Review

One year in review 2016: Sjögren's syndrome

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ABSTRACT

Sjögren's syndrome (SS) is a complex heterogeneous disease charactered by a broad spectrum of clinical and serological manifestations, including non-Hodgkin's lymphoma (NHL).

Last year, 2015, was an exciting year for research into SS with novel insights into disease pathogenesis, clinical aspects and long-term outcomes. In addition, the use of biologic therapy in SS is rapidly expanding, with new evidence emerging regarding potential therapeutic targets. In this article, we will provide an overview of the recent literature on the pathogenesis, clinical features and novel treatments of SS.

Introduction

Sjögren's syndrome (SS) is a complex heterogeneous disease characterised by a broad spectrum of clinical and serological manifestations, including non-Hodgkin's lymphoma (NHL).

2015 was an exciting year for research into SS. New classification criteria for SS are moving toward acceptance by both ACR and EULAR, the European League Against Rheumatism. Novel insights into disease pathogenesis are coming out, especially on lymphomagenesis. Preliminary results from the "Big Data Sjögren Project" have been presented at several international meetings taking a "high-definition" picture of primary SS at diagnosis by merging international SS databases. In this scenario, potential therapies targeting pathways or molecules recognised as being involved in the pathogenesis of SS have been introduced in the treatment of SS and preliminary results have been published during the year.

In this article, following the previous paper of this "one year in review" collection (1), we will provide an overview of the recent literature on the pathogenesis, clinical features and novel treatments of SS.

New insights into the epidemiology of SS

The geoepidemiological distribution of systemic autoimmune diseases is very variable in global population, in terms of prevalence of specific diseases, M/F ratio, organ involvement and disease severity. An international study group, led by Ramos Casals, using the largest web search engine (Google), has collected and analysed all the items related to "systemic autoimmune disease"; among 394.827 selected, confirming that SS represents one of the most common systemic autoimmune diseases, with the wider M/ F ratio (1:10) (2).

Incidence and prevalence rates of primary Sjögren's syndrome (pSS) vary widely according to study design and geographical area. A meta-analysis of studies published between 1993 and 2013 evaluated SS incidence rate (IR) and prevalence rate (PR). The selected six studies evaluating IR reported heterogeneous data and provided a pooled estimate of 6.92 cases per 100,000 person-years with an IR ratio between females and males of 9.29. A geographic heterogeneity was observed among studies, with a higher IR in those performed in Asia (6.57 per 100,000 person years) compared to those performed in Europe (IR between 3.9 and 5.3 per 100,000 person-years) and US (3.9 per 100,000 person-years). On the other hand, PR data were based on the meta-analysis of eighteen studies. The pooled PR was 60.82 cases per 100,000 inhabitants, though with great heterogeneity due to study design (pooled PR of 43.03 considering only population-based studies) and geographical area (71.22 in studies performed in Europe, 44.85 in those performed in Asia and 170 in the only study performed in South America). The PR female/male ratio was 10.72, which appeared to be higher than IR ratio. This could be due to different methods used to evalu-

ate IR and PR in the analysed studies. Analysis performed after stratification of the studies confirmed the great influence of classification criteria on results. This meta-analysis also confirmed the peak incidence of pSS in women aged between 55 and 65 years (3).

Defining the real PR of SS is also very important to establish whether the disease is to be considered rare or not. In this setting, an analysis of 3 studies performed in Europe evaluating large population-based studies of pSS diagnosed according to American-European Consensus Group (AECG) classification criteria showed a pooled PR of 38.95 per 100,000 inhabitants, which would confirm that pSS can actually be considered a rare disease in Europe (4).

An interesting study performed in Taiwan by Kuo *et al*. evaluated the risk of developing pSS in subjects with familiarity for autoimmune diseases. Interestingly, first-degree relatives of patients with pSS had a risk ratio (RR) of 12.37 of developing the disease as compared to general population. RR of pSS was 666.75 in twins, 18.99 in siblings, 11.31 in offspring and 12.46 in parents of patients affected. The risk increased in subjects with more than one relative affected pSS and was also higher for developing more other diseases than pSS (2.95 for rheumatoid arthritis, 6.25 for systemic lupus erythematosus (SLE), 2.39 for systemic sclerosis and 3.38 for multiple sclerosis). These data could be explained both by a genetic susceptibility of family clusters and a common exposure to environmental agents which can contribute to the development of autoimmune diseases (5).

A prospective study was performed by Kvarnström *et al*. at Karolinska University Hospital in Stockholm during a five-year period and showed an overall IR of 3.1 per 100,000 person-years with a female/male ratio of 14/1. The authors also evaluated the frequency of extraglandular manifestations of the disease, confirming arthralgias, fatigue, myalgia and Raynaud's phenomenon as the most frequent symptoms, while haematologic, neurological, renal and pulmonary involvement appeared to be less frequent (6).

New insights into the pathogenesis of SS

Both genetic and non-genetic factors are involved in disease susceptibility and initiation of disease process, leading to a dysregulation of the epithelial cells and to an autoimmune response, and appear to have a role even in disease progression. In this paragraph we will provide a bird's eye review of the most significant contribution published in the recent literature on SS pathogenesis.

A number of different regions of the genome, both within and outside of the major histocompatibility complex (HLA), have been implicated SS. As for most autoimmune diseases, some HLA class II loci have been demonstrated to be associated with pSS. During the last months, Huang *et al*. (7) showed a strong association between HLA- DRB1*0803 and Chinese population. Furthermore, they sequenced the 2nd exon of the HLA- DRB1 locus and showed that amino acid variations within the binding pocket P7 and P9 were associated with the susceptibility to the disease. Beyond genetics, epigenetic alterations, in particular those related to DNA methylation, have been shown to play key roles in the pathogenesis of SS. Miceli-Richard *et al*. (8) performed a genome-wide DNA methylation study in blood CD4$^+$ T cells and in blood CD19$^+$ T-cells of patients with SS and demonstrated that alteration of methylation was mainly present in B cells as compared to T cells. Moreover, dysregulation of methylation involved some specific pathways that play a key role in the pathogenesis of the disease, including Interferon regulated genes, mainly in seropositive patients.

Another interesting contribution, during the last few months came from Markeljevic *et al*. (9) who firstly reported the association between low platelet serotonin level in patients with SS and polymorphism in the serotonin transporter gene (5-HTT), demonstrating that variants of the 5-HTT gene (in particular the presence of HTTVNRTin2 SS genotype) significantly contribute to decreased platelet serotonin levels in these patients.

Regarding the novel insights into the innate immune related events that are involved in the pathogenesis of SS, growing evidence over the last decades points towards a significant role of the Interferon (IFN) system in determining the disease process. Hall *et al*. (10) investigated the different patterns of IFN activity in labial salivary glands from a large cohort of pSS patients. Upregulated IFN activity was associated with a more severe disease phenotype characterised by salivary dysfunction, ocular dryness, higher focus score, leukopenia, SSA antibodies, high antinuclear antibodies (ANA) titer and hypergammaglobulinaemia. Of interest, both the focus score and hypergammaglobulinaemia were the most significant predictors of high IFN activity. Furthermore, they stratified patients with high IFN activity into the 3 predominant IFN pathway groups: type I-predominant, type II-predominant and mixed type I-II IFN activity. They found no different clinical features between the different IFN patterns except for the focus score, which was highest in type-II predominant patients. Such findings reflect the need to define the activity of inflammatory pathways in disease-relevant target tissues in order to stratify pSS patients and select IFN-targeting therapies according to the specific pathway.

Concerning type I and II IFN signatures in pSS pathogenesis, Nezos *et al*. (11) evaluated their role in the induction of SS clinical phenotypes, including lymphoma development. Over-expression of both type I and type II interferon inducible genes was observed in peripheral blood and minor salivary gland tissues of pSS patients, with a predominance of type I IFN signature in peripheral blood and a type II IFN signature in minor salivary gland tissues. Additionally, in minor salivary glands tissues of pSS with lymphoma, a lower IFNα but higher IFNγ and type II IFN inducible gene transcripts compared to pSS patients without lymphoma and healthy controls were observed, suggesting a role of IFNγ/IFNα ratio in salivary gland biopsies as a novel histopathological biomarker for the prediction of in situ lymphoma development. Concerning the role of the so-called IFN signature in the induction and perpetuation of pSS, Alunno *et al*. (12) examined the

role of the interferon -inducible protein 16 (IFI16) in pSS pathogenesis. Higher serum levels of IFI16 were depicted in pSS patients as compared to healthy donors and, of interest, IFI16 serum concentration was directly correlated with disease duration and focus score and inversely with age at diagnosis. Moreover, IFI16 positivity was associated with positivity for rheumatoid factor. Furthermore, SS minor salivary glands displayed marked expression and cytoplasmic mislocalisation of IFI16 by acinar and ductal epithelial cells as well as infiltrating lymphocytes and peri/intralesional endothelium compared to normal minor salivary glands or non-specific chronic sialadenitis.

Another important point is the role of cytokine networks alterations in the pathogenesis of pSS. Recent studies have shown that a local and systemic over-expression of interleukin (IL)-18 and IL-22 in pSS patients. In particular, Ciccia *et al*. (13) examined the dysregulation of IL-22 signaling, modulated by IL-18, in pSS patients and in pSS-associated lymphoma. According to their data, aberrant expression of IL-22 receptor, induced by IL-18, was observed among tissue and circulating myeloid cells of pSS patients and macrophages of non-Hodgkin lymphomas tissues of pSS patients. Furthermore, they demonstrated an IL-18 dependent aberrant expression of IL-22R on cells of haematopoietic origin that seems to be a specific immunological signature of patients with pSS and pSS-associated lymphoma. Concerning the role of IL-1 cytokine family in the pathogenesis of pSS, recent studies have clarified the importance of IL-33, a molecule constitutively expressed in the nucleus of epithelial and endothelial cells but also induced in inflamed tissues, which acts as a nuclear factor and as an extracellular cytokine through the IL-33/soluble transporter (ST) 2 axis. Jung *et al*. (14) evaluated the expression of IL-33 in sera and salivary tissues of pSS patients and discovered an elevated expression of the cytokine and its receptor in both samples. Furthermore, they reported that the production of IL-33 mRNA by salivary epithelial cell could be promoted by IFN-γ stimulation, suggesting a potential role in the pathogenesis of pSS. Concerning the IL-1 family of cytokines, IL-36 cytokines (including Il-36α, IL-36β, IL-36γ and IL-6 Ra) are novel members that seem to be crucial in the control of IL-23/IL-17/IL-22 axis that has been demonstrated to be one of the key mediators of pSS pathogenesis. Ciccia *et al*. (15) demonstrated an increased expression of IL-36α in serum and salivary glands of patients with pSS and a correlation with disease activity, suggesting a potential role in monitoring the activation of the immune system in these patients.

A third aspect that needs to be considered in the pathogenesis of pSS is the role of salivary gland epithelium in the initiation and perpetuation of local autoimmune responses. Barrera *et al*. (16) demonstrated that in pSS the ectopic localisation of salivary mucins in the extracellular matrix of salivary glands, due to a loss of apical-basolateral acinar-cell polarity, could be involved in the expression of proinflammatory cytokines. Oligosaccharides present in mucins, such as sulpho-Lewis residues, seemed to act as damage-associated molecular patterns (DAMPs) that are recognised by TLR4 in epithelial cells, inducing a significant increase in CXCL8, TNF-α, IFN-α, IFN-β, IL-6, IL-1β and initiating a pro-inflammatory response that could attract inflammatory cells to amplify and perpetuate inflammation and thereby contribute to the development of a chronic state characteristic of pSS.

Moving to the role of acquired immunity in the devolopment of pSS, Yaciuk *et al*. (17) have demonstrated that RNA-binding auto-antigens like La/SSB have a key role in positive selection of T regulatory (Treg) cells during their thymic maturation in mice. The presentation of La/SSB by dendritic cells (DC)s leads to positive selection of specific Treg cells. To confirm this observation, the authors removed T-Cell Receptor (TCR) rearrangements or TCR loci in other murine models, preventing positive selection of Tregs. These mice consequently developed serologic and clinical signs of autoimmunity, mainly in lungs. It is important to underline that positive Treg selection was dependent on the amount of peptide presented by DCs, with positive selection occurring only for lower peptide-presenting DCs.

Immunologic selection process is normally driven by antigen-presentation during T and B cell development. Different mechanisms of B cell selection might be involved in pSS, one of which could be represented by an increased N-glycosylation process of the Ig H chain V region which can drive B cell selection in an unconventional way. (18)

Sudzius *et al*. performed a large analysis on the distribution of different lymphocyte populations in peripheral blood (PB) of pSS patients and depicted a reduced number of $CD3^+$ cells, consisting of both $CD8^+$ and $CD4^+$. A different distribution of various cell populations between seropositive and seronegative patients was also evident, as well as an association between cellularity and specific parameters such as SG biopsy focus score, EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI), EULAR Sjögren's Syndrome Patient Reported Index (ESSPRI) and Schirmer's test. It is important to underline that a reduced number of Th17 cells was evident in pSS patients, as result of a tissue redistribution of such cells. Also B-cell Activating Factor (BAFF) levels negatively correlated with the number of T and B cells in PB of seronegative patients, suggesting a negative feedback control of BAFF secretion by monocytes (19). Despite the crucial role of BAFF expression for maturation and activation of B cells, Seror *et al*. found that pSS patients responding to Belimumab, an anti-BAFF monoclonal antibody, had lower baseline levels of natural killer (NK) cells both in PB and in SGs. Furthermore, non-responder patients showed an increase in NK cells after treatment, thus suggesting different types of pSS subsets with distinct underlying physiopathologic mechanisms (20). Sjöstrand *et al*. were able to identify highly conserved sequences in the promoter of BAFF gene which are bound by IFN Regulatory Factors (IRFs). More specifically, they identified IRF1 and IRF2 as potent activators of BAFF expression, and IRF4 and

IRF8 as repressors. This could explain, at least partially, the increased levels of BAFF in patients with SLE and pSS (21). Finally, the activation of autoreactive B cells specific for SSA-antigen in salivary glands of pSS patients might be also fostered by an impaired migration of invariant NKT (iNKT) cells to inflamed sites (22).

Additional data on immune system cells distribution in patients with pSS can be acquired from the work by Szabò *et al*. who focused their analisys on T follicular helper cells (Tfh) and B cells. Naïve B cells seem to be increased in PB of pSS as a possible consequence of impaired B cell tolerance checkpoints. Memory B cells levels were instead reduced compared to healthy controls (HC). This could be explained by an increased amount of memory B cells switching into plasma cells. As more data are published about Tfh cells, their prominent role in pSS is becoming progressively more evident. They are essential for B cell maturation and Ig production. Tfh cells appear to be increased in seropositive patients and in subjects with extraglandular manifestations and their concentration correlated with serum levels of IgG and rheumatoid factor (RF). These data seem to confirm the tight co-operation between Tfh cells and B cells in SS (23, 24). An increased number of naïve B cells in pSS patients was also shown by Karlsen. Furthermore, the authors analysed the expression of Toll-like receptor (TLR)-7 and -9 in B cells from pSS patients and failed to demonstrate increased levels of expression, though TLR-7 an -9 activity could be actually increased in pSS patients without an up-regulation of the receptors. These data are in contrast with previously published studies (25).

Liu *et al*. showed that Tfh cells are relatively more abundant in PB of pSS patients and that their frequency correlates with ESSDAI and with serum level of anti-La/SSB antibodies. The number of these cells is effectively reduced by co-culture of peripheral blood mononuclear cells (PBMCs) from pSS with human umbilical cord mesenchymal stem cells (hUCMSs), probably through an up-regulation of indoleamine dioxygenase (IDO) and IL-10 (26). Th17 cells represent a T cell subtype recently attracting more interest and their role in the development of pSS and other inflammatory diseases is well established (27). Lin *et al*. showed that IL-17 KO mice did not develop experimental SS after immunisation with SG proteins compared to Wild Type mice, while Th17 cells transfer was able to induce sialoadenitis and reduced salivary flow. These data confirm the central role of Th17 cells as initiators of the inflammatory process in SS (28).

Furthermore, Pertovaara *et al*. have investigated the activation of various Signal Transducer and Activator of Transcription (STAT) proteins in PBMC of patients with pSS and compared these data with HC. STAT proteins have a role in signal transduction induced by several stimuli, including inflammatory cytokines. It was shown that STAT-5 is constitutively activated (*i.e*. phosphorylated) in PB T cells, B cells and monocytes of patients with pSS and its levels correlated with serum IgG and anti-La/SSB antibodies, but also - in $CD4^+$ T cells - with IFNγ, IL-4 and tumour necrosis factor (TNF)-α levels. A correlation was demonstrated between STAT-5 activation and the entity of the inflammatory response but not with sicca symptoms (29).

New insights into the clinical features of SS

Primary SS is generally considered a benign disease, mainly characterised by glandular involvement (30); however, in the last year a greater interest has arisen in mortality, morbidity, cardiovascular risk and hospitalisation especially in subgroups of patients with very active disease and/or systemic involvement. In addition, the heterogeneity of the disease has been emphasised, highlighting the crucial need of the early identification of both the glandular and extraglandular manifestations of the disease.

From this perspective, in order to better characterise the heterogeneity and severity of SS clinical manifestations in different patient subgroups and to facilitate the early recognition of the disease systemic manifestations the international study group, the SS-EULAR task force, has recently published a systematic review of the literature exploring prevalence and presentation of joints, skin, lung and kidney involvement in SS (31). Moreover, Colafrancesco *et al*. characterised muscle involvement in course of SS. Analysing in a multicentre study a cohort of 1320 patients, it was found that 1.3% of the population studied showed muscle weakness, in associations, in minor part with myalgia, alterations of myonecrosis (CPK) serum level and in electromyography. Interesting histopathological correlation performed on 13/17 symptomatic patients: 5/13 had a biopsy compatible with myositis (PM-like) and 1/13 compatible with inclusion body miositis-like (IBM). So it seems that in rare cases of myositis associated with SS, with CPK and EMG abnormalities, muscle biopsy and histology examination were mandatory, in order not to overlook a real PM- overlap syndrome, misinterpreted as extraglandular involvement of SS (32).

In addition, Quartuccio *et al*. (33) described two forms of cutaneous vasculitis in SS: cryoglobulinaemic purpura (CV) and hypergammaglobulinaemica purpura (HGV), analysing possible correlations with clinical and serological specific subset of SS patients. Over 652 patients, cutaneous vasculitis group with HGV (40/652) was characterised by a higher incidence of RF seropositivity, leukopenia, MGUS and anti-Ro/SSA; the group with CV instead was characterised by MGUS, hypocomplementaemia C4, SSB and peripheral neuropathy. Lymphoproliferative risk between the two subgroups and the SS general population was higher only in patients with CV, confirming that the presence of cryoglobulins has to be considered a negative prognostic factor, in particular as regards the lymphoproliferative risk, unlike the HGV.

Finally, a recent study conducted by Atisha-Fregoso *et al*. (34) analysed the main causes of hospitalisation of 170 patients with SS recruited from 2000 to 2013 recognising as leading causes of hospitalisation disease activity and severity and infections; subset of patients with higher risk for hospitalisation were

those with extraglandular involvement and severe organ damage.

Regarding cardiovascular risk in SS, interesting, and partly unexpected, results emerged from the study of the Italian study group on SS by Bartoloni Bocci *et al*. (35); the authors analysed 1,343 patients showing a higher significant incidence of cerebrovascular (ischaemic stroke) and cardiovascular events (heart attacks) comparing with healthy controls, only in part related to a higher prevalence of hypertension and hypercholesterolaemia.

These data were confirmed in a meta-analysis published Singh *et al*. (36) including approximately 7888 patients with SS, from 10 different studies. The mortality rate resulted higher in SS than in healthy controls; the authors attributed this data to three main causes: cardiovascular events, infections and malignant lymphoproliferative disorders. Interesting statistically significant correlation resulted between the increased risk of mortality and already known disease activity indices and lymphoproliferative evolution such as glandular swelling, extraglandular involvement, hypocomplementaemia and cryoglobulinaemia. Risk for venous tromboembolism in SS was also highlighted (37).

In addition to disease severity, many studies conducted in 2015 have sought to evaluate the impact of the disease on quality of life with particular regard on pain, mood and sexual functions. More specifically, three studies by van Leeuwen *et al*. (38), Karageorgas *et al*. (39) and Choi *et al*. (40) focused on fatigue and pain in patients with SS. In the first paper the authors investigated the role of the psychological profile of 300 patients with SS in the development of symptoms related to asthenia, often disproportionate to the damage and the activity of the disease. Administering different questionnaires designed to study specific profiles, the authors identified four psychopathological clusters of patients: functional, dysfunctional, alexithymia and self-sufficient; the results showed a higher incidence of fatigue in alexithymia and dysfunctional clusters and less in functional and self-sufficient group, indicating how a proper educational and behavioural approach can be useful in this subset of patients. The second study confirmed in 106 patients the association between fatigue and a higher incidence of anxiety disorders, depression and sleep as well as non-specific fatigue and painful symptoms (such as malaise, myalgias, arthralgias). In the third study, similar results were obtained by Choi *et al*. (40) who analysed the association between fibromyalgia (FM) and activity/damage in SS in a cohort of 100 patients with SS, showing that FM was associated with higher ESSPRI and more severe depression.

New insights into lymphomagenesis

It is well known, that patients with pSS, present a relative risk for non-Hodgkin's lymphoma (NHL) and MALT- NHL (salivary glands and gastrointestinal tract), estimated at 10 to 15 times when compared to the general healthy population.

The goal of the most recent studies was to characterise genetic, serologic, clinical and histological subsets of patients with pSS at increased risk of lymphoproliferative evolution; many of the so called adverse prognostic factors have now been determined, including recurrent glandular swellings, cryoglobulinaemia, hypocomplementaemia, lymphopenia, positivity for SSA/SSB and rheumatoid factor antibody. This was confirmed in a multicentre Italian study by Quartuccio *et al*. including 548 patients with pSS fulfilling the criteria AECG. The authors identified two subsets of patients: 342 patients positive for both minor salivary gland histopathology and for anti-Ro/SSA and/or anti-La/SSB and 206 patients positive for histopathology but negative for autoantibodies. They analysing also clinical, serological and laboratory features focusing, in particular, on risk factors for lymphoma and showed that the negative status for anti-Ro/SSA and/or anti-La/SSB represented a protective factor for evolution toward lymphoma in patients with pSS (41).

In line with the former results, Nocturne *et al*. demonstrated in 101 SS patients with NHL demonstrated that rheumatoid factor represented an independent risk factor for lymphoma development and emphasised the role of disease activity (ESSDAI) as an unfavourable prognostic factor of lymphoproliferative evolution in SS patients (42).

In addition, Papageorgiou *et al*. (43), in a cohort of 77 SS patients with NHL, analysed the impact of SS disease activity (ESSDAI) on patients' overall survival (OS) and event-free survival (EFS), defining as "event" relapse of haematologic disease, treatment failure, progression of disease, histologic transformation or death. The results showed that patients with a higher ESSDAI score at lymphoma diagnosis had a greater risk for death (OR = 5.241, 95% CI: 1,034 to 26,568) or for event (OR = 4.317, 95% CI: 1146–9699, p=0.008). In addition, An unfavourable International prognostic index (IPI) score (high-intermediate/high) was associated with high risk of death and event (OR = 13.867, 95% CI: 2.656–72.387 and OR = 12.589, 95% CI: 3.911–40.526, respectively), worse EFS (log-rank p<0.001, HR = 8.718, 95% CI: 3.477–21.858), as well as with worse OS (log-rank p<0.001, HR = 11.414, 95% CI: 2.414–53.974).

Regarding NHL pathogenesis, a special interest has arisen for BAFF production, other cytokines and chemokines including CCL11 and CXCL13, and for genetic abnormalities leading to an aberrant over-activation of the NF-kB pathway and ultimately, to a B-cell abnormal proliferation.

Papageorgiou A *et al*. (44, 45) evaluated the prevalence of the mutation His159Tyr of the BAFF-R gene in patients with pSS compared to those with other autoimmune diseases and healthy controls, concluding that His159Tyr mutation of BAFF-R (receptor) was increased in pSS patients with lymphoma onset, occurring at younger age, between 31 and 40 years. The authors linked the BAFF-R mutation to an increased downstream signaling through activation of NF-kB pathway and found that the expression of the p52 protein, the active form of the NF-KB2 protein, was significantly increased in pSS-lymphoma-BAFF-R His159Tyr-derived B cells compared to both SS-lymphoma-BAFF-RWT- and healthy controls.

In fact, a wide variety of genetic al-

terations that affect the activity and/or expression of NF-kB signalling have been described in patients with NHLs. Nocturne *et al*. (46), in this regard, have assessed the association between a functional variant of TNFAIP3, the rs2230926 at the germline level and pSS-associated lymphoma in an independent cohort of European patients with pSS from the UK and performed a meta-analysis with the French cohort. The TNFAIP3 gene encodes the A20 protein, a central gatekeeper of NF-kB activation. Among two large cohorts of European subjects, the authors confirmed that the germline missense variant rs2230926 resulted in impaired control of the NF-kB activation pathway and was associated to pSS-NHL.

Among the other cytokines and chemokines implicated in lymphomagenesis, a growing attention is paid to CXCL13 and CCL11 serum levels. These chemokines were evaluated in 385 patients of the ASSESS cohort of whom 22 pSS patients with NHL by Newcastle study group (47). Patients with lymphoma presented higher levels of CXCL13 when compared to patients without lymphoma and a trend for an association with CCL11. In addition, CCL11 and CXCL13 positively correlated with three B-cell biomarkers: RF, kappa/lambda free light chain and beta-2 microglobulin and with disease activity. This study highlights the continuum between chronic B-cell activation, disease activity and lymphomagenesis.

New insights into the diagnosis of SS

SS is an heterogeneous disease, characterised by a broad spectrum of clinical and serological manifestations. Therefore, due to its complexity the diagnosis of SS remains challenging. Lately, at the American College of Rheumatology (ACR) 2015 annual conference, Caroline Shiboski presented the new ACR classification criteria for SS, as resulting from an International Clinical Alliance supported by US National Institutes of Health (NIH). These criteria are now very close to acceptance by both ACR and European League Against Rheumatism (EULAR), possibly replacing the provisional ACR 2012 criteria and the American-European Consensus Group (AEGC) criteria used in past decades and are meant to be able to identify homogeneous SS patients for clinical trials. In fact, great concordance was noticed between the new and the AECG criteria. The new classification criteria, however, weighted each single item giving three points to minor salivary glands biopsy (MSGB) focus score (FS), three points to the positivity for anti-Ro/SSA antibodies while one point is given to a Schirmer's test ≤5 mm in 5 minutes, an ocular staining score major or equal to 5 and to a salivary flow rate (SFR) ≤0,1 ml per minute. A cut-off of 4 points is required to classify a patient as having SS.

Among the items included in the classification criteria, MSGB and serology still represent key tools for the identification of patients affected by SS. In fact, a critical reappraisal of the diagnostic and prognostic value of both MSGB and anti-Ro/SSA antibodies has been performed during the past months (41, 48-52). The general idea is that traditional diagnostic tools, routinely used in clinical practice may also offer the possibility of recognising different phenotypes of SS, allowing a prognostic stratification of the patients. Before to be able to do that, has stated in two recently published papers, there is a crucial need for a general standardisation of the diagnostic procedures. Fisher *et al*. (53) and Costa *et al*. (54) have recently summarised some of the potential pitfalls in the MSGB procedure and in the histopathological interpretation and scoring of MSGB as a diagnostic tool for SS. Fisher *et al*., more specifically, pointed out how standardisation of slice preparation procedure is still needed, using multiple cutting levels and an area of at least 4 mm^2, recognising the presence of non-specific chronic sialoadentis, the presence of germinal centre like structure and fibrosis. With the increasing number of actual and proposed clinical trials in SS, the authors reviewed also the literature that might support the role of histopathology as a biomarker for stratification and response (53).

When considering MSGB as a prognostic instrument, the focus score interpretation represents a key issue. A recent paper retrospectively evaluated MSGB of 794 primary SS patients scored with both CM and FS on MSGB, testing association with clinical, instrumental and laboratory features. They found that the FS was associated with xerostomia, salivary glands enlargement, haematological involvement, CNS involvement, leukopenia, hypergammaglobulinaemia, monoclonal component, ANA, RF and other SS specific antibodies. When performing binary logistic regression FS was associated with xerostomia, salivary glands enlargement, hypocomplementaemia, hypergammaglobulinaemia, monoclonal component and positivity of autoantibodies and that a FS≥3 was associated with high risk of NH lymphoma (50). In addition, Risselada *et al*. (51, 55) showed reduced survival for the patient group with FS≥3 (p=0.009) in a Kaplan-Meier curve analysis and increased HR for NH lymphoma on Cox's proportional hazard regression analysis, both on univariate analysis and when adjusted for autoantibody profile and Ig levels.

Similarly, a renewed interest in SS serology as a valuable tool for the diagnosis of the disease arisen recently. In order to identify phenotypic subset of patients with SS with particular activity/severity of disease, Baer *et al*. (52) have analysed the sera of 3297 patients satisfying ACR criteria or AECG for SS. From the results obtained, patients Anti-La/SSB + and Anti-Ro/SSA- were found to be a small percentage (2%) of the global cohort and there were no specific clinical or laboratory correlations able to define subsets different from seronegative patients.

Among novel tools for the diagnostic and prognostic assessment of patients with SS, salivary gland ultrasonography (SGUS) has been proposed as a reliable and repeatable technique, evaluating either the presence of disease related characteristics but, possibly, also the disease evolution over the time and response to treatment. In a recent paper, Cornec *et al*. (56) pointed out that being an operator-dependent procedure, specific training and formation to standardise the SGUS procedure should be developed.

In a recent meta-analysis examining the diagnostic properties of SGUS in

comparison to classification criteria, SGUS showed a sensitivity of 69%, specificity of 92%. This paper underlines the potential of SGUS as a viable alternative to MSGB for its non-invasive nature, even if the low sensitivity may leave space to misclassification, thus recommending MSGB for those patients with negative SGUS but high clinical suspicion (57). In 2015, studies on SGUS on SS showed that higher SGUS scores were significantly more prevalent in patients with anti-Ro/SSA and/or anti-La/SSB, pathological Schirmer's test, pathological stimulated and unstimulated salivary flow, higher dry mouth VAS, and also a trend for significance for higher focus score on MSGB in the population with higher SGUS scores (58).

SGUS has also shown a sensitivity of 66% and a specificity of 98% in distinguishing patients with SS and symptoms of less than 5 years from subjects with no-SS idiopathic sicca syndrome (59). The ability of SGUS of differentiating SS from other rheumatological diseases such as Undifferentiated Connective Tissue Disease (UCTD) and IgG4 disease was also confirmed (60, 61). Regarding the possibility of using SGUS to monitor glandular involvement during the follow-up, several preliminary contributions have been published lately.

Cornec *et al*. analysed the changes from baseline in major salivary glands size and ecostructure by SGUS performed 6 months after treatment with either Rituximab or placebo (TEARS trial). They found that the echo-structure, graded on a 0–4 scale previously proposed by Cornec *et al*. was significantly improved in the parotid glands of Rituximab group (62). In addition, Takagi *et al*. (63) analysed 168 patients with SS and 140 controls found that using different salivary stimulating agents the change in SFR was positively correlated with treatment duration in both group, being negatively correlated with baseline SGUS score in SS patients and with baseline SFR in the control group.

In conclusion, great progress has been made for the diagnosis of SS. However, despite the efforts of the international community, the concept of an early diagnosis for SS remains an open issue. From this perspective, in a matched case-control study, Theander *et al*. identified autoantibody positivity for either one or more SS-related autoantibodies [antinuclear antibodies (ANA), rheumatoid factor (RF), antiRo60/SSa, anti-Ro52/SSa, antiLa/SSB] in 84% of sera collected up to 20 years before disease diagnosis. This confirmed that the production of autoantibodies may starts many years before clinical disease onset, but also that carrying autoantibodies increased the chance of developing the disease over time, with high odds ratios especially for anti-La/SSB (OR 34) and Ro60/SSA (OR 30), and possibly predicted a more severe clinical course. When analysing also age at diagnosis, the pre-diagnostic positivity for the above mentioned autoantibodies was significantly higher in patients diagnosed before the age of 40 when compared to those diagnosed between 40–60 years or over 60 years, possibly identifying a separate disease subgroup (64).

New insights into biomarkers of SS

Moving forward an early recognition of SS, a number of interesting studies have been published during the past months searching for novel biomarkers for the disease.

Interesting data emerged from a number of studies exploring the potential clinical significance of novel antibodies directed against the modified proteins (post-translational changes) or against new epitopes of antigens already known. It is the case of the two studies, respectively conducted by Bergum *et al*. (65) from University of Bergen and Wolska *et al*. (66) from the US study group.

In the first study the authors identified specific antibodies against carbamilate proteins (anti-CarP) in 78 patients with SS exploring any eventual correlation between these autoantibodies and SS activity indices. The authors found that 27% of the patients tested positive for IgG-antiCarP. In addition anti-CarP were positively correlated with IgG/IgM reumatoid factor, with a focus score greater than or equal to 3, with presence of germinal centres, and with a higher degree of patient reported sicca symptoms as registered by OSSDI. Anti-CarP antibodies derived from post-translational modifications by specific enzymes activated in peculiar conditions, in particular autophagy in systemic autoimmune diseases; therefore, further studies are needed to evaluate whether these antibodies against modified epitopes would acquire a role in the SS diagnostic and/or prognostic significance as anti-cyclic citrullinated peptides in rheumatoid arthritis or they would be considered only an epiphenomenon of autoimmune/inflammatory systemic activations. In the second study the authors analysed sera from 235 patients with SS in order to evaluate the role of antibodies against TRIM38 proteins. Anti-TRIM38 positivity was significantly associated with the presence of anti-Ro60, anti-Ro52, anti-La, rheumatoid factor and hypergammaglobulinaemia. Clinically, anti-TRIM38 were associated with higher ocular surface staining scores, lower Schirmer's test scores and lower labial salivary gland biopsy focus scores ≥3.0 (66).

Regarding the "omics" markers Delaleu *et al*. (67) applied the proteomics approach to saliva samples of 48 SS versus 24 no-SS controls (12 with rheumatoid arthritis and 12 healthy controls). The authors identified 61 proteins differently expressed in the study populations: among them, increased clusterin, interleukin 5(IL5), interleukin 4(IL4) and reduced fibroblast growth factor 4(FGF4) effectively discriminate between SS and non-SS patients.

The technique of high-abundance protein depletion allowed Deutsch *et al*. (68) to remove highly abundant albumin, salivary alpha-amilase and immunoglobulins G from saliva, to better analyse less-abundant salivary proteins by one- and two-dimensional electrophoresis and mass-spectrometry. This approach allowed the authors to identify 79 proteins with >2-fold increased or decreased expression in SS patients, with 10 of them presenting >3-fold different expression and 55/79 being newly reported. These proteins were related to defensive response, catabolic processes, response to stress processes and cell mobility.

Kageyama *et al*. (69) performed the first metabolomics analysis of saliva in patients with primary SS: using a gas chromatography-mass spectrometry analysis; they first identified 88 metabolites that were then quantified in each subject, thus showing that 41 of them were significantly reduced in primary SS patients compared to healthy controls, possibly reflecting structural damage of the salivary glands.

From an epigenetic approach, Gourzi *et al*. (70) measured the levels of let7b, miR16, miR181a, miR200b-3p, miR20-5p, miR223 and miR483-5p microRNAs in minor salivary glands (MSG), peripheral blood mononuclear cells (PBMC) and long-term cultured non neoplastic salivary gland epithelial cells (SGEC) of both anti-Ro/SSa and anti-La/SSB double positive SS patients and healthy patients with sicca symptoms. The study showed a de-regulated expression of miR16, miR223, miR200b-3p, miR483-5p in patients affected by SS when compared to healthy controls. Moreover levels of Ro52, Ro60 and La/SSB mRNAs were associated with the expression of let7b, miR181a, miR16 and miR200b-3p microRNAs.

In conclusion, despite in their infancy, overall these preliminary data reinforce the general concept that in a near future we will be able to identify novel biomarkers for specific SS subsets, opening new avenues for targeted therapies in SS

New insights into the treatment of SS

To date, therapeutic strategies for pSS are mainly empirical, principally symptomatic and none of proven effectiveness in modifying the disease course, at least at glandular level. In recent years, a growing interest as arisen for using biologic drugs in SS, including abatacept, rituximab and belimumab, but the clinical studies conducted so far were not unanimous in reaching the primary endpoints of efficacy.

In fact, rituximab (RTX) failed at least in part to reach primary endpoints in RCTs, however, it has shown efficacy in improving disease activity and patients' symptoms in the vast majority of the open-label studies so far conducted. From this perspective, Meiners *et al*. (71) evaluated the response to retreatment of 15 patients, after recurrence of symptoms, undergoing two cycles of RTX therapy (each course consisted of 1000 mg RTX intravenously), with mean time between infusions of 103 months (60 to 136). The most significant results were that retreatment was well-tolerated and the significant improvement of subjective and objective parameters (ESSDAI, stimulated whole saliva, B-cells, rheumatoid factor, IgG levels, patient global disease activity and VAS for oral drynes) was obtained also in the second course.

Analogously, an open-label phase II studies of belimumab was recently conducted (72). The 30 enrolled patients had to present systemic complications, or disease duration ≤5 years or at least one biomarker of B cell activation. Belimumab appeared overall effective, as 60% of patients achieved the primary endpoint at 28 weeks, with an improvement in some predominantly objective clinical signs (parotid swelling), systemic activity and B-biomarkers (Ig levels, rheumatoid factor titre and cryoglobulins), while the improvement in the patient's symptoms was quite limited, especially for fatigue and pain VAS scores. Moreover salivary flow and Schirmer's test did not change. Only one case of severe pneumococcal meningitis occurred and the safety of the drug was in line with data from lupus patients.

The 52-week extension of this study for 19 patients confirmed the clinical and biologic efficacy of Belimumab: interestingly, 3/4 of the not-responding patients at W28 who continued the study responded at W52 (73).

Additional data from the BELISS study show that belimumab can induce changes in salivary glands infiltrate, decreasing the lymphocytic cells, Chisholm score, BAFF-expressing cells in the foci and the B-cell/T-cell ratio. Nevertheless, the only predictor of response to treatment seems to be the low count of natural killer (NK) cells both in peripheral blood and salivary tissues (74). Belimumab can reduce the overexpressed number of transitional and naive CD27- B cells (the subsets charactered by BAFF-dependent survival) in peripheral blood of pSS patients, increase the BAFF-receptor (BAFF-R) expression in CD27- memory B cells and downregulate the circulating BAFF levels, even if it is an early and transient reduction. Balancing the feedback interaction mechanisms between circulating levels of BAFF and BAFF-R, belimumab restores peripheral B cell homeostasis (75). Since the values of BAFF are not long-term suppressed, a treatment option could combine therapies targeting BAFF with B cell depletion approaches (76).

Exploring the latest research frontiers, beneficial effects from the proteasome inhibitors (PIs), like bortezomib (BTZ) and next generation PIs, currently used in the treatment of haematological malignancies, were observed in many models of autoimmune diseases. The proteasomes have a role in multiple cellular processes, including MHC-mediated antigen presentation, cytokine and cell cycle regulation and apoptosis: the PIs inhibit the activation of nuclear factor (NF)-κB and transcriptional regulation of pro-inflammatory cytokine and induce apoptosis of activated immune cells. In pSS the PIs could act on B lymphocytes, whose susceptibility to therapeutic response may be influenced not only by the increased turnover of protein and by the increased activity of the proteasome in these cells, but rather by the stabilation of pro-apoptotic proteins leading to cell death. For the same reason they could also act on T lymphocytes and APC, altering their activation, the expression of surface receptors and suppression of cytokine release (77).

To encourage the use of targeted biological therapies for SS, a critical reappraisal on how to design future RCTs in SS has appeared crucial. From this perspective, Oni *et al*. (78) have studied how different eligibility criteria can affect the number of potential recruited patients. In this paper, 688 patients from the UK Primary Sjögren's Syndrome Registry (UKPSSR) were selected, representing a real-life patient cohort, and taking into account the eligibility criteria of some previous/current clinical trials, the authors assessed which was the percentage of real patients that could have been in-

cluded in clinical trials. The results showed that 75.2% of patients fulfilled the eligibility criteria for BELISS (trial of belimumab); only the 46.3% and 41.4% of patients, fulfilled respectively the TEARS (Tolerance and Efficacy of Rituximab) and TRIPSS (Remicade) inclusion criteria, whereas the ETAP (tocilizumab), ASAPIII (abatacept) and TRACTISS (rituximab) protocols allowed the enrolment of only the 35% and 26% of SS patients.

In a similar way, Devauchelle-Pensec *et al*. (79) evaluated the proportions of patients from the ASSESS cohort (Assessment of Systemic Signs and Evolution in Sjögren's Syndrome), who would have been eligible for the RCTs concluding that. Considering that in previous clinical trials, eligibility criteria included short disease duration, systemic involvement, high mean VAS scores for dryness, pain, and fatigue, and biological evidence of activity, the authors concluded that only a small proportion of patients would have been enrolled.

The second open question regards the endpoints that should be taken into account for clinical trials.

The European League Against Rheumatism (EULAR) SS Patient Reported Index (ESSPRI) and the EULAR SS Disease Activity Index (ESSDAI) have been recently validated (80). To provide support for the use of the EULAR outcome measures, it has been demonstrated that they are also useful as health outcome tools for clinical and economic analysis: investigating the relationship between EULAR indexes and quality of life, it has been emphasised that higher scores of EULAR outcome measures correlate with poorer health status and that this correlation was strongest for ESSPRI (81). In addition, through a post hoc analysis of data from the two trials on rituximab (TEARS trial and a single centre study of Rituximab) and from a multicentre study of Infliximab, Cornec *et al*. (82) have created a composite index capable of detecting therapeutic responses in patients with pSS, the Sjögren's Syndrome Responder Index (SSRI). They included both patient-reported measures (VAS scores) and objective measures (unstimulated whole salivary flow and ESR), defining an SSRI-30 response as a ≥30% improvement in at least two of the five outcome measures chosen. SSRI-30 has proved useful as the measures were sensitive to change, since were significantly improved by rituximab compared with placebo at any of the time points in both trials, but showed no significant difference between treatment groups in the trial of Infliximab, supporting the clinical observation that infliximab was not effective.

At this stage of clinical research in the field of SS therapy, more studies are needed in order to better identify targeted treatments for specific subsets of patients and composite endpoints able to catch more precisely patients clinical responses.

Conclusions

In this article we have revised the most recent literature on SS pathogenesis, clinical and serological features and treatments. Overall, recent contributions emphased the heterogeneity of the disease and its complexity. There is a critical need to identify molecular pathogenetic pathways underlying the disease and its different subsets in order to recognise novel potential therapeutic targets and select those patients who may respond and benefit from biologic treatments

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