Review

One year in review 2019: Sjögren's syndrome

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ABSTRACT

Primary Sjögren's syndrome (pSS) is a complex and heterogeneous disorder characterised by a wide spectrum of glandular and extra-glandular features. Novel insights into disease pathogenesis and the discovery of novel biomarkers are allowing us to characterise the disease not only phenotypically on the basis of clinical presentation, but also on the basis of the endotype. Ultimately, a better stratification of patients may pave new avenues for novel targeted therapies, opening new possibilities for the application of personalised medicine in pSS.

Introduction

Primary Sjögren's syndrome (SS) is a female dominated, chronic systemic autoimmune disease with a diverse clinical picture, extending from exocrine involvement to extraglandular manifestations. Many efforts have been made during the last few months to define the complexity of the disease, its pathogenetic pathways and ultimate searching for novel biomarkers that may allow an earlier diagnosis and a more precise therapeutic intervention. In this review, following the others of this series (1-4), we will summarise the most recent literature on SS clinical presentation, diagnosis and treatment. The "leit motif" this year will be to start from SS phenotypic characterisation, as emerged also from big data, and then move towards basic science presenting the most recent highlights in disease pathogenesis and biomarkers. We will conclude with an overview on the state of the art of pSS therapy leading to future developments in this field. In this perspective we have performed a Medline search of English language articles published in the PubMed database from 1st January 2018 to 31st December 2018. The following key words: Sjögren's syndrome, dry eye, dry mouth, pathogenesis, diagnosis, salivary gland ultrasonography, biomarkers, antibodies, biopsy, clinical manifestations, lymphoma, therapy formed the data sources.

New insights in pSS clinical presentation

Primary SS is a multisystem disorder that does not behave as a single disease. In the following paragraphs we have summarised the most recent literature on glandular and extra-glandular disease manifestations highlighting the complexity and the heterogeneity of the disease presentation and the challenges for targeted therapies in pSS patients.

Glandular manifestations

Dry eye and dry mouth are the hallmarks of pSS and are associated with several oral and ocular damage manifestations, including tooth loss (5) and voice problems (6), chronic pain, impairment of quality of life and disability (7). In particular, dry eye is one the most frequent symptom reported by SS patients and about 85% of patients complain of severe or very severe dry eye, as reported in a recent analysis of a Spanish SJOGRENSER registry. In this study, 94% of the patients complained of daily, persistent, troublesome dry eye, 92% had the sensation of sand in the eye and 16% developed corneal ulcers. Interestingly, evaluation of an association between dry eye severity and extra-ocular involvement depicted a significant association between severe and very severe dry eye and inflammatory articular involvement (8). The reported prevalence of dry eye in Chinese patients appears to be lower than in other countries may be because the assessment methods of sicca symptoms were different. Furthermore, the proportion of severe dry eye was related to older age (9). Severity of dry eye may hamper the quality of life

of patient as demonstrated in SS women enrolled in the SICCA cohort (10), where participants with reported symptoms of dry eyes manifested higher odds of depression compared to symptom-free participants.

A better understanding of the pathogenetic mechanisms underlying dry eye development may open up novel therapeutic strategies. In SS patients, dry eye has been associated with two main pathogenetic mechanisms including aqueous tear deficiency and increased tear evaporation. These two mechanisms may coexist in the same patient while recently the involvement of Meibomian gland dysfunction (MGD) in pSS dry eyes has been also highlighted. Sullivan *et al*. demonstrated that symptoms of dry eye were increased ~10-fold in primary and secondary SS patients as well as in subjects with non-SS related MGD, in comparison to normal controls. Mechanisms of androgen deficiency and down regulation of insulin-like growth factor 1, contributing to MGD, leading eventually to the development of dry eye, were hypothesised in women with SS (11). A prospective age- and gender-matched case-control study focusing in MGD, disclosed differences in ocular symptoms and signs, between SS and non-SS aqueous-deficient dry eye patients by Keratograph and LipiView, two novel non-invasive quantitative instruments (12). The destruction of the meibomian gland in the upper tarsus was significantly more severe in SS patients than in the control group. The authors pointed to these changes in the upper eyelid as being responsible for the differences between SS and non-SS subjects. In addition, SS patients had a higher degree of MGD with an inconsistent lesser symptom of ocular dryness, which may be related to a lower corneal nerve fibre density, thus suggesting that MGD may be considered as an adjunctive mechanism associated with SS-associated dry eye (12). Moreover, the intensity of ocular discomfort and severity of ocular surface damage scores were higher in SS patients with MGD compared to non SS subjects with MGD (13). Kang *et al*. evaluated the manifestations of MGD in patients with SS, non-SS dry eye and non-dry eye controls and their correlation with disease severity by the infrared camera system of the Keratograph 5 M (14). In accordance with previous studies, patients with SS tended to have a higher degree of MGD and higher incidence of severe gland dropout and gland obstruction than those with non-SS dry eye, exhibiting poorer meiboscores and meibomian gland expression in comparison to controls, leading to a higher impairement of meibomian gland function compared to non-dry eye controls (14).

Retinal involvement, may be subclinical and thus underdiagnosed in SS. Conigliaro *et al*. evaluated morphological and functional visual abnormalities in a cohort of systemic lupus erythematosus (SLE) and SS patients and and tried to determine potential associations between retinal disorders and disease activity, organ involvement and therapy (15). The structural analysis was performed by spectral-domain optical coherence tomography (SD-OCT) and detected a reduction in total posterior pole retinal thickness as well as the temporal-inferior and the nasal-inferior sectors in SS patients, compared to both healthy controls and SLE patients. In addition, in SS patients, the fundus perimetry differential sensitivity was reduced and the mean defect values were higher than in healthy subjects being more prevalent in those SS patients who received a cumulative hydroxychloroquine (HCQ) dose more than 1,000 g. The thickness of peripapillary retinal nerve fibre layer (pRNFL) in the inferior quadrants was compromised compared tocontrols due to dry eye disease or optic neuropathy. The authors discussed the possibility that the observed thinning might be due to HCQ treatment or apoptosis of retinal ganglion cells induced by anti-Ro antibodies. However, no difference both in the total retinal and pRNFL thickness was detected between anti-Ro-positive and -negative patients (15). SS affects corneal structure causing biomechanical changes, specifically in corneal hysteresis (CH) which can be demonstrated by the ocular response analyser (ORA), a non-contact tonometer that measures the corneal biomechanical properties. Lower CH in SS patients, may be caused by corneal alterations related to dry eye or by inflammatory phenomena produced by a tear rich in immune complex, cytokines and active oxygen species, as well as direct inflammation of the stroma (16). The evaluation by *in -vivo* laser scanning confocal microscopy (IVCM) of the corneal epithelium in non-SS dry eye and SS dry eye showed morphological alterations either on the superficial corneal epithelium associated with local alterations of the underlying epithelial surface with decreased cell densities in the epithelial layers of both, thus confirming previous studies and highlighting the valuable role of this technique for the assessment of eye involvement in pSS (17, 18).

Extraglandular manifestations

It is widely recognised that pSS is a complex systemic disease with a scattered spectrum of systemic manifestations. Generally, systemic pSS features, are mild and do not affect severely the overall survival of patients. On the other hand, some of them are producing discomfort in pSS patients, leading sometimes to disability. An important example is small fiber neuropathy (SFN), a frequently under-diagnosed PNS complication in SS often causing paresthesia, allodynia and burning sensation. In SFN, nerve conduction studies are unremarkable and the diagnosis is established by specific assays performed on skin biopsy, that essentially describe the density of small fibers within the skin. This assy presents high sensitivity and specificity reaching to 80–90%. According to a large cohort study performed at the Johns Hopkins Rheumatic Disease Research Core Center, in the US, SS patients with biopsy-proven SFN often experience neurologic symptoms before the onset of sicca syndrome, adding SFN in the initial symptoms of the disease (19).

In a recent report (20) from a Spanish registry, the authors analysed a sub-population of patients with severe disease activity, defined if at least one ESSDAI domain was scored as "high". The mortality rate of this cohort was 20% and even higher when more than one domains were involved. Unsur-

prisingly, cytopenia, RF positivity, hypocomplementaemia and positive cryoglobulins were associated with severe systemic involvement, in line with previous studies. Moreover, SS patients displayed a higher rate of hospitalisation compared to controls, mainly due to comorbidities, such as cardiovascular and musculoskeletal degenerative disorders (21). One of the most prevalent causes of death in SS patients was the presence of cardiovascular (CV) events. It is now well known that patients with SS, like most systemic autoimmune diseases, present a significantly higher risk of developing cardiovascular events. It is still uncleare, however, if this comorbidity is a consequence of the disease itself or it is associated with conventional CV risk factors (such as hyperlipidaemia, hypertension, diabetes, mellitus, cigarette smoke, etc.) (22, 23). A meta-analysis performed on ten studies, including in total more than 150,000 patients confirmed an increased rate of cardiovascular events, but not cerebrovascular in pSS patients compared to controls. Some traditional risk factors, including hypertension and hyperlipidaemia, were more prevalent in pSS patients than controls (24). The same authors also published another meta-analysis of 9 studies showing that patients with pSS have a significantly increased intima-media thickness (IMT) and arterial stiffness measured by pulse-wave velocity (PWV), both reliable surrogate markers of atherosclerosis (25).

A large Taiwanese cohort study showed that both pSS and secondary SS (sSS) patients have a significantly higher risk of developing aortic aneurysms and dissection (26). It is likely that both the inflammatory process linked to the disease and the increased prevalence of traditional risk factors contribute to an acceleration of the atherosclerotic process, eventually leading to overt cardiovascular events and significantly influencing the patients' prognosis. These observations, however, need to be confirmed, since the standardised mortality ratio of pSS patients with no risk factors for lymphoma development does not differ from the general population.

Sleep impairment was also found to be associated with a higher risk of carotid plaque formation, independently of other CV risk factors (27).

The development of non-Hodgkin's lymphoma is considered one of the most severe complications of SS, and it accounts for a significant proportion of the increased mortality rate caused by the disease (28). For this reason, important scientific efforts have focused on risk stratification and prediction of the development of lymphoma in SS patients, in order to achieve an early diagnosis and treatment and, in the future, to potentially prevent the evolution towards overt malignant disease. The most common type of lymphoma that develops in SS patients derives from mucosa-associated lymphoid tissue (MALT). Several clinical and laboratory feature of SS patients have been identified as potential predictors of lymphoma development although no definite consensus has been reached. An interesting study by De Vita *et al*., investigating a cohort of SS patients, reported that the most valuable predictors of lymphoma development are features of heavy MALT involvement, such as persistent glandular swelling and cryoglobulinaemia, ranking independently of the EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) score (29). With the evolution of bioinformatics, new analytical models to predict the development of lymphoma are being used. Some of the main limitations of common algorithms are the abnormal distribution of variables and the limited power, in case of non-linear relationships between variables, which are both common characteristics of clinical parameters. To overcome these limitations, Baldini *et al*. have employed a new analytical method which created a semantic connectivity map preserving non-linear associations of variables (30). Using Auto CM, the author highlighted that rheumatoid factor was strongly associated to MALT-NHLs development, besides traditional lymphoproliferative risk factors (*i.e*. salivary gland enlargement, low C4, leukocytopenia, cryoglobulins, monoclonal gammopathy, disease duration). By applying data mining analysis, a predictive model with a sensitivity of 92.5% and a specificity of 98% was obtained. If the analysis was restricted to the seven most significant variables, the sensitivity of the model was 96.2% and its specificity 96%. The authors also confirmed the associations between autoantibody positivity and several pSS clinical manifestations, highlighting the importance of serological biomarkers in distinguishing pSS patients with predominant glandular manifestations and no or mild extra-glandular features from those with a more severe clinical presentation. Interesting data reported also from a large multicentre project (Sjögren Big Data Project) which analysed the immunological profiles of 10,500 primary SS patients and its impact on the clinical phenotype of patients (31). Among the large amount of data obtained, some are worth mentioning. Patients with double anti-Ro and anti-La positivity show higher ESSDAI score in constitutional, lymphadenopathy, cutaneous, renal and haematological domains compared to those with single anti-Ro or anti-La positivity. This study confirmed previously demonstrated associations, including those between cryglobulinaemia and vasculitis, as well as low complement levels and increased risk of lymphoproliferative disorders.

New insights into pSS biomarkers: novel antibodies, proteomics and imaging biomarkers

Several efforts have been recently made to identify valuable biomarkers, useful in the early diagnosis/stratification of pSS, when curative interventions are still possible (32). The presence of novel tissue specific autoantibodies (TSAs) including salivary protein-1 (SP-1), parotid secretory protein (PSP), carbonic anhydrase 6 (CA6) have been described in the early stages of pSS. These early markers are found before the classic autoantibodies, such as SS-A/Ro, SS-B/La, ANA, and RF (33, 34). Bunya *et al*. (35) evaluated the prevalence of these novel pSS autoantibodies in the DRy Eye Assessment and Management (DREAM) cohort that included patients with pSS but also patients with no history of SS or other autoimmune diseases and negative traditional pSS autoantibodies and patients

with no history of pSS, but a history of other autoimmune diseases. Data from this multicentre, prospective study demonstrated that one of the novel candidate autoantibodies, SP-1, is associated with underlying SS and that novel autoantibodies may be associated with a worse ocular surface disease. Similarly, Karakus *et al*. (36) investigated the value of these novel autoantibodies in differentiating the SS-related dry eye from non-SS dry eye and reported that anti-CA6 was the most prevalent novel autoantibody in patients with dry eye, and was associated with younger age and more severe disease. The specificity of these novel autoantibodies, however, needs to be tested in larger studies, since patients with fibromyalgia who also complain of xerostomia and sicca symptoms, are positive for one or more of the TSAs (35). Future longitudinal studies are needed to evaluate their utility in screening patients with dry eye for SS. Another potentially interesting autoantibody that has been evaluated in SS is anti-calponin 3, reported to be associated with pSS patients and neuropathies (37).

Besides novel autoantibodies, the idea of looking at different sources beyond blood for reliable biomarkers in primary SS is also opening new avenues in the diagnostic and prognostic stratification of the disease. In line with previous studies (38), in the past few months novel data have supported the work hypothesis of looking at primary SS biomarkers in patients' tears and saliva. Versura *et al*. (39) reported that the concentrations of tear proteins LACTO and LIPOC-1 possess a significantly higher accuracy, compared with the traditional ocular clinical tests for reaching pSS diagnosis. Thus, these proteins can be considered as candidate biomarkers of SS. Similarly, Jazzar *et al*. (40) described significant differences in salivary levels of S100A8/A9 in subgroups of patients with SS subdivided on the basis of their risk for lymphoma development.

Finally, in addition to -omic biomarkers, imaging biomarkers have gained a growing attention (41, 42). Salivary gland ultrasonography is a non-invasive tool, valuable for the morphologic characterisation of major salivary glands. Several studies have been performed to optimise a scoring system to be utilised as universally accepted diagnostic standard (43, 44). The existing scores showed similar diagnostic accuracy and, therefore, international efforts are undergoing towards the standardisation refinement of the specificity of this tool, particularly when used in primary SS differential diagnosis with mimickers (*i.e*. IgG4RD and sarcoidosis) (45). Ultrasound elastography, which allows the evaluation of the elasticity of the parenchyma, appears to provide complementary information regarding major salivary gland involvement in pSS that can be used for distinguishing the disease from control groups. Cindil *et al*. (46) evaluated 58 pSS patients with B-mode and elastography, suggesting that the strain ratio measurement increases the diagnostic effectiveness of pSS by providing high sensitivity, specificity, and negative predictive values. The possibility of using SGUS to monitor damage accrual in salivary glands, remains an open issue; in fact the authors did not observe any significant difference between the disease duration and the elasticity scores or strain ratios in pSS group. Similarly, Kimura-Hayama *et al*. (47), correlated the stiffness of parotid and submandibular glands with ESSPRI, ESSDAI, non-stimulated whole salivary flow rate, C3 and C4 levels, anti-Ro/La antibodies, salivary inflammatory chemokines and cytokines (CXCL13 CXCL10, CXCL8, CCL2, IL-10 and IL-6) and pro-fibrotic (CXCL14, CCL28, tumour necrosis factor-related apoptosis-inducing ligand and TGF β) and fibrosis in the minor salivary gland and found that an increased shear wave velocity correlated with salivary flow, ESSDAI, C4 levels, salivary CXCL13 and CXCL10 but not with age and fibrosis. The authors concluded that salivary elastography might reflect more chronic glandular inflammation than fibrotic changes.

An emerging potential application of salivary gland ultrasonography is its use for monitoring the response to therapy. Recent data from the literature show that the SGUS pattern tend to remain unchanged during the follow-up (48). However, the use of biological drugs is paving new paths for potential applications of SGUS. SGUS change following biologic treatments has been investigated in two recent trials. In a multicentre, randomised, double-blind, placebo-controlled trial 'Tolerance and Efficacy of Rituximab in Primary Sjögren's Syndrome' (TEARS), it was shown that the glandular parenchyma echo-structure was improved in rituximab-treated patients, more evidently in the parotids compared to the submandibular glands, whereas the size of the glands and the resistive index remained unchanged (49). The degree of SGUS abnormalities seem to represent also an interesting prognostic factor for the treatment response, with regard to oral and ocular dryness (50). In a substudy of the TRACTISS randomised, double-blind, multicentre and multi-observer phase III trial, the authors reported that the glandular definition improved in the rituximab arm, comparing the effects of rituximab *versus* placebo on SGUS (51), with regard to the visibility of the salivary gland posterior border. None of the other components showed a significant improvement at weeks 16 and 48, following rituximab treatment.

In conclusion, significant efforts have been made and are ongoing to search novel valuable biomarkers in pSS. It is highly likely that the new insights into the disease pathogenetic mechanisms may further improve their armamentarium, ultimately fostering a shift from phenotypic to endotypic patients stratification.

New insights into pathogenesis

Epigenetics

Genetic predisposition, environmental factors and epigenetic mechanisms contribute to the disease initiation and progress. Genetic factors associated with pSS are particular HLA-DR allele subtypes and specific gene polymorphisms including STAT4, IL-12A, TNIP1, IRF5, BLK, and CXCR5. Most of these genetic mutations are far from DNA coding regions and they are suspected to modify disease susceptibility genes by altering their expression indirectly via an action on the epigenetic machinery (52).

In pSS, several epigenetic mechanisms are altered, including DNA demeth-

ylation that predominates in epithelial cells, an abnormal expression of microRNAs, and abnormal chromatin positioning-associated with autoantibody production. Herein, we will cite some of the most recent data on DNA methylation, miRNAs and IFN signature in pSS (52).

DNA methylation is the most commonly studied epigenetic mark in pSS. It constitutes in the transfer of a methyl group by DNA methyltransferases (DNMTs) from S adenosyl-methionine (SAM) to the carbon-5 position of the cytosine residue in CpG dinucleotides, generating 5-methyl cytosine (5-mC). DNA methylation on a gene promoter leads to the silencing of the gene expression, while DNA demethylation of a gene promoter is linked to transcriptional activation and gene expression. Early investigations in pSS have assessed methylation at specific CpG sites in candidate genes or determined global methylation without single CpG site resolution in different cell and tissue types. More recently, epigenome-wide association studies (EWASs) have applied the Infinium HumanMethylation 450 (HM450k) BeadChip array (53). The first EWAS in pSS was published by Altorok *et al*. who investigated differentially methylated CpG sites in naïve CD4+ T cells and found hypomethylation at multiple sites of lymphotoxin (LT)-α gene, which encodes for an essential protein contributing in the formation of ectopic lymphoid tissue. IFN-induced genes such as STAT1, IFI44L and IFITM1 and genes encoding members of solute carrier proteins were also found to be hypomethylated (54). Hypomethylation at IFN-induced genes was also observed in different cell types and was more pronounced in patients with anti-Ro/SSA and/or anti-La/SSB antibodies as demonstrated in to other EWASs (55, 56). These studies revealed that important genes contributing to the sustainability of the autoimmune response in pSS, are regulated by epigenetic factors.

Mavragani *et al*. investigated the promoter methylation and gene expression of long interspersed nuclear element-1 (LINE-1) in minor salivary gland biopsies from patients with pSS and controls. LINE-1 is a retroviral-like, endogenous DNA sequence, with the ability to transpose within the genome. Transposons have the ability to act in distant regions of the DNA sequence and modify them epigenetically. The authors found hypomethylation of the LINE-1 promoter with concomitant LINE-1 mRNA overexpression in pSS salivary glands, which correlated with the presence of IFN-α assessed by immunohistochemistry and suggested that lymphoid-specific helicase (LSH) and DNA methyltransferase (DNMT)3A should be investigated as candidate upstream mediators of decreased L1 promoter methylation and increased L1 expression (57).

The immune system as well as target tissue cells (plasmatic, β-pancreatic, fibroblast-like synoviocytes, thyroid follicular and epithelial cells of the lachrymal glands, salivary glands, intestine, bronchioles and renal tubules) share the characteristics of secretory cells that is the extended endoplasmic reticulum (ER). Their main function is the production and secretion of several components, including glycoproteins that are involved in antigenic presentation, including the histocompatibility complex (MHC) class I and II molecules. All these proteins are synthesised and modified in the ER. Therefore, disturbances in the normal functions of this organelle such as protein folding, protein quality control, calcium homeostasis and redox balance, promote the accumulation of unfolded or misfolded proteins, a condition known as ER stress (58). ER stress appear to play a significant, but still not well defined, role in the tissue injury of autoimmune diseases. The expression, promoter methylation and localisation of the inositol-requiring enzyme 1α/X box-binding protein 1 (IRE1α/XBP-1) pathway components, which regulate genes involved in biogenesis of the secretory machinery in labial salivary glads (LSGs) of pSS patients were studied by Sepulveda *et al*. A significant decrease of IRE1α, XBP-1u, XBP-1s, total XBP-1 and GRP78 mRNAs was observed in LSGs of SS patients and was correlated with increased methylation levels of their respective promoters, as well as the protein levels for IRE1α, XBP-1s and GRP78 that were decreased. Treatment of cells IFN-γ led to an anticipated decrease of mRNA as well as protein levels of XBP-1s, IRE1α and GRP78. Decreased mRNA levels for IRE1α, XBP-1 and GRP78 can be partially explained by the hypermethylation of their promoters and is consistent with chronic endoplasmic reticulum stress, a finding that may give us more insights into the glandular dysfunction observed in the LSGs of pSS patients (59).

Given the female preponderance in pSS, the X chromosome has attracted the attention of research as a particularly promising candidate for genetic studies. Mougeot *et al*. sought to determine how epigenetic DNA methylation changes could explain an X chromosome dose effect in SS for women with normal 46,XX genotype and investigate the relevant interplay to this dose effect, between X linked genes, genes controlling X chromosome inactivation (XCI) and genes encoding associated transcription factors. They identified 58 upregulated X-chromosome genes and found XIST and its cis regulators RLIM, FTX, and CHIC1, and polycomb repressor genes of the PRC1/2 complexes to be upregulated (60).

miRNAs

Another face of epigenetics in pSS is represented by microRNAs that have been widely reported as differently expressed in saliva and salivary glands in pSS (61-64). As previously reported, miRNA-146a and miR-155 are the most essential regulators in pSS (4). Studies demonstrated that miR-146a expression is increased in PBMCs of pSS patients compared to healthy controls and this overexpression is linked with immune functions such as cellular migration, cytokine production and phagocytosis (65). More recently, Talotta *et al*. demonstrated that higher expression of salivary mi146a may represent a marker of the disease, and salivary miR17, 18a and 146b may be altered in patients with pSS. These findings were associated with abnormal ultrasound and ESSPRI scores as well as anti-La/SSB positivity (66). In another study, Kapsogeorgou *et al*. suggested low miR200b-5p lev-

els in minor salivary glands as a novel predictive and possibly pathogenetic mechanism-related factor for the development of SS-associated NHL, since its expression is impaired years before the lymphoma clinical onset (67). The differential expression of miR-17-92 cluster, which encodes 6 microRNAs (miRNAs) including miR-17, miR-18a, miR-19a, miR-19b, miR-20a and miR-92a, among varying histological stages of labial minor salivary gland (MSG) tissues in patients with pSS was investigated by Yan *et al*. (68). They proposed that increased expression levels of miR-18a and reduced expression levels of miR-92a were associated with advanced clinical stages of pSS. The authors suggested that these molecules may aid the diagnosis of pSS (68). Oral dryness and altered rheology in pSS patients has also been attributed to miRNA-mediated mechanisms focusing on the mucin O-glycosylation pathway. Among 754 miRNA analysed, 126 miRNAs were identified to be significantly de-regulated in pSS compared to controls. This deregulation was inversely correlated with the impairment of salivary flow rates. Among them, the GALNT1 gene responsible for mucin-7 glycosylation was found to be deregulated in pSS compared to controls (69).

Dysregulation of the adaptive immune system

• *Toll-like receptors*

A crucial point in the pathogenesis of SS is the epithelial cell activation by innate immunity signals through toll-like receptors (TLRs), whose activation has been described both in murine models of early disease and SS patients. Recently, the involvement of TLRs have been investigated: TLR2-4 and TLR7-9 have been associated with the induction of inflammation in exocrine glands of SS patients. The endosomal TLRs, especially TLR7 and 9, are the ones involved in recognition of self-nucleic acids, and may be responsible for initiating responses leading to autoimmunity. Upregulated levels of TLR7 and 9 mRNA in PBMC from patients with pSS have been reported, but recent analyses of TLR7 and 9 revealed similar expression levels in B cells between patients with pSS and healthy controls at both mRNA and protein levels. In addition to these studies on PBMC and B cells, TLR2, 3 and 4 mRNA expression in salivary gland epithelial cell (SGEC) lines derived from patients with SS were found significantly higher, compared with control SGEC lines (70, 71). The TLR activities produce cytokines such as type I IFN or induce the activation of the nuclear factor kappa B (NFκB) pathway. Previous studies identified the overexpression of type I IFN-inducible genes in the salivary glands and peripheral blood from pSS patients, suggesting that the type I IFN pathway is a key player in the pathogenesis of pSS (72).

Ha *et al*. (73) reported that a TLR3 ligand significantly increased IL-28A, and especially increased the IL-29 mRNA expression in the NS-SV-DC cells. Thus, TLR3-mediated events may induce IFN-λs and type I IFN expression, in the salivary glands. Moreover, the stimulation with IFN-λs in the salivary gland epithelial cells significantly enhanced TLR3 gene expression, suggesting that TLR3 and IFN-λ pathways reciprocally stimulate their expression.

Shimizu *et al*. confirmed that TLR7 (and its downstream signalling for type I IFNs) are strongly expressed in both ducts and MNCs (especially pDCs) of labial salivary glands from pSS patients. These results indicate that TLR7-dominant innate immunity correlates with the development of sialadenitis in pSS (74). In conclusion, TLRs are expressed and functional in salivary tissue, and TLRs in peripheral blood cells of SS patients are upregulated and hyper-responsive to ligation. However, the ligands that activate TLRs in the context of SS are unknown; also, the role of TLR activation in SS is poorly understood and further studies are required to elucidate the mechanisms that govern TLR regulation and activation, both in exocrine tissue and in the periphery.

A critical component of the innate immune response is represented by the inflammasome. Inflammasomes are large intracellular complexes that induce inflammation in response to exogenous and endogenous damage signals and are linked to TLRs. Several inflammasomes have been described in the scientific literature in relation with SS; the most extensively characterised is NLRP3 (Nod-like receptor family protein 3) inflammasome, the oligomerisation of which is activated by P2X7 receptor (P2X7R) activation. Experimental evidence demonstrated the presence of P2X7R in salivary glands tissue in pSS's patients (75). P2X7R-NLRP3 inflammasome complex activation regulates production and release of inflammatory cytokines IL-1β and IL-18, which are able to cause tissue damage and maintain a state of chronic inflammation in several tissues (75). These evidences suggest an interesting scenario for the initiation and amplification of the innate immune response in pSS. Recently, Vakrakou *et al*. demonstrated a systemic activation of NLRP3 inflammasome in SS. They found increased levels of various NLRP3 inflammasome-related mRNA transcripts in patients' PBMC and described a higher cumulative NLRP3 score in SS patients, especially in those with severe disease manifestations (76).

• *INF signature and cytokine production*

Previous studies have clearly shown that pSS patients display higher expression of type I and type II IFN-regulated genes in both the affected salivary tissue and peripheral blood. One of the most definitive genome-wide association studies in pSS patients, identified single nucleotide polymorphisms in many IFN-inducible genes that are implicated in innate immunity and, in particular, HLA-alleles, STAT4, IRF5, IL-12A and TNIP1 (77). Bodewes *et al*. assessed the relationship between systemic IFN type I and II activity and disease manifestations in pSS. They showed that systemic IFN activation is associated with higher activity only in the ESSDAI biological domain but not in the other domains or the total score. Their data suggested that the ESSDAI biological domain score is a sensitive endpoint for trials targeting either IFN pathways (78). Using flow cytometry, Davies *et al*. analysed MAPK/ERK and JAK/STAT signalling networks in peripheral blood mononuclear cells from pSS patients upon stimulation with IFN-2ab. Type I IFN in-

duced gene expression was found to be negatively correlated with INF-2ab induced phosphorylation of STAT3 S727 in T cells and positively with pSTAT1 Y701 in B cells, thus indicating a direct involvement of both pathways in SS pathogenesis (79).

Congenital heart block (CHB) is related to anti-Ro/SSA and anti-La/SSB antibodies. The role of macrophages for the induction of tissue injury in the affected hearts has been established in previous studies. However, the interplay between type I IFNs that are found to be upregulated in both pSS and SLE, diseases hosting mothers bearing children with CHB, and the activation of macrophages is poorly understood. To this end, Clancy *et al*. studied the IFN-stimulated genes, and the phenotype of macrophages in the affected foetal cardiac tissue. TLR ligation of macrophages with hY3 led to the upregulation of a panel of IFN transcripts, including sialic acid-binding Ig-like lectin 1 (SIGLEC1), a receptor on monocytes/macrophages. Using bioinformatic approaches, it was shown that CD45+CD11c+ and CD45+CD11c– human leukocytes, sorted from the CHB hearts, expressed highly type I IFN response related genes including SIGLEC1. Siglec-1 expression was identified in the septal region of the affected fetal hearts. These findings suggest a link between IFN, IFN-stimulated genes, and the inflammatory and possibly fibrosing components of CHB, making the Siglec-1-positive macrophages as a major effector cell, in the process of tissue injury in CHB (80).

Ushio *et al*. studied the macrophages in the salivary glands in thymectomised NFS/sld mice that present spontaneous Sjögren's-like picture and compared them with those of (non)-thymectomised NFS/sld mice that exhibit no inflammatory lesions in the salivary and lacrimal glands. The study group exhibited a unique population of tissue resident macrophages bearing the marker CD11bhigh and producing CCL22. CCL22 upregulated the migratory activity of $CD4^+$ T cells by increasing CCR4, the receptor of CCL22, and enhancing eventually the production of IFN-γ from T cells. The authors suggested that CCL22 may impair the local immune tolerance in the target organ of this SS model. These findings propose that specific chemokines and their receptors may serve as novel therapeutic or diagnostic targets for pSS (81).

IL-12 is a pro-inflammatory cytokine inducing the production of interferon-γ (IFNγ), thus favouring the differentiation of T helper 1 (Th1) cells. Liaskou *et al*. evaluated the IL-12 signalling pathway in primary biliary cholangiitis (PBC), studying human monocytes from patients with PBC, alongside those with primary pSS. Patients with primary sclerosing cholangitis (PSC) were used as disease controls. T regulatory cells of both PBC and pSS exhibited a lower threshold for IL-12 stimulation, suggesting the importance of this cytokine on Treg differentiation through the STAT4 pathway (82).

Previous studies have clearly shown that SG epithelial cell respond to poly(I:C), IFNα, IFNγ and TNF-α stimulation, producing high levels of BAFF, CXCL9 and CXCL10. These findings, however, cannot address the question if chronic immune activation of the glandular epithelium can initiate the tissue lesion in pSS. To this end, Wang *et al*. demonstrated that the activation of the NF-κB pathway in KRT14+ epithelial cells results in features that resemble the early histopathological phases of pSS. These features include the presence of both T-cell dominated periductal infiltrates organised in foci, and non-occluded lymphoepithelial lesions. Elevated mRNA levels of the pro-inflammatory cytokines IFNα, IL-6, TNF-α, IFNγ and the chemokines CXCL10 and CXCL13 in the glandular tissue were demonstrated in A20-/- mice, a mouse model for SS. Both findings are similar to those described in the tissue injury of early pSS in humans (83).

In another study, Lee *et al*. showed that the JAK inhibitor figotinib regulated the IFNα- and IFNγ-induced pSTAT-1Y701, pSTAT-3Y705, as well as, the protein inhibitor of activated STAT-3 (PIAS-3) in human SGECs. The drug was also studied in murine SGECs and found to induce in a similar manner the IFNγ-induced pSTAT-1Y701, pSTAT-3S727 and PIAS-1. These findings suggest that JAK inhibition may serve as a novel therapeutic approach for pSS, through the down regulation of the STAT pathway (84).

Imgenberg-Kreuz *et al*. analysed the transcriptome of CD19+ B cells from patients with pSS and healthy controls to decipher the B cell-specific contribution to pSS. RNA-sequencing identified 4047 differentially expressed autosomal genes in pSS B cells. Upregulated expression of type I and type II interferon (IFN)-induced genes was observed, establishing an IFN signature in pSS B cells. Among the top upregulated and validated genes were CX3CR1, encoding the fractalkine receptor which is shown to be involved in the regulation of B-cell malignancies, as well as CCL5/RANTES and CCR1 (85).

IFN-inducible protein 16 (IFI16) belongs to a protein family that constitute the type I signature and, therefore, is upregulated after IFNα treatment. The protein serves also as an innate immune sensor that forms filamentous oligomers when activated by double-stranded DNA (dsDNA). Anti-IFI16 autoantibodies have been found in patients with pSS and associate with severe phenotypic features. Antiochos *et al*. showed that IFI16 is present in a filamentous state in the target tissue of SS and demonstrated that this property of DNA-induced filament formation contributes to its status as an autoantigen in SS. This study, suggests that tissue-specific modifications and immune effector pathways might play a role in the selection of autoantibodies in rheumatic diseases (86).

The role of T cells: focusing on Th17

T cells are the main lymphocytes infiltrating the SS exocrine salivary glands and play a key role in the induction and perpetuation of the disease. In 1983 it was first reported that the majority of the infiltrating T cells in SS salivary glands were $CD4^+$ cells polarised toward a T helper (Th) 1 phenotype. It is now known that different T cell subsets are involved in the SS pathogenesis, such as regulatory T (Treg) cells and Th17.

In an interesting work, Fonseca *et al*. have shown that activated PD-1+ICOS+ T follicular helper cells (Tfh) cells in peripheral circulation of SS patients are

strongly associated with disease activity (*i.e.* ESSDAI score) suggesting that these cells may be involved in the SS pathogenesis, particularly in those patients presenting with more severe clinical symptoms. They also found a close correlation between the follicular regulatory T cells (Tfr)/Tfh ratio and ectopic lymphoid structures (ELS) formation in SS salivary glands. Since ELS contribute to the disease process and are frequently associated with a more severe disease course, the authors suggest that the Tfr/Tfh ratio may be considered as a specific marker of SS (87).

Gao *et al*. have studied the implication of tissue-resident memory (Trm) $CD8^+$ T cells in the pathogenesis of SS. Trm are CD69+CD103+/- T cells and reside mainly in the epithelial barrier tissues. Most probably, they migrate to the salivary glands to protect against viral infections through degranulation and production of interferon-γ (IFNγ). The authors analysed Trm in both human tissue and in the p40-/-CD25-/- mice suggesting a pathogenetic role of $CD8^+$ T cells for the development of SS symptoms in salivary glands. In fact, they observed that the percentage of $CD8^+$ T cells with a tissue-resident phenotype was significantly higher than that of $CD4^+$ T cells in the SS labial salivary glands, with the number of Trm $CD8^+$ cells being higher in salivary glands compared to peripheral blood in the same SS patients. Since SS can develop in patients with primary biliary cholangitis, they also investigated whether p40-/-CD25-/- mice with primary biliary cholangitis could develop symptoms of SS. First, they found that p40-/-CD25-/- mice with severe autoimmune cholangitis also have features of human SS. Then they observed that in p40-/-CD25-/- mice, the majority of T cells infiltrating the salivary glands were $CD8^+$ Trm INFγ-producing cells. They demonstrated the primary role of CD8+ Trm cells in SS pathogenesis of this mouse model by showing that following knocking down of CD8a, SS manifestations in salivary glands were improved following by a restoration of saliva secretion (88).

Alunno *et al*. studied the recently discovered angiogenic T cells (Tang) and their role in pSS pathogenesis. By performing FACS analysis, they found a close correlation between increased number of Tang cells in pSS blood and the clinical symptoms (ESSDAI). In particular, they observed that the majority of these Tang cells present a senescent phenotype, as they were CD4-/CD8-/CD28-. By immunofluorescent studies, they demonstrated that Tang cells were localised mostly in periductal and perivascular infiltrates and their presence was correlated with SDF-1/CXCR12 expression in the labial salivary glands of pSS patients (89).

Th17 cells are a lineage of pro-inflammatory $CD4^+$ T cell subsets, which have been associated with SS pathogenesis. Since Th17 and Treg cells are reciprocally regulated by cytokines, Luo *et al*. reported the link between the Th17/Treg cell ratio and IL-2, which is known to promote Treg generation, thus inhibiting Th17 differentiation. They found that an increased number of Th17 cells but not Treg cells correlates with reduced IL-2 and increased IL-6 expression in pSS patients. This finding strongly highlights the role of a Treg-independent, upregulation of Th17 differentiation, most probably linked to an IL2 deficiency in pSS. Moreover, they found that the level of phosphorylated STAT5 (pSTAT5) was decreased while pSTAT3 was enhanced both in the circulation and SS target organs. Interestingly, *in-vitro* treatment with IL-2 leads STAT5 to compete with STAT3 in binding onto IL17a locus, resulting in a decreased Th17 differentiation. This finding indicates an alternative mechanism of immune suppression in SS mediated by IL-2 (90).

Another group investigated the direct effect of IL-2 on the Th17/Treg cell balance in the peripheral circulation of pSS. The group firstly observed a reduced level of Tregs in pSS, as compared to healthy individuals, whereas the level of Th17 was similar in the two groups. Then, stratifying patients based on disease activity, they observed that patients with high disease activity present the lowest Tregs level. Notably, both Tregs and the ratio Th17/Treg were restored following a low dose-IL2 therapy. In the long-term follow-up, patients treated with IL-2 had a significant reduction in glucocorticoid and disease-modifying anti-rheumatic drug (DMARDS) use. The authors suggested that more attention should be paid to immunomodulation rather that immunosuppression for the treatment of pSS (91).

By performing TCRαβ sequencing from single cell sorting, Voigt *et al*. analysed the Th17 and Th1 TCR repertoires in the SS labial salivary gland (LSG) biopsies. They found that in pSS LSG an increased frequency of activated Th17 cells but only a limited repertoire diversity of TCRα/β in activated Th1 cells were observed. Moreover, they showed that the Th1 and Th17 TCR repertoire of LSG pSS patients had a restricted diversity in the CDR3 regions, expressing predominantly TRBV3-1/J1-2(CFLFLSMSACVW), TRBV20-1/J11 (SVGSTAIPP*T), and TRAV8-2/J5 (CVVSDTVLETAGE), the latter found only in pSS patients. The suggested that the limited and clonal expanded repertoires could indicate a restricted antigenic exposure for the effector T cell (92).

Another interesting observation regarding IL-17 was made by Hauk *et al*. The authors showed that the neuropeptide vasoactive intestinal peptide (VIP) improves the abnormal immune status by down regulating IL-17A expression in the exocrine glands and enhancing the secretory function of exocrine glands by up regulating aquaporin 5 (AQP5) expression. Decreased AQP5 expression on the apical membranes of acinar cells causes a water transport disorder, which worsens salivary secretion (93). Li *et al*. investigated the effect and mechanism of VIP on the immune response and exocrine gland function in SS in a non-obese diabetic (NOD) mouse strain and analysed the expression of IL-17A and AQP5. The mice group treated with intraperitoneal VIP was characterised by significantly fewer infiltrating lymphocytes in comparison to the control group, thus suggesting that exogenous VIP may reduce lymphocytic infiltration in the salivary glands. Moreover, IL-17A expression in CD4 T cells was higher in SS mice in comparison to the control group and significantly decreased after VIP therapy. Interestingly,

VIP adminstration was associated with a significant upregulation of AQP5 expression in the submandibular glands of SS mice (94).

Besides TH17, multiple cytokines can affect or mediate effector T cell functions. IL-7 is a pleiotropic cytokine produced by non-haematopoietic cells and increased levels of both IL-7 and IL-7 receptor (IL-7R) have been detected in several autoimmune and inflammatory diseases. In SS patients, increased levels of IL-7 and IL-7Rα-expressing T cells have been demonstrated to directly correlate with the severity of salivary gland inflammatory infiltrate and of xerostomia. The pathogenic effect of IL-7 is mainly mediated by enhanced Th1 and Tc1 response in submandibular glands and by the increased production of IFNγ and TNF-α, two cytokines that are essentially required for the induction and development of SS. Indeed, neutralisation of IFNγ and TNF-α considerably improved salivary secretion, reduced leukocyte infiltration and down-regulated CXCL9 and CXCL13 expression in submandibulary glands, thus reinforcing the essential role of cytokines in disease induction and persistence. Collectively, these results suggest the potential of targeting IL-7/IL-7R pathway and Th1/Tc1 effector cytokines as therapeutic strategies for this chronic autoimmune condition (95). In a recent study, intraperitoneal administration of a blocking antibody against the IL-7 receptor α chain (IL-7Rα) to female NOD mice that exhibited newly onset clinical SS significantly improved hyposalivation and leukocyte infiltration of the submandibular glands (96). Moreover, the signalling cascade of IL-17 requires Act1 (adaptor for IL-17 receptors) to propagate downstream signalling events in tissue cells. By a mass spectrometry analysis, Zhang *et al*. demonstrated that Act1 was able to directly interact with and suppress STAT3 activation in Th17 cells. They observed that a deficiency of Act1 resulted in hyper IL-23-induced STAT3 activation in naive $CD4^+$ T cells and increased IL-21 expression in B cells. IL-23R deletion or blockade of IL-21ameliorated SS-like diseases in Act1-/-mice. These results indicate that Act1 is a critical checkpoint in immune homeostasis via negative regulation on STAT3 activation during IL-23-dependent Th17 response and IL-21-driven B cell function. Thus, IL-21 blocking antibody might be an effective therapy for treating SS-like syndrome in patients with Act1-deficiency (97).

The role of B cells

Numerous studies support the involvement of B cells in the pathogenesis of SS. The presence of pathogenic autoantibodies before the onset of the disease highlights the key early role of B cells for the development of SS. In addition, B cells are found within the target organ of pSS, the salivary glands, and can occasionally form ectopic germinal centres which are often associated with more severe symptoms. Finally, SS is associated with high incidence of B cell lymphomas.

Miles *et al*. focused their attention on a population of innate-like B cells (B1a) which inhibit the response to self as they maintain the tolerance to apoptotic cells (ACs). B1a B cells appear to play a role in the SS pathogenesis since it is known that the disease develop once the tolerance to ACs is broken. The authors demonstrated that 80% of splenic and 96% of peritoneal B cells are B1a B cells that respond to ACs and secrete IL-10. Interestingly, B1a B cells isolated following an *in-vivo* inflammatory immune response are still able to induce antigen-specific T cells to secrete IL-10, promoting Treg cell responses to self-antigens and therefore preventing an inflammatory autoimmune response. On the other hand, by producing self-reactive natural antibodies (Nabs), the B1a B cells induce a rapid clearance of apoptotic fragments. It was also demonstrated that either TLR9 or TLR7 stimulation induce a higher release of IL10 and Nabs by B1a B cells, accelerating the clearance of dying cells by macrophages and therefore inhibiting the macrophage proinflammatory responses (98).

Takei *et al*. investigated the possible alteration of peripheral B cell subclasses and their association with clinical and immunological characteristic in pSS. They employed FACS analysis and firstly observed that an increased number of $CD19^+$ B cells correlates with higher IgG serum level and hypergammaglobulinaemia. Moreover, an altered proportion of peripheral B cell subsets was observed in pSS patients. In fact, the proportion of CD38highIgD$^+$ and CD38highIgD- B cells was significantly increased in pSS patients, while that of $CD27^+$IgD- B cells was reduced. Interestingly, the proportion of CD38high-IgD$^+$ B cells positively correlated with specific parameters such as the ESSDAI score, IgG serum level and pathogenic autoantibodies. This data suggest that CD38highIgD$^+$ B cells play an important role in the pathogenesis of pSS by regulating B cell activation (99).

A study by Koelsch *et al*. focused on the possible link between posttranslational modification in the IgG V region and the autoreactive B cell selection and proliferation in patients with SS. By sequencing the V-region of antibody-secreting single cells from minor salivary glands, they identified an increased frequency of N-glycosylated sites acquired by SHM (acN-glyc) in SS compared to NHS. The presence of these sequences were associated with an increased number of replacement mutations and lowered selection pressure. Since Ig N-glycans can bound bacterial lectins triggering a nonspecific B cell activation, the authors suggested that this finding indicate a possible mechanism for a break of tolerance that leads to Ig autoreactivity and potentially an increased risk of lymphoma in patients with SS. They also demonstrated that the removal of the acN-glyc motive from a self-reactive antibody could nearly abolish the binding to autoantigens, highlighting the role of N-glycosylated sites for the selection and proliferation of autoreactive B cells in SS (100).

SS patients have a 15–20 fold higher risk to develop non-Hodgkin's B cell lymphoma. The onset of lymphoma in SS has been linked with an increased proportion of circulating CD21–/low B cells, suggesting that these B cells may represent the initial reservoir for transformed clones. Glauzy *et al*. analysed the reactivity of antibodies produced by CD21-/low B cells isolated from SS blood by PCR-based techniques. They

observed the highest frequency of expanded clones in CD21-/low B cells from the blood of SS patients, suggesting that these monoclonal expansions may originate from this B cell subset. By sequencing SS monoclonal expansions, analysing the mutations and by performing a lineage tree analysis for the heavy chains, the authors concluded that the BCR underwent a strong antigenic expansion. Then, by testing the reactivity of these antibodies, they found that they were autoreactive and one in particular was reacting against a ribosomal self-antigen. Finally, they investigated whether the autoreactive recombinant antibodies originated from unmutated self-reactive clones that underwent SHM by reverting mutated antibody heavy- and light-chain genes to their original unmutated sequences, and observed that one of these BCR sequences remained autoreactive. Based on these results, the authors hypothesised that the autoreactive antibodies may originate from clones activated by self-antigens that induce their proliferation and acquisition of SHM, thus enhancing BCR affinity for self (101).

New insights into therapy

It is still quite controversial how to better treat SS patients, since there are no unique and effective treatments available at the moment (102). Understanding the pathogenetic mechanisms of the disease will help to identify novel molecules that may play a pivotal role in future therapies for SS. Recently, a number of biologics have been developed and are under investigation in pSS. Among them, despite failing to demonstrate efficacy in randomised clinical trials, to date, rituximab has been the most frequently used. Indeed the therapeutic objectives in pSS range from controls of sicca symptoms, pain and fatigue to the control of systemic complications and tissue damage. So far, however, treatment of patients' subjective symptoms has mostly relied on symptomatic agents or saliva stimulating agents (103, 104). Regarding pilocarpine, this drug still represents an adjunctive treatment for SS dry mouth leading to relevant subjective improvement of xerostomia. The efficacy of continuous administration of pilocarpine in salivation as attested by the Saxon test and symptom improvement was recently showed (105). The authors explored also molecular biological mechanisms of action of pilocarpine administration in a mouse model of SS. They demonstrated a significant increase of gene and protein expression of muscarinic acetylcholine receptor 3 (M3R) in mouse salivary glands following continuous the administration of pilocarpine in comparison to a single administration while the expression of AquaPorin5 (AQP5) and other salivary gland markers did not change. In patients, salivation and the relief of symptoms were maintained during pilocarpine administration even at lower doses than recommended (105).

Recently, interventional sialendoscopy has been described as an alternative tool to control the number of episodes of sialadenitis in SS patients. The procedure is minimally invasive and allows the endoscopic visualisation of the salivary duct system and the treatment of inflammation and obstruction. A pilot study compared the effectiveness of interventional sialendoscopy with a cycle of intraductal steroid irrigations to that of sialendoscopic steroid duct irrigation alone in patients with SS (106). Objective clinical improvement with post-operative reduction in the mean number of episodes of glandular swelling and subjective improvement of oral dryness were depicted in more than 70% of patients in both groups. However, at multivariate analysis, sialendoscopy combined with intraductal steroid irrigations was associated with higher reduction of the pain component of ESSPRI in comparison to interventional sialendoscopy alone (106).

As far as antimalarials are concerned, efficacy and tolerability of this therapy and its immunomodulatory properties have been recognised, although the exact mechanism by which antimalarials might influence disease-related damage in autoimmune disease is still unknown (107). In a recent study analysing a wide Latino-American SS cohort, antimalarial use ranged from 44% to 80% and disease-related damage accrual was demonstrated in 45% of patients, mostly due to the ocular domain, parotid swelling and malignancy (108). Comparing patients with and without use of antimalarials, it was demonstrated that the prevalence of comorbidities were similar, while patients who used antimalarials had a longer disease duration, used more frequently glucocorticoids (GCs) and immunosupressors (IS) and had a lower Sjögren's Syndrome Disease Damage Index (SSDDI) score. In particular, comparing patients with a SSDDI ≥3 versus SSDDI<3, use of antimalarial therapy resulted protective for damage accrual in the pulmonary domain (108). Immunosuppressors are commonly used to treat systemic manifestations of SS although their long-term safety and tolerance are still uncertain. A retrospective cohort study assessed causes of withdrawal of IS and GC in more than on -hundred SS patients routinely followed (107). More than 65% of pSS patients received GC and/or IS and these patients were characterised by more frequent parotid enlargement, lymphadenopathy, arthritis, autoimmune cytopenias, autoimmune hepatitis and higher median cumulative ESSDAI score. There were no differences in age, disease duration and damage accrual among patients with and without use of CG and/or IS either no difference in comorbidity prevalence (hypertension, diabetes mellitus and dyslipidaemia) or in serological features. However, no erosive arthritis and higher cumulative ESSDAI score were identified to be significantly associated with IS use at multivariate analysis (107). The main reasons for IS withdrawn were disease improvement and patient's own decision. Prevalence of toxicity was moderate (18%). The most used drug was antimalarials and the main cause of its withdrawal was patient's own decision due to adverse events whereas discontinuation due to lack of efficacy and to non-compliance were documented later during the follow-up. In addition, toxicity in 18% of the patient and adverse events were mostly related to non-ophthalmologic reasons, mainly due to skin rash and gastrointestinal symptoms (107).

Given the current state of the art in the use of conventional DMARDs, there

certainly is a strong need for novel therapeutic approaches and new clinical study designs in pSS. It is likely that new insights into disease pathogenesis may open new avenues in the disease therapy.

Conclusions

Tremendous efforts have been made in pSS clinical and basic research paving novel pathways for individualised treatments. New insights into pathogenesis and a better patients stratification will hopefully allow us to move from phenotypic to endotypic stratification, ultimately leading to ameliorate patient assessment and management.

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