Review

Sjögren's syndrome: one year in review 2022

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ABSTRACT

Primary Sjögren's syndrome (pSS) is a complex disabling systemic autoimmune disorder. The hallmark of pSS is the T-cell-mediated hyperactivation of B-cells, evolving from asymptomatic conditions to systemic complications and lymphoma development. On tissue level, the typical feature is the lymphocytic infiltration of the salivary gland by B-, T- and antigen presenting cells, as mirrored by the diagnostic cornerstone role of minor salivary gland (MSG) biopsy. B-cells show multiple possible roles in disease pathogenesis, from autoantibody production, to antigen presentation, and cytokine production. B-cells hyperactivation is supported by genetic risk factors, T-cell dependent and independent mechanisms, and the presence of different pathogenic B-cell subsets must be reminded.

Many aspects have been investigated in the last year regarding genetic and epigenetics, B- and T-cell role in pSS pathogenesis, their interaction with salivary gland epithelial cells (SGECs) and in their direct or indirect use as biomarkers and predictors of disease development, activity, and lymphomagenesis.

In this review, following the others of this series, we will summarise the most recent literature on pSS pathogenesis and clinical features focusing in particular on new insights into pSS molecular stratification and therapeutic advances in the era of precision medicine.

Introduction

Primary Sjögren's syndrome (pSS) is a complex and heterogeneous autoimmune disease a disease with multiple clinical faces potentially evolving toward non-Hodgkin's lymphoma mucosa-associated lymphoid tissue (NHL-MALT) with an unknown aetiology. Even though many genetic, environmental, and hormonal potential causes have been investigated, no causal association currently exists that might explain the aberrant immune response targeting multiple epithelial structures that in turn give pSS its characteristic presentation. In this review, following others of this series, we will summarise the most recent literature on pSS pathogenesis and clinical features focusing in particular on new insights into pSS genetics and epigenetics, innate and adaptive immune system abnormalities, as well as disease-related glandular and extra-glandular manifestations. In the final session we will provide an update on SS therapy in the era of precision medicine. Hopefully, novel insights into pSS pathogenesis will pave the way to new therapeutic approaches to the disease improving patients management and prognosis.

Epigenetics in Sjögren's syndrome

Epigenetic abnormalities, affecting imprinted gene regulation, have been added in the last decade to the list of possible processes involved in pSS pathogenesis. The intricacies involved, however, are just beginning to give in to scientific investigation. In this part, we will focus and expand on last year's epigenetic-oriented developments in pSS (Table I).

DNA methylation

DNA methylation is a ubiquitous epigenetic mechanism that hampers the binding of transcription factors to the promoter of a gene, resulting in its silencing. It refers to the addition of a methyl group to the fifth carbon of a cytosine residue located adjacent to a guanine residue (CpG) in the DNA strand inducing chromatin condensation (1). In pSS, DNA methylation is the most widely

Table I. Epigenetic modifications in pSS.

Epigenetic modifications	Novel data
DNA Methylation (3-6)	Hypomethylation of INF-related genes Hypomethylation of NOTCH signalling pathway-related genes
Histone modification (7)	Tri-methylation of histone 3 at the lysine residues (lymphoma)
miRNAs (9-12)	Downregulation of hsa-miR-145-5p (type I IFNs and MUC) Increased of miR 155 5p (apoptosis, TCR signalling pathway) Upregulation of miR-146a-5p (Th17 cell differentiation)
Non-coding RNAs (13-16)	GABPB1-AS1 and PASM3-AS (serum IgG) CTA-250D10.23 lncRNA (CXCL13)

studied epigenetic mark and methylation imbalances either at specific CpG sites of immune related genes or on an epigenome-wide basis have been repeatedly demonstrated (2). Adding to the already known hypomethylated status of circulating B and T cells of pSS patients, it was recently shown that pSS monocytes follow a similar hypomethylation predominant pattern, as well (3, 4). Using the Infinium HumanMethylation850 (HM850k) BeadChip array on separated monocytes ($CD14^+$) from 11 pSS patients and five sex- and age-matched controls, it was shown that, among many, the most differentially hypomethylated genes were the IFN-related genes and those involved in the Notch signalling pathway (5). It is noteworthy, that the overlap in the methylation profile between monocytes and salivary gland epithelial cells consists of genes associated with the cell cycle, the cell senescence, and the IL-17 signalling pathway. Prompted by the strong evidence attesting to a differentially methylated genome between pSS patients and controls, Chi *et al*. attempted to determine whether there is a genetic master control of those methylation variations. Making use of the methylation and genotype profile of a large number of minor salivary gland biopsy tissues of pSS patients and controls, the authors showed that differentially methylated regions were associated with short ranged (± 250 kb) SNPs at specific loci known to affect the DNA methylation patterns (meQTLs). Causal inference testing showed that 19 of the 26 pairs display a causal mediation relationship while almost half of those reside in the MHC region, implying that alterations in the DNA methylation pattern could account for the increased genetic risk conferred by the MHC. However, most of the differentially methylated regions, especially at non-MHC locations, were not associated with a nearby SNP (6).

Histone modification

A vast array of post-translational chemical modifications (acetylation, methylation, ubiquitylation, and phosphorylation) on the histone N-terminal tails at lysines, arginines, serines, and threonines residues is another epigenetic regulator of gene expression. These types of modifications alter the physical state of the chromatin from a euchromatic (accessible to transcription factors) to a heterochromatin (restricted access to transcription factors) state and *vice versa*. The different physical properties of the chromatin created by the various chemical marks on the protruding histone tails is the epigenetic mechanism least investigated in pSS. However, recently Ningning *et al*. added to the field by exploring the dynamic changes and interplays of the tri-methylation of histone 3 at the lysine residues 4, 9, 27, 36 and 79 in two pSS patients, before and after the diagnosis of lymphoma. As observed in the study by a chromatin immunoprecipitation sequencing (ChIP-seq) in longitudinal tissue biopsies, the trimethylation of lysine of histone 3 showed no differences in residues 36 and 79, a decrease in 9 and 28, and an increase in residue 4 that seems to be the most vital, since it correlates with all other histone modifications. Finally, those findings combined with an RNA sequencing analysis revealed a correlation between the gene expression profile and the different histone patterns (7).

Non-coding RNAs (ncRNA)

Non-protein coding RNAs are regulatory, functional untranslated transcripts. Among all sub-categories of ncRNAs, the long non-coding RNAs (lncRNAs) (>200 nt) are the most abundant and the microRNAs (<200 nt) are the most extensively studied. During the last decade numerous studies have shown dysregulated miRNAs and to a lesser extent lncRNAs in PBMCs, salivary gland tissue, tears and saliva of SS patients (8). These studies are limited by their small sample size and lack of reproducibility on similar study design strategy. Recent scientific aspirations, however, vastly exceed the differential expression of ncRNAs in SS, instead attempting to untangle their specific functional role.

Jara *et al*. showed recently that hsa-miR-145-5p, an miRNA with anti-inflammatory properties that is known to be downregulated in SS, is the molecular bridge between type I IFNs and glandular dysfunction/inflammation through the overexpression of mucin 1(MUC1) and toll-like receptor 4 (TLR4). Overactivation of the type I interferon pathway, known to play an important pathogenetic role in SS, may lead to the downregulation of hsa-miR-145-5p, which in turn drives the overexpression of MUC1 and TLR4 and is contributing to the perpetuation of the aberrant immune response (9). Similarly, Jingli *et al*. investigated the role of miR-155-5p, a miRNA that has been also found overexpressed in SS patients. Stimulation of salivary gland epithelial cells (SGECs) with interferon-γ, increased the levels of miR-155-5p while also inducing apoptosis, effects that were reversed with the miR-155-5p knockdown. This response seems to be mediated by the overactivation of the NF-κB pathway through the elimination of one of its inhibitors, Arrestin β2 (10). Another group conducting an miRNome analysis of T cell receptor (TCR)-activated $CD4^+$ T cells from PBMCs of SS patients before and after treatment with mesenchymal stem cells (MSCs), also found the same miRNA (miR-155-5p) among others to be differentially expressed (11). In particular, miRNA-155-5p upregulation was

further enhanced after MSC treatment, while gene ontology term enrichment implicated it in the TCR signalling pathway. On the other hand, upregulation of miRNA-5096 and miRNA-7150 and downregulation of miRNA-22-3p and miRNA-125b-5p in the pSS group were reversed by the MSC co-culture (11). Another functional pathway modulated by an miRNA known to be upregulated in PBMCs of SS patients was investigated recently. MiR-146a-5p was shown to promote T helper 17 (Th17) cell differentiation through a disintegrin and metalloprotease 17 (ADAM17) dependent IL-23/IL-23 R activation (12).

Unlike miRNAs, research regarding the differential expression and putative functional role of lncRNAs has not received extensive attention in SS. Contributing to the field, Xiaochan *et al*. revealed the differentially expressed lncRNAs in 30 well characterised SS patients and controls. A transcriptome sequencing from more than 1000 up or down-regulated lncRNAs, found GABPB1-AS1 and PASM3-AS to be the most important. The results were validated by RT-qPCR, which also uncovered a correlation of GABPB1-AS1 expression levels with the percentage of circulating B cells and the serum IgG levels of SS patients (13). Similarly, in another study, a bioinformatics analysis of the gene expression profile data of minor salivary gland biopsies from SS patients and sicca controls, identified 14 lncRNAs as the master regulators of the invading, infiltrating SS-specific immune cell subpopulations. Among them, CTA-250D10.23 lncRNA was identified as the most significant associated with infiltration events, and a functional annotation analysis revealed a relevance to chemokine signalling pathways (14). Of added interest, a strong correlation between CTA-250D10.23 lncRNA and the serum levels of chemokine CXCL13, which serves as a histologic and clinical biomarker of the disease, was also shown (15, 16).

From epigenetic abnormalities to molecular stratification

Epigenetic abnormalities of whole blood samples from a European cohort of over 300 patients, and a similar number of age and gender-matched healthy volunteers have been recently analysed by Soret *et al*. (18) along with transcriptomic, genomic, flow-cytometry data, cytokine expression and clinical parameters to provide a molecular classification scheme for SS patients based on the multi-omic profiling that may foster promising novel approaches to new treatment modalities. The authors identified four groups of patients with distinct patterns of immune dysregulation. Patients from Cluster 2 displayed a healthy-like profile with no increase in the IFN modules and minimal activity of inflammation-related gene modules. The three other clusters (C1, C3, C4) exhibited a prominent IFN gene signature. Patients from C4 exhibited a more severe clinical phenotype compared to the others with an inflammatory transcriptomic signature particularly linked to cytokine signalling from the acute phase response. C4 was also characterised by a massive lymphopenia and high levels of neutrophils. Noteworthy, patients included in the C4 cluster showed the most predominant hypomethylation. The hypomethylated genes were mostly associated with the neutrophil degranulation pathway. Exclusively to this molecular subgroup, hypermethylated CpGs were detected corresponding to 25 genes implicated in the immunological synapse, platelet multimodal function, and PD-1 signalling. The other 2 molecular groups (*i.e*. C1 and C3), characterised by a type I predominant interferon response, revealed a much lower number of differentially methylated regions, but a more predominant gene-promoter region localisation. Finally, a minimum number of differentially methylated regions were detected in the C2 group of pSS patients (17).

Likewise, unsupervised integrative analysis of whole blood transcriptome and methylome data of more than 700 patients with various systemic autoimmune diseases (including pSS), revealed 3 common pathologic clusters (inflammatory, lymphoid, interferon) and one undefined/normal. The inflammatory cluster was defined by differentially methylated modules driven by monocytes and neutrophils, the lymphoid cluster was defined by T and natural killer (NK) cell functions, and the interferon cluster was defined by interferon, viral, and dendritic cell functions. Intriguingly, each patient's cluster assignment was stable over time, unaltered after treatment or throughout the course of the disease (18).

In conclusion, the implication of epigenetics on autoimmune diseases is becoming gradually apparent. The identification of novel pathogenetic mechanisms and biomarkers for prognosis, stratification, and response to treatment as well as the discovery of new therapeutic targets might all become a reality in the future, given that we grasp a better understating of integrative gene expression data. It is a high time for the field of epigenetics to shift its research orientation, focusing on previously under or unexplored areas including a) the effect of epigenetics on immune subpopulations within the inflammatory lesions of the salivary gland and/or other affected tissues; b) epigenetic alterations upon the immune regulatory component either at the tissue or the blood level (*e.g*. T regs, B regs); c) epi-trascriptomics sudies (RNA methylation); d) functional epigenetic studies on B cells from pre-lymphoma and lymphoma pSS patients to record the landscape of the B cell component, and finally e) co-cultures of immune cells with epithelial cells to study the net effect of drugs modifying the epigenome on either cell type or by affecting their interaction.

Take home messages

- pSS monocytes follow an hypomethylation predominant pattern similar to that observed in circulating lymphocytes B and T with differentially hypomethylated genes including IFN-related genes and those involved in the Notch signalling pathway (3-5).
- Genetic control of differential methylation appeared as a risk factor for SS, especially at the MHC (6).
- A tri-methylation of histone 3 at the lysine residues has been associated with the diagnosis of lymphoma (7).

- Differentially expressed mi-RNAs and lncRNAs have been associated to glandular infiltrate severity, B-cell hyperactivity and chemokine signalling (9-16).

Epigenetics along with other multiomic data allowed to reclassify pSS patient revealing the existence of three pathologic clusters (inflammatory, lymphoid, interferon) and one undefined/normal (17-18).

B- and T-cell role in pSS pathogenesis, their interaction with salivary gland epithelial cells

A complex interaction between innate, adaptative immunity and salivary gland epithelial cells (SGECs) is crucial in pSS pathogenesis, with SGECs playing both an active and passive pathogenetic role in disease onset and development (19).

Rivière *et al*. explored the role of the IL7/IFNγ axis in the interplay between SGECs and T cells in pSS. In their study, pSS patients showed higher levels of serum IL7 and decreased IL7-R expression, potentially reflecting its internalisation after binding. IL7 serum levels were associated with B-cell activation biomarkers, IFN-induced chemokines and disease activity markers (*i.e*. SSA/SSB/RF positivity, low c4 levels, serum CXCL13, lymphopenia, a past or current history of lymphoma). A higher expression of IL7-RNA was found in pSS SGECs. These cells, if stimulated with type 1 and 2 interferons, would produce IL7, whereas IL7 stimulation increased activation of T-cells and IFNgamma secretion. Moreover, results on PD1 and ICOS expression on T-cells suggest a role of IL7 in their differentiation into T follicular cells (Tfh). Thereby, the authors postulated an active role of an IL7/IFNgamma loop axis involving T-cells and SGECs in the pathogenesis and organisation of ectopic lymphoid structures in pSS (20).

Pringle *et al*. focused on the link between the degree of glandular lymphocytic infiltration and the levels of salivary sodium, the last known to be consistently increased in pSS, without a yet defined biological reason. The results highlight a correlation between stimulated parotid gland salivary sodium levels and the degree of CD20+ B-cell infiltrate. Moreover, epithelial sodium channel (ENaC), responsible for saliva transport, was localised on the apical membrane of luminal striated duct cells in non-pSS controls or FS- pSS specimens, whereas in FS+ pSS patients its expression was absent. Since no difference was found between LELs+ and LELs- tissue specimens, a soluble factor secreted by B-cells, rather than the actual physical tissutal invasion or epithelial hyperplasia, might cause ENac dysregulation. According to the authors, the measurement of salivary sodium, in addition to other clinical and laboratory features, might support pSS differential diagnosis; moreover, they suggest that its correlation with B cell infiltrate might be further studied if feasible as surrogate for salivary gland biopsy and correlating with lymphoma evolution risk (21).

Take home message

- The interplay between SGECs and T and B infiltrating cells (*i.e*. IL7/IFNγ axis, sodium levels) is crucial in pSS pathogenesis (19-21).

From pathogenesis to traditional and novel biomarkers in pSS

Autoantibodies anti-Ro52/SSA and Ro60/SSA represent the most traditional biomarkers for pSS and have been correlated with patients' younger age at diagnosis, more severe lymphocytic infiltration of the salivary glands and a higher mean ESSDAI (22). Recently, a particular interest has arisen in defining the significance and role of single anti-Ro/SSA specificities.

Robbins *et al*. (23) showed that Ro52+Ro60+ patients had a higher frequency of anti-SSB/La and hypergammaglobulinaemia than those with either isolated anti-Ro60 or isolated anti-Ro52.

Zampeli *et al*. (24) found that double positive patients presented more frequently an enlargement of their salivary glands leukopenia, hypergammaglobulinaemia, low C4 and RF and lymphoma. They observed that patients who were positive only for anti-Ro52 had a milder disease characterised by sicca and arthritis.

New surrogated biomarkers of pSS disease (*e.g*. cytokines or chemokines) have been also proposed, with an insight on their relationship with disease activity and risk of lymphoma development.

Chatzis *et al*. (15) demonstrated that serum CXCL13 and the number of CXCL13 positive cells per tissue area of MSG biopsies was higher in pSS patients with severe MSG infiltrates, expressed as high values of FS. Moreover, this chemokine serum levels were found to be upregulated before the clinical onset of lymphoma in pSS, and therefore might be involved in earlier stages of lymphomagenesis.

The same author defined salivary gland FS value ≥4 as an independent lymphoma risk factor, connecting it to a statistically shorter time interval from pSS to lymphoma diagnosis and more frequent B-cell originated manifestations (*e.g*. salivary gland enlargement, monoclonal gammopathy, RF positivity) (25).

Bharaj *et al*. (26) stratified pSS patients according to a three-stage inflammatory severity index based on FS value and GCs presence in MSG biopsy (S1:FS≤1; S2: FS≥2; S3 FS≥3 and GCs), and described the connection between index, other histological features (*i.e*. atrophy, adipose tissue presence), the incidence and composition of the main immune cell infiltrating population ($CD4^+$and $CD8^+$ T-cells, $FoxP3^+$ T regulatory cells, $CD74^+$ class II MHC expressing antigen presenting cells, $CD68^+$ macrophages, $CD20^+$ B-cells, and $CD138^+$plasma cells) and clinical-serological characteristics (SSA/SSB/RF positivity, salivary flow). The evaluations proposed in this study give further hints on pSS evolvement on a histopathological level, supporting the importance of tissue evaluation for patient stratification, since distinct inflammatory disease stages could be driven by different cell populations and benefit from different targeted therapeutic agents.

Pontarini *et al*. (27) extensively disserted on the importance of recognising and enquiring the heterogeneity of pSS disease when analyzing clinical, laboratory, and histological markers of

disease severity and risk of lymphoma development. Focusing on the heterogeneity of salivary gland immunopathology in pSS, the authors remarked the role of FS and ectopic lymphoid structures as more relevant histopathological stratifiers. The immunological mechanisms underlying their formation was discussed, pointing at the usefulness of possible surrogated markers (*e.g.* CXCL13) and a "liquid biopsy" approach in pSS. Moreover, the complex role of T follicular cells (Tfh), T peripheral helper cells (Tph) and T follicular regulatory cells (Tfr) in ectopic lymphoid structures was remarked, completing a thorough evaluation on prediction of disease severity, lymphoma development, clinical response and proof of therapeutic efficacy.

Many authors concentrated their researches on lymphocytic subpopulation in pSS, their association with disease activity and autoimmune profile, and on their usefulness in distinguishing pSS from sicca syndrome.

Mielle *et al.* focused on the quantification of regulatory IL-10 producing B-cell subtype ($B10^+$ cells) and for the first time on their function in patients with pSS. They demonstrated the preservation of B10+ cell frequency in pSS and of their ability to promote Treg differentiation in similar level to healthy subjects, supporting this cellular subtype as a useful mirror of B-cell regulatory function assessment (28).

Dupré *et al.* (29) further studied Tfh and Tph in pSS, confirming the expansion of circulating Tfh ($CD4^+CXCR5^+PD1^+$) and of Tph cells (Tph, $CD4^+CXCR5^-PD1hi$) in pSS also in their activated form ($ICOS^+$). Moreover, a positive moderate correlation between ESSDAI score and both percentage and the activation of Tfh was found. A positive moderate correlation was also defined between percentage of plasmablast and the percentage of Tfh and Tph, as well as between activated Tfh and hypergammaglobulinaemia. Higher median percentages of activated Tph were found in RF and anti-SSB positive pSS patients. This study extends the knowledge on Tfh and particularly on Tph cells, highlighting their association with B-cell biomarkers and disease activity, thereby supporting their role as potential new therapeutic targets. Loureiro-Amigo et al. used advanced lymphocyte profiling confirming a profound imbalance in the distribution of circulating T- and B-cells, with a decrease in $CD4^+$ T-cells (mainly naïve and central memory CD4 subsets), diminished CD4/8 ratio and memory cells and an increase in activated T cells and naive B-cells in pSS. Moreover, they showed a good accuracy of the ratio between non-switched memory B-cells to activated $CD4^+$ T-cells in discriminating between pSS and sicca syndrome also in seronegative patients (30).

The same group analysed and supported the role of CXCL13, BAFF, IL21 and IL22 as biomarkers of pSS activity, finding correlation with B cell activity surrogate biomarkers and/or ESSDAI. Moreover, they demonstrated that increased IL21 levels related to abnormal naïve/memory B-cell ratio, while IL22 levels related with increased circulating activated $CD4^+$ T cells. The measurement of serum PD-L2 levels in combination with CXCL13 and BAFF allowed discrimination between pSS patients and sicca syndrome patients in this study (31).

Barcelos *et al.* evaluated lymphocytes subpopulations in SSA-positive and SSA-negative pSS patients in comparison to sicca patients and healthy controls, relating the results to ESSDAI. Anti-SSA positive patients expressed increased levels of IL21 producing $CD4^+$ and $CD8^+$ T-cells, CD24HiCD38Hi B-cells, naïve B-cells, and $IgM\pm CD38^{++}$ plasmablasts, and lower levels of memory B-cells (including $CD24hiCD27^+$) compared to sicca patients and healthy controls. Compared to anti-SSA negative pSS patients, only IL21 $CD4^+$ cells were increased, while $CXCR5^+$Tfh17 absolute and percentage levels were decreased. Moreover, in this study population there was a positive correlation between anti-SSA positivity, disease activity (*e.g.* SG swelling), ESSDAI levels and $IL21^+CD4^+$ and $CD8^+$ cells, and $CXCR5^+$Th1 cells levels (32). Similar results were reported by Szabó *et al.* (33), who found an increased frequency of activated circulating Tfh cells ($CD4^+CXCR5^+PD1^+ICOS^+$) in anti-SSA positive pSS population compared to healthy individuals. The percentage of Tfr resulted similar to healthy controls, while absolute cell count of circulating Tfr was significantly decreased, as found also by Barcelos *et al.* In both studies, when anti-SSA positivity was considered, anti-SSA positive patients showed higher proportions and absolute cell numbers of Tfr. (32, 33). Szabó *et al.* confirmed an increase of transitional and naïve B cells in pSS, and a decrease of switched and un-switched B memory cells, the latter more pronounced in seropositive patients. Moreover, they extendedly evaluated the correlation between Tfh, Tfr and B-cells in terms of quantity and subsects. Finally, they tested *in vitro* the combined neutralising action of anti-human CD40/TNFRSF5 and anti-human IL21 antibodies on Tfh/B cell interactions and autoantibody production, with encouraging results (33).

These studies further support the key role of follicular T-cell/B-cell axis in disease pathogenesis, development of aberrant humoral immunity, disease activity, and possible correlation with prognosis and response to treatment.

Early biomarkers enabling an early recognition of pSS have been recently investigated also in tears and saliva as well as in salivary gland tissue by means of new OMICS techniques. Urbanski *et al.* (34) investigated the tears metabolomic signature of patients with newly-diagnosed pSS and of patients with no-pSS dry eye syndrome. They found that serine, aspartate and dopamine concentrations were decreased in pSS, while six phospholipids (LysoPC C18:1, C18:2, C16:1, SM C16:0, C22:3, and PCaa C42:4) were increased in pSS, compared to the non-pSS Sicca group. In saliva, Sembler-Møller *et al.* (35) showed that the combination of upregulated salivary TRIM29 levels and anti-SSA positivity allowed to differentiate pSS patients from non-pSS patients with a sensitivity around 99%. Similarly, Garreto *et al.* (36) found that the expression of the serine protease dipeptidyl peptidase-4/CD26 (DPP4/CD26) was increased in pSS saliva

and might be involved in cytokines and chemokines production. Moreover, matrix metalloproteinase-9 (MMP9) and some serine proteases such as neutrophil elastase (ELANE), cathepsin G (CTSG), and myeloblastin (PRTN3) were found to be increased in pSS saliva too. Finally, Sandyia *et al*. (37) described an over-expression of salivary free light chains in pSS patients with respect to controls.

In the glands of pSS patients, Oyelakin *et al*. (38) observed an increased expression of genes associated with immune processes such as B cell proliferation, regulatory T cell differentiation, regulation of and chemokine-mediated signalling (CXCL9, CXCL10, CXCL11, CXCL13). Intriguingly, an enrichment of differentially expressed genes (DEGs) commonly associated with repression of the PD-1/PDL-1 pathway was observed. This findings are in line with recent reports of the development of a Sjögren's like syndrome in cancer patients treated withPD-1/PD-L1 checkpoint inhibitors. On the other hand genes which were downregulated in pSS were highly enriched in normal salivary gland and they are involved in processes necessary for proper salivary gland function.

Verstappen *et al*. (39) analysed DEGs in paired parotid (PSGs) and labial salivary gland (LSGs) tissue and peripheral blood mononuclear cells from biopsy-positive, biopsy-negative pSS and non-pSS sicca patients. The top 20 up-regulated genes in biopsy-positive pSS patients were mostly B-cell or T-cell related with a strong correlation between PSGs and LSGs. Particularly gene signatures observed in both glands of biopsy-positive pSS patients included IFN-α signalling, IL-12/IL-18 signalling, CD3/CD28 T-cell activation, CD40 signalling in B-cells, double negative type-2 B-cells, and FcRL4$^+$ B-cells. None of these signatures were found to differ significantly in biopsy-negative pSS compared with non-SS sicca patients. They further assessed gene signature in peripheral blood mononuclear cells (PBMCs) showing that IFN-α signalling and DN2 B-cell signatures were also enriched in pSS patients' PBMCs.

Take home messages

- Recently, a particular interest has arisen in defining the significance and role of single anti-Ro/SSA specificities (23, 24).
- New surrogated biomarkers have been proposed for pSS disease activity and lymphoma risk (CXCL13, FS>3, ectopic lymphoid structures (15, 25-27).
- Many authors concentrated their researches on lymphocytic subpopulation and cytokines in pSS and their usefulness in distinguishing pSS from sicca syndrome (*i.e*. regulatory IL-10 producing B-cell subtype, expanded Tfh (CD4$^+$CXCR5$^+$PD1$^+$) and of Tph cells (Tph, CD4$^+$CXCR5-PD1hi, serum PD-L2 levels in combination with CXCL13 and BAFF; IL21 producing CD4$^+$ and CD8$^+$ T-cells) (28-33).
- New OMICS salivary and tears biomarkers are apparently able to distinguish pSS from sicca, thus enabling pSS early recognition (*i.e*. tears aspartate and dopamine concentrations, salivary TRIM29 levels; salivary serine protease dipeptidyl peptidase-4/ CD26 (DPP4/ CD26), salivary matrix metalloproteinase-9 (MMP9) and neutrophil elastase (ELANE), cathepsin G (CTSG), myeloblastin and free light chains (34-38).
- Finally, IFN-α signalling and DN2 B-cell signatures are enriched in pSS patients' PBMC and glandular tissues (39).

Glandular involvement

Dryness symptoms represent the most typical manifestations of the disease and characterise almost all pSS patients regardless of age and disease stage (40). Recently, attention focused on different symptoms related to systemic mucosal involvement which greatly impair patient quality of life. Genital symptoms, including vulvar and vaginal dryness, dyspareunia, itching, genital pain and increased susceptibility to infection, are frequently reported by pSS women. In particular, vaginal and cervical atrophy are frequent in pSS with sexual dysfunction and strictly correlate to depression and mood changes (41). However, the aetiology of vaginal dryness remains unclear. To overcome this issue, a recent cross-sectional study including about 200 pSS women demonstrated that patient-reported vaginal dryness was significantly associated with older age, postmenopausal status, peripheral neuropathy, oral and ocular dryness, EULAR Sjögren's Syndrome Patient-Reported Index (ESSPRI) score and SF-36 mental and general health. In particular, the evidence of independent association of vaginal dryness with oral and ocular dryness and neuropathic abnormalities might suggest a common etiopathogenic pathway of these symptoms (42). This suggests that sexual dysfunction should always be evaluated in pSS women and specific treatment addressing vaginal dryness, dyspareunia and depression should be considered, if necessary. Interestingly, recent evidences suggest that autoimmune diseases, as systemic lupus erythematosus, predispose to an absence of smell function and diminished smell sensitivity. In pSS, progressive exocrine gland damage with loss of secretions may be associated to reduced sense of smell. In this setting, olfactory function including smell threshold, identification and memory were investigated in a cohort of Chinese pSS patients with a mean disease duration of 4.5 years (43). Interestingly, a significant decrease in olfactory function, both as identification and memory functions, was observed in pSS patients compared to healthy controls. Moreover, hyposmia was more prevalent and anosmia was present exclusively in pSS patients. Disease activity and dryness had negative impact on olfactory function and impaired smell function was associated with organ involvement and autoantibody positivity (43).

Nevertheless, xerostomia and xerophthalmia are the predominant symptoms of pSS and represent the principal features leading to its suspicion, with reported positive and negative predictive values of 54-77% and 94-98%, respectively (44). Moreover, these symptoms are associated with significant impaired daily function, physical and emotional distress, decreased quality of life and long term adverse outcomes. Primary SS patients with dry eye, be-

sides olfactory dysfunction, may present ocular surface epithelial damage, assessed by ocular staining score, which is significantly associated with younger age, longer duration of disease, unstable tear film and meibomian gland function (45). Moreover, patients may complaint voice and swallowing dysfunctions secondary to oral dryness with a consequent impairment of quality of life, as demonstrated by Graf *et al*. (46).

A recent retrospective study assessed the long-term treatment outcomes of dry eye in patients with and without underlying pSS (47). At baseline, pSS dry eye patients had worse ocular parameters (including lower Schirmer's results, higher ocular surface staining and higher tear osmolarity) compared with the non-SS dry eye cohort. Interestingly, with proper escalation of dry eye treatment, both SS and non-SS dry eye patients achieved significant improvement of objective dry eye parameters. Nearly half of patients had resolved conjunctival staining, a third resolved corneal staining and a quarter had no ocular surface staining by their final visit. During follow-up, 10% of patients experienced vision-threatening corneal complications (epithelial defects/ulceration and corneal melt/perforation) and eleven patients lost vision due to corneal haze/scarring with no difference between the two cohorts. Of consequence, assessment of glandular function represents an undoubted cornerstone for the diagnosis and assessment of the disease. In this setting, salivary gland ultrasonography (SGUS) emerged as an important toll in the evaluation of salivary gland involvement in pSS patients being less invasive, cheaper and with far fewer risks than salivary gland biopsy (SGB). Recently, sonoelastography (SE), a developing technique which assesses the tissue elasticity or stiffness property of parenchymal elasticity by colour scale coding the different degrees of mechanical-elastic deformation, has been evaluated in a cohort of 79 pSS patients with median symptom duration of 6 years (48). The elasticity scores in the pSS group were significantly higher than those in the disease controls with significant difference between parotid and submandibular scores. Interestingly, correlation analysis depicted higher elasticity scores of parotid glands in patients with disease duration >10 years in comparison to patients with disease duration ≤5 years and 5-10 years respectively. Moreover, total score of parotid and submandibular glands correlated with rheumatoid factor level but not with anti-SSA/SSB antibodies. Although larger prospective studies are needed to better define the usefulness of SE in pSS patients, this novel technique performed simultaneously with B-mode ultrasound may represent a feasible adjunct tool in the diagnosis of pSS.

Extra-glandular involvement

Beyond exocrine glandular involvement, pSS patients may develop many extra-glandular manifestations, which may represent the first presentation of the disease and appear before the onset of the characteristic features of dryness. Moreover, extra-glandular involvement influences the prognosis of the disease, so several studies focused on different systemic domains, among which lung involvement represents one of the most important. Interestingly, the high prevalence of pulmonary abnormalities in pSS patients encouraged to investigate the percentage of unrevealed pSS in a prospective population with interstitial lung disease (ILD) and one or more interstitial with autoimmune features (IPAF) classification criteria or xerophtalmia. Shirmer's test, ocular Staining Score (OSS) and minor SGB were performed in a blinded manner by experienced specialists (49). Among 334 patients with ILD, 67 had at least one IPAF criteria and a significantly higher proportion of patients with positive SGB, pathologic Schirmer's test (both 53.7%) and OSS (43.3%) met pSS criteria (40%), suggesting that occult pSS must be evaluated in this group of patients (49). However, in the last year, the majority of studies mainly focused on the identification of prognostic factors associated with increased risk and worse outcome of lung involvement in pSS patients. Older age, male sex, smoking and extent of pulmonary involvement were confirmed factors associated with poorer prognosis or lower survival (50-52). Nevertheless, several drawbacks should be considered in the interpretation of such results. Among these, the retrospective design of all studies, the different settings of cohort enrolment, including outpatients (50, 51) or more severe hospitalised patients (52), the non-homogenous instrumental methods to detect pulmonary involvement or to define the clinical and respiratory function variables and the low number of patients included in some studies (51), surely do not allow to drawn definite conclusions.

Although carried out on a very low number of patients, a study by Dong *et al*. may deserve attention as focused on the analysis of 15 female pSS patients with lymphocytic interstitial pneumonia (LIP), a typical, rare, ILD pattern in pSS which main pathological features include extensive and polyclonal infiltration of interstitial lymphocytes with widened interlobular/alveolar space (53). In comparison to a previous cohort of 206 pSS-ILD patients, pSS-LIP patients were characterised by higher prevalence of hypergammaglobulinaemia and antibody positivity, including anti-Ro52, anti-Ro60 and anti-La. Pulmonary function tests demonstrated a more favourable respiratory function in pSS-LIP with higher total lung capacity and diffusion capacity of carbon monoxide. HRTC patterns helped to distinguish between acute phase (characterised by ground-glass opacity) and later stages (presence of cysts), allowing the adequate treatment.

Among systemic manifestations associated with adverse prognosis in pSS, neurological involvement gained considerable attention. A study involving 205 consecutive Chinese pSS patients depicted neurological involvement, both central and peripheral, in 19% of patients and patients with neurological involvement were characterised by significant higher prevalence of anti-SSA antibodies. However, methodological approach to detect neurological involvement, including suggestive symptoms and/or variable diagnostic tests, and the heterogeneous definition

of clinical neurological phenotypes hamper the results of this study (54).

Thus, in order to overcome these limitations which hamper to define the true prevalence of neurological involvement in pSS, a recent multi-centre Italian study applied a standardised protocol to evaluate prevalence and features of peripheral neurological involvement in a large cohort of 1695 pSS patients (55). Prevalence of peripheral nervous system was rare (3.7%) and the main manifestations were pure sensory neuropathies or axonal sensorimotor polyneuropathies. This subset of patients exhibited a more active disease profile and were more frequently treated with immunosuppressant therapies. Intriguingly, clinical and serological negative prognostic factors, including purpura, extra-glandular manifestations, leukopenia, low complement and cryoglobulinaemia, principally characterised patients with sensorimotor polyneuropathy, while subjects with pure sensory neuropathy displayed a milder phenotype, thus suggesting that different pathogenic pathways may underlie peripheral nervous system involvement in pSS (55). On the other hand, central nervous system involvement is very rare in pSS and difficult to detect due to non-uniform definition and variable imaging features. In this setting, abnormalities of brainstem auditory evoked potentials, a test employed to detect central nervous system involvement, were recorded in about 16% of pSS patients (56). However, the very low number of patients included highly hamper the relevance of the results and further studies are needed to define this important issue in pSS.

Moreover, during the last 12 months, literature focused on characterisation of common extra-glandular manifestations, including cutaneous, generally poorly characterised due to the low number of patients included in most cohorts and the variability of cutaneous manifestation definition, and articular. A recent epidemiological French study on a population of 685 pSS included in two French cohorts (the ASSESS and diapSS) and the cohort enrolled in the TEARS randomised trial, depicted a prevalence of cutaneous signs defined according to ESSDAI domain of 4.1%, 5.3% and 6.4%, respectively (57). In the ASSESS cohort, patients with active cutaneous involvement in the ESSDAI domain had higher prevalence of peripheral neuropathy and muscular involvement compared to the control group. As widely known, articular involvement has been reported as presenting manifestations in 40% of pSS patients and is mainly characterised by a symmetrical arthritis or arthralgia generally involving small joints. However, in a recent retrospective analysis of a cohort of 148 pSS patients, Jarrot *et al*. identified a subset of 29 (20%) female patients presenting with axial articular manifestations, of which 23 (79%) fulfilled psoriatic arthritis and ankylosing spondylitis classification criteria (58). This subset of patients was characterised by younger age and higher frequency of peripheral articular manifestations in comparison to patients without axial involvement. Prevalence of extra-glandular manifestations, ESSDAI scores and antibody status were similar between the two groups. Interestingly, radiographic sacroiliitis was reported in 65% of patients, among which 47% had bilateral involvement associated with anterior syndesmophytes (26%) and presence of HLA-B27 (13%). A common genetic background and the involvement of interleukin-17 axis, implicated in the pathogenesis of both ankylosing spondylitis and pSS, may explain this association, thus suggesting to consider inflammatory axial involvement in young pSS with suggestive symptoms (58).

Although poorly characterised, muscular inflammatory involvement represents an important systemic manifestation in pSS being associated with the highest grade in ESSDAI score. To better characterise and define prevalence, features and response to treatment of myositis, a population of 395 pSS patients with at least 60 months of follow-up from the multicentre prospective ASSESS cohort was selected. Myositis was confirmed only in 4 pSS (1%) (59). Disease duration in patients with confirmed myositis was 3-fold longer than patients without and patients with suspected myositis were characterised by more frequent articular and peripheral nervous involvement than other groups. Interestingly, two of the four myositis patients fulfilled criteria for sporadic inclusion body myositis which may be considered as a late complication of the disease (59).

Finally, cardiovascular (CV) involvement was confirmed as an important co-morbidity in pSS patients. Increased prevalence of accelerated subclinical atherosclerosis, namely endothelial dysfunction, characterised a small cohort of pSS patients in comparison to healthy controls without CV risk factors and, of note, correlated with disease activity, anti-Ro positivity and inversely with ADMA levels, further supporting the strict relationship between systemic inflammation and atherosclerosis in these patients (60). On the other hand, estimation of long-term CV risk in pSS represents an unmet need and the performance of traditional CV algorithms in the disease has never been investigated. A recent study evaluated the performance of two risk algorithms, including the "Progetto Cuore" and the Reynolds Risk Score (RRS), and factors contributing to CV risk score in an Italian cohort of 68 pSS patients free from CV events (61). The mean 10-year estimated risk of CV events was 4.3 (4.9 SD) % and 4.1 (4.8 SD) % according to RSS and "Progetto Cuore", respectively. Interestingly, both inflammatory parameters, as C reactive protein and ESSDAI, and traditional CV risk factors, namely hypertension and dyslipidaemia, were identified as significant predictors of 10-year CV risk score (61).

Toward a disease phenotype

The identification of pSS subgroups characterised by common peculiar genetic, serologic and/or clinical features may allow a better characterisation of the disease and may drive targeted therapies toward the application of precision medicine in pSS. In this setting, the possibility to phenotype disease clusters represents a future open challenge and, recently, increasing research aimed to investigate and characterise patient subgroups according to specific disease-related features.

Higher frequency of anti-RNP and anti-centromere antibodies (ACA) identify a subset of younger pSS patients characterised by a more severe phenotype with higher frequency of extraglandular manifestations, in particular lung involvement, in comparison to anti-RNP and ACA negative patients in a retrospective cohort of 333 new-onset pSS patients (62). These results were partially confirmed in a recent analysis of a cohort of Korean pSS patients with both anti-Ro52 and ACA positivity which were characterised by higher frequency of xerostomia, Raynaud's phenomenon, liver involvement and lower frequency of leukopenia, hypergammaglobulinaemia, RF and anti-Ro/SSA in comparison to ACA-negative patients (63). Hypergammaglobulinaemia represents a distinct serologic feature of the disease characterising about one half of pSS patients. A recent analysis of the Korean Initiative pSS (KISS) cohort confirmed that 47% of pSS patients display hypergammaglobulinaemia at baseline (64). Immunoglobulin levels changed following treatment with hydroxychloroquine and glucocorticoids and, after adjusting for age and drug use, persistent hypergammaglobulinaemia was associated with salivary flow impairment and solid organ damage after 3-year follow-up (64).

The relationship between serologic status and specific disease phenotypes needs further analysis. In a Turkey cohort of 375 patients, seronegative patients for ANA, RF, anti-Ro and anti-La were not statistically different from seropositive in terms of patient age, age at diagnosis, sex distribution, clinical features and laboratory parameters except for higher prevalence of hypergammaglobulinaemia in seropositive ones (65). On the contrary, an analysis of a wide cohort of 1569 patients demonstrated that triple seronegative (anti-Ro/SSA, anti-La/SSB, RF but ANA positivity) patients had lower prevalence of peripheral nervous system involvement in comparison to seropositive (66). Moreover, quadruple seronegative (anti-Ro/SSA, anti-La/SSB, RF, ANA) patients presented less frequently with lymphadenopathy and lymphoma, displaying a milder clinical picture mainly characterised by glandular and peri-epithelial manifestations (66). However, the different cutoff of antibody concentration in the two studies hamper data comparison.

Interferon (IFN)-γ signature may identify a distinct disease phenotype. A recent analysis of a small pSS cohort depicted that patients with elevated IFN-γ levels are younger, with shorter time to disease diagnosis and characterised by more active disease in comparison to patients with normal IFN-γ levels (67). Similarly, age at disease onset may identify specific clinical phenotypes. Interestingly, in the huge cohort of 12,753 pSS patients included in the Big Data Consortium (68), for each 1-year increase in the age at diagnosis, the frequency of positive antibodies decreased as well as glandular and lymph node involvement while pulmonary and peripheral system involvement significantly increased, thus suggesting that age at diagnosis may be considered an important parameter to identify specific disease cluster and to guide targeted follow-up and treatment.

Mossel *et al*. attempted to distinguish clinical phenotypes based on SGUS score (69). Patients with a total SGUS score ≥15 had a longer disease duration, higher ESSDAI, disease damage and frequency of glandular and serological dysfunction but, interestingly, reported lower ESSPRI fatigue and pain compared to SGUS negative patients.

Finally, latent class analysis and latent transition analysis may represent novel tools for classification of pSS patients according to symptom clusters. Latent class analysis applied in a prospective Korean pSS cohort using patient reported outcome identified three distinct subsets of disease presentation with different laboratory and clinical features, including high symptoms burden, dryness dominant and low symptom burden (70). At the 5-year follow-up, class membership was characterised by temporal stability and baseline class predicted salivary gland function and disease damage (70). Similar analysis was performed by Gairy *et al*. in a cross-sectional survey of real-world quantitative data. Latent class analysis identified five different clinical clusters including high burden (multiple organ involvement and highest frequency of pain and fatigue), low burden and three subsets defined by higher articular involvement and different extent of other organ system involvement (71).

In conclusion, pSS represents a systemic disease, with different clinical and serologic features which may identify specific clusters with different expression and prognosis. Moreover, the disease may significantly impair patient quality of life. The ESSPRI, a validated tool for estimate oral and ocular dryness, pain and fatigue, could be influenced by different factors. Sandoval-Flores *et al*. demonstrated an association between ESSPRI score >5 and higher prevalence of depression and lower non-stimulated whole salivary flow (72). The impact of pSS diagnosis on daily life is not entirely negligible, as shown by Bejerano *et al*. (73). In a cohort of252 patients, an impairment of allwork productivity and activity impairment domains was detected, including time lost due to health, decrease in work productivity and activity of daily living (ADL). Moreover, xerostomia, arthritis, and depression were significantly and independently associated with ADL impairment, mainly in patients treated in public centers, maybe as reflection of socioeconomic condition (73). Oral dysfunctions related to the disease, including mouth ulcers, trouble speaking and dysphagia, rather than systemic involvement seem to significantly impair quality of life (74, 75). In this setting, resilience, the process of adapting positively to stressful life events, has gained relevance in the last years. An Italian study analysed for the first time resilience in a cohort of 74 pSS patients by the Resilience Scale (RS-14) (76). SS patients displayed a moderate resilience with no significant difference compared to controls. An inverse relationship was found between resilience and mood disorders, as anxiety and depression. Greater resilience was associated with a better perception of the quality of life and general health, less fatigue and a more physically active lifestyle. Resilience was not correlated with socio-demographic and disease

features nor with disease activity, damage and ESSPRI (76).

Primary Sjögren's syndrome and SARS-CoV-2 infection

Data addressing specifically the impact of COVID-19 infection in pSS remain scarce as the majority of studies refer to patients with different systemic autoimmune diseases. However, a recent interesting review specifically focused on the effect of COVID-19 on the disease and of immunosuppressive therapies on disease outcome and on the emerging evidence of long-term xerostomia after SARS-CoV-2 infection (77). Data analysis of Big Data Consortium patients followed after SARS-CoV-2 infection demonstrated that 52% of patients remained symptomatic for post-COVID-19 symptoms for more than 4 weeks and 29% were symptomatic for more than 12 weeks, thus fulfilling the definition of post-COVID-19 syndrome (78). Moreover, more than 40% of pSS patients reported the persistence of several aymptoms, including anxiety/depression (59%), arthralgias (56%), sleep disorder (44%), fatigue (40%), anosmia (34%) and myalgias (32%). Age-sex adjusted multivariate analysis identified elevated C-reactive protein levels, use of hydroxychloroquine and antiviral agents, hospitalisation, mean length of hospitalisation and requirement of supplemental oxygen as factors associated with a higher risk of developing post-COVID-19 syndrome (78).

Take home messages

- Sicca symptoms, including genital symptoms related to vulvar and vaginal dryness, significantly impair quality of life of pSS patients and are strictly correlated to depression and mood changes (41).
- Salivary gland sonoelastography, if performed simultaneously with B-mode ultrasound, may represent a novel feasible adjunct tool in the diagnosis of pSS (48).
- Older age, male sex, smoking and extent of pulmonary involvement represent main factors associated with poorer outcome in pSS (50-52).
- In pSS with peripheral nervous system involvement, sensorimotor polyneuropathy is associated with higher frequency of clinical and serological negative prognostic factors, including purpura, extra-glandular manifestations, leukopenia, low complement and cryoglobulinaemia, in comparison to pure sensory neuropathy, thus suggesting a different pathogenic pathway (55).
- Primary SS patients with active cutaneous involvement in the ESSDAI domain have higher prevalence of peripheral neuropathy and muscular involvement (57).
- Primary SS patients with muscular involvement have longer disease duration and increased frequency of articular and peripheral nervous involvement (59).
- Traditional cardiovascular risk factors and inflammation significantly contribute to 10-year risk of cardiovascular events in pSS (61).
- Specific demographic, serologic and clinical parameters may allow the identification of peculiar disease subsets (62-66).
- Latent class analysis and latent transition analysis recently gained attention as novel tools for classification of pSS patients according to symptom clusters (70, 71).
- About one-third of pSS patients with precedent SARS-CoV2 fulfill classification criteria for post-COVID 19 syndrome (78).

Novel insights in pSS treatment

Most of the recent publications investigating novel potential treatments for SS report data from *in vitro* and animal models of the disease. The immunomodulating effects of mesenchymal stem cells (MSC) remain one of the most intensively explored areas of the latest years. The injection of human MSC isolated from healthy labial glands or umbilical cord into non obese-diabetic (NOD) mice, seem to improve salivary secretion, the differentiation of T regulatory (Treg) cells and the production of IL-10 by T cells, while reducing inflammatory infiltrate in salivary glands, the differentiation of T helper 17 (Th17) cells and also the amount of circulating anti-SSA/Ro and anti-α-fodrin autoantibodies (79, 80). Ecto-mesenchymal cells from wild-type mice can enhance the differentiation and expansion of myeloid-derived suppressor cells (MDSC) which, in turn, are capable of suppressing $CD4^+$ T cell proliferation. There is evidence that MDSCs are deficient in experimental models of SS and the restoration of their suppressing abilities by MSCs, likely through a complex mechanism involving toll-like receptor (TLR) signalling, alarmin A100A4 and autocrine activity of IL-6, may represent a potential target for cell treatment in autoimmune diseases (81).

Multiple potential therapeutic targets to address sicca syndrome in SS patients have been identified in animal models. Jasmer *et al*. (82) were in fact able to improve salivary flow and reduce lymphocyte infiltration by intraperitoneally injecting NOD mice with a selective inhibitor of $P2Y_2$ nucleotide receptor, which is known to be involved in immune cell homing. Similarly, the inhibitor of lymphocyte extravasation cenerimod, which acts by modulating sphingosine-1-phosphate receptor 1 ($S1P_1$), was able to reduce the inflammatory infiltrate of salivary glands by T cell and plasma cells in a murine model of SS (83). B-cells also seem to be the target of artesunate, an orally-administered artemisisin derivative that seems to suppress B-cell activating factor (BAFF)-induced activation of NFκB, thus improving salivary flow (84) and of paeniflorin-6'-O-benzene sulfonate (CP25), likely inhibiting CXCL13 signalling via JAK-STAT1/2 (85). Moreover, butyrate can increase salivary flow and reduce glandular infiltrate in murine model of SS, perhaps by increasing the number of IL-10-producing-B cells (B10) and reducing IL-17-producing B cells, both *in vitro* and *ex vivo* (86).

Circulating anti-Ro60 autoantibodies represent one of the key features of SS, from the diagnostic point of view but also in terms of association with clinical features of the disease. Ro60 autoantigens, however, seem to have a pathogenic role as well. In fact, there is evidence that the blockade of the interaction between Ro60 and major histocompatibility complex (MHC) class II

I-A^{g7} improves glandular function and reduces inflammatory infiltrate in NOD mice (87). Another promising strategy to modulate the immune response in SS emerges from a recent paper by Killian *et al*., who demonstrated that by administering an interferon (IFN) vaccination strategy to a MRL/lpr mouse model, a significant production of anti-IFNα antibodies, along with amelioration of glandular inflammation and disease activity, in the absence of significant adverse effects can be achieved (88).

Few new studies have been carried out on the local treatment for dry eyes. Briefly, a prospective study comparing 0.03% tacrolimus and 0.05% cyclosporine did not find any significant difference between arms (89), while a partial improvement was demonstrated after switching from cyclosporine A 0.05% anionic emulsion to 0.1% cationic emulsion, though with no control group (90). Interestingly, however, a trial suggested that the irrigation of salivary glands by sialendoscopy can significantly improve salivary flow, xerostomy and ESSPRI, likely through a mechanical action that opens ducts, removes debris, microliths and mucus (91).

In terms of systemic treatments, a retrospective study on hydroxychloroquine, commonly employed to treat milder forms of the disease, confirmed its efficacy on cutaneous manifestations and as a steroid-sparing agent (92). A phase IIb randomised controlled trial investigating the effect of ianalumab (BAFF receptor inhibitor) in SS found a significant dose-dependent improvement of ESSDAI, with no significant adverse events compared to placebo (93). Unfortunately, a phase II trial of seletalisib (inhibitor of phosphatidylinositol 3-kinase delta) failed to reach its endopoints due to little enrolment. However, a trend towards an improvement in ESSDAI and ESSPRI was observed (94). A prospective, open-label study assessed efficacy and safety of oral 24 week administration of iguratimod, which inhibits the activity of NF-κB, downregulates the production of inflammatory cytokines, including TNF-α, IL-1β, IL-6, IL-8, and IL-17 and the production of immunoglobulin by B cells, in a small cohort of pSS patients with disease duration ≤5 years (95). Significant reduction of disease activity, in particular in the articular, biological and haematological ESSDAI domains, was observed after 24 weeks of treatment in comparison to baseline. Moreover, IgG and rheumatoid factor levels significantly decreased. On the other hand, no significant effect was observed on ESSPRI, quality of life, unstimulated salivary flow and lacrimal gland function (95). Conversely, a randomised, placebo-controlled trial demonstrated a significant improvement of ESSPRI but not ESSDAI scores following 24 weeks of iguratimod therapy in a cohort of 66 pSS with longer disease duration (96). Finally, a very interesting trial was carried out by Posada *et al*. (97). The authors administered a fusion protein between a human IgG1 Fc domain and a fully functional RNase enzyme to pSS patients. The rationale of such treatment strategy lies on the hypothesis that such protein may digest circulating RNA autoantigens, thus preventing their presentation to immune cells. Despite a down-regulation of IFN-inducible genes was expected, an up-regulation was instead observed. However, although an up-regulation of IFN-inducible genes is expected to associate with disease worsening, from a clinical perspective, treated patients showed an improvement of ESSPRI and fatigue scores (97). These results confirm that the mechanisms linking IFN signature and pSS pathogenesis are complex and intertwining.

The main limit of current research on effective therapies is the low recruitment of patients in clinical trials due to low disease activity in the majority of pSS cohorts. Thus, research is currently focusing on validation of different disease-specific responder indexes to be employed in clinical trials to extent patient recruitment. These novel proposed scores include different domains that encompass some major disease features, including systemic activity, saliva and tear function, patient-reported outcomes and biological features. The Composite of Relevant Endpoints for Sjögren's Syndrome (CRESS) and the Sjögren's Syndrome Tool for Assessing Response to Treatments (STAR) will be future tools to be employed in new prospective trials involving both patients with systemic activity and with disabling symptoms but without disease activity. These tools, as the STAR, will be validated in prospective trial conducted by the NECESSITY Consortium (98).

Take home messages

- Mesenchymal stem cells have immunomodulating effects improving salivary flow and reducing glandular inflammation in mice models of SS (79-81).
- Experimental, orally-administered, drugs and administration of an interferon vaccination strategy seem to improve salivary flow and disease activity and reduce glandular infiltrate in mice models of SS (88).
- Hydroxychloroquine confirmed its efficacy on cutaneous manifestations and as a steroid-sparing agent (92).
- Ianalumab, a BAFF receptor inhibitor, and iguratimod, an inhibitor of NF-κB activity, seem to improve disease activity in recent phase IIb trials (93, 95).
- New composite indexes need to be validated in prospective trials to improve recruitment of patients in clinical trials and the management of the disease (98).

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