology, Department of Medical Sciences, Uppsala University, Uppsala, Sweden.
30 Department of Internal Medicine, Hospital Ramón y Cajal, Madrid, Spain.
31 Department of Rheumatology & Clinical Immunology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands.
32 Department of Radiology and Cancer Biology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan
33 Clinical Unit of Rheumatology, University of l’Aquila, School of Medicine, L’Aquila, Italy.
34 Rheumatology Department, Brest University Hospital, Brest, France.
35 Otorhinolaryngology/Head and Neck Surgery, Klinikum rechts der Isar, Technical University Munich, Munich, Germany.
36 Centre for Experimental Medicine and Rheumatology, Queen Mary University of London, London, UK.
37 Federal University of São Paulo, Sao Paulo, Brazil.
38 Department of Clinical Science, University of Bergen; and Department of Rheumatology, Haukeland University Hospital, Bergen, Norway.
39 Rheumatology Division, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (USP), São Paulo, Brazil.
40 Hospital Privado Universitario de Córdoba, Instituto Universitario de Ciencias Biomédicas de Córdoba (IUCBC), Instituto De Investigaciones En Ciencias De La Salud (INICSA), Universidad Nacional de Córdoba (UNC), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) - Córdoba - Argentina.
41 Rheumatology Department, Kasr Al Ainy School of Medicine, Cairo University, Cairo, Egypt.
42 IRCCS Galeazzi Orthopedic Institute, Milan, Rheumatology Unit, University of Messina, Italy.
43 Department of Rheumatology, Montpellier University Hospital and University of Montpellier, Montpellier, France.
44 German Hospital, Buenos Aires, Argentina.

*The members of the Sjögren Big Data Consortium are listed in Appendix 1

Address reprint requests to: Dr. Manuel Ramos-Casals, Servei de Malalties Autoimmunes Sistèmiques, Hospital Clinic, C/Villarroel, 170, 08036-Barcelona, Spain. Phone: 34-93-2275774.
FAX: 34-93-2271707. e-mail: mramos@clinic.ub.es

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 (RF) 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 be 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). 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. 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. 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, which evaluates 12 domains or organ systems, and clinESSDAI, 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\textsuperscript{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
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\textsuperscript{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)\textsuperscript{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\textsuperscript{21} (nearly 10% of Ro+ patients may be ANA negative\textsuperscript{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. 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 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. 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 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. In contrast, recent studies have reported a lower frequency of abnormal diagnostics tests (Schirmer test, UWSF and salivary gland biopsy) in isolated La carriers. 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 Q81*02, an association even more prominent and extended to QA1*0501 when patients were stratified according to the presence of La/SSB autoantibodies, 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, and we found recently that RF was associated with a higher ESSDAI score both at diagnosis and at the end of follow-up. 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%. 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.

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)\textsuperscript{7,32}, and recently Shiboski et al\textsuperscript{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)\textsuperscript{34}, although two studies\textsuperscript{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\textsuperscript{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

P. Brito-Zerón, C. Morcillo (Autoimmune Diseases Unit, Department of Medicine, Hospital CIMA - Sanitas, Barcelona, Spain); P. Brito-Zerón, A. Flores-Chavez, M. Ramos-Casals (Sjögren Syndrome Research Group AGAUR, Laboratory of Autoimmune Diseases Josep Font, IDIBAPS-CELLEX, Department of Autoimmune Diseases, ICMiD, University of Barcelona, Hospital Clinic, Barcelona, Spain); N. Acar-Denizli (Department of Statistics, Faculty of Science and Letters, Mimar Sinan Fine Arts University, Istanbul, Turkey); F. Ng (Institute of Cellular Medicine, Newcastle University, Newcastle Upon Tyne, UK); M. Zeher, Ildike-Fanny Horvath (Division of Clinical Immunology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary); A. Rasmussen, K. Sivils, H. Scofield (Arthritis and Clinical Immunology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK, USA); R. Seror, X. Mariette (Center for Immunology of Viral Infections and Autoimmune Diseases, Assistance Publique – Hôpitaux de Paris, Hôpitaux Universitaires Paris-Sud, Le Kremlin-Bicêtre, Université Paris Sud, INSERM, Paris, France Paris, France); T. Mandi, P. Olsson (Department of Rheumatology, Malmö University Hospital, Lund University, Lund, Sweden); X. Li (Department of Rheumatology and Immunology, Anhui Provincial Hospital, China); C. Baldini (Rheumatology Unit, University of Pisa, Pisa, Italy); J.E. Gottenberg (Department of Rheumatology, Strasbourg University Hospital, Université de Strasbourg, CNRS, Strasbourg, France); D. Danda, P. Sandhya (Department of Clinical Immunology & Rheumatology, Christian Medical College & Hospital, Vellore, India); L. Quartuccio, L. Corazza, S De Vita (Clinic of Rheumatology, Department of Medical and Biological Sciences, University Hospital "Santa Maria della Misericordia", Udine, Italy); R. Priori, E. Bartoloni (Department of Internal Medicine and Medical Specialties, Rheumatology Clinic, Sapienza University of Rome, Italy); G. Hernandez-Molina, J. Sánchez-Guerrero (Immunology and Rheumatology Department, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, México City, Mexico); A.A. Kruize, E. van der Heijden (Department of Rheumatology and Clinical Immunology, University Medical Center Utrecht, Utrecht, The Netherlands); V. Valim (Department of Medicine, Federal University of Espírito Santo, Vitória, Brazil); M. Kvarnstrom, M. Wahren-Herlenius (Department of Medicine, Solna, Unit of Experimental Rheumatology, Karolinska Institutet, and Karolinska University Hospital, Stockholm); D. Sene (Service de Médecine Interne 2, Hôpital Lariboisière, Université Paris VII, Assistance Publique-Hôpitaux de Paris, 2, Paris, France); R. Gerli (Rheumatology Unit, Department of Medicine, University of Perugia, Italy); S. Praprotnik (Department of Rheumatology, University Medical Centre, Ljubljana, Slovenia); D. Isenberg (Centre for Rheumatology, Division of Medicine , University College London , UK); R. Solans (Department of Internal Medicine, Hospital Vall d’Hebron, Barcelona, Spain); M. Rischmueller, S. Downie-Doyle (Department of Rheumatology, School of Medicine, The University of Western Australia, Crawley, Australia); S-K. Kwok, S-H. Park (Seoul St. Mary’s Hospital, The Catholic University of Korea, Seoul); G. Nordmark (Rheumatology, Department of Medical Sciences, Uppsala University, Uppsala, Sweden); Y. Suzuki, M. Kawano (Division of Rheumatology , Kanazawa University Hospital , Kanazawa , Ishikawa , Japan); R. Giacomelli, F. Carubbi (Clinical Unit of Rheumatology, University of l’Aquila, School of Medicine, L’Aquila, Italy); V. Devauchelle-Pensec, A. Saraux (Rheumatology Department, Brest University Hospital, Brest, France); M. Bombardieri, E. Astorri (Centre for Experimental Medicine and Rheumatology, Queen Mary University of London, UK); B. Hofauer, A. Knopf (Hals-Nasen-Ohrenklinik und Poliklinik, Technische Universität München, München, Germany); H. Bootsma, A. Vissink (Department of Rheumatology & Clinical Immunology, University of Groningen, University Medical Center Groningen, the Netherlands); J.G. Brun, D. Hammenfors (Department of Rheumatology, Haukeland University Hospital, Bergen, Norway); G. Fraile (Department of Internal Medicine, Maimonides Medical Center, Brooklyn, NY, USA).
b) Members of the French ASSESS Cohort

J. Sibilia (Rheumatology Centre National de Référence des Maladies Auto-Immunes Rares, Institut National de la Santé et de la Recherche Médicale UMR_S_1109, Fédération de Médecine Translationnelle de Strasbourg, Strasbourg University Hospital, Université de Strasbourg, Strasbourg, France); C. Miceli-Richard, G. Nocturne (Rheumatology, Bicêtre Hospital, Institut National de la Santé et de la Recherche Médicale U-1012, Université Paris Sud, Assistance Publique des Hôpitaux de Paris, Paris, France); J. Benessiano (Centre de Ressources Biologiques, Bichat Hospital, Assistance Publique des Hôpitaux de Paris, Paris, France); P. Dieude (Rheumatology, Bichat Hospital, Assistance Publique des Hôpitaux de Paris, Paris, France); J-J. Dubost (Rheumatology, Clermont-Ferrand Hospital, Clermont-Ferrand, France); A-L. Fauchais (Internal Medicine, Limoges Hospital, Limoges, France); V. Goeb (Rheumatology, Amiens University Hospital, Amiens, France); E. Hachulla (Pierre Yves Hatron, Internal Medicine, Lille University Hospital, Lille, France); C. Larroche (Internal Medicine, Avicenne Hospital, Assistance Publique des Hôpitaux de Paris, Bobigny, France); V. Le Guern, X. Puéchal (Internal Medicine, Cochin Hospital, Assistance Publique des Hôpitaux de Paris, Paris, France); J. Morel (Rheumatology, Montpellier University Hospital, Montpellier, France); A. Perdriger (Rheumatology, Rennes University Hospital, Rennes, France); S. Rist, Rheumatology, Orléans Hospital, Orléans, France; O. Vittecoq (Rheumatology, Rouen University Hospital, Rouen, France); P. Ravaud (Centre of Clinical Epidemiology, Hotel Dieu Hospital, Assistance Publique des Hôpitaux de Paris, Institut National de la Santé et de la Recherche Médicale U378, University of Paris Descartes, Faculty of Medicine, Paris, France).

c) Members of the Spanish GEAS Cohort (SS Study Group, Autoimmune Diseases Study Group GEAS, Spanish Society of Internal Medicine SEMI):

B. Díaz-López (Department of Internal Medicine, Hospital Universitario Central de Asturias, Oviedo), C. Feijoo, (Department of Internal Medicine, Hospital Parc Tauli, Sabadell), L. Pallarés (Department of Internal Medicine, Hospital Son Espases, Palma de Mallorca), M. López-Dupla (Department of Internal Medicine, Hospital Joan XXIII, Tarragona), R. Pérez-Alvarez (Department of Internal Medicine, Hospital do Meixoeiro, Vigo), M. Ripoll (Department of Internal Medicine, Hospital Infantia Sofia, Madrid), B. Pinilla (Department of Internal Medicine, Hospital Gregorio Marañón, Madrid), M. Akasbi (Department of Internal Medicine, Hospital Infantia Leonor, Madrid), B. Maure (Department of Internal Medicine, Complejo Hospitalario Universitario, Vigo), E. Fonseca (Department of Internal
Medicine, Hospital de Cabueñes, Gijón), J. Canora (Department of Internal Medicine, Hospital Universitario de Fuenlabrada, Madrid), G de la Red (Department of Internal Medicine, Hospital Espíritu Santo, Barcelona), A.J. Chamorro (Department of Internal Medicine, Complejo Hospitalario de Ourense, Ourense), I. Jiménez-Heredia (Department of Internal Medicine, Hospital de Manises, Valencia, Spain), P. Fanlo (Complejo Universitario de Navarra), P. Guisado-Vasco (Hospital Quirón, Madrid), M. Zamora (Hospital Virgen de las Nieves, Granada).
REFERENCES


33 Shiboski CH, Baer AN, Shiboski SC, *et al.* Natural History and Predictors of Progression to Sjogren’s Syndrome Among Participants of the Sjogren’s International Collaborative Clinical Alliance Registry. *Arthritis Care Res (Hoboken)* 2018; **70**: 284–94.


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.