

# Phenotyping Sjögren's syndrome: towards a personalised management of the disease

P. Brito-Zerón<sup>1,2</sup>, S. Retamozo<sup>2,3,4</sup>, M. Ramos-Casals<sup>2,5</sup>

<sup>1</sup>Autoimmune Diseases Unit, Department of Medicine, Hospital CIMA-Sanitas, Barcelona, Spain;

<sup>2</sup>Sjögren's Syndrome Research Group (AGAUR), Laboratory of Autoimmune Diseases Josep Font, IDIBAPS-CELLEX, Department of Autoimmune Diseases, ICMiD, Hospital Clínic, Barcelona, Spain;

<sup>3</sup>Hospital Privado Universitario de Córdoba, Instituto Universitario de Ciencias Biomédicas de Córdoba (IUCBC), Córdoba, Argentina.

<sup>4</sup>Instituto de Investigaciones en Ciencias de la Salud, Universidad Nacional de Córdoba, Consejo Nacional de Investigaciones Científicas y Técnicas, (INICSA-UNC-CONICET), Córdoba, Argentina;

<sup>5</sup>University of Barcelona, Spain.

Pilar Brito-Zerón, MD, PhD

Soledad Retamozo, MD

Manuel Ramos-Casals, MD, PhD

Please address correspondence to:

Dr Manuel Ramos-Casals,

Department of Systemic

Autoimmune Diseases,

Hospital Clínic,

University of Barcelona,

Villarroel 170,

08036 Barcelona, Spain.

E-mail: mramos@clinic.ub.es

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## ABSTRACT

*Sjögren's syndrome (SS) is a systemic autoimmune disease that mainly targets the exocrine glands. The disease overwhelmingly affects women around 30-60 years old, and more than 95% of patients present with oral and/or ocular dryness, although they may also develop a wide number of organ-specific systemic manifestations. The variable presentation is often linked to the influence of multiple personal determinants. In this review, we analyse the main geoepidemiological, immunological and histopathological determinants involved in the phenotypic expression of SS. With respect to sicca involvement, some patients (Asian, young-onset diagnosis, males and Ro-carriers) present with a less pronounced involvement in contrast to others with more pronounced dryness (seronegative, isolated La-carriers). With respect to the risk of developing systemic disease/ poor outcomes, we propose a phenotype-driven prognostic classification into patients at low risk (elderly-onset diagnosis, seronegative, isolated La-carriers), moderate risk (Black/African-american, young-onset diagnosis, Ro-carriers) and high risk (males, high focus score or presence of germinal centres in histopathological studies, RF-carriers, cryoglobulinaemic and hypocomplementaemic patients). Phenotype-based clustering of systemic autoimmune diseases may help physicians to offer a more personalised, cost-effective medical care of patients affected by these complex chronic diseases.*

## Introduction

There is an increasing interest in phenotyping chronic complex diseases. Diabetes mellitus, cardiovascular or chronic pulmonary diseases, or cancer, are diseases often considered hetero-

geneous phenotypic syndromes composed by multiple disease subtypes with a common underlying pathophysiology. To understand their heterogeneity, several studies have tried to identify clinical, imaging, histopathological and/or laboratory disease-related phenotypes that are often analysed using univariate and multivariate regression analyses (1). However, this statistical approach may be confounded by the overlap existent among the different disease subtypes. Network and cluster analysis have the potential to provide a more appropriate statistical approach to understand disease complexity, rather than focusing on individual components of disease (2), identifying differences that are not apparent when disease subsets are analysed in a pair-wise manner (3). The improved analysis of phenotypic heterogeneity is leading to identify well-defined subpopulations, and therefore, contribute to identify better ways of treating the different subsets of a disease with a major impact on both quality of care and health-care costs.

Sjögren's syndrome (SS) is a complex, systemic autoimmune disease in which immune-mediated inflammation causes secretory glandular dysfunction, leading to dryness of the main mucosal surfaces (4). Although the etiopathogenesis of the disease is unknown, the main hypothesis is based on the effect of multiple, mainly unknown, environmental factors affecting an individual with a specific genetic susceptibility. The disease overwhelmingly affects middle-aged women, but also children, men and the elderly. And despite sicca symptoms are among the most-frequent ocular and oral complaints seen by GPs, the disease is often under- or misdiagnosed. Beyond sicca syndrome, SS may be a serious disease, with an excess of

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mortality mainly due to systemic disease and haematological cancer. The variable presentation often linked to the influence of multiple personal determinants may result in diagnostic delays of up to 10 years with patients often visiting various specialists (5).

In this review, we analyse the main geoepidemiological, immunological and histopathological determinants involved in the phenotypic expression of SS, underlying the importance of phenotyping complex autoimmune diseases for offering a personalised, more effective medicine.

### **Sjögren's phenotype: clinical scenarios**

SS may be expressed in many guises. There are three predominant clinical scenarios, which may occur at the time of the diagnosis or later during the follow-up, and that are often overlapped: the DPF triad (dryness, pain and fatigue), organ-specific systemic involvement and cancer. The first scenario has an overwhelmingly effect in the health-related quality of life (HRQOL) of SS patients, while the other two have a great influence in organ dysfunction and survival.

#### *The DPF triad*

Sicca symptoms, mainly oral and ocular dryness, are the key phenotypic signal of the disease, occurring in more than 95% of patients; using the 3 questions included in the 1993/2002 classification criteria, these symptoms have a positive and negative predictive values of 54–77% and 94–98%, respectively (6, 7). Xerostomia is the subjective feeling of oral dryness, and other oral symptoms include soreness, adherence of food to the mucosa, and dysphagia. Reduced salivary volume interferes with speaking or eating and may facilitate local infection, tooth decay and periodontal disease. Xerophthalmia is the subjective feeling of ocular dryness, referred by the patient as itching, grittiness, soreness, photosensitivity, ocular fatigue and/or reduced visual acuity. Diminished tear secretion may lead to chronic irritation and destruction of conjunctival epithelia (*keratoconjunctivitis sicca*),

with a greater susceptibility to ocular infections. In patients referred to ophthalmology departments for the study of dry eyes, SS is diagnosed in around 15% of cases (8). There is no doubt that oral and ocular dryness are the principal symptoms leading to a suspicion of SS, especially when they present in tandem, as is reported by nearly 90% of patients (9); this is supported by the results of the ROC curve analysis carried out for the development of the 1993 European classification criteria of SS (6). Additional sicca symptoms that often coexist with oral and ocular dryness include hoarseness, non-productive cough, cutaneous dryness and dyspareunia in women.

Patients with SS often present with a couple of additional chronic symptoms closely associated with dryness: widespread or generalised pain, and fatigue/weakness (4). A large percentage of SS patients present with a clinical scenario overwhelmingly dominated by these symptoms, which are not life-threatening but have a serious impact on the quality of life (10) and that have been reported in more than 80% of patients with SS (11). The environment plays a key role in exacerbating these symptoms. Physicians should be alert to women reporting a dramatic change in their quality-of-life due to the abrupt onset of these symptoms (8), which may be closely related to other non-specific symptoms such as sleep disturbance, anxiety and depression, whose prevalence in primary SS patients is around 15%, 20% and 40%, respectively (8).

The DPF symptoms are best assessed using the ESSPRI score (12) and are stronger predictors of HRQOL impairment than systemic involvement (assessed by the ESSDAI) (13). Some authors have proposed alternative names for this specific SS phenotype, such as dry eyes and mouth syndrome (14) or sicca asthenia polyalgia syndrome (15). Greater intensity of dryness, fatigue and pain seems to go in tandem with less systemic involvement and identification of immunological markers, making it difficult in some cases to distinguish between SS and functional somatic syndromes (8). Segal *et al.* (16) found that physical impairment

was greater and pain more severe in seronegative primary SS patients, while Ter Borg *et al.* (17) found that patients with widespread pain had a lower prevalence of autoantibodies and systemic features. Recent data have changed the notion that proinflammatory cytokines are directly involved in fatigue reported by SS patients, since although serum levels were elevated in patients compared with healthy controls, cytokine levels inversely correlated with fatigue (18). A careful assessment is essential in these patients, as these symptoms are also characteristic of other processes (hypothyroidism, neoplasia, primary depression) and, above all, of functional somatic syndromes like fibromyalgia and chronic fatigue syndrome, which have a very significant epidemiological overlap with primary SS (16, 19).

#### *Systemic Sjögren's syndrome*

Some patients with SS may present with systemic features unrelated to involvement of the mucosal surfaces (8). A long list of extraglandular features is reported, with a wide variety of clinical presentations, histopathological scenarios, and outcomes, although the two internal organs that have the greatest influence on the survival of patients with SS are the lungs and the kidneys. Although severe, life-threatening systemic involvement has been rarely reported in SS, cryoglobulinaemic vasculitis (CV) is the life-threatening condition more frequently reported in patients with SS, with involvement of vital organs such as the kidneys, the lungs and the gastrointestinal tract. Other severe involvements include myelitis, ganglionopathy, pulmonary arterial hypertension, severe thrombocytopenia or autoimmune haemolytic anaemia.

Systemic manifestations are the first “visible” presentation of the disease. These features may appear before the onset of the characteristic features of dryness, or in patients reporting mild symptoms, often neglected by the patient or physician or both (8). The best example is the development of a fetal congenital heart block in the baby of a pregnant women which leads to the discovery of underlying maternal anti-

Ro antibodies; a significant percentage of these asymptomatic mothers will develop SS (20). An early diagnosis of these patients is essential and requires a multi-step sequential diagnostic process using a close workup in collaboration with the specialty involved in each organ-specific involvement. This clinical approach requires diagnostic tests not only to confirm adequately systemic involvement, but also to rule out other etiologies not directly related to SS. Coexisting laboratory abnormalities may also support a clinical suspicion of SS, including a typical laboratory triad (cytopenia, raised erythrocyte sedimentation rate and hypergammaglobulinaemia). In all these patients, a positive result for salivary gland biopsy or for Ro/La autoantibodies will lead to an early diagnosis of SS several years before the onset of an overt sicca syndrome. In a pre-diagnostic immunological study, Theander *et al.* (21) reported that the number of positive autoantibodies was 2-fold higher in the patients with early-onset disease compared to those presenting with a late onset. An early diagnosis will help to prevent systemic complications (chronic organ damage, lymphoma) by ensuring their timely treatment (8).

### Cancer

Lymphoma is one of the worst complications that physicians should expect in SS patients. In 1978, Kassan *et al.* (22) estimated a 44-fold higher risk for non-Hodgkin lymphoma (NHL), although the SIRs estimated by subsequent studies were overwhelmingly lower, with a pooled 14-fold higher risk reported in a recent meta-analysis (23). In the studies based on the fulfillment of the 2002 criteria, the SIRs for B-cell lymphoma range between 7 and 9 in population-based studies and between 16 and 48 in hospital-based studies; the majority of these studies were focused only on B-cell lymphoma and most of them used an old terminology (NHL) (24). Although the vast majority of cells infiltrating the salivary glands are T cells, the majority of lymphomas arising in SS are of B-cell origin (in a recent study, with a ratio between B and T-cell lymphomas of 15:1) (24). Among B-cell lymphomas,

the different subtypes not only have a different frequency, but also have differing clinical presentations and, logically, a different prognosis. Three subtypes of lymphoma alone account for more than 90% of reported B-cell lymphomas in primary SS: MALT lymphoma as the most frequent (around 60%), followed by DLBC and MZ lymphoma (24). Plasma-cell myeloma is rare, although a recent study has described an increased risk in primary SS patients presenting with monoclonal gammopathy (25). The main prognostic factors identified at SS diagnosis enhancing the risk of lymphoma included systemic activity, cytopenias and cryoglobulin-related immunological markers (26), although a recent study have demonstrated that the weight of these factors differed among each subtype of B-cell lymphoma (24). Non-B-cell haematological cancers, including myeloid neoplasia/leukaemia, Hodgkin disease and T/NK-cell lymphoma, represent 25% of the SS-related haematological cancers; in these patients, risk factors include systemic activity, cytopenias (anaemia, thrombocytopenia and leukopenia) and cryoglobulins at SS diagnosis (24).

Patients with primary SS should be closely followed also for the enhanced risk of developing some types of solid cancer. Some studies have analysed the risk for specific types of solid cancer and found a lower risk for the development of colon and breast cancers and a higher risk for thyroid cancer. We have recently reported an enhanced risk for the development of thyroid, lip/oral cavity and stomach cancers (SIRs of 5.17, 4.81 and 2.53, respectively) in primary SS patients (24). The highest risk was for thyroid cancer, in which some risk factors (predominantly female involvement, low frequency of smoking, association with autoimmune thyroiditis) are clearly shared with primary SS (24). The finding of the higher risk for cancers of the oral cavity and stomach in women with primary SS was also interesting, since the oral cavity is overwhelmingly involved in primary SS and the stomach is the most-frequent extraglandular extranodal site of lymphoma involvement in primary SS. A recent study have reported that

severe intestinal dysbiosis is a prevalent finding in SS and is associated both with clinical and laboratory markers of systemic disease activity as well as gastrointestinal inflammation (27), and further studies are warranted to elucidate a potential causative link between severe intestinal dysbiosis and cancer in SS patients.

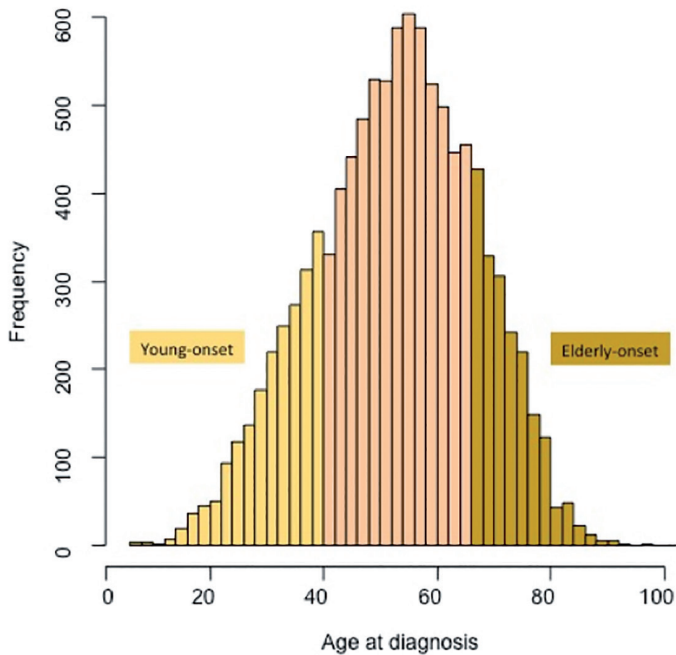
### Epidemiological determinants

#### *Ethnicity phenotypes*

Only two studies have evaluated the influence of ethnicity on the phenotypic expression of SS. The first reported a two-fold higher prevalence of the disease in patients with non-European ethnicity backgrounds (28), while the Sjogren Big Data Cohort study have reported significant variations between ethnic groups (9). In this study, the disease was diagnosed a mean of seven years earlier in Black/African American (BAA) patients compared with White patients, a trend also reported by Maldini *et al.* in the Parisian multi-ethnic cohort (28). The female:male ratio also varied significantly, with the highest ratio in Asian patients (27:1) and the lowest in BAA patients (7:1), as well as for the prevalence of sicca symptoms, with the lowest frequencies being reported in Asian patients, a finding previously reported that was related to cultural differences (29). Hispanic and White patients were more likely to have abnormal results compared with other ethnicities, and Maldini *et al.* (28) reported a similar trend in the multi-ethnic Paris cohort. With respect to systemic activity, the highest ESSDAI scores are reported in BAA patients, followed by White, Asian and Hispanic patients (30). A recent study has reported significant genetic differences, especially in the MHC region, for SSA/SSB autoantibody production and salivary gland focus score in non-European ancestry between European and Asian SS patients, although subphenotype differences did not explain most of the ancestry differences in genetic associations (31).

#### *Young-onset phenotype*

Several studies carried out in small series of patients have suggested a key influence of the age at diagnosis in



**Fig. 1.** Population pyramid of 10,300 patients with primary SjS included in the Sjögren Big Data International Cohort according to the age of diagnosis: young-onset and elderly-onset diagnosis of the disease.

SS phenotype in the two extremes of the population-aged pyramid (Fig. 1). Young onset of the disease is overwhelmingly defined on the basis of an age at diagnosis below 35–40 years, and elderly onset by an age at diagnosis higher than 65–70 years.

Patients diagnosed before the age of 35–40 are a differentiate subset of the disease (21). In 1998, we reported that young-onset patients had a lower degree of salivary gland involvement (dry mouth and parotid enlargement) and a higher frequency of immunologic markers (anti-Ro and low C4 levels) (32, 33), and Haga *et al.* also reported higher positivity of the Ro/La autoantibodies, RF and hypergammaglobulinaemia (34). In the study by Theander *et al.* (21), patients diagnosed before age 40 showed a significantly higher prevalence of prediagnostic autoantibodies including ANA (96%), RF (87%), Ro 60/SSA (91%), Ro 52/SSA (65%) and La/SSB (57%) and also had higher titres and a higher number of autoantibody specificities in the same samples. Due to the close association between systemic disease and seropositive phenotype (RF/Ro/La carriers), it seems that there is a subset of patients that could be prone to be diagnosed earlier. Ethnicity determinants may play a role in this early presentation. Maldini *et al.* (35) found a younger age at di-

agnosis and an increased frequency of polyclonal hypergammaglobulinaemia and positive Ro/La antibodies in non-European patients, and we have recently reported that patients from ethnic groups with the highest frequencies of positive anti-Ro antibodies (Asian, Hispanic and Black/African American) also had the youngest ages at diagnosis (30). We also found that patients with a younger age at diagnosis showed a lower frequency of dry eyes and positive ocular tests, and a higher frequency of ANA, RF, anti-Ro/La autoantibodies and low C3 levels (30). With respect to systemic activity, the highest scores were reported in patients with young-onset disease (<35 years), especially in comparison with the scores reported for patients with older onset (>65 years) for both the ESSDAI and clinESSDAI (30). With respect to the ESSDAI domains, a younger onset was associated with an enhanced risk of presenting activity at diagnosis in the constitutional, lymphadenopathy, glandular, cutaneous, renal, haematological and biological domains.

#### *Elderly-onset phenotype*

With respect to the diagnosis of the disease at older ages, the differences are not as much remarkable than that reported for the young-onset subset. In 1998, we report similar frequencies of

glandular, extraglandular and immunological features in patients diagnosed at elderly ages (36), while Tishler *et al.* (37) reported that patients diagnosed at older ages showed milder clinical symptoms with fewer immunological manifestations. A recent study has also confirmed that an elderly diagnosis of the disease was associated with a similar frequency of abnormal diagnostic tests (parotid sialography, ocular tests, minor salivary gland biopsy) and ocular/oral symptoms (38). In the largest reported cohort, we have recently found some significant differences in patients with an elderly diagnosis (>70 years) who showed a higher frequency of positive oral tests and a lower frequency of anti-Ro/La autoantibodies and low C3 levels, although the most striking differences were reported for the organ-by-organ systemic activity, since an older onset was associated with an enhanced risk for presenting activity at articular, pulmonary, muscular and peripheral nervous system domains (30).

#### *Male phenotype*

Although very infrequent (only 7% of cases included in the largest reported series), men affected by SS present with a very specific phenotype (39). Ocular involvement should be evaluated carefully in men since they are more likely to present with serious ocular complications, are less likely to have a known diagnosis of SS at presentation and tend to report a shorter duration of dry eye symptoms and severe ocular complications (40, 41). In fact, in the Sjögren Big Data cohort, men showed a lower frequency of reported dry eye (30). With respect to systemic features, previous studies reported a lower frequency of Raynaud's phenomenon in men with SS (39), while recent studies have reported a higher frequency of interstitial lung disease or lymphadenopathy (42) and vasculitis or interstitial nephritis (41). In the Big Data cohort, male gender was associated with a higher systemic activity at diagnosis including higher mean ESSDAI and clinESSDAI scores, with the domains that were more active in men than in women being lymphadenopathy, glandular, pulmonary, PNS and CNS (30). With respect to the im-

munological profile, ANA and Ro/La autoantibodies were often reported less frequently in men (39-41).

But the key phenotypic feature more homogeneously reported across the studies in men affected by SS is the significant association of male gender with poor outcomes (neoplasia and death). Lymphoma is diagnosed earlier (43) and with a higher frequency (42, 44-46) (3.44-fold higher risk) (47) in comparison with women. In a recent study, the SIR estimated for all cancers combined was higher in men than in women (2.29 vs. 1.87), and we also found that all SIRs for the main haematological groups of cancer were significantly higher in men than in women (43 vs. 36 for multiple myeloma and immunoproliferative diseases, 59 vs. 16 for Hodgkin lymphoma and 18 vs. 5 for non-Hodgkin lymphoma, respectively) (24). In addition, male gender has been associated with a 2–3-fold higher risk of death (48, 49).

#### *Geographical phenotypes*

Few studies have evaluated the potential role of geographical determinants in primary SS. The study by Maldini *et al.* (35) was the first to report a differing clinical and immunological pattern of SS expression in French patients with a non-European background. We have recently reported that geolocation may influence the phenotypic expression of primary SS at diagnosis, including significant geoepidemiological variations in the prevalence of sicca symptoms, the frequency of abnormal diagnostic tests and the positivity of the main immunological markers (9). We confirmed a north-south geographical gradient with respect to a lower frequency of ocular involvement and a higher frequency of cryoglobulinaemic-related tests (cryoglobulins and hypocomplementaemia) in northern compared with southern countries. For salivary gland involvement, the highest rates of abnormal results (including biopsy) were found in European patients from northern countries, while in America and Asia the highest rates were reported in patients from southern countries. A similar inverse gradient was observed with respect to autoantibodies (ANA,

Ro, La): highest frequencies were reported in European southern countries while in America and Asia, the highest frequencies were reported in northern countries. We have also reported the influence of geoepidemiological migration on the phenotypic expression of primary SS confirming significant differences between SS-related features of ethnic migrant and native populations pertaining to the same ethnicity (9).

#### **Immunological determinants**

##### *Seronegative phenotype*

A seronegative phenotype of the disease is defined on the basis of the absence of positive autoantibodies included in the corresponding classification criteria. Therefore, a seronegative phenotype on the basis of the 1993 European criteria is defined as patients with negative ANA, RF, Ro and La autoantibodies, even in the absence of positive salivary biopsy. In the recent 2002/2016 sets of criteria, seronegative patients must be biopsy-proven patients with negative Ro/La (2002 criteria) (7) or negative Ro (2016 criteria) (50) antibodies. In comparison with seropositive patients, the clinical phenotype of these patients is characterised by three main findings: i) no significant differences in the frequencies/abnormal results of signs and symptoms of glandular involvement; ii) higher frequency of fatigue and pain; and iii) lower frequency of systemic involvement (14, 17, 51-54). The best study was published by Quartuccio *et al.* (54) that compared 342 biopsy-proven patients carrying anti-Ro/La antibodies and 206 seronegative biopsy-proven patients, who showed an older age at diagnosis and a lower frequency of parotid swelling, purpura, leucopenia, lymphoma, hypergammaglobulinaemia, ANA, RF, low C3, low C4, and cryoglobulins. We have recently replicated this study in the Big Data cohort (2,073 seronegative vs. 3,172 seropositive biopsy-proven patients) and found that seronegative phenotype was associated with a diagnosis at older ages, higher frequency of abnormal oral diagnostic tests and lower frequency of ANA, hypocomplementaemia, rheumatoid factor and cryoglobulinaemia in comparison with seropositive patients (55).

Biopsy-proven patients with primary SS without circulating anti-Ro/La antibodies have a specific phenotypic profile at disease diagnosis characterised by an older age, a higher frequency of sicca symptoms, a lower frequency of abnormal diagnostic tests and a milder immunological profile, and also had a lower risk of lymphoma and a lower level of B-cell expansion (54). A key determinant that should be always evaluated in seronegative SS patients is the coexistence of functional somatic syndromes such as chronic fatigue syndrome (CFS) and fibromyalgia (FM). The frequency of the overlap of FM in SS patients ranges from 15 to 35% (17, 19, 56-58), and their concomitant presence has been statistically associated not only with the seronegative phenotype (17, 57), but also with depression (58, 59), fatigue (56, 60, 61), widespread pain (17, 61) and a 10-fold enhanced risk for work disability (62). With respect to CFS, one-third of CFS patients with sicca symptoms fulfilled the diagnostic criteria for SS, although all had a seronegative phenotype (51). Seronegative patients present with a phenotype that fit into a spectrum of disease which tended more towards functional somatic syndromes such as CFS and fibromyalgia.

##### *RF-driven phenotype*

Early studies including small series of SS patients reported an independent statistical association between RF positivity and the main clinical and immunological features of the disease (63). Recent studies have enhanced the key role of RF influencing the SS phenotype, with a statistical association with an earlier diagnosis (21), prediction of development of full SS (64), ocular scores (65), severe parotid scintigraphy dysfunction (66), ectopic germinal centres (67), systemic or severe disease (68-70) and lymphoma development (71, 72). In addition, data from the Big Data Cohort showed that RF was detected in nearly half our patients, who showed a specific phenotype consisting of a young age at diagnosis, a higher frequency of abnormal diagnostic tests, a higher mean ESSDAI score, and a higher frequency of systemic activity in

the glandular, articular, cutaneous and haematological domains (see the current supplement). In spite of this close and evident association with a more active and severe disease phenotype, RF has been excluded in the two last sets of classification criteria. Thus, RF detection in primary SS is clinically useful, especially for the diagnosis of some subsets of patients with SS, such as those with systemic/severe manifestations or with circulating cryoglobulins.

#### *Ro-driven phenotype*

Anti-Ro antibodies, which are detected in 70–80% of patients, are the key immunological markers of SS. In nearly 10–20% of patients carrying anti-Ro antibodies, ANA may be negative (73) and anti-Ro antibodies may be present many years before the onset of symptoms or the diagnosis of SS (early SS) (21). In the Big Data cohort, we found anti-Ro antibodies in 73% of our patients, a figure very close to that found for ANA (9). Various studies have correlated the presence of anti-Ro with most SS-related systemic and immunological features (63). A recent study by Quartuccio *et al.* compared Ro/La+ and Ro/La- patients (54) and found a younger age at diagnosis and a higher frequency of systemic features, immunological markers and lymphoma, while we have recently found that anti-Ro antibodies at diagnosis also correlated with a higher activity score in the articular, cutaneous and renal domains in a Spanish multicentre study (74). We have also confirmed a specific Ro-phenotype in the Big Data international cohort 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 (see current supplement).

#### *La-driven phenotype*

##### • *Booster effect in Ro-carriers*

In the international Big Data cohort, anti-La antibodies were detected in 45% of patients, overwhelmingly associated with the concomitant presence of anti-Ro antibodies (95% of cases) (9). Probably for this reason, the phenotype of La

carriers was very similar to that reported for Ro carriers. However, few studies have analysed whether the disease phenotype of Ro+ carriers is influenced by the concomitant presence or not of anti-La antibodies. Lochter *et al.* (75) 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 (17, 76); unfortunately, the recent study published by Baer *et al.* does not compare Ro+La vs. isolated Ro (77). In the Big Data cohort, 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 (78).

##### • *Specific La-driven phenotype*

A recent interest in characterising SS patients carrying isolated La autoantibodies has been emerged after the exclusion of this subset of patients from the recently proposed European/American classification criteria (50). This exclusion was based on the manuscript published by Baer *et al.* (77) in the SICCA cohort reporting lower ocular staining and salivary focus scores in isolated La carriers in comparison with Ro+ carriers (with or without associated La); however, isolated La carriers also showed a higher frequency of dry mouth, higher median Schirmer test and a higher frequency of abnormal UWSF results than the seronegative group, suggesting that patients with isolated La may have an intermediate phenotype between Ro+ and Ro- patients for SS-related glandular involvement. Two further studies have analysed the phenotype of isolated La carriers also including systemic involvement, although comparison with Baer's study is not straight forward as these studies included patients fulfilling the 2002 criteria (only about half of the participants in the SICCA cohort met 2002/2012 criteria). The first study was published by Danda *et al.* in US patients with primary SS (79), and found no significant differences in the main results of the diagnostic tests, an

earlier age at diagnosis, and a lower frequency of positive salivary biopsy, RF and systemic activity between carriers of isolated La antibodies and Ro+ carriers. The second study was carried in the international Big Data cohort and confirmed most of the results reported by Danda *et al.* (see current supplement). In comparison with biopsy-proven seronegative patients, isolated La carriers had an age at diagnosis 5.5 years lower, with a lower frequency of oral dryness but a significantly higher frequency of abnormal oral diagnostic tests, while with respect to Ro+ carriers, isolated La carriers showed a higher frequency of ocular dryness, a lower frequency of positive minor salivary gland biopsy and a significantly lower frequency of ANA, RF, hypocomplementaemia and cryoglobulinaemia. In the largest reported cohort of patients with primary SS fulfilling the 2002 AE classification criteria, only 3% of patients had isolated anti-La/SS-B antibodies. This small subset of patients had a specific clinical and immunological profile that mixed some features characteristic of both immunonegative patients and patients carrying anti-Ro/SS-A antibodies. When considering the practice of medicine, the gold standard for SS clinical diagnosis remains expert opinion as there is no pathognomonic diagnostic test, and we agree with Danda *et al.* that the best clinical diagnosis for this subset of patients is SS; however, and for clinical trials where homogenous populations are desirable, such patients could be probably excluded or analysed separately (79). The etiopathogenic central role of the La autoantigen in SS, confirmed by several studies published in the last 20 years, also supports the inclusion of La autoantibodies into the typical immunological spectrum of SS (80-84).

#### *Cryoglobulin-driven phenotype*

Twenty years ago, we reported the close association between cutaneous leukocytoclastic vasculitis, hypocomplementaemia, chronic hepatitis C virus (HCV) infection and cryoglobulins in patients with SS (85), and in 2007, we demonstrate the key prognostic role of cryoglobulins in patients with primary

SS without HCV infection (86). Since then, several studies have confirmed the close association between cryoglobulins and systemic disease (87). However, these studies have only analysed either the presence of serum cryoglobulins (with or without symptoms) or purpura/vasculitis (associated or not to cryoglobulinaemia). Many patients with cryoglobulinaemia remain asymptomatic, and the percentage of patients with circulating cryoglobulins who develop cryoglobulinaemic vasculitis (CV) varies between 2% and 50%, with a vasculitic expression that ranges from a benign disease (arthralgias and uncomplicated cutaneous purpura) to a life-threatening systemic vasculitis. In a recent study, we have evaluated the complete spectrum of clinical and immunological features currently integrated in the definition of CV according to the internationally accepted classification criteria (87). In patients with primary SS and positive cryoglobulins, CV was reported in 35% of cases (86% in those fulfilling CV criteria compared with 11% in those who did not). We have confirmed the strong link between CV and systemic disease also in the Big Data cohort: patients with CV had the highest frequency of activity in all organ domains except two (muscular and CNS), and mean ESSDAI scores were 2-fold higher at diagnosis and 3-fold higher at the end of follow-up compared with the scores of patients negative for cryoglobulins (see current supplement). In another study, Quartuccio et al have reported that the ESSDAI and the ClinESSDAI scores were significantly higher in cryoglobulin-positive patients especially in the constitutional, lymphadenopathy, glandular, cutaneous, peripheral nervous system and haematological domains (69).

Several studies have confirmed cryoglobulins as a strong predictor of lymphoma development in patients with primary SS (72, 86, 88, 89). We have confirmed the association between cryoglobulinaemia and lymphoma, which was stronger in patients with CV (HR 7.47) than in those without CV (HR 2.56); the more clinical/immunological CV-related markers the SS patient has, the higher the risk of lymphoma,

while patients negative for cryoglobulins were protected against lymphoma (HR 0.39) (87).

Patients with SS should be tested at diagnosis for cryoglobulins, RF, C3/C4 complement factors and serum immunoelectrophoresis, and should be evaluated both for systemic activity (ESSDAI) and for vasculitis (fulfillment of CV criteria), since those with concomitant CV at diagnosis are at high risk of developing an adverse outcome.

#### *Hypocomplementaemic-driven phenotype*

Together with cryoglobulins, hypocomplementaemia is the other key immunological prognostic factor in SS. Previous studies in multicentre national cohorts reported 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 (cryoglobulinaemia, rheumatoid factor) (86, 90); recently, Shiboski *et al.* (91) have also reported that sicca patients with hypocomplementaemia were 6 times more likely to progress to definite SS. In addition, hypocomplementaemia is also closely associated with lymphoma development and death (92), although there are more studies reporting association with lymphoma development for C4 hypocomplementaemia (47, 72, 93-96) than for C3 hypocomplementaemia (47, 93, 96, 97), as well as with poor survival for C4 hypocomplementaemia (48, 49, 52, 86, 98) than for C3 hypocomplementaemia (48, 49, 52).

No study has analysed a differentiated role of having C3 or C4 hypocomplementaemia in SS patients. In the Big Data cohort, we have analysed separately the phenotype associated with either low C4 or low C3 values, and we found significant differences. Patients with C4-hypocomplementaemia were older and had an enhanced frequency of positive salivary biopsy, while those with C3-hypocomplementaemia were younger and had a lower frequency of sicca symptoms. Both subsets of patients showed higher mean ESSDAI scores and a close association with

systemic activity that was more pronounced in C3-hypocomplementaemic patients. 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 for the worse outcomes (lymphoma and death) (see current supplement). Probably, the different degree of association between hypocomplementaemia and cryoglobulinaemia (cryoglobulinaemia is more frequently associated with C4 consumption) could explain the differences with previous studies, since the frequency of cryoglobulinaemia is strongly influenced by geographical and ethnicity determinants (9).

#### **Histopathological determinants**

Focal lymphocytic sialadenitis, defined as multiple, dense aggregates of  $\geq 50$  lymphocytes in perivascular or periductal areas in the majority of glands evaluated, is considered the clue histopathologic feature for a diagnosis of SS. The key requirements for a correct histological evaluation are an adequate number of informative lobules and the determination of an average focus score (a focus is a cluster or aggregate of at least 50 lymphocytes), although the reliability of the assessment of the focus score may vary between pathologists. Minor salivary gland biopsy remains a highly specific test for the diagnosis of SS: although invasive, it is a safe procedure that is associated with few adverse local effects (99).

#### *Focus score*

The focus score (FS) is the central histopathological measure in SS. In order to calculate the FS, the total number of foci in the specimen is divided by the glandular surface area, and multiplied by 4, to give the number of foci per 4 mm<sup>2</sup>. Above a FS of 10, foci are typically confluent, and a 'ceiling' score of 12 is often applied (99). Several studies have reported a severe systemic phenotype in patients presenting a high FS in the salivary gland biopsy, with much more consistency than that reported for the Chisholm-Mason classification. These studies have correlated FS with a higher frequency of parotidomegaly,

	PHENOTYPIC DETERMINANTS	Sicca syndrome	Abnormal dx tests	Immunological markers	Systemic disease	Poor outcomes
<b>Ethnicity</b>	Asian	↓				
	Hispanic		↑			
	White		↑			
	BAA				↑	
<b>Demographic</b>	Young-onset disease	↓	↓	↑	↑	
	Men	↓		↓	↑	↑
	Elderly-onset disease		↑	↓		
<b>Immunological</b>	Seronegative	↑		↓	↓	↓
	Isolated La carriers	↑	↑↓	↓	↓	
	Isolated Ro carriers	↓	↑↓	↑	↑	
	Ro+La carriers		↑	↑	↑	
	RF carriers		↑	↑	↑	↑
	Cryoglobulinemic			↑	↑	↑
	C3-hypocomplementemic	↓		↑	↑	↑
	C4-hypocomplementemic		↑	↑	↑	↑
<b>Histopathological</b>	High focus score			↑	↑	↑
	Germinal centers		↑	↑	↑	↑↓

Fig. 2. Geoepidemiological, histopathological and immunological determinants that play a key role in determining SjS phenotype enhancing the risk (red), lowering the risk (green) or with differing results among the studies (orange).

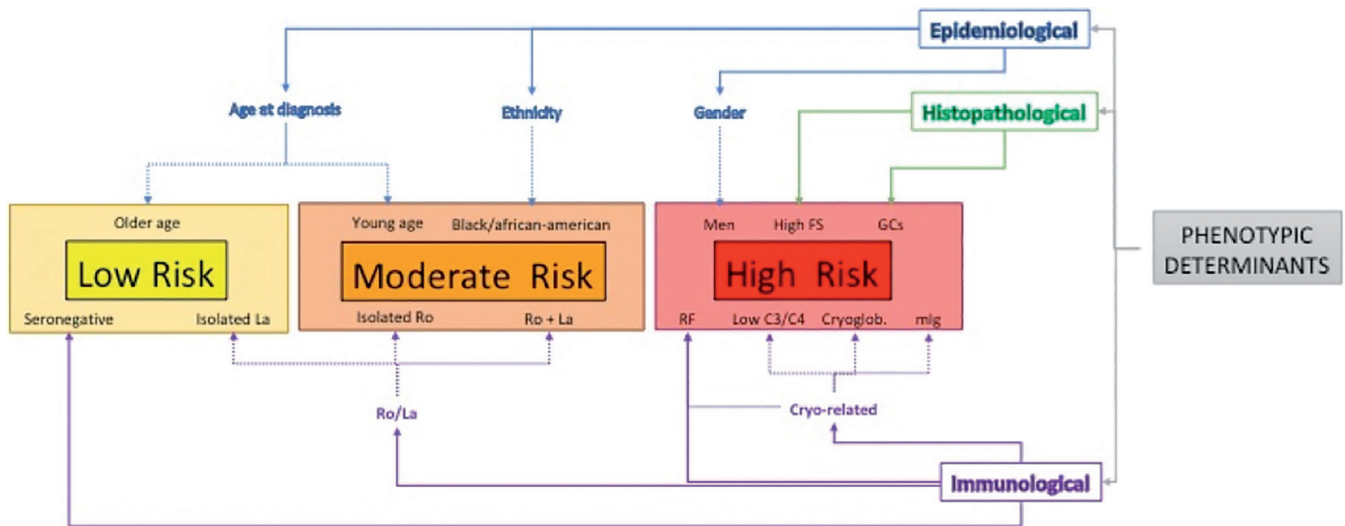


Fig. 3. Phenotypic-driven prognostic algorithm for the risk of SjS patients to develop systemic/severe disease according to the main phenotypic determinants.

systemic disease and ESSDAI scores, positive immunological markers, risk of lymphoma and lack of response to rituximab (100-103).

**Germinal centres**

Germinal centres are structures that arise in B-cell follicles of secondary lymphoid organs as a response to antigenic stimulation. While detection in secondary lymphoid tissue is usually relatively simple, recognition of these structures in salivary gland biopsies of

SS patients is generally more difficult (104). GC-like structures were firstly observed in a large series of primary SS by Salomonsson *et al.* in 2003 (105), who suggested that GCs could play a role in recruiting inflammatory cells and perpetuating the autoimmune glandular damage. The frequency of GC has been reported in 135 (24%) out of 559 patients included in 4 studies, with a frequency ranging from 17% to 30% (105-108). The main reported associations showed a correlation between GC

and FS, parotid enlargement, systemic involvement, hypergammaglobulinaemia, ANA, RF and Ro/La autoantibodies (67, 107-110). The close association with lymphoma development reported by Theander *et al.* (106) has not been confirmed by subsequent studies (104, 111). Some genetic variations may explain why ectopic GC-like structures are present in some SS patients, supporting the hypothesis that GC+ and GC- patients may be distinct disease phenotypes (112).



## Conclusion

After reviewing the literature published in the last 20 years, including very recent data from large international cohorts of patients, the amount and quality of evidence is enough to confirm that geoepidemiological, histopathological and immunological determinants play a key role in determining SS phenotype (Fig. 2). An overall analysis of the reported results identifies a first phenotypic classification of patients according to the degree of glandular involvement, both for the frequency of dryness (subjective feelings) as for the frequency of abnormal diagnostic glandular tests disclosing abnormal glandular function (objective tests). Thereby, some determinants (Asian ethnicity, young-onset diagnosis, males and Ro-carriers) drive a phenotype with a less pronounced glandular involvement, in contrast to others associated with a more pronounced dryness and glandular dysfunction (seronegative, isolated La-carriers). In addition, seronegative patients present with a phenotype that may also fit into a spectrum of disease which tended more towards functional somatic syndromes such as CFS and fibromyalgia, due to the enhanced presence of chronic fatigue and pain. With respect to the risk of developing systemic disease and/or poor outcomes, we propose a phenotypic-driven prognostic classification into patients at low risk (elderly-onset diagnosis, seronegative, isolated La-carriers), moderate risk (Black/African-american, young-onset diagnosis, Ro-carriers) and high risk (males, high focus score or presence of germinal centres in histopathological studies, RF-carriers, cryoglobulinaemic and hypocomplementaemic patients) (Fig. 3). This classification may have clinical implications for the management of patients with SS in the daily practice. Patients with a phenotype disease limited to mucosal surfaces may require only annual evaluation, with a routine physical examination that should include evaluation of mucosal surfaces to discard local complications, laboratory tests that should be made routinely each year including complete blood count, metabolic parameters and renal and liver tests, a key role of

specialties involved in the care of the main mucosal surfaces affected (ophthalmology, odontology/oral medicine, gynaecology) and a principal role in coordinating healthcare of these patients for primary care physicians. In contrast, patients with determinants that prone to a more systemic/severe phenotype should be followed every 3-6 months, with a close physical examination including examination for major salivary glands, peripheral adenopathies and visceromegaly, and following a multidisciplinary approach also including those specialties in the main internal organs involved (pneumology, neurology, haematology, nephrology) under the coordination of highly-specialised units on autoimmune diseases; these patients should be tested at diagnosis (and during follow-up) for cryoglobulins, RF, C3/C4 complement factors and serum immunoelectrophoresis, and should be evaluated both for systemic activity (ESSDAI) and for vasculitis (fulfillment of CV criteria). Phenotype-based clustering of systemic autoimmune diseases may help physicians to offer a more personalised, cost-effective medical care of patients affected by these complex chronic diseases, since not all patients with SS require the same follow up.

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