# Review

# One year in review 2015: Sjögren's syndrome

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**ABSTRACT**

*Primary Sjögren's syndrome (pSS) is a chronic autoimmune disease mainly characterised by the inflammation of exocrine glands; however, a broad spectrum of systemic manifestations may characterise the disease. Recently, pSS has been the object of considerable immunologic and clinical research which has led to significant advances in the diagnosis, prognostic assessment and management of the disease. Herewith, we provide a critical digest of the recent literature on this topic.*

## Introduction

Primary Sjögren's syndrome (pSS) is a chronic autoimmune disease mainly affecting lachrymal and salivary glands, however, during disease progression any organ or mucosal surface may be involved. Thus, pSS presents as a heterogeneous non-organ-specific autoimmune entity, encompassing a wide spectrum of clinical manifestations, serological abnormalities and scattered complications. Lately, pSS has been the object of considerable immunologic and clinical research which has led to significant advances in the diagnosis, prognostic assessment and management of the disease. Herewith, we provide a critical digest of the recent literature on this topic. We performed a medline search of English language articles published from the 1st January 2013 to 31st December 2014 using the following key words: *Sjögren's syndrome, classification criteria, pathogenesis, diagnosis, clinical manifestations, ultrasonography*, *clinimetry*, *outcome*, and *treatment*. We reviewed all the articles and selected the most relevant papers.

## I. Novel insights into pSS pathogenesis

Traditionally, a combination of genetic and environmental factors have been implicated in the pathogenesis of pSS, leading to the dysregulation of the epithelial cells and to an aberrant inflammation and autoimmune response. The T- and B- lymphocytic infiltrates of the salivary and lachrymal glands and a B cell hyperactivity have been widely considered as the hallmarks of the disease; however, lately, great efforts have been made in an attempt to better clarify the innate and adaptive mechanisms involved in the disease initiation and perpetuation.

The importance of the dysregulation of the innate and adaptive immune systems in the pathogenesis of pSS has recently emerged from the new genome-wide association studies (GWAS). In the large-scale genetic study conducted by Lessard *et al*. (1) the already known associations in the HLA region were replicated. In particular, HLA-DQA1*0501, HLA-DQB1*0201 and HLA-DRB*0301 were identified as the strongest genetic risk factors for pSS. Moreover, in this study also non-HLA risk loci were identified including: interferon regulatory factor 5 (IRF5), signal trasducer and activator of transcription 4 (STAT4) and interleukin (IL) 12A, all participating in the interferon (IFN) signalling, the B lymphocyte kinase (BLK) (a non-receptor tyrosine kinase) and chemokine receptor type 5 (CXCR5), that are important for B cell function and antibody production and the TNFAIP3 interacting protein 1 (*TNIP1*) gene that is involved in the negative regulation of the NF-kB pathway. Moreover, Nordmark *et al*. (2) also demonstrated, in a population from Scandinavia and the UK, that two polymorphisms in the *TNIP1* gene were associated with antibody-positive pSS.

In addition, Altorok *et al*. (3) performed the first epigenome-wide DNA methylation study in patients with pSS and identified DNA methylation changes in

several genes in naïve $CD4^+$ T cells that can be involved in the pathogenesis of the disease, such as the gene encoding for the lymphotoxin α and genes of the IFN signature pathway.

The trigger role of a viral agent in patients with a genetic predisposition to pSS has been traditionally hypothesised. In this regard, the pivotal role of the tertiary lymphoid structures (TLS) in the development of the autoimmunity process has been recently emphasised. It is possible that the formation of TLS may be physiologically primed by chronic viral infections but, in genetically predisposed individuals, they become the site of the break of tolerance against self-antigens, with the consequent differentiation of autoreactive B cells (4). Recently, Croia *et al*. (5) examined the expression of latent and lytic forms of Epstein-Barr virus (EBV) in the salivary glands (SG) of pSS patients and patients with chronic non-specific sialoadenitis suggesting that both latent EBV infection and EBV reactivation are a specific feature only of SG with germinal centre (GC)-like structures. So, ectopic lymphoid structures (ELS) seem to represent a protective niche for EBV. It is also interesting to note that plasma cells infected with EBV displayed an anti-Ro52 reactivity. This suggests that a humoral anti-EBV immune response takes place within the ELS and favours the break of self-tolerance, due to the molecular mimicry between pSS auto-antigens and viral proteins. Szymula *et al*. (6) investigated for the first time the role of molecular mimicry in the activation of Ro60 reactive T cells. On the basis of their data, they suggest that rather than a specific infection, a dysregulated immune response towards commensal oral and gut bacteria may be involved in the pathogenesis of autoimmune disease like pSS.

Regarding the novel insights into the innate immune related events that are supposed to initiate the pathogenetic process in pSS, Manoussakis *et al*. (7) have demonstrated that the defective clearance of the apoptotic cells seems to be related to serologic abnormalities (such as inhibiting IgG anti-apoptotic cells and hypocomplementemia) rather than to a primary dysfunction of the phagocytic cells. Fragoulis *et al*. have shown an impaired activity of DNasi1 in pSS (8) suggesting that the impaired enzymatic activity of DNase1, together with the increased capacity of circulating IgG to bind the secondary necrotic material, may determine an increased uptake of this material by phagocytes in pSS. In turn, the internalisation of immune complexes containing nuclear antigens by circulating phagocytes may contribute to the activation of the IFN I pathway. In recent years, the so called "*IFN-signature*" has acquired growing importance in the pathogenesis of the disease considering the over-expression of IFN I-inducible genes in the peripheral blood and in the salivary glands (SG) of pSS patients (9). Over the past two years, several contributions have analysed the presence of polymorphisms of transcription factors of the IFN I pathway, including IRF5 and STA4, the role of monocyte-derived dendritic cells (moDC) in the IFN-signature and the importance of Toll-like receptors (TLRs) in linking ambiental factors to the immune system (10). Moreover, it has been clarified the role of the INF I system in inducing the expression of Fas/FasL and the expression of Ro52, an intracellular protein involved in the ubiquitination, that translocates in the nucleus initiating apoptosis and most importantly, its role in the induction of the adaptive immune response (11-13). More specifically, in the study by Kyriakidis *et al*. (14), it was demonstrated that TLR3 signalling in salivary gland epithelial cells (SGECs) induces, through the IFNβ pathway, the up-regulation of Ro52 and, to a lesser extent, of Ro60 and La. The late induction of Ro52 is also accompanied by a nuclear redistribution of the protein. This mechanism activates the synthesis of auto-antigens in SGECs that become visible through the epithelial cell apoptosis. Moreover, Ro52 is implicated in the negative regulation of TLR3 pathway, by means of the ubiquitination and subsequent degradation of interferon regulatory factors (IRFs). According to Aqrawi *et al*. (15), in genetically susceptible individuals, the hyper-expression of Ro-52 on the ductal epithelial cells, induced by inflammation and in particular by the IFN I, can lead to the breakage of tolerance and to the production of auto-antibodies, contributing to the disease progression.

Concerning the role of innate immunity in pSS pathogenesis, another area of research was the role of the inflammasome in the early phases of the disease. Baldini *et al*. (16) explored the expression of the P2X7 receptor (P2X7R)-NLRP3 inflammasome complex in minor salivary gland biopsies of patients with pSS. P2X7R is a purinergic receptor that acts as an ATP-gated ion channel and plays a role in the regulation of the inflammatory process. They found that P2X7R expression and the gene expression levels of the inflammasome components were significantly higher in salivary glands from individuals with pSS than in controls (both sicca syndrome individuals and healthy controls). These findings corresponded to an increased expression of IL-18 in the saliva of pSS patients. Moreover, the expression of both the P2X7R and the inflammasome components was increased in patients with anti-Ro/SSA positivity and correlated with focus score (FS). So these results suggest an involvement of the P2X7R-inflammasome-caspase-1-IL-18 axis in the development of the SG damage in pSS, again emphasising the importance of the innate immunity in the pathogenesis of the disease.The evidence of a role of P2X7R in the pathogenesis of the disease is also confirmed by a work by Lester *et al*. (17) in which it was identified that a gain-of-function haplotype of the P2X7R gene (1405G) was a risk factor for the development of seropositive pSS and exhibited a negative epistatic interaction with HLA DR3, the major genetic risk factor for pSS. Xie *et al*. (18) have demonstrated a significant up-regulation of P2X7R on peripheral blood mononuclear cells after ATP stimulation in pSS patients compared to controls. The up-regulation of P2X7R after ATP stimulation was correlated to increased levels of IL1β in the supernatant of the cells. Moreover, in this study the expression level of P2X7R was positively correlated to the scores of anxiety and depression in pSS patients, so it was hypothesised that P2X7R can be involved in the pathogenesis of psychi-

atric disorders, representing a link between nervous and immune system.

A third aspect that it is worth mentioning is the amount of novel evidences further emphasising the central role of the epithelial cell in pSS pathogenesis. From this perspective, Lisi *et al*. (19-24) have investigated the effects of proinflammatory cytokines on the release of proangiogenetic factors (*i.e* growth regulated oncogene-alpha (GRO-α) and CXCR2) and nerve growth factor β (NGFβ) in the SG of pSS patients. They found that both CXCR2 and GRO-α, as well as the NGFβ, were stimulated by IL6 rather than by TNF-α. They also demonstrated that the activity of the GRO-α/CXCR2 system in pSS was mediated by the activity of the disintegrin and metalloproteinase ADAM17. Moreover, ADAM17 determined the release of the endogenous epidermal growth factor receptor (EGFR)-ligand, amphiregulin (AREG). and the ADAM17/AREG/EGFR axis drove the production of proinflammatory cytokines through the ERK1/2 activation in pSS.

Moving towards the novel insights into adaptive immunity, a great effort has been made in deeply characterising the role of different T-cells subsets in pSS. In this regard, Alunno *et al*. (25) have demonstrated that double-negative (DN) T cells are expanded in the peripheral blood of patients with long-standing pSS compared to healthy controls; they produce IL-17 and are also present in the SG infiltrate. In another work by the same group (26), it emerged that the levels of circulating DN T cells at the onset of pSS were comparable to those of healthy controls and lower than those of the patients with long-standing disease. On the contrary, in the early disease they found an increased number of circulating $CD4^+$ Th17.

Another important subset of T cells involved in the pathogenesis of autoimmune diseases are the T regulatory (Treg) cells. It is possible that a dysfunction or a depletion of Treg might contribute to the generation of autoimmune disorders like pSS. A new marker of Treg has been recently identified, the glucocorticoid-induced TNF receptor-related protein (GITR). CD25low-GITR+ cells are detectable in the peripheral blood of pSS patients and they express a regulatory phenotype (FoxP3, TGFβ, IL10), as in healthy donors. Interestingly, in agreement with their regulatory function, circulating CD25low-GITR+ cells are expanded in patients with inactive disease, compared with patients with active disease and healthy controls. At the same time, CD25low-GITR+ cells can also be found in a great number in the SG infiltrates, apparently in contrast with the idea that a depletion of Treg characterises autoimmune diseases. Indeed, it is interesting to note that the grade of lymphocytic infiltration in SG is positively correlated with both FoxP3 and IL-17 in patients with a mild-moderate inflammation, while Th17 clearly predominate on Treg cells in patients with a high degree of inflammatory infiltrate. This is probably due to the plasticity of Treg, which, in the context of an inflammatory environment, can change into IL-17 producing cells (27).

Another subset of T cells involved in the crosstalk between T and B cells are the follicular T helper cells (Tfh). Tfh are a subset of T cells that derives from T $CD4^+$ naïve lymphocytes under the stimulus of IL-12 secreted by dendritic cells. The main characteristic of Tfh is the expression of B-cell lymphoma (Bcl-6) transcription factor which regulates the expression of receptors (CXCR5, CXCR4, CCR7) driving Tfh towards the B-cell follicles, rich in CXCL13. Moreover, Bcl-6 regulates the expression of co-stimulatory molecules important in the T-B cell interaction. Szabo *et al*. (28, 29) found an elevated ratio of peripheral Tfh, compared to healthy controls, only in pSS patients with extra-glandular manifestations. Tfh values were positively correlated with circulating IgG levels, with SSA/SSB positivity and with the titers of autoantibodies; in addition they seemed to correlate with the severity of the GC and with the development of extra-glandular manifestations during the disease course. Jin *et al*. (30) found that SG Tfh are increased in pSS patients compared to controls and their percentage is positively correlated to the level of $CD19^+CD27^+$ memory B cells and $CD19^+$CD27 high plasma cells. Therefore, it seems clear that Tfh participate to the pathogenesis of pSS by promoting B-cell maturation. In addition, Gong *et al*. (31) have recently demonstrated that SGECs are able to induce the differentiation of Tfh from T $CD4^+$ naïve cells, through IL-6 secretion and that the expression of the inducible T cell co-stimulator (ICOS) enhances IL-21 production by Tfh.

As mentioned above, B cell hyperactivity represents the key pathogenetic moment in pSS. In fact, hypergammaglobulinaemia, autoantibody production, disturbances of B cell subpopulations, GC formation in the SG and an increased risk of developing B cell lymphoma are distinctive features of pSS (32-35). Lahiri *et al*. (36) demonstrated for the first time that BR3 is involved in the survival of SGECs due to an autocrine effect of B cell activating factor (BAFF). Corsiero *et al*. (37) studied the qualitative alterations of B cell subpopulations in the peripheral blood of pSS patients and healthy controls. First of all, they demonstrated that in the peripheral blood of pSS patients there is a significant accumulation of autoreactive naïve B cells and this can suggest that early B cell tolerance checkpoints are impaired in pSS. Moreover, they found that pSS patients show a reduction of circulating unswitched memory B cells, that is related to the migration of this subpopulation to the SG infiltrate where they represent the major B-cell subtype. It is important to know that switched memory B cells in pSS display anti-nuclear immunoreactivity, thus highlighting that there is an accumulation of autoreactive memory B cells in B-cell maturation. Finally, data from this study support the evidence that also later B-cell tolerance checkpoints are impaired in pSS. In fact, impaired mechanisms of autoreactive B cell follicular exclusion allow the clonal selection and affinity maturation of the autoreactive naïve and memory B cells, within the ectopic GC.

A decrease of unswitched memory B cells in pSS patients was also confirmed by Roberts *et al*. (38) in a study in which they examined the B-cell memory phenotypic and gene expression profile in pSS patients, sicca syndrome individuals and healthy controls. The number

of unswitched memory B cells also showed an inverse relation with clinical and serological abnormalities in pSS patients and, in particular, it was associated to a higher titer of autoantibodies. In this study, it also emerged that the unswitched memory B-cell gene profiles distinguished patients with pSS from healthy controls, suggesting that also the function of this B-cell subpopulation is altered. In particular, some evidences suggest that there could be a defect in BCR signalling in these cells from pSS patients. Another interesting result that emerges from this work is that the same alterations of B-cell phenotypic and gene expression profile also characterise a subgroup of sicca syndrome patients who do not fulfill the criteria for a diagnosis of pSS. So, this suggests that these individuals with a *B cell signature* could be in a preclinical phase of pSS.

## II. Novel insights into pSS classification criteria, activity and damage indices

For research purposes, several sets of classification criteria have been proposed since the 1980s but none of these has been validated and universally accepted (39-42). Consequently, in 1993, for the first time, a preliminary European criteria set was validated and adopted universally as classification criteria (43). However, these criteria, which focused on the three hallmarks of the disease (sicca symtoms, chronic exocrinopathy, systemic autoimmunity), raised objections concerning the biased misclassification of patients who could fulfill the subjective items for ocular and oral symptoms but not the histological or the autoimmunity criterion. To overcome such limitations, in 2002 the European criteria were re-examined in a revised version, the American Consensus Group (AECG) criteria set (44). According to AECG criteria, which maintained the previous European scheme of six items, either the minor salivary gland biopsy or serology for anti-Ro/SSA and anti-La/SSB had to be positive to classify a patient as affected by pSS. Moreover, each single item was defined more accurately. The AECG represent the most commonly used tool to classify patients with primary and secondary SS in clinical trials, epidemiological studies and in clinical practice, given their high sensibility and specificity. However, according to results derived from clinical settings, the higher specificity of the AECG criteria in comparison with preliminary criteria might lead to the exclusion of a considerable proportion of patients with classical features and long-term outcome complications of pSS (45). Moreover, the addition of rheumatoid factor in the classification criteria could be useful in differentiating patients with sicca symptoms sustained by an autoimmune systemic disorder, especially in anti-Ro/SSA and anti-La/SSB negative patients (45). Recently, the American College of Rheumatology (ACR)/ Sjögren's International Collaborative Clinical Alliance (SICCA) endorsed new classification criteria for pSS (46). According to the ACR/SICCA criteria, for pSS diagnosis 2 out of the following 3 are required:

i) positive anti-Ro/SSA and anti-La/SSB or positive rheumatoid factor and ANA ≥1:320;

ii) ocular staining score ≥3 (sum total score 0–12; 0–6 score for staining of the cornea with fluorescein, 0–3 score for staining of both the nasal and temporal conjunctivae with lissamine green);

iii) focal lymphocytic sialadenitis with FS ≥1 in labial gland biopsy.

The ACR/SICCA criteria do not target the general population but individuals suspected to be affected by pSS and are aimed to reveal the most typical and advanced subset of pSS patients, underscoring the subjective complaints of the disease (47). Rasmussen *et al*. recently compared the performance of the new ACR and the AECG classification criteria for pSS and found concordant results when applied to an homogeneous cohort of patients with sicca symptoms, providing no clear evidence for increased value of the new ACR criteria over the old AECG criteria from the clinical and biological perspective (48). Conversely, a difference in terms of accessibility was seen. Indeed, while the ACR criteria require evaluation by a practitioner specialised in eyes and lip biopsy, the AECG criteria involve simple questionnaires and objective, inexpensive tests which are easy to carry out. In addition, the AECG criteria gave more space to patient's subjective symptoms allowing clinicians to precisely select those subjects who most deserve to be considered for a diagnostic work-up for pSS (49). Even if the ACR criteria were not validated in patients with secondary SS in the SICCA cohort, a recent study supports their validity and application in patients with systemic autoimmune diseases (50). A major limitation of the AECG criteria rely on the employment of obsolete objective tests like sialography and scintigraphy. In this setting, it has been demonstrated that the addition of salivary gland ultrasonography (SGUS) to both criteria sets increases sensitivity without changing or slightly decreasing specificity, notably gaining diagnostic performance (47, 51-56). The incorporation of SGUS as an alternative to any of the three ACR classification items does not modify their diagnostic ability and could replace a more painful or invasive test (57, 58). However, the employment of SGUS as an adjunctive item in classification criteria needs further validation and standardisation.

The heterogeneous nature of pSS covers a broad spectrum of manifestations ranging from benign but disabling sicca symptoms to potentially severe extraglandular systemic manifestations, usually affecting 20–40% of patients. In this setting, great efforts have been recently made to provide valid and objective tools to be used in the assessment of subjective manifestations, like dryness, pain and fatigue, and in the measurement of disease activity and damage (59, 60). In recent years, different indexes have been developed for the evaluation of patients' perception of the disease, such as the Profile of Fatigue and Discomfort (PROFAD) and the Sicca Symptoms Inventory (SSI) (59). These tools evaluate the psychosocial impact of the disease and disease-related functional impairment, providing an evaluation of patients' quality of life. However, their usefulness in clinical practice has been debated due to their complexity. To overcome this issue, the EULAR SS Patient Reported

Index (ESSPRI) has been designed to measure the three main symptoms of SS patients (dryness, pain and fatigue) and recently validated in a large prospective international validation study (61, 62). The ESSPRI has been demonstrated to be a simple index, to possess a good construct validity and sensitivity to change, suggesting its validity as an outcome measure in clinical trials and clinical practice (62).

In order to evaluate systemic disease activity, the disease activity index (SSDAI) and the Sjögren' Systemic Clinical Activity Index (SCAI) were the first activity indexes to be proposed (59). However, their lack of exhaustiveness and complexity limited their use. Recently, the EULAR SS Disease Activity Index (ESSDAI) has been proposed (63). The ESSDAI includes specific organ-by-organ definitions and is more exhaustive than SSDAI and simpler to use in comparison to SCAI. Activity and patient-reported indexes have been shown to be valid and reliable. The activity index had a larger sensitivity to change in patients whose disease activity improved in comparison to patient-reported scores, amongst which ESSPRI had a significantly higher sensitivity to change (62). Seror *et al*. compared all disease-specific indexes and depicted a low correlation between systemic activity scores and patients symptoms, suggesting that these two components are different facets of the disease (62, 64). Moreover, the ESSDAI seemed to be the most valuable score for distinguishing currently active patients from inactive non-systemic or past-systemic patients (62). Interestingly, it has been demonstrated that a high baseline systemic activity (ESSDAI ≥14) is associated with a higher risk of death for systemic complications, suggesting the usefulness of ESSDAI as a prognostic outcome measure in SS patients (65, 66). Moreover, the presence at diagnosis of cytopenia, hypocomplementaemia and cryoglobulinaemia, all laboratory markers of a poorer prognosis), strongly correlated with a higher cumulative ESSDAI score during follow-up, suggesting that ESSDAI may be used to identify a subset of patients requiring closer follow-up (65). ESSDAI was also shown to be able to monitor responsiveness after rituximab treatment in a randomised clinical trial involving pSS patients (67). A recent study aimed to evaluate the relationship between EULAR indexes and quality of life demonstrated that higher scores of EULAR outcome measures correlated with poorer health states and that this correlation was strongest for ESSPRI (68-70). Finally, the definition of disease activity levels of ESSDAI, minimal clinically important improvement (MCII) and patient-acceptable symptom state (PASS) with ESSPRI was provided in a large prospective multicentre study (71). Low activity (ESSDAI≤5), moderate activity (5≤ESSDAI≤13) and high activity (ESSDAI≥14) levels of disease were defined. A decrease in the ESSDAI score of at least three points was defined as MCII and it may represent an outcome measure in future studies. Finally, PASS was defined as an ESSPRI <5 points and MCII of ESSPRI as an index decrease of at least one point or 15%. According to the results of this study, it may be hypothesised to include in clinical trials homogeneous cohorts of patients with at least moderate disease activity and to define response to treatment as an improvement of ESSDAI of at least three points. Inclusion of patients with unsatisfactory symptoms may allow the evaluation of the effect of new symptomatic therapies in pSS patients. Indeed, since disease activity and patient-reported symptom indexes poorly correlated, the choice of ESSDAI or ESSPRI as outcome measures in the evaluation of a new pSS therapy efficacy will depend on which component of the disease is the main target of the treatment (62, 71).

## III. Novel insights into pSS clinical manifestations

During the last two years, many relevant contributions have been published describing the heterogeneous clinical presentation of pSS. The four most significant areas of research that have been explored lately are essentially:

i) the assessment of the disease severity in large national cohorts,

ii) the analysis of the long-term mortality and its predictors,

iii) the differential diagnosis between pSS and the IgG4 disease and

iv) the evaluation of the cardiovascular comorbidity and its impact on the disease course.

The prevalence of severe systemic manifestations in pSS was described, in particular, in two large cohorts of Spanish and Italian patients, both using the ESSDAI definitions to assess the disease activity in each single organ (65, 72). Interestingly, as shown in Table I, the prevalence of severe systemic manifestations in the two cohorts was very similar for each individual organ or system. Moreover, both studies highlighted that a younger age at diagnosis was associated to severe systemic pSS manifestations as well as low C3/C4 levels and cryoglobulins.

Focusing the attention on the single organ involvement in pSS, renal involvement, and neurological involvement have lately been studied in detail. Goules *et al*. (73), more specifically, have estimated the prevalence and described the clinical features and the outcome of clinically significant renal involvement in a population of 715 patients with pSS. The kidney, in pSS, is potentially targeted by two distinct immunopathologic pathways: activated lymphocytes infiltrate tubu-

**Table I.** Prevalence of severe manifestations in large cohort of patients with pSS.

| | Ramos-Casals (65) (921) | Baldini (72) (1115) |
|---|---|---|
| Follow-up (months) | 75 | 70 |
| Age at the diagnosis (mean) | 53.8 | 51.6 |
| Articular (moderate/high) (%) | 9.5 | 11 |
| Skin (moderate/high) (%) | 8.1 | 9.5 |
| Lung (moderate/high) (%) | 3.7 | 5.4 |
| Peripheral nervous system (moderate/high) (%) | 4.7 | 5.3 |
| Central nervous system (%) | <3 | <3 |
| Kidney (moderate/high) (%) | <3 | <3 |

lar epithelium, resulting in interstitial nephritis, or an immune complex mediated glomerulonephritis. Moreover, although very uncommon, as described by Shavit *et al*. (74), patients with pSS can also present with atypical ANCA-MPO-related glomerulonephritis (GN). In the study by Goules *et al*. (73), 4.9% had clinically significant renal involvement; half of them presenting with GN, nearly 40% with interstitial nephritis (IN), while the remaining patients presented both entities. This study demonstrated that patients with pSS and renal involvement had significantly reduced survival compared to those without renal involvement, with LNH being the leading cause of death. Notably, death and lymphoma occurred more frequently in the GN patients than in the interstitial nephritis patients; the majority of GN patients developed lymphoma within 3 months of the appearance of renal disease, suggesting that GN can be a paraneoplastic phenomenon. Cryoglobulinaemia was found to mark serologically GN patients confirming the previous data on worsening survival ratio in pSS patients. Interestingly, the major proportion of patients that developed chronic renal failure was the IN group despite the notion that this kind of renal involvement evolves slowly and without functional damage. However, this observation could be a consequence of the reduced survival of GN patients.

Regarding peripheral involvement Brito-Zeron *et al*. (75) published a retrospective study in which 55 patients with pSS-related peripheral neuropathy were analysed. The most common form observed was the axonal sensorimotor polyneuropathy, characterised by a good evolution and in which a conservative approach should be recommended unless patients present an acute onset of the disorder. Sensory neuropathy is the most disabling form, with a poor response to treatment, and notably, precedes the pSS diagnosis in a large proportion of patients. Multiplex mononeuropathy is strictly linked with a high systemic activity profile, characterised by cryoglobulinaemic vasculitis, salivary gland swelling and cytopenia. In spite of an excellent therapeutic response, patients with multiplex mononeuropathy have the poorest survival. In summary, peripheral neuropathy in pSS patients is common and associated with a poor prognosis.

As far as mortality in pSS was concerned, a very important contribution came from the Spanish Group of Autoimmune Diseases (66). In their study they analysed the long-term outcome of 1045 patients and correlated the baseline activity with survival in this large cohort of patients. After a mean follow-up of 117 months, 115 (11%) patients died. The adjusted standardised mortality ratio for the total cohort was 4.66 (95% CI 3.85–5.60), and survival rates at 5, 10, 20 and 30 years were 96%, 90%, 81% and 60%, respectively. The main baseline factors associated with overall mortality in the multivariate analysis were male gender, cryoglobulins and low C4 levels. The authors also found that patients with pSS, who present at diagnosis with high systemic activity (ESSDAI ≥14) and more than one laboratory predictive marker (lymphopenia, anti-La, monoclonal gammopathy, low C3, low C4 and/or cryoglobulins) were at a higher risk of death.

Similarly, Horvath *et al*. (76) retrospectively evaluated the mortality ratios in a cohort of 547 pSS Hungarian patients, observing a negative effect of cryoglobulinaemia on survival ratios; additionally, the presence of vasculitis and lymphoproliferative diseases at the time of diagnosis increased the risk of mortality. Finally, Palm *et al*. (77) found that mortality was significantly increased after 10 years of disease among patients with lung involvement compared with those without lung involvement. Regarding cancer and pSS, a meta-analysis has recently been published that clearly demonstrates the association between pSS and the overall risk of malignancy; however it is still unclear whether this is due to excess occurrence of non-Hodgkin's lymphoma (NHL) and further studies are needed to better explain this association (78). Lymphoma development, was associated with an excess in the overall disease mortality. This observation confirms previous data and highlighted the importance of the early identification of the 'high-risk' pSS population with a specific need for closer monitoring as well as tailored and probably more robust therapeutic management. Two different groups tried to identify predictors of lymphoma in SS, approaching the same problem from different points of view. Risselada *et al*. (79) tried to determine the relationship between NHL and the disease activity and severity assessed by the ESSDAI score. The authors confirmed the associations between NHL and parotid enlargement, purpura, peripheral nervous system, GN, low C4 and IgM-kappa monoclonal compenent. However, no association was detected between the ESSDAI score and NHL development. Quartuccio *et al*. (80, 81) confirmed the value of cryoglobulinaemia, low C4, anti-La antibodies and leukopenia as serological markers for NHL. The authors tried to stratify the risk of NHL evolution in patients with prelymphomatous conditions such as salivary gland swelling and cryoglobulinaemic vasculitis using the same serological markers. Interestingly they found that among pSS patients with SW, only those with a positivity for the mentioned biomarkers presented an increased risk of evolution towards NHL.

The hot topic of the differential diagnosis between pSS and IgG4-related sialoadenitis has been analysed in several papers (82). Mavragani *et al*. (83) found that 7.5% of a cohort of 133 pSS patients presented raised IgG4 serum levels in association with IgG4RD-reminiscent clinical, serologic, and histopathologic features. The authors wondered whether this high-IgG4 pSS group represented a misclassified IgG4-RD group or a distinct pSS subtype.

Finally, the fourth line of research emphasised the prevalence and impact of cardiovascular morbidity on pSS (84-87). More specifically, a very recent study by an Italian group showed that, similarly to other autoimmune diseases, patients with pSS may be characterised by an increased risk of major cardiac and cerebrovascular events in comparison with healthy subjects. Bartoloni *et al*., observed in a large cross-sectional, retrospective study, a significantly higher prevalence of cardiovascular

events in patients with pSS with respect to the general population. Furthermore, cardiovascular events were more common in patients with more severe and extensive disease and with greater need to use glucorticoids and immunosuppressive therapies to control disease manifestations. Patients with leukopenia had a six-fold higher risk of developing angina compared to those with a normal white blood cell count, underlining the fact the disease itself has a role in promoting this kind of complications. Notably, concerning cardiovascular risk factors hypertension and hypercholesterolemia were more prevalent in the pSS population, whereas smoking, obesity and diabetes were less (84).

## IV. Novel insights into diagnostic tools for SS

During the last two years, two main areas of interest have been investigated in order to improve the diagnostic algorithm of pSS. On the one hand, a critical appraisal of the minor salivary gland biopsy and its value in the prognostic stratification of pSS patients has been pursued. On the other hand, novel diagnostic tools have been extensively tested and novel biomarkers have been searched for. More specifically, to improve the diagnostic performance and the reproducibility of the minor salivary gland biopsy (MSGB), a national validation study has been promoted (88). At the same time, the prognostic value of routinely performed MSGBs has been independently analysed by Carubbi *et al*. (89) and Risselada *et al*. (90) correlating the presence of FS and of germinal centre-like in the MSGBs with the severity of the disease and an increase in lymphoma risk. In parallel, novel biomarkers and novel diagnostic tools have being investigated. Some of them, although promising, are still in their infancies, especially proteomic and genomic studies (91-94).

Among novel diagnostic tools, a great interest has arisen for *in vivo* confocal scanning laser microscopy (CSLM) as a non-invasive method to evaluate the involvement of the lachrymal functional unit in pSS. To date, the study of the conjunctiva at the cellular level has been made by more invasive methods (impression or brush cytology and immunohistochemistry staining or flow cytometry); CSLM is a new technology that allows the *in vivo* assessment of many ocular surface diseases and anterior-segment disorders. At the end of 2014, Gabriellini *et al*. (95) examined the cornea in patients with pSS and patients with no-SS dry eye and they found a significant decrease in corneal thickness and in epithelium cell density as well as a more frequent superficial keratocyte activation and sub-basal nerve abnormalities in pSS.

However, the most promising novel diagnostic tool for pSS is probably SGUS which appears to be an easy, non-invasive and highly specific imaging technique for the assessment of salivary gland involvement in pSS (58, 96, 97). Even if its role in the early stages of the disease is still debated (52), SGUS has been proposed as a valid alternative to other standard procedures considering its characteristics: with a higher specificity with respect to sialometry and salivary scintigraphy and a similar diagnostic accuracy of sialography (55, 98). It is worthy of note that, when used in association with traditional tests, SGUS also improves the diagnostic sensitivity of the AECG criteria from 77.9% to 87.0% (52).

Among the US parameters evaluated, the parenchymal inhomogeneity appeared to be the most useful diagnostic tool with a high specificity and a good sensitivity: US inflammatory findings (hypoechoic areas and hyperechoic bands) showed a significant correlation with clinical, histologic and laboratory data. In fact they were more frequently observed in patients with a low unstimulated and stimulated salivary flow, with a positivity serological profile (positivity for antinuclear antibodies, anti Ro-SSA and/or anti-SSB and/or rheumatoid factor) and with FS ≥1 (54, 97). In addition, the real-time sonoelastography (RTS) due to its capacity in quantifying tissue rigidity of salivary glands has been recently proposed by Dejaco *et al*. in combination with the B-mode SGUS to assess the glandular damage in pSS patients with promising results (99).

All these results have further supported the routine use of SGUS in the diagnostic algorithm of pSS (57, 96, 100). To this end, an international expert group has been created to elaborate a new scoring system for SGUS by using OMERACT filters.

## V. Novel insights into pSS treatment

To date, therapeutic options for pSS patients are mainly empirical and evidence-based recommendations for the treatment of extraglandular disease manifestations are lacking. No therapy has been demonstrated to significantly affect disease course and, at the moment, evidence-based therapy for pSS is mainly limited to symptomatic drugs for dryness. In this setting, in a recent retrospective study it has been demonstrated that cevimeline, a parasympathomimetic drug, has a better profile of efficacy and tolerability in comparison to pilocarpine (101).

To date, literature evidence regarding the role of conventional immunosuppressive therapy is limited. Hydroxychloroquine (HCQ) is the most used drug to treat articular involvement in pSS patients. However, evidence regarding its efficacy is scarse. Indeed, a recent randomised clinical trial demonstrated that HCQ was not better than placebo in improving dryness, pain and fatigue in a large cohort of pSS patients (102). Over a 5-year follow-up, the use of HCQ was associated with a significant improvement of the atherogenc index (*i.e*. reducton of total cholesterol and increase of high density lipoprotein). Of interest, the positive effect of HCQ on lipid profile was observed also in SS patients not receiving hypolipidemic agents, suggesting that chronic HCQ use may be associated with an improvement of cardiovascular risk in pSS (103).

The effect of leflunomide (LEF), an immunomodulatory agent commonly used in rheumatoid arthritis (RA), on eye dryness has been evaluated in a cohort of RA patients with secondary SS. Despite the efficacy on disease activity, a 3-month LEF use was associated with a significant deterioration of eye dryness in RA patients with secondary SS with respect to RA patients without sicca syndrome. Moreover, LEF use

was associated with a major risk of ocular adverse effects, including peripheral ulcerative keratitis and keratocojunctivitis sicca with punctate in RA patients with secondary SS (104).

In recent years, the use of biologic agents in pSS has rapidly expanded. In particular, given the central role B-lymphocytes in pSS pathogenesis, the anti-CD20 biologic agent rituximab, inducing B cell depletion by several mechanisms, was considered a potential therapeutic option for pSS patients. Evidence-based data on the clinical benefit of rituximab administration in pSS patients are conflicting. Gottenberg *et al*. demonstrated an improvement of systemic disease manifestations, including parotid swelling, pulmonary and articular involvement, six months following the first treatment cycle in a cohort of 78 pSS patients (105). No data were available on the evolution of dryness, pain and fatigue. In addition, both the median ESSDAI and the median daily dose of prednisone decreased 6 months after rituximab administration (105). Likewise, an Italian study by Carubbi *et al*. reported a significant improvement in ESSDAI and other parameters in a cohort of forty-one patients with early pSS and high disease activity (ESSDAI ≥6) at 120 weeks following rituximab administration with respect to conventional DMARDs (106). Of interest, a significant improvement in objective (unstimulated salivary flow and Schirmer's test) and histologic (Chisholm and Mason grading and FS) parameters was observed in a rituximab treated cohort (106).

Conversely, two clinical studies failed to demonstrate a significant clinical benefit following rituximab treatment (107, 108). In detail, a recent randomised trial on 120 pSS patients concluded that rituximab was not superior to placebo in improving disease-associated symptoms or disease activity at week 24. However, an improvement in visual analogue scale (VAS) fatigue score was more common in the rituximab cohort at earlier time points (107). Similarly, a statistically significant improvement in some symptoms, including oral dryness and discomfort and fatigue, was observed in a small pSS cohort at 26 weeks following a single course of rituximab. However, no significant improvement was reported on other parameters, including joint pain, salivary flow, tear production and quality of life as measured by the SF-36 (108). All trials, however, demonstrated a good safety profile of rituximab therapy in SS patients (105-108).

Interestingly, De Vita *et al*. described a female patient with a SS-related lymphoproliferation and cryoglobulinaemic vasculitis sequentially treated with the monoclonal antibody against BAFF, belimumab, and rituximab after failure of each drug singularly administered (109). A complete and persistent regression of both lymphoma and skin ulcers with normalisation of cryoglobulins and rheumatoid factor was reported following treatment administration (109).

Another biologic therapy evaluated as potential therapeutic target in pSS aimed to interfere with B-cell activation. An open-label phase II study on 30 SS showed encouraging results at 28 weeks following belimumab administration, with more than half of the patients achieving improvement in subjective symptoms of dryness, fatigue and pain and objective activity scores and/or in laboratory markers of disease-related immune system dysregulation, including serum levels of free light chains of immunoglobulin, β2-microglobulin, monoclonal component and C4 levels. In addition, the mean ESSDAI and ESSPRI significantly decreased and the safety profile was good while objective measures of dryness did not change (110).

In recent years, the role of T lymphocytes in disease pathogenesis has been clearly demonstrated. Indeed, it has been demonstrated that T cells are involved in the formation of inflammatory infiltrated in salivary glands of pSS patients and to increase B-cell activation. Consequently, studies evaluated the effect of T-cell targeted therapies in SS. The cytotoxic T lymphocyte antigen 4 abatacept has been judged effective, safe and well tolerated in pSS patients in three clinical trials (111-113). Specifically, an open-label prospective study on 15 primary pSS patients demonstrated an improvement in quality of life measures and in several clinical and laboratory parameters 24 weeks after drug administration (111). However, objective measures of dryness did not change during treatment (111). On the contrary, a 1-year open-label study on 32 RA patients with secondary SS demonstrated a slight increase in saliva and tear volumes 24 weeks after abatacept administration (112). In line with these findings, histopathologic revision of minor salivary gland biopsy samples obtained before and after 8 doses of abatacept in 10 pSS patients showed a significant decrease in the total number of lymphocytic foci and in the density of all infiltrating lymphocytic subpopulations. In addition, saliva secretion tended to increase reaching statistical significance when the data were adjusted for disease duration (113).

No clinical trials evaluating the effect of anti-TNF agents and anti-IL-6 receptor antibody tocilizumab have been published the last two years. Case series reporting the beneficial effect of tocilizumab in a 52-year-old woman diagnosed with ulcerative colitis who developed pSS during infliximab therapy, and in a 38-year-old SS woman with relapsing transverse myelitis, have been published (114, 115). Similarly, no clinical trials have been published on further biologic agents except reviews exploring IL-1, IL-10, IL-17 and CD6 as possible targets for the treatment of SS (116-119). A summary of clinical trials on the effect of biologic therapy in pSS is reported in Table II.

New drugs for pSS treatment are currently being developed. A protein polymer-based platform consisting of diblock copolymers composed of Elastin-like polypeptides fused with FKBP12 was evaluated in non-obese diabetic mouse, a classic animal model of SS. This nanoparticle formulation significantly suppressed lymphocytic infiltration in the lacrimal glands suggesting its potential role as future promising tool for the treatment of pSS patients (120).

Inhibition of p38-MAPK pathway has been investigated as a potential treatment strategy for SS-related dry eye. In particular, p38-MAPK pathway inhibitor lacrimal gland injection sig-

**Table II.** Clinical trial on biologic agents in SS patients.

| Drug | Author (reference) | Study | Patients (n) | Dosage | Median follow-up | Efficacy | Adverse events |
|---|---|---|---|---|---|---|---|
| Rituximab | Gottenber (105) | Prospective multicentre | 78 | 1 g days 1/15 (67 pts) 375mg/m$^2$/ weekly for 4 weeks (11 pts) | 34.9 months | At 6-month: - median ESSDAI decrease -corticosteroid dosage reduction | - infusion reactions - delayed serum sickness-like disease - serious infections – cancer |
| | Carubbi (106) | Prospective, multi-centre | 19 RTX 22 DMARDs | 1 g days 1/15 repeated every 24 weeks | 120 weeks | In RTX group: - higher decrease of ESSDAI, VAS global disease activity, VAS pain, physician global assessment; - Schirmer's test and histological improvement | No adverse events |
| | Devauchelle-Pensec (107) | RCT, double-blind | 122 | 1 g days 1/15 | 24 weeks | No significant difference primary end-point; improvement in VAS fatigue score in RTX | RTX group: - severe infections - cytopenia - purpura - allergic events |
| | St Clair (108) | Prospective, open-label, single arm | 12 | 1 g days 1/15 (single course) | 52 weeks | At week 26 modest improvement in pts reported symptoms, lacrimal and salivary function | - vaccination reactions - cancer |
| Belimumab | Mariette (110) | Open-label, phase II | 31 | 10 mg/kg, at week 0, 2 and 4 then every 4 weeks | 28 weeks | Primary endpoint achieved in 60% of patients; significant ESSDAI and ESSPRI decrease; salivary flow and Schirmer's test unchanged | - infections - cancer |
| Abatacept | Meiners (111) | Open-label, phase II | 15 | 10 mg/kg on days 1, 15 and 29 then every 4 weeks | 48 weeks | Median ESSDAI/ESSPRI decrease HR-QoL improvement; salivary flow/lacrimal function unchanged | - mild infusion n reaction - dizziness and hypotension - mild infections |
| | Tsuboi (112) | Open-label, prospective, observational multicenter | 32 | 500 mg (<60 kg) 750 mg (>60 kg) at weeks 0, 2, 4, and every 4 weeks | 52 weeks (interim analysis 24 weeks) | Slight increase of saliva and tear volume at week 24 | - infections |
| | Adler (113) | Open-label, prospective, observational | 11 | 500 mg (<60 kg) 750 mg (>60 kg) at weeks 0, 2, 4, 8, 12, 16, 20, 24 | 28 weeks | Significant reduction of lymphocytic foci at biopsy and increase of saliva secretion | - lupus-like skin lesions - diverticulitis |

DMARDs: disease-modifying anti-rheumatic drugs; ESSDAI: EULAR Sjögren's syndrome disease activity index; pSS: primary Sjögren syndrome; ESSPRI: EULAR Sjögren's Syndrome Patient Reported Index; pts: patients; RA: rheumatoid arthritis; RCT: randomised clinical trial; RTX: rituximab; VAS: visual analogue scale.

nificantly increased tear production and reduced lymphocyte infiltration in a mouse model of pSS (121).

It is interesting to note that, although the role of vitamin D deficiency on primary SS pathogenesis and clinical presentation remains unclear, reduced vitamin D levels at the earliest stages of the disease have been found in these patients, suggesting a potential benefit of vitamin D in SS (122).

Referring to topical therapy, trials on the amino acid analogue of 2 (1H)-quinolinone rebamipide, cyclosporine, botulinum neurotoxin type A, autologous serum eye drops, and anti-IL-1 agent anakinra have recently been published (123-128).

Administration of a 2% rebamipide ocular suspension in thirty SS patients led to significant improvement of fluorescein staining and lissamine green-staining score and of tear film break-up time (BUT) at week 4, in association with subjective symptom improvement (123).

Twenty-six pSS patients treated with one drop of 0.05% cyclosporine twice a day reported significant improvements in all subjective symptoms at 1 week in comparison to a control group. In addition, significant improvements in eye redness, Schirmer's test and BUT were depicted at 1 week. No adverse effects related to the drug occurred (124).

Botulinum neurotoxin type A injection in 24 SS patients lead to an increase in the Schirmer score in all cases. However, a loss of efficacy was depicted after a mean time of 4.5 months (125).

In the same way, a significant improvement of ocular symptoms, ocular surface staining scores and BUT was reported in pSS patients following autologous serum eye drops administration (126, 129).

Amino acids enriched tears were tested in 20 patients by the administration of sodium hyaluronate (SH) plus L-glycine, L-proline, L-lysine hydrochloride, and L-leucine. At 1-month follow-up, a significant improvement of BUT score was observed in these patients compared to subjects treated with SH only. Efficacy of treatment was maintained at 3-month of follow-up in patient treated with amino acids enriched tears sug-

**Table III.** Studies on topical eye medications in SS patients.

| Drug | Author (reference) | Dosage | Composition | Patients (n) |
|---|---|---|---|---|
| Rebamipide | Arimoto (123) | 1 drop per eye, 4 times daily for 4 weeks | 2% suspension | 30 |
| Cyclosporine A | Deveci (124) | One drop twice/daily | 0.05 suspension | 26 |
| Botulinum neurotoxin type A | Bukhari (125) | A single subcutaneous injections of 0.1 mL 2 mm inferior to the lid margin medial to the lower punctum of both lower eyelids | 3.3 U/0.1 ml | 24 |
| Autologous serum eye drops | Hwang (126) | Eye drops 8 times/daily for 4 weeks | 50% autologous serum plus 0.1% SH | 20 pSS and 14 secondary SS |
| | Cho (129) | Serum eye drops were applied six times/daily in addition to preservative-free artificial tears four times/daily | Four different formulations:<br>-100% autologous serum<br>-50% serum with normal saline<br>-50% serum with SH<br>-50% serum with ceftazidime | 22 SS patients on a total of 85 patients |
| Amino acids enriched tears | Aragona (130) | 1 drop x 5 times/daily | SH (0.15%) plus L-glycine (0.1%), L-proline (0.0752%), L-lysine hydrochloride (0.014%), and L-leucine (0.0108%) | 20 |

pSS: primary Sjögren's syndrome; SH: sodium hyaluronate.

gesting that, although SH is one of the most common substances employed as tear substitute, the addition of amino acids may be of additional benefit (130). Table III summarises studies on topical treatment in pSS patients.

In conclusion, noteworthy contributions to our understanding of pSS have been made over the past two years. Novel insights into the pathogenesis of the disease, as well as data coming from multicentre studies exploring the clinical presentations of the disease and its subsets, have opened new avenues for the treatment of the disease. It is likely that in the near future, novel therapeutic strategies will be available for pSS that will ultimately improve prognosis and long-term outcomes of pSS patients.

## List of abbreviations

pSS: primary Sjögren's syndrome
GWAS: genome wide association
IRF5: interferon regulatory factor 5
STAT4: signal transducer and activator of transcription 4
IL: interleukin
INF: interferon
BLK: B lymphocyte kinase
CXCR5: chemokine receptor type 5
TNIP1: TNFAIP3 interacting protein 1
TLS: tertiary lymphoid structures
EBV: Epstein-Barr virus
GC: germinal centre
ELS: ectopic lymphoid structures
moDC: Monocyte-derived dendritic cells
SG: salivary glands
SGECs: salivary gland epithelial cells
FS: focus score
GRO-α: Growth-regulated oncogene alpha
NGFβ: nerve growth factor β
EGFR: epidermal growth factor receptor
ERK: extracellular-signal-regulated kinases
DN: double-negative
GITR: glucocorticoid-induced TNF receptor-related protein
T reg: T regulatory
Tfh: follicular T helper cells
BAFF: B cell activating factor
AECG: American European Consensus Group
ACR: American College of Rheumatology
SICCA: Sjögren's syndrome International Collaborative Clinical Alliance
SGUS: salivary gland ultrasonography
PROFAD: profile of fatigue and discomfort
SSI: sicca symptoms inventory
ESSPRI: EULAR SS Patient Reported Index
SSDAI: SS Disease Activity Index
SCAI: Sjögren's Systemic Clinical Activity Index
ESSDAI: EULAR SS Disease Activity Index
MCII: minimal clinically important improvement
GN: glomerulonephritis
NHL: non-Hodgkin's lymphoma
MSGB: minor salivary gland biopsy
CSLM: confocal scanning laser microscopy
RTS: real-time sonoelastography
HCQ: hydroxychloroquine
LEF: leflunomide
RA: rheumatoid arthritis
SH: sodium hyaluronate

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