Review

One year in review 2021: Sjögren's syndrome

G. Cafaro[1], R. Bursi[1], L.G. Chatzis[2], G. Fulvio[3], F. Ferro[3], E. Bartoloni[1], C. Baldini[3]

[1]*Rheumatology Unit, Department of Medicine and Surgery, University of Perugia, Italy;*
[2]*Department of Pathophysiology, Athens School of Medicine, National and Kapodistrian University of Athens, and Institute for Autoimmune Systemic and Neurological Diseases, Athens, Greece;*
[3]*Rheumatology Unit, Department of Clinical and Experimental Medicine, University of Pisa, Italy.*

Giacomo Cafaro, MD
Roberto Bursi, MD
Loukas G. Chatzis, MD
Giovanni Fulvio, MD
Francesco Ferro, MD
Elena Bartoloni, MD
Chiara Baldini, MD, PhD

Please address correspondence to:
Chiara Baldini,
Rheumatology Unit,
Department of Clinical and Experimental Rheumatology,
University of Pisa,
via Roma 67,
56126, Pisa, Italy.
E-mail: chiara.baldini74@gmail.com

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ABSTRACT

Sjögren's syndrome (SS) is a multifactorial systemic autoimmune disease of unknown aetiology characterised by a wide spectrum of different clinical manifestations and scattered complications. Recently, great efforts have been made to elucidate mechanisms involved in the pathogenesis of the disease in order to identify exploitable therapeutic targets in SS. Similarly, novel insights have enabled to better define disease phenotypes and different outcomes. Ultimately, the discovery of new potential therapeutic targets and a better stratification of patients are paving new avenues for novel treatment options and treat-to-target therapeutic approach. In this review, we will provide a critical digest of the recent literature published in 2020 on SS pathogenesis, clinical manifestations and novel treatment options.

Introduction

Sjögren's syndrome (SS) is a multifactorial systemic autoimmune disease of unknown aetiology characterised by a wide spectrum of different clinical manifestations and scattered complications (1).

A still undetermined interplay of environmental, genetic, and epigenetic processes accounts for the initiation, perpetuation, and sustainability of the autoimmune inflammatory response towards the affected epithelium. Recently, novel insights have been published on SS pathogenesis, clinical phenotypes, different outcomes and therapy. Following the other reviews of this series (2-5), in this annual review we will summarise the most relevant contributions in the field published during 2020.

New insights into SS pathogenesis

Genetics, epigenetics and environmental factors in SS

Original data emerged in the last months confirming that the key players in SS pathogenesis are indeed the persistent activation of type I interferon system (IFN) together with autoreactive B and T cells and disease-associated autoantibodies thus offering interesting targets for individualised therapeutic approaches in SS (5). Several genetic polymorphisms and epigenetic dysregulation have been described in SS (6, 7). A growing body of research focuses on epigenetic alterations in SS, with the altered patterns of DNA methylation and the non-coding RNAs (ncRNAs) forming the core of investigations. Herein, we focus on recent developments in epigenetics-oriented research in SS.

DNA methylation, a cellular process responsible for gene repression, is the most intensively studied epigenetic modification in SS. The procedure is actively catalysed by a group of enzymes, known as the DNA methyltransferases (DNMTs) (8) responsible for the addition of a methyl residue onto the fifth carbon of the cytosine ring of CpG dinucleotides. If DNA methylation takes place in the promoter of a given gene, it foils the binding of transcriptional factors that are key to the building of mRNA, leading to the genes' silencing (9). In SS, heavily methylated or hypomethylated genes of interest have been investigated extensively during the last 10 years (10). More recently, hypothesis-free, telomere to telomere methylation studies, known as Epigenome Wide Association Studies (EWAS), utilising high throughput techniques, have been carried out in patients with SS disclosing important alterations in the methylation of minor salivary gland epithelial cells and peripheral lymphocytes, when compared to sicca controls. Indeed, hypomethylation of the promoters of interferon-inducible genes (STAT1, USP18, IFI44L, IRF5, TNFAIP8) in different cell types and genes involved in the trafficking of solutes typified most studies, reinforcing the importance of interferons and transmembrane

transporters in the pathophysiology of the disease. Under this prism, Karagianni *et al*. investigated the methylation status of five selected gene loci in the saliva of 16 SS patients (11). Compared to 10 sicca controls, a hypomethylated state that can epigenetically regulate the expression of the H19 imprinting control region (ICR) was revealed. This finding was correlated with C4 hypocomplementemia. Loss of H19 imprinting has been implicated in various types of cancer, but its importance in autoimmunity is unknown (11).

Interestingly, quantitative differences in DNA methylation have been observed between two alleles of a polymorphic loci, forming an epigenetic mechanistic channel between DNA sequence variations (SNPs) and disease predisposition. The genetic loci influencing methylation patterns across wide genome areas, able to forge an allelic asymmetry in DNA methylation in the presence of nucleotide polymorphisms, are known as methylation quantitative trait loci or meQTLs. These sequences are usually located inside or around the genomic regulatory elements (cis regulation), but they can also be found more distantly, even in another chromosome (trans regulation). Arvaniti *et al*. explored the connection of cell-specific SS-associated SNPs and their effect on DNA methylation marks on peripheral blood mononuclear cells (PBMCs), CD4 and CD8 T cells, and cultured salivary gland epithelial cells (12). They showed that methylome-changing SNPs are usually located within the introns and in close proximity to transcription start sites. The most effective variants are those associated with histone marks of active promoters. Both cis and trans regulation was evident, dampening the importance of physical distance between meQTLs and target genes. The pathophysiologic pathways regulated by SS-associated methylome altering SNPs were also investigated with bioinformatic tools, showing the impact of epigenetic phenomena on various proinflammatory cascades important in SS, including the IFN-γ signalling (12).

Adding to the growing list of methylome-based applications, Björk *et al*. proposed an alternative to the reliance of the interferon system activity assessment on mRNA-based scores, exploiting DNA methylation analysis of 3 interferon related genes in SS patients (13). This novel methodology generated scores with a strong correlation to RNA sequencing based IFN scores, able to correctly classify all but one of SS analysed patients into high and low IFN signature groups, using whole blood for methylome assessment. This suggests that DNA methylation patterns can be employed to assess INF signature on historical cohorts, in which RNA samples may not be readily available (13).

Recent studies have shown that several forms of ncRNA, including microRNAs (miRNAs), short interfering RNAs (siRNAs), piwi-interacting RNAs (piRNAs), circular RNAs (circRNAs), as well as long (>200 nt) ncRNAs (LncRNAs) are implicated in epigenetic processes. Of them, microRNAs are the most intensively studied in SS. More than 50 miRNAs have been found variably expressed in different type of cells in SS patients (14). Indeed, recently it was shown that, in contrast to blood, SS saliva miRNA expression profile may be able to discriminate Sjogren syndrome patients from sicca controls. However, the results of the study require validation, given the small number of patients (15).

Many putative functions have been ascribed to miRNAs in SS, including a role in immune tolerance disruption, enhancement of pro-inflammatory signalling, lymphomagenesis, as well as, the recent implication of miRNAs in the suppression of CD4+ T-cells from SS patients induced by co-culture with normal mesenchymal stem cells (14, 16). Recently, added in the growing list is apoptosis, since 2 miRNAs (miR-1207-5p and miR-4695-3p) regulating apoptotic mechanisms as well as the expression of TRIM21, the autoantigen of Ro52, have been found down-regulated in SS (17). In this line, transfection of those miRNAs in cultured human submandibular gland (HSG) cells resulted in down regulation of both antiapoptotic genes and TRIM21, while microRNA inhibitors reversed the process conferring a pro-apoptotic phenotype in these cells similar to that found in SS (17). However, in general, the complexity of the miRNA/mRNA regulatory axis has precluded the precise understanding of their role in the pathogenesis of the disease. To this end, Pilson *et al*., focusing exclusively on ocular inflammation, demonstrated that miR-744-5p that is upregulated in cultures of primary human conjunctival epithelial cells (PECs), can transduce the production of various pro-inflammatory cytokines via downregulation of Pellino3 (PELI3), a known negative regulator of inflammation (18). Undoubtedly, the highlight of this year's research on miRNAs in SS was the study by Cortes-Troncoso *et al*. who suggested a functional relationship between immunopathology and glandular dysfunction. The influence of immune cells on epithelial cell gene expression was carried out by T cell derived exosomes transferring the miR-142-3p. When delivered and engulfed in the neighbouring epithelial cells, miR-142-3p was found to restrict cAMP production, alter calcium homeostasis and lead to a decrease of protein production, ultimately resulting in glandular cell dysfunction (19).

Another type of conserved across species, non-coding RNAs are the LncRNAs which consist of more than 200 nucleotides and have been recently found important in epigenetically regulating immune responses. In SS, research has been mainly focused on the LncRNAs transcription profile, delineating how specific LncRNAs can affect both interferon signalling in T-cells and the transition to aerobic glycolysis ("Warburg Effect"), necessary for cellular expansion and proliferation. In 2020, the role of nuclear paraspeckle assembly transcript 1 (NEAT1), a lncRNA known to act as a mediator of inflammasome activation, was investigated by Ye *et al*. It was shown that even though NEAT 1 was found upregulated in peripheral T cells of SS patients, clinical correlations were opposite between CD4+ and CD8+ T-cells, with the CD4 T-cells showing a positive correlation as opposed to CD8 cells. Additionally, studying Jurkat cell lines disclosed that NEAT1 affected the expression of NEAT1-induced factors, such as CXCL8 and TNF-α, by altering

the expression of p-p38 and p-ERK1/2 of the MAPK pathway. These experiments revealed the mechanism behind NEAT 1 proposed implication in SS (20). Another study investigated the interplay of LncRNAs and mRNAs in SS, in a 2-step process (detection and validation). LncRNAs were differentially expressed in PBMCs of SS patients with NRIR and BISPR being the most upregulated compared to healthy controls. Furthermore, co-localisation and co-expression analysis revealed that the mRNAs correlated with the upregulated LncRNAs, associate predominantly with immune signalling pathways (NF-κB, JAK-STAT) and with the cascade involved in the cellular aspects of metastasis studied primarily in cancer (LOXL2) (21). Finally, in the study of Chen *et al*., plasma levels of lnc-DC, a dendritic cell-specific LncRNA, was also found upregulated in SS compared to healthy and disease controls including lupus and rheumatoid arthritis patients. An interesting finding was that longitudinally assessed samples revealed a decrease in Inc-DC plasma levels after treatment (22).

Adding to the complexity of epigenetic modifications in SS, an emerging player has been recently proposed, the Circular RNAs. Circular RNAs are non-coding RNAs created by back-splicing of exons from pre-mRNA that may act as a miRNA hub, since they pack multiple miRNAs binding sites. Recently, Li *et al*. showed that compared to sicca controls, circ-IQGAP2 and circ-ZC3H6 were found upregulated both in salivary gland biopsies and plasma exosomes of SS patients, correlating well with important clinical manifestations and histologic activity of SS, and thus serving as potential disease biomarkers (23).

Besides genetics and epigenetics, environmental factors have been investigated as trigger factors in SS(24). Among them an increasing interest in SS dysbiosis of the gut and oral microbiomes has emerged (25, 26).

Several reports have described microbiome compositional differences in SS patients compared to sicca and healthy volunteers (27, 28). Human gut is mainly inhabited by two phyla of bacteria, Firmicutes and Bacteroidetes, the latter mostly dominated by Bacteroides and Prevotella genera (29). During 2020, new evidences have been published showing that Bacteriodetes increased in pSS gut microbioma, while Firmicutes/Bacteroidetes ratio was reduced (30). Of interest, data showed that genus of Prevotella was significantly increased and that tear secretion was strongly affected by Prevotella (30). Noteworthy, to investigate potential roles of the SS-associated species in the pathogenesis of SS, Alam *et al*. (31) analysed SS oral microbiota and tested in vitro if selected species of oral bacteria could induce functional and phenotypic changes in human submandibular gland tumour (HSG) cells. The authors showed that Prevotella melaninogenica uniquely upregulated the expression of MHC molecules, CD80, and IFNλ in HSG cells. Concomitantly, P. melaninogenica efficiently invaded HSG cells ductal cells and the areas of infiltration were heavily infected with bacteria in the labial salivary glands with focal sialoadenitis. In conclusion, although the specific role of microbiota in SS etiopathogenesis remains unclear, collectively, dysbiotic microbiota may initiate the deregulation of salivary gland epithelial cells and the IFN signature through bacterial invasion into ductal cells.

From molecular signature to exploitable therapeutic targets in SS

Traditionally, great amount of evidence has shown that IFNs play a central role in the pathogenesis of SS. An overexpression of type I IFN, the 'so-called' type I IFN signature, has been described in peripheral blood mononuclear cells, and has been associated with the development of SS extra-glandular disease manifestations. By contrast, a lower expression of type I IFN has been described in patients with a milder disease phenotype limited to glandular involvement (32).

From this perspective, James *et al*. (33) have recently investigated whether molecular profiles including IFN, inflammation and other signatures might be used to separate patients with SS into distinct clusters. By using gene expression microarray the authors identify three different clusters of SS patients. Cluster 1 showed no significant elevation of IFN or inflammation modules. Cluster 2 showed strong IFN and inflammation modular network signatures, as well as high plasma protein levels of IP-10/CXCL10, MIG/CXCL9, BLyS (BAFF) and LIGHT. Cluster 3 samples presented moderately elevated IFN modules, but with suppressed inflammatory modules, increased IP-10/CXCL10 and B cell-attracting chemokine 1/CXCL13 and tended to have increased MIG/CXCL9, IL-1α, and IL-21. Anti-Ro/SSA and anti-La/SSB were present in all three clusters.

Expression of IFNs has also been described in salivary gland epithelial cells (SGEC). Gene expression analysis identified an upregulation of interferon signalling pathway and genes involved in immune responses (HLA-DRA, IL-7 and B-cell activating factor receptor) in SGECs of SS. SGECs in SS release several soluble cytokines that induce an increase in B-lymphocyte activation and survival. Reviere et al. (34) demonstrated that targeting a single cytokine (*i.e*., BAFF, IL-6) did not inhibit this effect, whereas leflunomide, BTK or PI3K inhibitors partially decreased B-lymphocyte viability in this model.

Moreover, according to Hong *et al*. (35), type I IFN seem to contribute to inflammasome-associated pyroptosis of the SGECs of SS patients, suggesting another pathogenic role of type I IFN in SS in terms of target tissue-SGECs destruction. Indeed, Hong *et al*. found that the expression of type I IFN signature genes was correlated with mRNA levels of caspase-1 and gasdermin D in SGECs.

Vakrakou *et al*. (36) found that the ductal epithelial cells of SS patients displayed a cell-intrinsic activation status related to a persistent activation of the AIM2 (absent in melanoma-2) inflammasome. The authors described marked cytoplasmic accumulations of damaged genomic DNA that co-localised with AIM2 in the specimens of SS patients (but not controls) likely due to an impaired DNase1 expression and to a consequent aberrant defective cytoplasmic DNA degradation.

Indeed SGECs play a pro-inflammatory role and Sisto et al. (37) showed that

TGFβ1/Smad2/3 signal transduction and the noncanonical TGFβ1/Erk1/2/EMT pathway, act independently in activating IL-17-dependent epithelial-mesenchymal transition in SGEC. EMT in turn plays a critical role in the progression of SG fibrosis in SS.

Looking at tissue target salivary gland progenitor cells (SGPCs) have been also studied. Wang *et al*. (38) assessed the extent of senescence of cells in a SGPC niche in SS patients' SGs, and its correlation with functional and clinical parameters analysing the expression of p16 and p21 as markers of senescence in both total SG epithelium and a SGPC niche. The authors found that SGs of SS patient contained significantly more p16+ cells both in the epithelium in general and in the basal striated duct (BSD) cells layer, than non-SS SGs. Significant correlations were found in SS patients between p16+ BSD cells and secretion of unstimulated whole saliva, stimulated whole saliva, stimulated parotid saliva, CD45+ infiltrate, ultrasound total score and ACR-EULAR classification score.

SGECs play a role in promoting B-lymphocyte activation within the target tissue. The leitmotif of several studies was therefore to study the interactions between SGECs from patients with SS or controls and B lymphocytes in order to search for important treatment targets in SS. Particularly, B cells and its interaction with of a subset of T cells called follicular B helper T cells (or Tfh cells) gained a lot of interest. First of all, several evidence reinforced the concept that CXCL13 and its receptor CXCR5 control the organisation of B cells within follicles of lymphoid tissues and CXCL13 serum level correlates with histomorphology in the SG infiltrates (39, 40). Moreover, ***XY et al.*** found that decreased memory CXCR3 + CCR9+ Th cells in blood of pSS patients may be due to a concerted action of overexpressed ligands CXCL10 and CCL25 at the site of inflammation in the salivary glands facilitating their preferential migration and positioning in the lymphocytic infiltrates.

Sun *et al*. (41) performed whole transcriptome sequencing of B cells from SS patients and explored Toll-like receptor 9 (TLR9) signalling to reveal the potential mechanism of B cell hyperactivation in SS. The authors confirmed that elevated epithelial stromal interaction (EPST1) expression in SS B cells promoted TLR9 signalling activation and contributed to the abnormal B cell activation, by promoting NF-ϰB signalling.

Concerning aberrant B lymphocytes Visser *et al*. (42) analysed the repertoire of B-cells located in striated ducts of SGs of SS patients. The authors found that intraductal and periductal B-cells were closely related to each other with intraductal B-cells being most likely derived from periductal B-cells. The authors speculated that in the early phases any activated B-cell can enter the striated ducts from the periductal infiltrate, irrespective of its antigenic specificity. Within the ducts, these B-cells may further expand and acquire driver-mutations toward lymphoma.

Verstappen *et al*. (43) performed a gene expression profiling of Fc receptor like 4 (FcRL4+) memory subset B cells postulating that these cells may contribute significantly to the epithelial damage. The authors found that genes coding for CD11c (ITGAX), T-bet (TBX21), TACI (TNFRSF13B), Src tyrosine kinases and NF-κB pathway-related genes were significantly upregulated in glandular FcRL4+ B cells when compared to FcRL4- B cells. The authors concluded that FcRL4+ B cells in pSS exhibit characteristics of chronically activated, pro-inflammatory B cells and that their gene expression profile may suggest increased risk of lymphomagenesis.

Finally, Pontarini *et al*. (44) identified in SG a Tfh-signature, with interleukin-21 (IL-21) and the inducible T-cell co-stimulator (ICOS) costimulatory pathway as the most upregulated genes particularly in ELS+SS patients, and in parotid MALT-L SS patients. Moreover, a unique subset of CD4+ T cells named peripheral T helper cells (Tph cells, PD-1hiCXCR5–CD4+ T cells) that also play a role as B-cell helper cells were significantly expanded in ELS+SS patients; these cells were identified as the main producers of IL-21, and closely correlated with circulating IgG and reduced complement C4. The authors speculated that IL-21+ Tfh and Tph cells under control of ICOS costimulatory pathway played a key role in promoting ectopic lymphoid structures (ELS) and B-cell mucosa-Associated lymphoid tissue (MALT) lymphomas (MALT-L).

Take home message

- Genetic and environmental factors in association with immunological profile may influence the different phenotypes of the disease (7).
- Hypomethylation of the promoters of interferon-inducible genes (STAT1, USP18, IFI44L, IRF5, TNFAIP8) in different cell types a reinforces the importance of interferons in SS pathophysiology (11).
- Dysbiotic microbiota may initiate the deregulation of salivary gland epithelial cells and the IFN signature through bacterial invasion into ductal cells (25).
- SGECs play a role in promoting B-lymphocyte activation within the target tissue (34).
- CXCL13 serum level correlates with histomorphology in the SG infiltrates (39, 40).
- Tfh-signature may be implicated in ELS+development and in parotid MALT-L (44).

New insights into SS clinical manifestation

Glandular involvement

Glandular involvement represents the most common clinical feature of SS and its assessment is widely considered as the cornerstone for the diagnosis of the disease. Recently, a critical appraisal of the available tools for the characterisation of glandular inflammation and dysfunction has been made. Particularly, great attention has been paid to the SG biopsy as well as to the salivary gland ultrasonography (SGUS). Regarding SG biopsy, Lucchesi *et al*. (45) have proposed the concept of area fraction (AF) defined as the area of the SG occupied by the inflammatory infiltrate as an additional parameter that can be estimated by the use of digital image analysis. the authors found that AF achieved a 30% improvement over the FS at generating consensus among

raters when used as a diagnostic cut-off concluding that that digitally calculated AF could be utilised additionally to the focus score to improve data harmonisation in large multicentre studies. Regarding ultrasonography, according to Jousse-Joulin *et al*. (46) SGUS seems to improve significantly the sensitivity of the 2016 ACR/EULAR classification criteria (from 90.2 % to 95.6%) when physician diagnosis was the reference standard. Moreover, according to Zabotti *et al*. (47) even less-expert sonographers could be reliable if adequately instructed in assessing SGUS scores. In addition, shear wave elastography (2D-SWE), an ultrasonography technique used to investigate the elasticity of soft tissues, increase the diagnostic accuracy of SGUS when applied to SG defined normal or nonspecific with conventional SGUS and may help to identify MALT-L in parotid glands (48).
From a clinical perspective, differently to salivary gland enlargement, which is associated with a worst prognosis for lymphoproliferative risk mainly in patients with early onset disease (≤ 35 years) (49), oral and ocular dryness are stable, have less important prognostic value but have been significantly associated with impairment of patient quality of life, leading to altered chemosensory function, poorer oral health and higher prevalence of halitosis and sleep disturbances (50-52). However, feeling of ocular and oral dryness is not always associated to a real reduction of lacrimal and salivary flow or to its severity. Indeed, a recent study demonstrated that non-SS sicca syndrome patients complain a worst quality of life in comparison to SS patients despite having significantly less pronounced clinical characteristics of dry mouth and slightly better saliva production rates (53). Inflammatory mechanisms have been hypothesised to contribute to dry eye symptoms. In this setting, lower intake of polyunsaturated fatty acids omega-3, molecules associated with inflammatory burden and immune response regulation, has been associated with higher Ocular Surface Disease Index score in a cohort of 108 SS patients (54).
Of course, an impaired glandular secretion is associated with long-term consequences. Ocular dryness may cause a wide spectrum of manifestations ranging from local discomfort to serious ocular damage, like cicatrising conjunctivitis, optic neuropathy, sterile corneal ulcer, corneal perforation and vision threatening lesions (55). A retrospective hospital-based case series of about 900 SS patients showed that corneal scarring and ulcerations, low Schirmer values, cataract, glaucoma and age at diagnosis were independent risk factors for developing severe visual impairment (56). Moreover, objective ocular sicca syndrome is more severe in patients with positive anti-centromere antibodies (57). Interestingly, ocular sicca symptoms are associated with a significant impairment of patient quality of life, often reported more burdensome than systemic manifestations of the disease, and patients experiencing vision-threatening ocular manifestations, like inflammatory keratolysis and scleritis, are more frequently male and have more than two-folds greater incidence of death, either from malignancy or direct results of disease complications (58, 59).
Likewise, reduced salivary flow increases the risk of various traumatic, inflammatory and infective conditions raging from pulpitis, aphthae, tongue fissuration to oral candidiasis, despite the latter seems to not directly influence the severity of xerostomia. The risk of dental caries, pulpitis, gingivitis, periodontitis and stomatitis is significantly higher in patients with SS with consequent higher costs related to increased frequency of dental visits (60). In this setting, oral discomfort associated with reduced salivary flow may partly explain the evidence that SS patients are more prone to stop smoking compared to healthy subjects (61).
Of course, lacrimal and salivary gland are not the only exocrine glands involved by the disease. Indeed, the disease may impair vaginal secretion and lead to pelvic floor distress and consequent sexual dysfunction, especially in women with a long-term disease (62). Moreover, patients may complain nasal dryness, decreased smell acuity and taste alteration mainly associated with neurosensory dysfunction (63).

Extraglandular manifestations

Being a systemic disease, SS may virtually involve any organ or system, but articular, ranging from arthralgia to erosive arthritis, pulmonary, renal and neurological system involvement are the most frequent. Surely, the systemic phenotype of SS is strongly influenced by different factors. A recent analysis of 10.000 SS patients included in the Sjögren Big Data Consortium revealed that higher disease activity at diagnosis mainly characterises male subjects, patients diagnosed before 35 years, Black/African Americans and patients from Southern countries. Interestingly, the frequency of involvement of each systemic organ also differed between ethnic groups, thus reinforcing the importance of genetic and environmental factors in influencing disease phenotype (64). In this setting, also the immunological profile may exert a key role in driving the systemic phenotype of the disease. Analysis of the same Big Data Cohort and of patients included in the Italian GRISS database revealed a specific disease phenotype correlated to isolated anti-La/SSB antibodies, mainly characterised by higher frequency of active patients (global ESSDAI score ≥1) in most clinical ESSDAI domains in comparison with the immune-negative and isolated Ro/SSA subsets but lower frequency of active patients with moderate or high activity (65). Moreover, in patients with concomitant anti-Ro/SSA and salivary gland biopsy positivity, the presence of anti-La/SSB may help in identifying a disease subset with distinct prognostic features, as higher risk of lymphoproliferative complications (66).
Finally, as chronic disease, SS is associated with relevant comorbidity during its course. An interesting analysis of a large German statutory health insurance fund demonstrated high prevalence of comorbidities, including hypertension, osteoarthritis, osteoporosis and depression, in SS population as compared to healthy controls (67). Moreover, an increasing burden of hospitalisation for serious infections, including opportunistic, skin, pulmonary, soft tissue and urinary tract infections, was recently observed in SS patients, in particular in elderly ones (68).

As far as single organ involvement is concerned, manifestations related to pulmonary involvement still represents a major challenge due to their variable course and prognosis. Respiratory tract involvement ranges from 9% to 24% across studies, depending on diagnostic criteria. Xerotrachea, cough and symptoms related to small airway involvement are the most frequent clinical manifestations but interstitial lung disease (ILD), reported in around 20% of patients, still represents the most serious pulmonary complication, mainly characterising older male patients, anti-Ro/SSA positive and with longer disease duration (69, 70). However, as recently highlighted, 10% to 50% of patients may develop ILD years before SS onset (69). Non-usual interstitial pneumoniae (NSIP) is the most common pathological and radiological ILD pattern, reported in up to 45% of patients, followed by usual interstitial pneumonia (UIP) and organising pneumonia (69).

In this setting, lymphocytic interstitial pneumonia (LIP), although occurring in about 4%-9% of cases, deserved recent attention as classically linked to seropositive SS and usually characterised by insidious onset of obstructive ventilation dysfunction (69, 71). Prognosis of ILD in SS patients is variable as no treatment has been demonstrated to significantly influence mortality. Five-year survival rates have been estimated to range from 83% to 89% (69). Recent studies and systematic review identified FEV1 and FVC <60%, HRCT score > 13 (graded according to the Schurawitzki method), a higher level of pCO2 in arterial blood gas sampling and higher number of reticulations in HRCT and lymphoblastic foci in biopsy as risk factors significantly associated with death in ILD-SS patients (72, 73). Finally, survival may be influenced by increased risk of solid lung neoplasia, mainly reported in male patients, as recently emerged in a retrospective study which enrolled more than 6.000 SS patients (74).

In the last year, analysis of nervous system (NS) involvement in SS gained considerable attention as, although uncommon, is associated with a negative influence on patient quality of life (75). The exact prevalence of neurological manifestation in SS is still a matter of debate as selection bias in different studies, symptom variability, methods used to diagnose NS diseases and new definitions of central NS manifestations hamper a proper analysis of data (76). Peripheral (P) NS is affected in 10%-20% of SS patients. Among PNS-related manifestations, in the last year small fibre neuropathy (SFN), reported in 3%-9% of patients, gained considerable interest. A recent study compared the clinical and serologic features of SS patients with SFN to SS patients with large (L) FN, as pure sensory and sensorimotor axonal polyneuropathy (77). Interestingly, prevalence of anti-SSA antibodies was lower in SFN group and a strong association with dysautonomic features was observed in the same group (77). Some Authors hypothesised that the neurosensory threshold dysfunction observed in these patients, rather than the sicca syndrome, may be associated with chemosensory impairment of taste and smell (51, 63). Similarly, a recent study demonstrated that limitation in swallowing and mastication complained by SS patients may be also associated to an impaired tongue strength and masticatory muscle activity examined by electromyography, as well as to temporomandibular disorder (78). Finally, different tools and imaging techniques, like single-photon emission computed tomography, have been employed to detect cognitive impairment in SS with different results due to lack of standardised method. A recent study explored prevalence of cognitive impairment in a cohort of SS patients by the Montreal Cognitive Assessment (MoCA) and the Automated Neuropsychological Assessment Metrics (ANAM), two easily applicable tests which have been used to detect early cognitive impairment in subjects with autoimmune diseases (79). Interestingly, Authors demonstrated a statistically significant difference in simple reaction time, learning, attention, mathematical processing, matching to sample-spatial work memory and delayed memory in SS compared to controls while no difference was observed in naming, attention, language, abstract thinking, delayed recall and orientation functions. These results suggest that cognitive function should be explored in the routine assessment of SS patients.

Renal involvement represents a quite common extra-glandular complication in SS patients with a prevalence ranging from 2% to 40% according to cohort analysed and diagnostic methods. Moreover, manifestations related to renal involvement are highly variable and several factors, as anti-SSB antibodies and early-onset disease, have been associated with higher risk of kidney involvement in these patients (80-82). Development of renal complication during disease course may affect patient prognosis and is associated with severe disease, systemic multiorgan involvement. In this setting, a retrospective cohort study in Taiwan explored the absolute incidence and relative risk of chronic kidney disease (KD) and end-stage renal disease (ESRD) in a wide SS cohort enrolled in a National Health Insurance Database (83). Although the risk of ESRD did not increase in patients in comparison to non-SS controls, a significantly increased risk of long-term CKD was observed in SS patients, in particular in females. Similarly, a retrospective analysis of 437 patients included in the Spanish Sjögrenser registry, showed that CKD develops in about 60% of patients, thus suggesting the importance of regular monitoring for renal complications in patients with SS(84) .

Finally, articular and digestive tract involvement in SS patients have been recently investigated. A retrospective analysis of the same Spanish Sjögrenser cohort depicted digestive tract involvement in about 16% of patients, especially as autoimmune disorders, and half of patients developed digestive tract involvement at the same time or after disease diagnosis (85). Chronic atrophic gastritis was the most frequent form, reported in about 30% of patients, followed by primary biliary cholangitis and autoimmune hepatitis. Pancreatic involvement was observed in 10% of patients and 7% had coeliac

disease. Interestingly, patients with digestive involvement were significantly older at SS diagnosis, more frequently women, had higher prevalence of autoimmune hypothyroidism and C3 hypocomplementaemia. Patients with pancreatic involvement had higher prevalence of central NS and renal involvement, Raynaud's phenomenon, lymphoma and hypocomplementaemia (85). Articular involvement may also be detected in SS at the time of disease diagnosis. In this setting, a retrospective study of two Italian SS cohorts demonstrated that 68% of patients display articular involvement at time of disease diagnosis (86). An oligo-polyarticular arthropathy of small joints (wrist and hand) was the most frequent articular picture observed. Although not associate with a B-cell chronic activation or systemic manifestations, joint involvement represent an important determinant of patients' quality of life as patients with articular manifestations reported higher VAS pain, VAS dryness and ESSPRI as compared to patients without (86).

Sjögren's syndrome and COVID-19

Few and conflicting data are available on the impact of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection on SS patients. Indeed, most of the National, as the CONTROL-19 Italian registry (87), and international registries do not differentiate SS as a single connective disease and SS patients represented a very small part of the enrolled patients, thus hampering a reliable analysis specifically restricted to the disease. Nevertheless, a recent study explored the personal experience of a cohort of 102 SS patients as a consequence of the COVID-19 outbreak impact on the healthcare system by a telephone consultation (88). The great majority of patients had their rheumatology consultation or laboratory tests related to SS cancelled. However, only 26% of patients their rheumatologist by telemedicine (either telephone or computer) during the closure and a disease relapse was the most frequent reason. In total, 22% patients experienced a disease relapse with articular manifestations being the most frequent. Disease activity and patient reported symptoms significantly worsened during the lockdown period. All patients practiced social distancing, most of those employed switched to smart working and different work settings impacted on the type of symptom worsening. Only two patients reported proven SARS CoV-2 infection, one manged at home with favourable outcome and one required hospitalisation without needing admission to intensive care unit.

Take home message

- Xerophthalmia and xerostomia are associated with a significant impairment of quality of life in SS patients (50-52).
- Vision-threatening ocular manifestations, like scleritis, are more frequent in male patients (55, 58, 59).
- In addition to inflammatory and autoimmune mechanisms, neurosensory dysfunction seems to contribute to mucosal dryness in SS patients (63).
- Higher disease activity at diagnosis mainly characterises male subjects, patients diagnosed before 35 years and Black/African Americans (64).
- Spirometry parameters, some HRTC findings and lymphoblastic foci in pulmonary biopsy have been significantly associated with death in SS patients with ILD (72, 73).
- Lower prevalence of anti-SSA antibodies and dysautonomic features strongly associate with SFN in SS patients with PNS involvement (77).
- Higher prevalence of impairment of some cognitive functions has been demonstrated in SS patients in comparison to control population (79).
- Although the risk of ESRD is not significantly increased in SS patients, up to 60% of patients may develop long-term CKD (84).
- Articular involvement has a significant impact on patient quality of life being associated with higher dryness and pain in ESSPRI domains (86).

New insights in SS treatment

Because the most common clinical manifestations of SS are dry eyes and dry mouth, despite not being major drivers of morbidity or mortality, a large share of recently published data on SS treatment are focused on this aspect. Along with other studies published in the past few years, new methods of local delivery of immunosuppressants have been investigated, in order to overcome the current limitations due to bioavailability and systemic toxicity. In fact, the injection of a depot formulation of rapamycin into the lacrimal glands of a murine model of sialoadenitis was able to reduce inflammation and improve tear production with significantly lower amounts of drug, compared to systemic administration (89). A randomised clinical trial comparing the effect of citric acid solution and malic acid lozenges as salivary stimulants confirmed their ability to improve secretion and oral-related quality of life, with the lozenges being more effective than the solution, maybe due to their stronger mechanical stimulation (90). Nevertheless, the increase of salivary secretion induced by pilocarpine does not seem to be effective in improving periodontal conditions, at least in the short period (91).

As an additional potential therapeutic approach for xerostomia, the use of the immunosuppressor fingolimod – currently approved for the treatment of multiple sclerosis and likely acting by reducing the migration of lymphocytes to target organs – on a murine model of SS showed a reduction of sialadenitis severity and improvement of saliva secretion (92).

In latest years a large amount of data has been accumulating showing that paeony extracts can exert an immunomodulatory action in SS models. In the last year multiple papers have been published, demonstrating that the paeony-derived molecule paeoniflorin-6'-benzene, also known as CP-25, was able to reduce the severity of sialadenitis and improve salivary flow in murine models of SS, probably by modulating B cell response and migration. However, it is still not clear what the target of the molecule is and more research on the topic is required before any conclusion can be drawn (93-95).

Because no drugs have been approved for the treatment of SS, most of the current therapeutic approaches are based on the experience and data acquired for oth-

er systemic autoimmune diseases. Despite immunosuppressants and immunomodulators are commonly used, very few randomised controlled trials (RCT) have been performed in SS. Hydroxychloroquine (HCQ) is usually prescribed in a good proportion of patients with SS, mostly in case of mild cutaneous and musculoskeletal involvement. A retrospective analysis performed on patients enrolled in the JOQUER trial demonstrated that treatment with HCQ is able to downregulate the expression of type I interferon-regulated genes and the circulating levels of IgG and IgM. However, no significant effect was demonstrated on disease activity parameters (96). Nonetheless, in other real-world studies HCQ was apparently able to improve sleep quality (97) and to reduce cardiovascular (CV) risk (98). Other small studies with conventional immunosuppressants have shown a potential benefit of mycophenolate mofetil on SS-related ILD in terms of improvement in forced vital capacity and no effect of sirolimus on SS-related thrombocytopenia, unlike systemic lupus erythematosus, in which it showed some benefit (99, 100). Conflicting data have emerged from studies investigating the efficacy of iguratimod, a drug approved for the treatment of rheumatoid arthritis in Japan and China, likely acting as a B-cell regulator. In fact, although an open-label pilot study showed a reduction of disease activity according to ESSDAI score, mostly in the articular, biological and haematologic domains, no significant changes were found in patients reported outcomes (PRO) (101). Conversely, a randomised controlled trial demonstrated a dramatic improvement of ESSPRI scores and patient's global assessment, along with IgG and ESR levels, but no differences in ESSDAI score (102). The two main RCTs recently published, evaluating the efficacy of abatacept and tocilizumab in SS did not reach their primary end-point. Only some effects on biological markers of the disease were found with abatacept (103, 104). Unsurprisingly, CT-P10 biosimilar of rituximab originator showed equivalent efficacy, compared to the originator (105).

Among some of the most difficult to treat manifestations of SS is SFN, usually managed symptomatically. In a recently published case series, Pindi Sala *et al*. suggested that intravenous immunoglobulins (IVIG) may provide a certain degree of efficacy, even in the long-term (106). However, also due to the cost of this treatment, larger trials are necessary before this therapeutic option can be considered on a larger scale. Along with SFN, fatigue is probably the main driver of poor quality of life in SS. Although no pharmacological treatments seem to be particularly effective for this aspect of the disease, a recently published double-blind RCT demonstrated that transcranial direct current stimulation (a neuromodulation technique that seems promising for the treatment of other conditions, such as depression, multiple sclerosis and Parkinson's disease) is able to improve fatigue compared to the sham group, similarly to studies employing aerobic exercise (107). Moreover, four months of resistance exercise demonstrated a significant improvement of quality of life in a RCT (108).

As more evidence accumulates failing to demonstrate the efficacy of currently available medications, the need to find new potential targets to treat the disease is driving research on SS. Some very interesting data have been published from *in vitro* and *in vivo* models of the disease in which new potentially targetable disease mechanisms have been identified.

Because circulating anti-Ro/SSA antibodies are known to bind RNA molecules, thus triggering an inflammatory response *in vitro*, treatment of SS patients with a fusion protein containing a catalytically active RNase was able to improve multiple PROs, although a counter-intuitive increase in circulating IFN-inducible genes was observed, deserving further research (109).

Treatment of SS animal models demonstrated that administering an analogue of B7-H4 – a co-stimulatory molecule involved in the promotion of Treg cells activity and whose blockade is known to worsen multiple models of autoimmune diseases – was able to reduce lymphocyte infiltration in salivary glands and an expansion of Treg cells (110). Disease improvement was also demonstrated by inhibiting bone morphogenetic protein (BMP)-6, high-mobility group box (HMGB)1 (which have both been associated to reduced salivary secretion(110, 111)), and transcription factor RAR-related orphan receptor (ROR)γt (112).

Some *in vitro* evidence suggests that Janus kinase (JAK) inhibitors may be effective in SS by modulating DNA hydromethylation induced by IFNs and reactive oxygen species(113). Other evidence supports a positive effect of IL-38 that may reduce the activity of Th17 cells(114). However, all these data need to be confirmed *in vivo*.

Although a beneficial effect of mesenchymal stem cells (MSC) in SS has been confirmed in multiple studies, the mechanisms underlying such activity are still mostly unexplored. Only recently, interactions between MSCs and myeloid-derived suppressor cells (MDSC) have been demonstrated. MDSCs are immature myeloid cells whose differentiation is hampered in autoimmune diseases, such as SS, SLE, RA and multiple sclerosis. In these conditions, rather than differentiating into mature cells, such as macrophages and dendritic cells, they proliferate and lose their suppressive capacity. MSCs seem to be able to restore the *in vitro* suppressive function of MDSCs via multiple pathways, such as TGF-β and cyclooxygenase (COX)2 (115, 116).

Take home message

- Pilocarpine has been demonstrated to be effective in increasing salivary secretion but not in improving periodontal conditions (91).
- Some recent experimental therapeutic approaches, like rapamycin, fingolimod and paeony extracts, have been demonstrated to improve lacrimal and saliva secretion in murine models of SS (92).
- HCQ has been demonstrated to downregulate the expression of type I interferon-regulated genes and the circulating levels of IgG and IgM but with no significant effect disease activity (96).
- Small studies have shown a potential benefit of mycophenolate mofetil on SS-related ILD in terms of improve-

ment in forced vital capacity (99, 100).

- Immunomodulatory drugs, including abatacept, tocilizumab and rituximab, seems to exert more beneficial effect on biological parameters than on disease activity (103-105).
- The *in vitro* efficacy of Janus kinase-inhibitors and mesenchymal stem cells needs to be confirmed in *in-vivo* studies (113).

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