How to assess treatment efficacy in Sjögren’s syndrome?

Arjan Vissink\textsuperscript{a}, Hendrika Bootsma\textsuperscript{b}, Frans G.M. Kroese\textsuperscript{b}, and Cees G.M. Kallenberg\textsuperscript{b}

**Purpose of review**

This article critically reviews the current views and discusses the future challenges with regard to assessing disease progression and disease activity in Sjögren’s syndrome, as a decrease of disease progression and activity is what an effective Sjögren’s syndrome therapy aims for. This topic has recently gained renewed attention as targeted treatment modalities have become available in primary Sjögren’s syndrome, while the lack of well established outcome parameters interferes with a straightforward comparison of the outcomes of the various trials.

**Recent findings**

Recent advances in how to assess changes in disease progression and activity objectively (via repeated biopsies of salivary glands, sialometry, sialochemistry, biomarkers, secretion and composition of tears, EULAR Sjögren’s Syndrome Disease Activity Index: ESSDAI) and subjectively (EULAR Sjögren’s Syndrome Patient Related Index: ESSPRI) have opened new ways to reliably assess the outcome of a particular treatment.

**Summary**

Newly applied tools are instrumental, both for clinical research and clinical practice, in reliably judging and comparing the value of well established and newly developed therapies in Sjögren’s syndrome.

**Keywords**

biologicals, ESSDAI, ESSPRI, saliva, Sjögren’s syndrome, tears, treatment efficacy

**INTRODUCTION**

Sjögren’s syndrome is an autoimmune inflammatory disorder of exocrine glands. It particularly affects the lacrimal and salivary glands. Dry mouth and dry eyes are frequently proffered as presenting symptoms [1,2]. These symptoms are in many cases accompanied by nonspecific symptoms, such as malaise and fatigue [3]. In addition, extraglandular manifestations, like purpura, polyneuropathy, and arthritis, can be present, even as presenting signs of the disease [2]. Lymphomas develop in 5–10% of Sjögren’s syndrome patients [4,5]. Sjögren’s syndrome affects mainly women with a female/male ratio of 9:1 and can occur at all ages.

In-vitro and in-vivo experimental data have pointed to new immunopathogenic mechanisms in primary Sjögren’s syndrome (pSS). The availability of targeted treatment modalities has opened new ways to selectively target these mechanistic pathways in vivo [6]. A problem of all studies assessing the efficacy of these new modalities is the large variety of outcome parameters used in the various studies which makes a proper comparison of results between studies difficult if even possible [7\textsuperscript{**},8\textsuperscript{*}]. Therefore, this study critically reviews the current views and future challenges with regard to measuring disease activity and disease progression in Sjögren’s syndrome.

**PROGRESSION AND DISEASE ACTIVITY IN PRIMARY SJÖGREN’S SYNDROME**

As mentioned in the introduction, the largest hurdle to be taken is to develop a reliable set of assessments...
**KEY POINTS**

- ESSPRI and ESSDAI are promising tools to assess disease activity and disease progression in pSS to monitor treatment efficacy.
- Salivary gland functioning in pSS patients is best assessed by measuring glandular secretions and should be included in monitoring progression and treatment efficacy of pSS.
- Tear osmolarity may evolve into a more potent tool for assessing the ocular component in pSS than lissamine green and Schirmer tests.
- Proteomic and genomic approaches in saliva and tears are potent tools to facilitate diagnosis, to monitor the effect of a particular treatment, and to contribute to understanding of pSS pathogenesis.
- Labial or parotid biopsies are needed for proper diagnosis, as they may indicate the likelihood of developing MALT or NHL lymphoma and thus may indicate which patients have to be closely monitored.
- Repeated (parotid) salivary gland biopsies may show what the treatment effect is at a tissue level.

by which the efficacy of a particular treatment approach can be assessed, meanwhile allowing evidence-based comparison of the various treatment approaches tested in pSS [6*,7**,8*]. Such a set of assessments probably also can be used to rate disease activity and disease progression in pSS.

**EULAR Sjögren’s Syndrome Disease Activity Index and EULAR Sjögren’s Syndrome Patient Related Index**

Evidence-based therapy for Sjögren’s syndrome is largely limited to treatments that improve sicca features [7**,9–11]. In addition, the variety of ad hoc outcome measures used to rate the effects of both symptomatic and disease-modifying treatments are mainly based on glandular symptoms [12*,13*]. Therefore, validated activity indexes are needed to assess the effectiveness of (new) targeted therapies [14–16]. For example, B-cell-targeted therapies have shown promising results for both systemic [17–22] and glandular features [17,21,23,24**], but, again, a variety of outcome measures has been used [7**,8*]. To solve these shortcomings, the European League against Rheumatism (EULAR) recently introduced a patient-administered questionnaire to assess patient symptoms (EULAR Sjögren’s syndrome Patient Reported Index: ESSPRI) and a systemic activity index to assess systemic complications (EULAR Sjögren’s Syndrome Disease Activity Index (ESSDAI) [12*,13*,25*] as there are for rheumatoid arthritis (RA) [26] and systemic lupus erythematosus (SLE) [27].

With regard to pSS, studies are in progress to assess the reliability and sensitivity to change of the ESSDAI and ESSPRI. Such studies are important as the usefulness of instruments designed to measure change over time or after therapeutic intervention is not only dependent on their validity and reliability, but also on their potential to detect clinically relevant changes [28–30]. Seror et al. [12*] retrospectively investigated the sensitivity to change of the ESSDAI over time in 96 patient profiles and reported that the internal and external responsiveness of ESSDAI was large, and changes over time were detected more accurately than with other known indices. Meiners et al. [31**] prospectively assessed the internal and external responsiveness to change of ESSPRI and ESSDAI in pSS patients treated with rituximab. The latter study revealed that ESSPRI and ESSDAI are, indeed, sensitive to measure the changes in disease activity after therapeutic intervention, which supports the usefulness of these indices for future clinical trials. The promising results from the retrospective analysis of Seror et al. [12*] and the prospective intervention study by Meiners et al. [31**] showed that, when validated, application of both instruments for outcome measurements in randomized controlled clinical trials should allow assessing all aspects of pSS (Fig. 1).

**Saliva**

Within the wide spectrum of clinical manifestations of Sjögren’s syndrome, salivary gland dysfunction is considered to be a key manifestation [32]. Dysfunction of the salivary glands results in changes of the amount and composition of the saliva. The autoimmune process itself is reflected by the presence of various components of the immune response, such as cytokines, chemokines, autoantibodies, in saliva. As such, sialometry is not only of diagnostic but also of prognostic importance. Moreover, as the amount and composition of saliva reflect the damage caused by the autoimmune process in the salivary glands, sialochemistry may be valuable in diagnosis, assessment of prognosis, and evaluation of treatment [17,24**,33–36]. Thus, sialometry and sialochemistry can be used as a diagnostic tool either by collecting whole saliva (the combined secretions of all salivary glands) [37] or by collecting glandular saliva (gland-specific saliva) [33,34,38,39]. Although unstimulated whole saliva is a major criterion for the evaluation of salivary gland dysfunction in Sjögren’s syndrome [37], when Sjögren’s syndrome...
develops, not all major salivary glands may yet manifest dysfunction, rendering whole saliva less valuable as a parameter for evaluating progression of the disease or therapeutic intervention than glandular saliva (Fig. 2) [35]. In contrast to whole saliva, analysis of gland-specific saliva can reveal sequential involvement of particular glands, reflecting the ongoing autoimmune process in individual major salivary glands. By using glandular saliva, patients with Sjögren’s syndrome may frequently be diagnosed at an earlier stage, and progression and effects of therapeutic intervention can be measured in a noninvasive way. For example, Pijpe et al. [36] showed that regeneration of salivary gland tissue by rituximab was accompanied by an increase in salivary flow and normalization of salivary composition (Figs 3 and 4).

**Biomarkers in saliva**

Although Kalk et al. [33,34] suggested stimulated submandibular/sublingual flow rate in combination with parotid sodium and chloride concentration as a reliable diagnostic test for Sjögren’s syndrome, recent progress in proteomics and genomics has shown that the proteomic and genomic profile reflecting the autoimmune process even might be more sensitive and specific in diagnosing Sjögren’s syndrome [40–42,43*,44*. In contrast to most other diseases studied thus far, in which the majority of reports studying the same disease produced inconsistent results to studies on biomarkers, proteomic and genomic analyses of saliva in pSS were more consistent [45,46].

It has been studied whether biomarkers that differ between pSS patients and healthy individuals are indeed specific for pSS or are also commonly expressed in saliva of patients with other autoimmune diseases [46]. As a first step to look into the specificity of biomarkers for diagnosing Sjögren’s syndrome, the Sjögren’s syndrome proteomic/genomic profile was compared with that of SLE patients (all not fulfilling criteria for secondary Sjögren’s syndrome). It was shown that three protein biomarkers, namely, cathepsin D, alpha-enolase, and beta-2-microglobulin (B2M), and three mRNA biomarkers in saliva, namely, myeloid cell nuclear differentiation antigen (MDNA), guanylate-binding...
protein 2 (GIP2), and the low-affinity IIb receptor for the Fc fragment of IgG (FCGR3B), were all significantly elevated in pSS patients compared with both SLE patients and healthy controls. The combination of cathepsin D, alpha-enolase, and B2M yielded a receiver operating characteristic (ROC) value of 99% in distinguishing pSS from healthy controls. The combination of the protein marker, B2M, and the mRNA biomarkers MNDA and GIP2 reached a ROC of 95% in discriminating pSS from SLE [43*,44*].

The next step has to be to further test the genomic and proteomic biomarkers in patients with pSS and RA, and to reveal biomarkers that can distinguish pSS from RA and from non-Sjögren’s syndrome sicca patients. A validation study [47] sponsored by National Institutes of Health (USA) is currently underway. Moreover, proteomic/genomic analysis of pSS, followed by gene ontology or functional pathway analysis, may also reveal molecular targets and related pathways associated with the disease pathogenesis. As such, these

FIGURE 2. Relation between disease duration, that is, the time from first complaints induced by or related to oral dryness until referral, and mean salivary flow rates (mean ± SEM). UWS, unstimulated whole saliva; SM/SL, submandibular/sublingual glands [35].
Ultrasound

The focus of ultrasound is not only how to diagnose pSS, but also how to rate the progression of the disease and thus might evolve into a tool to objectively assess a treatment effect [48–51,52–53]. Moreover, although the ultrasound image in major salivary glands affected by Sjögren’s syndrome is rather characteristic, a current limitation is still that the image is characteristic for the advanced stages of pSS and not for the very early stages of pSS. The better performance of submandibular ultrasound in diagnosing Sjögren’s syndrome is in agreement with the observation that submandibular/sublingual glands are earlier involved in Sjögren’s syndrome than the parotid glands as based on sialometrical studies [33–35,38]. Moreover, parotid glands seem to have a greater reserve capacity than submandibular glands as, on a histopathological level, changes can already be observed in the parotid glands when the salivary flow is not yet significantly affected [33–35,38]. This is also in line with parotid ultrasound findings, as changes in parotid salivary composition preceed changes in parotid flow showing that the ductal system is primarily affected by the disease process.

Salivary gland biopsy

A widely accepted criterion for histological confirmation of Sjögren’s syndrome is focal lymphocytic sialoadenitis in labial salivary glands [54,55]. In a normal population, labial biopsy results in 6–9% false-positive diagnoses, and 18–40% of patients with clinically diagnosed Sjögren’s syndrome have a negative labial biopsy [54–56]. An alternative to a labial biopsy is an incision biopsy of the parotid gland [57]. A major advantage of parotid biopsies over labial biopsies with regard to disease progression and assessing the effect of an intervention treatment is that parotid gland tissue can be harvested easily, repeated biopsies from the same parotid gland are possible, and the histopathological results can be compared with other diagnostic results derived from the same gland (e.g. secretory function, sialographic appearance, and ultrasound) [36]. There are, however, also some differences between labial and parotid biopsies as, for example, in contrast to labial glands, lymphoepithelial lesions (LELs) are often observed in parotid gland tissue of Sjögren’s syndrome patients [58]. Finally, by performing parotid biopsies as a routine diagnostic procedure for Sjögren’s syndrome, lymphomas can be found at an earlier stage [59,60]. However, very rare, B-cell MALT lymphomas are occasionally found in labial biopsies of Sjögren’s syndrome patients [61,62]. Recently, it has been shown that
the presence of germinal center-like structures diagnostic of salivary biopsies for pSS might be a highly predictive and easy-to-obtain marker for lymphoma development. This observation allows the risk stratification of patients and the possibility to initiate preventive B-cell-directed therapy [62]. It emphasizes the need to obtain salivary gland biopsies as a method to predict the risk of lymphoma [63].

Tears
Similarly to saliva, the amount and composition of tears are not only influenced by damage of the lacrimal glands, but also reflect the autoimmune response itself. Regarding the dry eye, the use of tear osmolarity in dry eye assessment has recently gained attention for diagnostics, monitoring disease progression, and treatment evaluation in pSS. Tear osmolarity values were shown to be greater in patients with dry eye syndrome related to pSS compared with control individuals, and positively correlated with the severity of dry eye [64,65]. Measuring osmolarity is simple and probably will become an integral part of dry eye assessments in the clinical setting. Osmolarity has the advantage of functioning as a noninvasive and easily performed objective clinical biomarker for dry eye severity [64]. Osmolarity values of greater than 308 mOsm/l are generally indicative of dry eye disease (mild ≈308 mOsm/l; moderate ≈320 mOsm/l; severe >355 mOsm/l).

Concentrations of autoimmunity response parameters as anti-Ro/SSA antibody and anti-La/SSB antibody in serum are significantly higher in pSS patients with severe keratoconjunctivitis sicca. Thus, serum anti-Ro/SSA antibody, serum anti-La/SSB antibody, and tear IL-17 are likely to be strongly involved in the clinical severity of keratoconjunctivitis sicca in pSS patients [66] and, thus, may have some potential as a disease progression and treatment outcome monitor in pSS.

Histological and functional changes of the lacrimal gland might be reflected in proteomic patterns in tear fluids [67]. In a tear study [68], multiple protein changes were reproducibly detected in pSS patients, including 10 potential novel biomarkers. Thus, the ability to probe the protein content of human tear fluid has enormous potential for deepening our understanding of ocular and systemic disease pathology and enabling novel noninvasive tear-based diagnostic technology using proteomic approaches and microfluidic homogeneous immunoassays [69]. For example, when using the latter immunoassay, Karns and Herr [69] were able to measure lactoferrin, a biomarker that is reduced in Sjögren’s syndrome, in a very low volume of tears (<1 μl) which brings the use of tears to monitor Sjögren’s syndrome disease activity and progression as well as to assess treatment outcome within reach for clinical application.

Serum
Serum may contain a variety of biomarkers related to the autoimmune process that can be used to diagnose Sjögren’s syndrome, to characterize disease activity and progression in Sjögren’s syndrome, to
recognize Sjögren’s syndrome patients who may develop extraglandular manifestations, to recognize Sjögren’s syndrome patients with an increased risk to develop MALT lymphomas or non-Hodgkin lymphomas, and to evaluate the effect of an intervention treatment with biologicals.

Presence of autoantibodies against Ro (SSA) and La (SSB) in serum is commonly used as an accepted criterion for diagnosing Sjögren’s syndrome [37]. Also autoantibodies directed against alpha-phodrin [70–72] and muscarinic acetylcholine receptors [73–75] have been proposed as markers for diagnosis and progression of Sjögren’s syndrome. Their role is still not clear as, for example, Potthoff et al. [72] showed that neither the severity nor the progression of Sjögren’s syndrome correlated with the presence of antialpha-phodrin antibodies in serum.

Increased serum levels of rheumatoid factor and circulating monoclonal immunoglobulins (in particular IgG), reduced levels of C4, and presence of cryoglobulins are frequently observed in Sjögren’s syndrome patients and may be indicative for patients at risk of developing extraglandular manifestations and lymphomas and thus can be used as a monitor to early recognize potential development of a lymphoma [5,76–78].

Regarding the use of serum in rating and understanding the effect of intervention treatment with biologicals, the decrease and re-increase in rheumatoid factor, γ-globulins, IgG and β2-microglobulin following B-cell depletion therapy with rituximab in pSS patients might be a useful serum parameter for assessing treatment effects [18,24**]. Analysis of changes in immune activation markers, such as cytokines involved in lymphocyte activation and inflammation, following rituximab treatment might be indicative for response to treatment and, possibly, for recurrence of disease activity (Pollard et al., unpublished observation).

Application of unbiased proteomic technologies may be useful in further unraveling the mechanisms of autoimmune diseases [79]. As discussed previously, first attempts have been made in proteomic and genomic analysis of saliva [40,41,43*,44*,47] and tears [67] in pSS, but no data are available yet about blood samples from pSS patients. However, recently it has been shown that interferon-α (IFN-α)-inducible protein 27 (IFI27) showed the most significant difference between Sjögren’s syndrome patients and controls in a DNA microanalysis using a low-density DNA microarray system with 778 genes [80*]. IFI27 gene-expression level was increased in patients with Sjögren’s syndrome compared with controls, and the level of IFI27 significantly correlated with serum IgG, β2-microglobulin, soluble interleukin-2 receptor, erythrocyte sedimentation rate, and antinuclear antibody titer. Thus, upregulation of IFN-inducible genes in Sjögren’s syndrome patients seems to be a systemic phenomenon and is in line with the notion that pSS patients have a type I IFN signature. Expression levels of IFI27 could thus be an effective and specific biomarker associated with Sjögren’s syndrome [80*].

CONCLUSION

During the last decade, much progress has been made regarding development and evaluation of tools to assess disease progression and disease activity in pSS in addition to facilitating pSS diagnosis. On the basis of the results described in this review and our own expertise, we propose the following disease progression and disease activity tools to assess disease activity and progression in pSS.

Overall, ESSPRI and ESSDAI are proper instruments to rate disease activity, to evaluate disease progression, and to assess the effect of treatment in pSS patients, although some final validation studies have to be completed before these tools can be generally applied in pSS. When looking at more organ-specific tools, sialometry, sialochemistry, ultrasound, and repeated biopsies are proper tools to assess the salivary gland functioning and regeneration. For lacrimal glands, breakup time and tear osmolarity are valid, noninvasive tools to assess in addition to Schirmer’s test and lisamine green staining. Finally, the proteomics and genomics approaches that are currently in progress may lead to the development of specific tools for the analysis of serum, saliva, and tears in pSS.

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None.

Conflicts of interest

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REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 344).

Clinical therapeutics


7. Most treatment in pSS is still symptomatic, although some progress has been made regarding targeted treatment modalities. This systematic review concluded that benefits can be expected from symptomatic treatment of sicca features with pilocarpine and cevimeline and from topical cyclosporine for the moderate-to-severe dry eye. Regarding biologicals, larger controlled trials are needed.


9. This review summarizes the current knowledge on treatment of pSS, with rituximab being the most promising treatment option to date. Rituximab (re)treatment was shown to be effective in reducing disease activity in pSS patients for about 6–9 months. Large randomized controlled trials are needed to assess the long-term effects of rituximab treatment, to determine which pSS patients will benefit most from rituximab and to assess which retreatment schedule should be followed.


12. The first study to prospectively evaluate the responsiveness of ESSPRI and ESSDAI in pSS patients treated with rituximab. Both ESSPRI and ESSDAI were shown to be sensitive to measure change in disease activity after therapeutic intervention, which supports the usefulness of these indices for future clinical trials.

13. The EULAR consortium developed a score for the assessment of symptoms in pSS, the EULAR Sjögren’s Syndrome Disease Activity Index (ESSDAI) and the Systemic Sjögren’s Syndrome Disease Activity Index (SSDAI). Clinical therapeutics

14. On the basis of the abstracted patient profiles, the authors showed that ESSDAI seemed to detect changes in activity more accurately than other disease activity indexes used in pSS. For pSS patients with stable activity, ESSDAI did not show erroneous improvement.


16. The EULAR consortium developed a disease activity index for pSS patients, the ESSDAI. The ESSDAI is composed of 12 organ-specific ‘domains’ significantly contributing to disease activity. Once validated, standardized evaluation of pSS with the ESSDAI is thought to facilitate clinical research and to be helpful as an outcome measure in clinical trials.


This study demonstrated the potential of a high-throughput protein microarray approach for the discovery of autoantibody biomarkers. The identified saliva autoantibody biomarkers (antitransglutaminase, antithrombin, anti-SSA, and anti-SSB) may lead to a clinical tool for simple, noninvasive detection of pSS at low cost.


This study showed submandibular ultrasound to be a promising technique to be used as a practical alternative to sialography in the classification of Sjögren’s syndrome.


This study showed salivary gland ultrasonography to be a very promising, non-invasive, and easy-to-use tool in the diagnostic work-up of Sjögren’s syndrome.


This retrospective study revealed an algorithm as how to treat MALT lymphoma in Sjögren’s syndrome. An initially high Sjögren’s syndrome disease activity likely constitutes an adverse prognostic factor for progression of lymphoma and Sjögren’s syndrome. Such patients may require treatment for both MALT lymphoma and Sjögren’s syndrome. In Sjögren’s syndrome patients with localized asymptomatic MALT lymphoma and low Sjögren’s syndrome disease activity, a ‘watchful waiting’ strategy seems justified. This algorithm has to be validated in prospective studies.


This study showed interferon-α (IFN-α)-inducible protein 27 (IFIT2) to be increased in Sjögren’s syndrome compared with controls and to be significantly correlated with serum IgG levels, IgG microglobulin, soluble interleukin 2 receptor, erythrocyte sedimentation rate and anticardiolipin antibody titer. Thus, upregulation of IFN-α-inducible genes in Sjögren’s syndrome patients seems to be a systemic phenomenon to play an important role in the pathogenesis of Sjögren’s syndrome. The expression level of IFIT2 could be an effective and specific biomarker associated with Sjögren’s syndrome.