13th International Symposium on Sjögren’s syndrome

Sjögren’s syndrome has its name after Henrik Sjögren, a Swedish ophthalmologist who in his doctoral dissertation from 1933 reported detailed clinical and histological findings in 19 women with xerostomia and keratoconjunctivitis sicca, of whom 13 had chronic arthritis [1]. Today, classification criteria are in use to better define patients involved in, for example, attempts to improve treatment measures [2, 3].

The 1st International Symposium on Sjögren’s syndrome (ISSS) was held in 1986 in Copenhagen, organized by Rolf Manthorpe. We are proud to host the 13th ISSS, and we are delighted to see that the meeting continues to grow and attract an impressive number of delegates. In May this year, world leading scientists, both clinical and experimental, and also patient organizations on Sjögren’s syndrome, will gather in Bergen on the west coast of Norway. We are exceptionally thankful to the excellent speakers who have confirmed their participation. The 13th ISSS will provide up-to-date information on a wide range of key topics. Areas covered include immunopathology, autoantibodies, new biomarkers, classification criteria and new diagnostic tools, extraglandular manifestations, genetics, animal models and personalized therapies. The ultimate aim is to provide better care for patients with Sjögren’s syndrome. In addition to the scientific discussion, the aim of this meeting is to encourage worldwide networking between various disciplines such as immunology, rheumatology, pathology, ophthalmology and dentistry. The full meeting programme is available at http://www.siccoa.org/issss2015/.

Most of what we know today about Sjögren’s syndrome emanate from studies of clinical observations, patient material/samples and experimental models. A number of such studies will be presented by the >200 abstracts we have received for the conference and published in this issue of the Scandinavian Journal of Immunology [4]. In addition, recent issues of the Scandinavian Journal of Immunology contained studies on Sjögren’s syndrome focusing on the involvement of cytokines [5, 6], immune cells [7–9] and genetics [10].

The meeting has graciously received funding from the Broegelmann Foundation, the Bergen University Fund, the Bergen Rheumatology Research Fund, the Scandinavian Foundation for Immunology and several companies listed in the programme. Once again, the 13th ISSS conference is a golden opportunity to share research achievements and exchange ideas that will inspire our future and coming work. We hope you enjoy the meeting and the wonderful venue on the western part of Norway!

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References
Abstracts

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Opening Lecture

A Tissue-Based Map of the Human Proteome

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Resolving the molecular details of proteome variation in the different tissues and organs of the human body will greatly increase our knowledge of human biology and disease. The Human Protein Atlas (www.proteinatlas.org) project employs an antibody-based proteomics approach to systematically map the distribution and relative abundance of human proteins in a multitude of human normal tissues, cancer and cells. In this talk, a map of the human tissue proteome based on an integrated omics approach that involves quantitative transcriptomics at the tissue and organ level, combined with tissue microarray-based immunohistochemistry, to achieve spatial localization of proteins down to the single-cell level, is presented. Our tissue-based analysis detected more than 90% of the putative protein-coding genes. This approach was used to explore the human organ proteomes and subproteomes including the druggable proteome and cancer proteome in 32 different tissues and organs. The combined data provide both basic knowledge regarding the gene expression landscape in the human body and a starting point for various biomedical research projects, including biomarker discovery efforts.

Session 1. Classification Criteria

S1.1 Proposed ACR–EULAR Classification Criteria for Sjögren’s Syndrome: Development and Validation

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We will describe development and validation of proposed consensus criteria for Sjögren’s Syndrome (SS) , developed jointly by the ACR-EULAR Sjögren’s Syndrome (SS) Classification Criteria Working Group. Criteria development followed methods approved by both ACR and EULAR and included the use of multicriteria decision analysis (MCDA) tools to quantify opinions of participating expert clinicians on relative rankings of component tests. Resulting draft criteria were further refined using clinical vignettes derived from randomly selected SS cases and non-cases from 3 established cohorts: the Sjo¨gren’s International Collaborative Clinical Alliance (SICCA); the Paris-Sud Kremlin-Bicêtre cohort; and the Oklahoma Medical Research Foundation cohort. Component tests included labial salivary gland biopsy with focal lymphocytic sialadenitis and focus score ≥ 1; anti-SSA(Ro) positivity; ocular staining score ≥ 5 (or VB ≥ 4) on at least one eye; Schirmer ≤ 5 mm/5 min on at least one eye; and unstimulated whole saliva flow rate ≤ 0.1 ml/min (after 15-minute test). Items were assigned weights based on the results of the MCDA and analysis of vignette results, and these form the basis of the candidate criterion score. The chosen cut-off value for defining a case was derived from an receiver operating curve (ROC) analysis of this score applied to cases and non-cases in the development cohort. Validation analyses were based on a similar approach applied to vignettes derived from a separate sample of patients, also representing all three cohorts.

S1.2 Patient Sub–Phenotyping in Primary Sjögren’s Syndrome

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Primary Sjögren’s Syndrome (pSS) is a chronic autoimmune syndrome with a complex and heterogeneous patient population. To date, there are no clinical parameters that are able to clearly sub-classify pSS patients. Further knowledge of the patient heterogeneity of the pSS population has implications for diagnosis, treatment and monitoring of clinical features and may also aid researchers in experimental design and data interpretation. The aim of this study was to determine whether there are any biological parameters that reflect the heterogeneity of the patients.

Patient data for this study were obtained from the UK Primary Sjögren’s Syndrome Registry (UKPSSR). Extensive clinical profiles are available for the patients, including demographics, disease activity and damage, past and current treatments and patient-reported outcome measures. All patients fulfilled the 2002 American European Consensus Group Criteria. Data from the standard haematological panel of tests as well as cytokine profiles were measured using a multiplexed bead based assay in serum samples contemporaneous to the haematological data were analysed.

Initially, hierarchical clustering was performed upon the clinical data based on 5 symptom categories; Pain, Fatigue, Dryness, Anxiety and Depression (patients n = 150). From the hierarchical clustering, 4 groups were identified that defined patients who were 1) high for all domains, 2) low for all domains, 3) high for fatigue symptoms, 4) high for dryness symptoms. Haematological and serum cytokine profiles were analysed to search for a biological determinant for the patient groupings. Principle components analysis identified measurements that together explained patient groupings. IgG, IgA and ESR contributed most highly to this component. Statistical modelling revealed that IgG alone could be a useful indicator of group membership. However, most serum cytokine measurements were a poor indicator of group membership. These results support that the symptom groupings are not simulated clusters but likely have a biological basis. This may provide useful measures for the stratification of groups for more personalised medical care, improved experimental design and interpretation.
S1.3
Fulfillment of Classification Criteria of Cryoglobulinemic Vasculitis (CryoVas) is Associated with a Higher Systemic Activity in Patients with Primary Sjögren Syndrome

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Objective: To evaluate the usefulness of the recently validated classification criteria for cryoglobulinemic vasculitis (CV) in a large cohort of Spanish and Italian patients with primary Sjögren syndrome tested for serum cryoglobulinemia.

Patients and Methods: The database of the two centers included 515 patients who fulfilled the 2002 classification criteria for primary SS and who were consecutively tested for serum cryoglobulins. Fulfillment of the 2014 validated classification criteria for CV, and their association with systemic activity, lymphoma and mortality was retrospectively evaluated.

Results: The cohort included 484 (94%) females and 31 (6%) males, with a mean age at diagnosis of 54 years. Positive serum cryoglobulins were detected in 65 (12%) patients, of whom only 21 (32%) fulfilled the 2014 classification criteria for CV. Compared to patients carrying serum cryoglobulins, who do not fulfill the criteria for CV, patients with CV showed a higher frequency of systemic activity in the constitutional (76% vs 27%, \(P < 0.001\)), cutaneous (100% vs 29%, \(P < 0.001\)), peripheral nervous system (71% vs 14%, \(P < 0.001\)) and hematological (86% vs 75%, \(P < 0.001\)) ESSDAI domains, with a higher global mean ESSDAI score (35.29 vs 16.23, \(P < 0.001\)).

With respect to laboratory parameters, patients with CV had a higher frequency of monoclonal gammopathy (68% vs 34%, \(P = 0.04\)), low C3 levels (55% vs 23%, \(P = 0.021\)), low C4 levels (95% vs 25%, \(P < 0.001\)), a higher frequency of type II cryoglobulinemia (86% vs 43%, \(P = 0.04\)) and a higher mean cryocrit level (6.58% vs 1.25%, \(P < 0.001\)). Kaplan–Meier curves disclosed a higher frequency of lymphoma development and a poor survival in patients fulfilling criteria for CV (log rank <0.05).

Conclusion: Among patients with primary SS carrying serum cryoglobulins, the fulfillment of criteria of cryoglobulinemic vasculitis is associated with a higher systemic activity, a higher frequency of immunological abnormalities (hypocomplementemia, higher cryocrit levels, monoclonality and type II cryoglobulins), a higher risk of development of B-cell lymphoma and a poor survival. The application of the classification criteria for CV is then useful in primary SS, identifying a high-risk subset of patients who require a closer follow-up and a more intensive therapy.
The items responsible for this increased sensitivity are both the introduction of hypocomplementemia and the separate consideration of different hematological disorders in the new classification criteria. In patients with SS-SLE, hypocomplementemia, leukopenia and arthritis appear to be the most frequent manifestations.

S1.5
Previous Diagnosis of Sjögren’s Syndrome as Rheumatoid Arthritis or Systemic Lupus Erythematosus

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Background: The diagnosis of Sjögren’s Syndrome (SS) is often difficult, and many patients are symptomatic for years without prior diagnoses before confirmation of SS. Overlapping clinical and serologic features of SS with other connective tissue disorders, in particular rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), may in part drive the misdiagnoses.

Methods: One thousand one hundred and ninety-one subjects with sicca were evaluated in a multi-disciplinary clinic and classified for SS based on the American-European Consensus Criteria. They were interrogated for a past history of suspicion or diagnosis of RA, SLE or SSc, and these diseases were confirmed or ruled out by applying the corresponding classification criteria if the patients responded affirmatively.

Results: Five hundred and thirty-one (44.6%) subjects reported previous diagnosis of RA or SLE as well as suspicion of RA, SLE or SSc; we confirmed the suspected diagnosis in 130, but the remaining 401 (75.5%) did not meet criteria for these diseases. Of those previously diagnosed with another illness, 183 (45.6%) met criteria for primary Sjögren’s syndrome. Rheumatoid factor was present in 31/71 patients with a previous diagnosis of RA compared to 185/830 without a history of RA diagnosis (P < 0.0001), while 118/147 with a diagnosis of SLE had positive ANA compared to 621 of 926 without the diagnosis (P = 0.012). Age also influenced prior diagnoses: people with suspected RA were older than those without the diagnosis (P < 0.0001), while patients with SLE suspicion were younger (P = 0.009). Presence of anti-Ro/SSA showed a gradient that was progressively less common in subjects who were pure SS, RA and/or SLE overlap with SS, misdiagnosed RA or SLE, and lowest in non-SS sicca.

S1.6
Primary Sjögren’s Syndrome is an Orphan Disease: A Meta-Analysis of Prevalence Studies in Europe

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Background: Primary Sjögren’s syndrome (pSS) is frequently considered as the second most frequent systemic autoimmune disease after rheumatoid arthritis. Several epidemiological studies have reported on the prevalence of pSS in Europe, but their results are highly heterogeneous. In Europe, a disease is considered rare (orphan disease) when it affects less than 1 individual in 2000 (or 50/100,000 inhabitants).

Objective: To assess the prevalence of pSS in Europe through systematic literature search and meta-analysis.

Methods: The systematic literature analysis was performed independently by 2 authors (DC and LC). The prevalence rates (PR) were analyzed according to study design (survey-based vs population-based studies), the case-ascertainment method (AECG criteria vs other disease definition), and the size of the background population. Meta-analysis was performed using the OpenMetaAnalyst software.

Results: Twelve articles reporting on the PR of pSS in European countries were retrieved. Their results were extraordinary heterogeneous (I > 99%, PR range 11.3 to 3790.1/100,000). The main factor explaining these discrepancies was study design. Survey-sample studies (which are considered of poor/moderate quality, n = 7) were performed on small populations (range 332–16,046) and concluded to the highest pSS PR: 148 to 3790/100,000. Conversely, population-based studies (n = 5) were performed on large populations (range 25,885–5,472,032) and concluded to much lower PRs: 11.3 to 86.4/100,000, overall 46.9/100,000 (95% CI 23.8–70.0). Sensitivity analyses were performed on population-based studies. The case-ascertainment method did not modify significantly PR: 3 studies used AECG criteria, with an overall prevalence rate of 49.0/100,000 (95% CI 9.3–88.7). The overall PR did not change significantly after excluding one study at a time.
Why and How: All You Need to Know About Parotid Gland Biopsies in Sjögren’s Syndrome

F. K. L. Spijkervet, A. Vissink, F. G. M. Kroese & H. Bootsma

Why: Salivary gland biopsy findings are a major criterion in both the AECG and ACR classification criteria sets for Sjögren’s syndrome (SS). Labial salivary gland biopsies have been shown to have restrictions compared to parotid gland biopsies for pathogenic research in SS. Furthermore, labial salivary gland biopsies are accompanied by 6–10% permanent sensory nerve morbidity, while no permanent sensory or motoric nerve morbidity is reported in parotid biopsies. In modern medicine, the risk on permanent morbidity from a diagnostic procedure should be avoided when possible. Parotid gland biopsies overcome the risk on permanent donor site morbidity and are compared to labial salivary gland biopsies superior in early detection and disease control of MALT-lymphoma associated SS. In addition, a parotid gland biopsy can be repeated in the same patient to evaluate new treatment options in the target organ. Last but not least it is useful to compare parotid gland saliva production, with ultrasound images in relation to histological parameters of the same parotid gland, the so called: saliva-ultrasound-biopsy axis.

How: Parotid gland biopsies can be taken under local anaesthesia during a 10-minute procedure, an operation time comparable to taking a labial salivary gland biopsy. An 1–1.5 cm incision below and mainly behind the earlobe is preferred for cosmetic reasons, without concessions to the diagnostic capacity of the parotid biopsy obtained. A short movie how to perform a state of art parotid biopsy technique will be shown. To our opinion, the parotid gland biopsy technique should be part of the toolbox of every SS-research team.


Towards the Standardisation of Salivary Gland Histopathology for Use in the Diagnosis of Primary Sjögren’s Syndrome and in Clinical Trials. Report of a Preliminary Workshop


Minor salivary gland (MSG) biopsy is widely used in the diagnosis of primary Sjögren’s syndrome (PSS) and evidence suggests it also has the potential to stratify patients, and to function as a biomarker in clinical trials. Despite this central role, it is apparent from anecdotal experience, audit and published data, that acquisition of tissue, processing and histological interpretation is variable. With the increasing number of clinical trials in PSS, we believe there is a need to (i) provide guidance on standardisation of salivary gland histopathology to ensure homogeneity of study populations, and (ii) understand its potential as a biomarker of response. A workshop was convened in Birmingham, UK, to address these issues. Day 1 focussed on the use of histopathology in the diagnosis of PSS and had attendees from rheumatology, histopathology and oral medicine. On Day 2, attendees and patient partners discussed the use of histopathology in clinical trials. Discussion addressed differences in the number of MSGs sampled and cutting levels in relation to sampling error, as well as variability in determining the presence of focal lymphocytic sialadenitis (FLS), the most characteristic feature of PSS, and in calculation of a focus score. Variation in the reporting of both germinal centre-like structures and
lymphoepithelial lesions, which may have prognostic importance and may stratification, was also discussed. In relation to clinical trials, the natural history of FLS and is reliability of assessment was discussed and a ‘core set’ of measures debated. This included the handling of non-FLS lymphocytic infiltrates, additional immunohistochemical stains, quantification of the size and organization of foci, and the need for observer standardisation. Presentations, break-out groups and round-table discussion resulted in a series of recommendations that will be refined through a Delphi process, and which will be a first-step towards standardisation.

S1.9
Salivary Gland Biopsies in Sjögren’s Syndrome: Focus Score is Related to Age
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Aims: Patients with Sjögren’s syndrome (SS) are usually classified according to the AECG criteria of 2002, requiring either positive autoantibodies (AA) or a positive focus score (FS) in combination with clinical findings and symptoms for inclusion. The newly suggested ACR criteria also require positive AA or FS. None of the classification criteria consider the age of the patient at inclusion. The aim of the present work was to investigate AA and FS related to age at the point of salivary gland biopsy.

Methods: One hundred and twenty seven consecutive patients referred mostly by rheumatologists for the evaluation of SS were included in the study after informed consent. Labial salivary gland (LSG) biopsies were taken from all patients by one oral and maxillofacial surgeon; at least 5 LSGs were excised from the lower lip following a standard procedure. Gland evaluation was performed by two oral pathologists and included area assessment, counting of foci, and evaluation of germinal centers (GCs). Aims: Patients and Methods: The 702 fictive vignettes created using data from 96 real cases of pSS for the study of development ESSDAI were used. The assessment by the 39 experts who participated to the development study of disease activity using a scale ranging from 0 to 10 was used as the “gold standard” for weighting domains in the robust regression model. The explanatory variables included all domain of ESSDAI except the biological domain. The ClinESSDAI was compared to original ESSDAI to assess whether it could be a surrogate of ESSDAI by assessing its reproducibility using ESSDAI as reference. The psychometric properties of ClinESSDAI (including construct validity, reliability and sensitivity to change) were then assessed and compared to ESSDAI, in the 395 patients of the EULAR Sjögren Task Force.

Results: Among the 127 patients referred for LSG biopsies, 12 were 70 years or older (mean age 74, range 70–84 years). One was excluded due to sarcoidosis. Five of the old patients had positive AA and were classified as having SS, only one having a positive FS. Among patients 40 years of age or younger at the age of LSG biopsy, (n = 19, mean age 34, range 18–40 years), 9 patients fulfilled the criteria, all having positive AA. In six of these young patients, a positive FS, varying from 1 to 4, was seen. GCs were seen in one young patient.

Conclusions: Six patients in the younger age group versus one in the older age group had a positive FS. Accordingly, SS developing at an older age may not be associated with lymphocytic infiltration. However, other morphological features besides FS (acinar atrophy, adipocyte infiltration) could be of diagnostic significance in these patients. We would like to suggest that the criteria for evaluating LSG biopsies in older patients should be discussed.

S1.10
Development of ClinESSDAI Score (Clinical EULAR Sjögren’s Syndrome Disease Activity Index) without Biological Domain: A Tool for Biological Studies
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Rational: ESSDAI is a validated and widely used activity index in primary Sjögren’s syndrome. In this context of biological studies that search new biomarkers, the presence of the biological domain may induce circular reasoning and collinearity of data.

Objective: To develop and validate the ClinESSDAI score, derived from the ESSDAI after deleting biological domain.

Patients and Methods: The 702 fictive vignettes created using data from 96 real cases of pSS for the study of development ESSDAI were used. The assessment by the 39 experts who participated to the development study of disease activity using a scale ranging from 0 to 10 was used as the “gold standard” for weighting domains in the robust regression model. The explanatory variables included all domain of ESSDAI except the biological domain. The ClinESSDAI was compared to original ESSDAI to assess whether it could be a surrogate of ESSDAI by assessing its reproducibility using ESSDAI as reference. The psychometric properties of ClinESSDAI (including construct validity, reliability and sensitivity to change) were then assessed and compared to ESSDAI, in the 395 patients of the EULAR validation cohort.

Results: In the multivariate model, each of the 11 areas was significantly associated with disease activity. The weight of the domains was slightly different from the fields of ESSDAI (table). The ClinESSDAI was an excellent surrogate of the original ESSDAI, since the ICC between the 2 scores were 0.98 [0.97; 0.98] for fictive vignettes and
0.90 [0.87; 0.92] in the EULAR cohort. Psychometric properties of ClinESSDAI were very close to that of ESSDAI.

**Conclusion:** The ClinESSDAI appears valid and very close to the original score. In this score, weights of some domains changed. This score allows an evaluation of disease activity independent of B-cell biomarkers. It will be useful in biological studies to avoid circular reasoning and colinearity of data, and in clinical practice when immunological tests have not been performed.

**S1.11 Performance of the Ocular Staining Score (OSS) vs. the van Bijsterveld Score in the Assessment of Sjögren’s Syndrome–Related Keratoconjunctivitis Sicca**

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**Background:** The assessment of keratoconjunctivitis sicca for the classification of Sjögren’s syndrome (SS) is done either by the van Bijsterveld score (vBS-AECG classification) or Ocular Staining Score (OSS-ACR classification). We present a direct comparison of the performance of the two scores.

**Methods:** We performed all tests for AECG and ACR classification in a multidisciplinary sicca clinic. Complete vBS and OSS evaluations were available for 716 participants of whom 587 were classified by AECG criteria as pSS (n = 257) or sicca (n = 330); 129 had other diseases or overlap/secondary SS. Analysis of concordance (vBS=OSS) or discordance (vBS≠OSS) was done for the 716 participants, but the correlations with classification criteria and clinical features were restricted to the pSS/sicca subset.

**Results:** The scores were concordant in 538 subjects (75.1%); discordance clustering in patients with higher vBS (P < 2.2x10⁻¹⁶). ROC comparison of vBS vs OSS showed the vBS cutoff of 4 has a sensitivity of 0.59–0.68 and specificity of 0.74–0.79; similar performance is observed for OSS scores of 4 and 5. Discordant participants were significantly more Ro (+), La (+), and biopsy (+) than the concordant cases (P = 1.7x10⁻¹⁰; 4.3x10⁻⁵; 1.8x10⁻⁶, respectively). The presence of the three additional points of the OSS was highly associated with participants meeting criteria for pSS (P = 1.73x10⁻¹³; (+) Schirmer’s (P = 6.5x10⁻¹⁰), Ro (P = 2.9x10⁻¹⁰), La (P = 8.8x10⁻⁶), biopsy (P = 3.4x10⁻¹⁰), and WUSF (P = 1x10⁻⁶). In all cases, the patches of confluent staining were the most highly associated with markers of disease severity and the corneal filaments were the best predictors of pSS.

**Conclusion:** The additional points of the OSS are good predictors of disease severity and pSS. Our results support a cutoff of ≥5 for the OSS; this would increase the specificity significantly without sacrificing sensitivity, a matter of great importance when applying the criteria for patient selection for clinical trials.
S1.12
DAS-28 Score is Useful to Evaluate the Effect of Biologicals on Articular Involvement in Primary Sjögren’s Syndrome

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Background: Arthralgia and arthritis are common features of disease activity in patients with primary Sjögren’s syndrome (pSS). Disease Activity Score including 28 joints (DAS-28) is validated for rheumatoid arthritis, but it is also used in other auto-immune diseases. This extrapolation of indications is very relevant in the current scientific debate on comparability of the efficacy of biologicals and biosimilars.

Objectives: To evaluate the usefulness of DAS-28ESR in pSS patients treated with rituximab or abatacept.

Methods: pSS patients treated with rituximab or abatacept within our previously reported studies1,2 were selected based on DAS-28ESR of ≥3.2 at baseline. Eighteen patients treated with rituximab were evaluated at baseline and at weeks 16, 24, 36, 48 and 60 after treatment. Thirteen patients treated with abatacept were evaluated at baseline and at weeks 4, 12, 24 (on treatment), 36 and 48 weeks (off treatment). All patients fulfilled the revised AECG criteria for pSS and were ACPA antibody negative. Generalized estimating equations were used to analyse DAS-28ESR. P < 0.05 was considered statistically significant.

Results: At baseline, the median DAS-28ESR score was 3.7 and 4.5 in the rituximab and abatacept groups, respectively. In the rituximab group, DAS-28ESR decreased significantly up to 48 weeks and returned to baseline values at week 60 (Figure 1A). In the abatacept group, DAS-28ESR decreased significantly up to 24 weeks and returned to baseline values at week 36 (Figure 1B).

Conclusions: DAS-28ESR is useful to evaluate biologic therapies in trials with pSS patients. DAS-28ESR has a good sensitivity to change and is valuable to monitor disease activity in pSS. DAS-28ESR has comparable course over time with the articular domain of the ESSDAI score. These findings are of particular relevance for the extrapolation of indications and comparison of biosimilars in the treatment of auto-immune diseases.

References:
Session 2. New Diagnostic Tools

S2.1

Is Salivary Gland Ultrasonography A Useful Tool in Sjögren’s Syndrome? A Systematic Review


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Objectives: Ultrasonography (US) has been shown to be a sensitive tool to diagnose major salivary gland abnormalities in primary Sjögren’s syndrome (pSS). This systematic review was performed to assess the metric properties of this technique.

Methods: PUBMED and EMBASE databases were searched. All publications between January 1988 and January 2013 were considered. Data were extracted from the articles meeting the inclusion criteria according to US definition of salivary gland scoring system and metric properties studied. The type and number of glands tested, study design, metric properties according to OMERACT filter (truth, discrimination, feasibility) were assessed.

Results: Of 167 publications identified with PUBMED and EMBASE, 31 met the inclusion criteria. The number of pSS patients varied among the studies from 16 to 140. The diagnosis of pSS was based in most of the cases on the American-European Consensus Group (AECG) classification criteria. The US examination was performed in suspected pSS only in studies in which the sensitivity ranged from 45.8% to 91.6% and specificity from 73% to 98.1%. The parotid and submandibular glands were most commonly studied. There was heterogeneity in regard to the definition of US in B mode and few studies used colour Doppler. Few studies reported US reliability and sensitivity to change in pSS.

Conclusion: US is a valuable tool to detect salivary gland abnormalities in pSS. Its reliability is poorly investigated, and there is considerable variation in the definition of US abnormalities. Further studies are required to validate and standardize US definition of salivary gland in pSS.

S2.2

Accuracy of Ultrasonographic Imaging of Major Salivary Glands in Diagnosing Sjögren’s Syndrome: A Systematic Review and Meta-Analysis

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Background: Ultrasonography is a promising technique in diagnosing Sjögren’s Syndrome (SS), but the diagnostic properties, sensitivity, specificity and diagnostic odds ratio (DOR) of ultrasonography are not established yet.

Objective: To systematically review studies on the accuracy of ultrasonography of major salivary glands in diagnosing SS.

Materials & Methods: Eight databases were searched. Two reviewers independently selected titles/abstracts and full-text articles. The quality of included articles was assessed with the QUADAS-2 tool. Publication bias, pooled sensitivity, specificity, DOR and 95% confidence intervals (95% CI) were calculated. Meta-regression analysis was performed.

Results: Thirty-seven studies and 33 ultrasonographic scoring systems were identified for quality assessment. High risk of bias was observed in ‘patient selection’, ‘conduct and interpretation of ultrasonography’ and ‘flow of patients and timing of tests’ in 78%, 70% and 51% of the studies, respectively. We included 29 studies in the meta-analysis. Publication bias was highly probable. Pooled sensitivity was 0.69 (95% CI: 0.67–0.71), specificity 0.92 (95% CI: 0.91–0.93) and DOR 33.89 (95% CI: 20.75–55.35). Significant heterogeneity was detected between studies (I² = 72.4%–89.1%). The low quality of studies and their clinical and methodological heterogeneity limit the interpretation of pooled effects. Meta-regression analysis revealed that studies with high risk of bias in ‘conduct and interpretation of ultrasonography’ and studies evaluating only the parenchymal homogeneity had a higher log DOR (1.09 and 2.49, respectively, P < 0.05).

Conclusions: Salivary gland ultrasonography has the potential to evolve to a viable, non-invasive alternative in the diagnostic work-up of patients with SS. However, due to the low quality of the included studies, further research is required to elucidate the properties of salivary gland ultrasonography in diagnosing SS.
Further Insights on Advanced Ultrasonographic Modalities in Primary Sjögren’s Syndrome

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Background: During the last years, there have been numerous reports and discussions concerning the value of major salivary gland ultrasound in patients with suspected primary Sjögren’s Syndrome (pSS). Modern ultrasonographic techniques, such as Virtual Touch Tissue Imaging (VTTI), Real Time Tissue Elastography (RTTE) and Virtual Touch Tissue Quantification (VTTQ), proved to be beneficial in the evaluation of salivary gland involvement in pSS.

Material and Methods: Diagnostic value of modern ultrasound techniques was evaluated in 50 patients with pSS, diagnosed according to the AECG criteria. Patients underwent ultrasonography of submandibular and parotid gland. Results of B-mode sonography (BMUS), RTTE, VTTI – each graded by using appropriate scoring systems – and VTTQ were compared to 50 patients with sicca symptoms but not fulfilling the AECG criteria. For the determination of intrarater reliability, three experienced sonographers scored every scan independently. For the determination of intrarater reliability, every scan was scored twice with a time interval of two weeks.

Results: In BMUS, 34/50 parotid glands in patients and 8/50 in control group had abnormal findings (P < 0.001). 38/50 patients with pSS showed abnormal findings in submandibular gland BMUS compared to 9/50 in patients without confirmed pSS (P < 0.001). In RTTE, parotid glands in patients with pSS did show higher scores (P = 0.012), whereas submandibular glands in the control groups showed higher scores (P = 0.001). VTTI did not show any significant difference (P = 0.080 and P = 0.750). In VTTQ, the parotid (mean: 2.99 m/s²) and submandibular glands (mean: 2.54 m/s²) showed significant higher values than parotid (mean: 2.16 m/s²) and submandibular glands (mean: 2.04 m/s²) in the control group (P < 0.001 and P = 0.008). Interrater reliability yielded Cronbach’s alpha values of 0.895 (BMUS), 0.751 (RTTE), 0.787 (VTTI) and 0.751 (VTTQ).

Conclusion: In contrast to BMUS, which proved to be useful in visualization and subjective assessment of structural glandular alterations in pSS, VTTQ enables the objective measurement of glandular stiffness and might therefore be considered in the diagnostic algorithm of pSS.

Automated Digital Analysis of Major Salivary Gland Ultrasound Images

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Background: Primary Sjögren’s syndrome (pSS) is an autoimmune chronic inflammatory disease mainly affecting the salivary and lacrimal glands, with symptoms such as dryness of the mouth and eyes as well as fatigue. The diagnosis is based on the objective findings of reduced secretion of saliva and/or tears, the detection of autoantibodies against Ro/SSA and/or La/SSB in serum, and the observation of focal mononuclear cell infiltration in minor labial salivary gland biopsies. In the recent years, interest in major salivary gland ultrasonography as a diagnostic tool for pSS has increased. Several scoring systems evaluating glandular homogeneity and echogenicity have been suggested, presenting a challenge for both researchers and clinicians. The aim of this study was to develop a reliable automated digital evaluation of ultrasound images, as a useful tool for the clinician and as an objective method for the researcher.

Methods: The parotid glands of patients (n = 26) fulfilling the AECG criteria (1) had previously been examined using a GE LogiqE9 with a linear high-frequency transducer (6–15 MHz) and the images evaluated using a simplified grading system (0–3) (2). The stored images were analysed digitally with a pilot version of the software developed for this study. Briefly, the software analyses local variability in grayscale values. The algorithm used for the digital analysis was developed using MATLAB (MathWorks, Natick, Massachusetts).

The patients were randomly selected from a previously characterized cohort (n = 97) where the ultrasound findings correlated with objective findings such as reduced saliva secretion, minor salivary gland inflammation and elevated autoantibody titers, as well as sicca symptoms of the mouth (3).

Results: Preliminary findings show an excellent correlation between the scores obtained with the simplified grading system (0–3) and the automated digital evaluation of local variability in grayscale values (P < 0.05, r = 0.816, n = 26). Mean digital score for images graded 0–3 was -9.833 (grade 0), -6.018 (grade 1), 2.751 (grade 2) and 6.850 (grade 3), respectively. Images will next be analyzed...
using a new algorithm to account for factors additional to the local variability in grayscale values.

**Conclusion:** The preliminary results of ultrasound image analysis show an excellent correlation between the automated digital analysis and the evaluation by a trained clinician. In future studies, automated analysis will enable an objective and reproducible analytic method for the researcher as well as provide a useful diagnostic and possibly prognostic tool for the clinician.

**References:**


**S2.5**

**Salivary Gland Ultrasonography: Towards a Simplified Scoring System for the Clinical and Functional Assessment of Patients with Primary and Secondary Sjögren’s Syndrome**


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**Objective:** To investigate whether salivary gland ultrasound (SGUS) evaluation may correlate with disease activity, salivary flow impairment, organ damage and patient-reported outcomes in primary (pSS) and secondary Sjögren’s syndrome (sSS).

**Methods:** Unselected patients with pSS and sSS (AECG 2002) were consecutively enrolled in this study and systematically evaluated. The ESSDAI and the SSDDI were used to assess disease activity and damage. All patients underwent sialometry and completed the ESSPRI. SGUS was carried out by the same radiologist recording: size, parenchymal echogenicity and inhomogeneity, number and location of hypo/anechoic area, presence of lymph nodes and calcifications. A modified version of the De Vita score was adopted.

**Results:** We included 139 patients: 100 with pSS (43 included at the diagnosis and 57 with an established disease), 21 with a concomitant Systemic sclerosis (sSS-Sc), 12 with Rheumatoid arthritis (sSS-RA) and 6 with Systemic Lupus Erythematosus (sSS-SLE). No differences between pSS and sSS were detected regarding mean SGUS score, fibrosis, presence of lymph nodes and calcifications. Significantly, higher SGUS scores were found in patients with a anti-Ro/SSA and anti-LA/SSB. The size of the glands significantly decreased over the time. We observed a moderate correlation between SGUS score and the ESSDAI ($r = 0.385$) and specifically, between the ESSDAI and the presence of hypoechoic areas (range: from $r = -0.400$ to $r = 0.504$). The correlation between the SGUS score and both the ESSPRI and the SSDDI was weak. However, salivary flow impairment moderately correlated with both the size of the glands and the presence of hypoechoic areas.

**Conclusion:** This study documented the value of the SGUS in the clinical and functional assessment of both pSS and sSS. Simplified scoring systems mainly focused on the presence of hypoechoic areas should enter in clinical practice to optimize the evaluation of SS patients.

**S2.6**

**Comparison of Scialoscintigraphic Uptake Among Bilateral Parotid and Submandibular Glands in Patients of Confirmed and Non–Confirmed Sjögren Syndrome (SjS)**

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**Background:** As for diagnostic tool of SjS, scialoscintigraphy is relatively non-invasive and useful one for the functional evaluation of salivary glands. Among parameters of the graphy, quantitative uptake in each of bilateral
parotid and submandibular glands is a major item of observation, both for diagnosis and for elucidating pathophysiology.

**Methods:** Patients who underwent scialoscintigraphy by order of the attending physician from 2011 to 2013 in our hospital were included. The patients were divided into those who fulfilled and not fulfilled ACR 2012 criteria. Till 20 min. after intravenous $^{99m}$Tc 370 MBq injection, RI accumulation in a region of interest (ROI) was observed at every 30 second, and peak accumulation data were used as uptake data. Uptake ratio (%) = uptake count at each ROI/total injected dose count*100.

**Results:** Sixty-four (55 females and 9 males, 23–90 years old) patients were included. Thirty-seven were diagnosed with SjS, and 27 were not (non-SjS). The uptake was significantly lower in SjS patients than in non-SjS patients, both in parotid and submandibular glands. When comparing uptakes of the 4 glands in non-SjS, although uptakes in parotid glands were higher than those in submandibular glands, no statistical difference was among the 4 (right parotid: 0.28 ± 0.28, left parotid: 0.24 ± 0.22, right submandibular: 0.14 ± 0.09, left submandibular: 0.16 ± 0.10). On the other hand, in SjS patients, uptake in submandibular gland was significantly lower than that in parotid gland in both sides (0.14 ± 0.16, 0.12 ± 0.13, 0.05 ± 0.06, 0.07 ± 0.06, in the same order).

**Conclusion:** Mean uptake ratio in SjS patients was around 0.13% in parotid, and 0.06% in submandibular gland; they were statistically significantly decreased to about a half level of those in non-SjS patients. Significantly lower uptake in submandibular gland than in parotid gland was in SjS patients, while no significant difference was in non-SjS patients. Submandibular glands would be involved more in SjS.

**S2.7 Sonoelastography Evaluation of Salivary Glands in Primary Sjogren's Syndrome**

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**Objective:** To explore the performance of sonoelastography (SE) in diagnosis and evaluation of primary Sjogren’s syndrome (pSS).

**Methods:** Sonoelastography examination of major salivary glands was conducted for 79 pSS patients and 39 disease control and 15 healthy subjects. Elastographic images were determined with a qualitative 4-point scoring method. The scores ranged from 0 to 16 obtained from the scores of bilateral parotid and submandibular glands. Receiver operating characteristic (ROC) curve was employed to evaluate the performance of qualitative elasticity scoring by sonoelastography, and the best cutoff value was determined.

**Results:** Scores of elasticity for pSS group were significantly higher than those of the non-pSS group ($P < 0.001$). The sum of the scores of all four glands provided the largest AUC-ROC (0.916, 95% CI 0.87–0.962), compared with that of bilateral parotid glands (0.857, 95% CI 0.794–0.919) and that of bilateral submandibular glands (0.783, 95% CI 0.704–0.863). The optimal cut-off value was 9 for combined evaluation of all four glands (81% sensitivity and 87% specificity, respectively). The elasticity scores of parotid glands were related with disease duration ($r = 0.244$, $P < 0.05$). Further, there was significantly difference between disease duration $≤5$ years and $>10$ years ($P = 0.007$) and between 5–10 years and $>10$ years ($P = 0.009$), but not for the disease duration $≤5$ years and 5–10 years ($P = 0.952$).

**Conclusion:** The qualitative assessment of salivary elasticity with sonoelastography is of diagnostic value in pSS. It is feasible in clinical practice.

**S2.8 Development of a Highly Sensitive Single-Cell Multiplex Technology for Early Detection of Sjogren’s Syndrome**

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One of the major missions of the Sjogren’s Syndrome Foundation is to develop better diagnostics to shorten the time of diagnosis for primary Sjogren’s syndrome (pSS) by 50% in five years. To accomplish this goal, we must discover signature biomarkers to predict early onset of Sjogren’s syndrome by 50% accuracy. Alternatively, we can build upon existing biomarkers with highly predictable rate using innovative technology with high sensitivity and specificity. To achieve this objective, we propose using Single-Cell Autoantibody Nanowells (SCAN) technology that can directly profile single B cells producing known antibody biomarkers such as anti-SSA/Ro52, anti-SSA/Ro-60, anti-SSB/La, and anti-muscarinic receptor type-III (M3R). SCAN is a soft lithographic technique that uses array of nanowells fabricated with polydimethylsiloxane to isolate individual cells for printing of corresponding molecules.
secreted by each cell. The array of nanowells is fabricated on glass slides containing 84,672 wells of 50 µm size nanowells or 248,832 wells of 30 µm size. In this study, lymphocytes were isolated from minor salivary glands (MSG) and peripheral blood of pSS, non-pSS, and healthy controls. Cells were labeled with Calcein (live cells) and CD20 + marker then placed into the nanowells. Capture or print slides were coated with immunoglobulins and hybridized by placement on the nanochip to capture autoantibodies such as anti-SSA/Ro52, anti-SSA/Ro-60, anti-SSB/La, and anti-M3R produced by individual B cells. Our preliminary data demonstrated that SCAN can simultaneously detect high frequency of anti-SSA/Ro-60, anti-SSB/La, and anti-M3R in MSG and peripheral blood cells of SS patients that are seronegative by conventional bioassays. These results indicate the high specificity and sensitivity that surpasses conventional techniques. Our data support a clear proof-of-concept that SCAN can be used to develop a diagnostic test that accurately detects biomarkers of SS and advances SS diagnoses.

**Materials and Methods:** A total of 81 Sicca and 50 SS patients were retrospectively evaluated for IgG4-RD-associated signs and symptoms. Additionally, IgG4 and total-IgG staining was performed on lip gland biopsies from all patients while IgG4 serum levels were evaluated on the Italian cohort.

**Results:** Only 2 of the Sicca patients had positive IgG4 staining but did not fulfill the diagnostic criteria. The serum analysis found 3 sicca patients with raised IgG4 (≥1.35 g/L) but none had a concomitant positive histology nor IgG4-RD related symptoms.

**Conclusions:** In our cohort of patients with sicca symptoms not fulfilling SS criteria no case could be accounted as an undiagnosed IgG4-RD based upon IgG4 staining on labial salivary gland biopsies. This study also suggests that in the absence of a clear clinical suspicious of an IgG4-RD, a routine IgG4 staining on labial salivary gland biopsies is not of clinical utility.

**S2.9 Routine Investigations for Circulating IgG4 and/or IgG4+ Salivary Gland Histopathology Fail to Reveal Undiagnosed Cases of IgG4 Related Disease in Sicca Patients not Fulfilling the Diagnosis of Sjögren’s Syndrome**

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**Background and objectives:** Immunoglobulin G4 (IgG4)-related disease (IgG4-RD) is an immune-mediated condition characterized by dense IgG4 + plasmacytic infiltration of diverse organs, fibrosis, tumefactive lesions and eosinophilia. Salivary and lacrimal glands are frequent targets that can present with enlargement and severe hypofunction. The diagnosis of IgG4-RD is challenging because of its serological and histopathological overlaps with other diseases, in particular, a differential diagnosis with Sjogren’s syndrome (SS) can sometimes be achieved only on the basis of a labial salivary gland biopsy.

In this study, we evaluated whether some of the patients with sicca symptoms not fulfilling SS classification criteria were in fact affected by IgG4-RD.

**S2.10 Characterization of Early and Progressive Autoimmunity in Sjögren’s Syndrome: The Incomplete Sjögren’s Syndrome Model**

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**Background:** Autoimmune diseases are often preceded by subclinical serologic and functional abnormalities that predate diagnosis by several years. The insidious and progressive nature of these incomplete syndromes results in a lag in diagnosis, preventive and therapeutic strategies, and likely contributes to damage accrual.

**Methods:** To develop a likelihood model of transition from incomplete Sjögren’s Syndrome (iSS) to primary SS (pSS), we identified 467 iSS patients and compared them to 364 pSS in a multi-disciplinary sicca clinic. We evaluated them for AECG classification measures, clinical and serological features (25 autoantibodies), and gene expression profiling of 64 IFN-inducible genes.

**Results:** Nineteen iSS subjects had anti-Ro antibodies (4.1%), and 33 (7.1%) were biopsy (+); the remaining 415 had non-SS-sicca. Ro(+)SS constituted a distinct subgroup from all Ro(-)iSS: they were younger (P = 0.001), less Caucasian (P = 1.95E10–7); more anti-La (+) (P = 8.8E10-6), and had more hypergammaglobulinemia...
(P = 0.02) and lymphopenia (P = 0.009). Furthermore, 14/19 had extraglandular manifestations. These differences in age, race, anti-La, hypergammaglobulinemia, and lymphopenia were also statistically significant in comparison with Biopsy(+)/iSS and Ro(-)/Biopsy(-)/iSS. Finally, Ro (+)/iSS subjects were significantly more similar to pSS than any other iSS. Another distinct subgroup of iSS subjects (n = 10) had (+) anti-dsDNA but did not meet criteria for SLE. Analysis of the IFN signature of all subjects is currently underway.

Conclusion: Patients with iSS may represent a forme fruste of SS, but it is plausible that some subsets, in particular Ro(+)/iSS and dsDNA(+)/iSS, will progress to primary SS and SLE or overlap syndromes, respectively. These iSS patients warrant careful follow-up to characterize the transition from iSS to full-blown pSS. Understanding the early events of disease has the strongest potential to lead to improvements in prevention, early diagnosis, and therapeutics.

S2.11 Long time Follow-up of Pediatric Sjögren’s Syndrome: The Rate of Patients who Developed Other Rheumatic diseases is not High

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Background: The natural disease course of Sjögren’s syndrome (SS) in children is unclear. A certain number of SS patients develop other rheumatic diseases. The rate of pediatric SS patients who develop other rheumatic diseases in their clinical courses is unknown.

Objective: To predict whether children with SS will probably develop other autoimmune diseases, we checked their clinical history and laboratory data.

Material and Method: Seventeen primary SS patients who visited Chiba University Hospital between 1989 and 2003, and followed up for the subsequent 10 years, were enrolled in the study. The data were collected retrospectively from clinical records.

Results: The anti-DNA antibody becomes positive in four patients. In three patients, the anti-RNP antibody also changed positively. Three patients fulfilled the criteria for juvenile systemic lupus erythematosus, and two patients for mixed connective tissue disease. They needed immune-suppressants. All of them were positive for the anti-SS-A antibody and had sicca symptoms.

Discussion: The rate of patients who developed other rheumatic diseases was only 17.6%, and they were no different in their symptoms at onset and with their laboratory data from other patients who did not. It is considered that they had SS and newly developed other rheumatic diseases.

Conclusion: We can diagnose SS in pediatric patients; however, we should consider that some of them could develop other rheumatic diseases. Further observation and analysis is needed to reveal any differences.

S2.12 Effect of Punctal Occlusion and Topical Cyclosporine on the Diagnosis of Sjögren’s Syndrome


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Background: The American College of Rheumatology (ACR) criteria, one of the two major classification systems for the evaluation of Sjögren’s syndrome (SS), uses the Ocular Surface Score (OSS) as part of the diagnostic criteria for keratoconjunctivitis sicca. The American-European Consensus Group (AECG) criteria include the van Bijster-veld score and Schirmer’s I test. Factors such as punctal occlusion and topical cyclosporine use may influence the ocular surface staining scores and Schirmer’s test and therefore influence the diagnosis of SS.
Methods: Cross-sectional, multi-specialty observational study of 533 patients with keratoconjunctivitis sicca (213 SS and 320 non-SS patients).

Results: SS patients with a low OSS and punctal occlusion were more likely to have a negative (normal) Schirmer’s test compared to those with a low OSS but without punctal occlusion (P = 0.018). There was a significant trend towards punctal occlusion and topical cyclosporine users to have a worse OSS.

Conclusion: Sjögren’s syndrome patients with punctal occlusion and a low ocular surface score were more likely to have a negative (normal) Schirmer’s test than those with a low ocular surface score and no occlusion. We hypothesize that this is due to increased tear volume creating a false-negative result which should be accounted for when considering the diagnosis of Sjögren’s syndrome, as a “false normal” Schirmer’s may be obtained in those patients with punctal occlusion. In our study, topical cyclosporine did not appear to have this effect on Schirmer’s testing. The worse OSS in those with punctal occlusion or cyclosporine use may represent selection bias. However, a prospective interventional study would be necessary to determine the alternative scenarios: that this is due to punctal plugs and cyclosporine truly having no effect on OSS, or that patients with xerophthalmia who undergo punctal occlusion subsequently increase their tear volume and “normalize” their OSS.

S2.13
Can Speckle Tracking Echocardiography Detect Left Subclinical Ventricular Dysfunction in Patients with Primary Sjögren Syndrome?

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Background: The aim of this study was to evaluate the left ventricular myocardial function using STE in patients with pSS and normal ejection fraction.

Methods: The study involved 49 outpatients who fulfilled the AECG criteria for pSS (14 males and 35 females; mean age 57 ± 6.9 years) and 49 healthy controls. CV risk profiles were assessed by means of ECG, conventional and stress trans-thoracic echocardiography with the measurement of CFR, carotid ultrasonography and PWV. Commercially available QLAB 9 software (Philips Medical System, USA) was used in order to assess end-systolic LV longitudinal strain (ε).

Results: None of the patients showed any signs or symptoms of CV disease, pulmonary involvement, or any other complication. The patients’ mean LVEF and E/A ratios were, respectively, 59.11 ± 6.35% and 0.94 ± 0.24, which were not significantly different from those of the controls (60.02 ± 6.04% and 0.96 ± 0.22); however, although within the normal range, their CFR was lower (median 2.70, IQR 2.40–2.90 vs 3.20, IQR 3.06–3.33; P < 0.0001). The results of the speckle tracking analysis were significantly different between the two groups, with global longitudinal strain deformation in the apical 4-chamber view (Long. ε 4c) being significantly lower in the pSS patients (Long ε 4c %: median 15.28, IQR 12.33–16.21 vs 19.84, IQR 19.34–20.40; P < 0.0001). Right and left pulse wave velocity (PWV) (PWV m/sec median 8.8, IQR 7.26–10.32 vs 6.86, IQR 6.66–7.10; P < 0.0001) and right and left coronary intima media thickness (cIMT) (cIMT mm: median 0.6, IQR 0.51–0.72 vs 0.53, IQR 0.50–0.60; P = 0.08) values were all higher in the pSS patients, but the differences of cIMT were not statistically significant.

Conclusion: LV myocardial longitudinal ε measured by means of speckle tracking echocardiography was impaired in pSS patients in the absence of any clinical evidence of CV disease and echocardiographic evaluations negative, suggesting a myocardial alteration.
Session 3. New Biomarkers for Sjögren's Syndrome

S3.1 Biomarkers in Sjögren’s Syndrome
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Primary Sjögren’s syndrome (pSS) is a chronic autoimmune disease with a diverse clinical picture, extending from a mild exocrinopathy to a severe, systemic, life-threatening disorder, whereas approximately 5% of patients develop B-cell non-Hodgkin lymphoma. Extraglandular systemic manifestations of pSS are common and can be roughly categorized as either periepithelial inflammation (lung or liver involvement, interstitial nephritis) or immune complex-mediated vasculitis (peripheral neuropathy, glomerulonephritis, purpura). The heterogeneity of clinical phenotypes and the diverse disease outcomes, which are most probably associated with complex pathogenetic mechanisms, hamper the effective treatment of patients with SS. Treatment of pSS patients remains symptomatic, and expensive biologic regimens have proven ineffective, thus highlighting the need for personalized therapeutic approaches. The development of such tailored therapeutic protocols requires the stratification of patients according to distinct clinical phenotypes and identification of the clinical, molecular and cellular biomarkers that characterize each phenotype and/or predict adverse outcome. Hence, research has lately been focused on the identification of biomarkers for pSS diagnosis and prognosis in affected tissues, saliva and serum. Indeed, patients predisposed to develop lymphoma can be identified at the time of pSS diagnosis by the expression of several clinical and laboratory parameters. Furthermore, histological parameters, such as extended infiltration of affected salivary glands, the organization of infiltrates to ectopic germinal centers and severe infiltration by certain types of mononuclear cells has been linked to adverse outcome. Finally, recent studies suggest that the proteomic and/or transcriptional expression pattern of salivary tissues and saliva, as well as exosomes, can discriminate patients with salivary dysfunction. These findings support the notion that the identification of biomarkers for disease diagnosis and prognosis is a feasible goal.

S3.2 Preliminary Proteomic Analysis of Saliva from Patients with Sjögren’s Syndrome
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Introduction: Xerostomia is characterised as a subjective dry mouth feeling, which can be a consequence of reduced or impaired salivary gland function. Xerostomia is a symptom of the complex exocrinopathy Sjögren’s syndrome (SS). The pathogeny of xerostomia in SS is only partially unravelled, but the pathological basis is an autoimmune epithelitis, which destroys the glandular parenchyma.

Objectives: The aim of this study was a preliminary proteomic analysis of the saliva from patients with SS.

Material and Methods: Unstimulated whole saliva was obtained during the morning from patients with SS, at the Hospital das Clínicas, SP. As controls, salivary samples from healthy volunteers were collected. The protein was precipitated with cetone 1:4 (v/v), and the protein concentration of the supernatants was determined by the BCA Protein Assay Reagent with bovine serum albumin (BSA) as a standard. The samples were stored at −80°C until use. The proteins were separated by SDS PAGE. All protein spots were cut out manually and digested with trypsin, reduced and alkylated, before their analyses by liquid chromatography/mass spectrometry (MS/MS) ion trap time-of-flight (LC/MS-ESI-IT-TOF). The protein identification was performed using a human data base (MASCOT).

Results and Discussion: Among the recognised proteins, we highlight some related to acute and chronic inflammation: Lactotransferrin, E3ubiquitin-protein ligase, Cystatin-S and Cystatin-SN. It was in line with the systemic immuno-inflammatory characteristics of SS. Conclusion: This preliminary analysis suggests that clinical alterations of the salivary glands might be reflected in the qualitative analyses of the salivary proteomes from these patients. Comprehensive analysis and identification of the proteomic content of human saliva may contribute to the understanding of oral pathophysiology and provide a foundation for the recognition of potential biomarkers of SS.
S3.3
Sjögren’s Syndrome Patients with Ectopic Germinal Centers Present with a Distinct Salivary Proteome
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Objectives: Clinical expression of Sjögren’s syndrome (SS) shows considerable inter-patient heterogeneity. In this context, per individual salivary proteomic profiles may provide a framework for the identification of disease-phenotype-driven biomarker signatures.

Methods: Using a 187-plex capture antibody-based assay, proteomic biomarker-profiles were generated from a SS-cohort comprising six clinically relevant disease phenotypes. Discriminant function analyses identified the most powerful biomarker signatures for correct recapitulation of a patient’s status with respect to hyposalivation and histopathological features of salivary gland inflammation. In addition, gene ontology (GO)-based network analyses allowed systematic interpretation of molecular patterns underlying these specific disease features.

Results: Presentation of hyposalivation was associated with significant alteration of a highly inter-correlated module of 20 biomarkers. Thereof a 4-plex signature allowed prediction of salivary gland function for more than 80% of the cases. With respect to histopathological features, the data showed most distinct alterations in context with the presence of ectopic germinal centers (GC). Selected from significant alteration in 13 analytes, pregnancy-associated plasma protein A, thrombospondin 1 and peptide YY recapitulated the presence or absence of tertiary lymphoid organization in 93.8% of the cases. Subsequent functional annotation of the proteomic profile as a whole showed specific chemotactic profiles and altered regulation of apoptotic processes to accompany ectopic lymphoid organization.

Conclusions: Accessible and repetitively collectable, biomarker-signatures from saliva harbor great potential for patient sub-classification and follow-up. The recently identified strong association between ectopic GC and increased risk of developing malignancies creates a strong incentive to consider the development of tools allowing periodical monitoring of patients with SS.

S3.4
Different S100 Protein Salivary Patterns Characterized Sjögren’s Syndrome Patients and Patients with Connective Tissue diseases and Sicca Syndrome
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Background: S100 proteins are calcium-binding proteins that induce a proinflammatory and thrombogenic response. Some members of the S100A protein family were found to be up-regulated in saliva and tears of Sjögren’s Syndrome (SS) patients.

Objectives: To evaluate the salivary presence of S100A8 and S100A9 in patients with sicca syndrome (ss) and autoimmune diseases.

Methods: S100A8 and S100A9 with its 2 isoforms (short and long) were studied using a proteomic approach in the saliva of 9 SS, 21 patients with connective tissue disease and (ss) and 20 patients without ss and with rheumatoid arthritis, systemic sclerosis and systemic lupus erythematosus and 16 healthy controls. The specimens were analyzed by HPLC coupled to electrospray-ionization mass spectrometry.

Results: Variable expression of S100 protein family members (S100A8, S100A9) was detected in patients with autoimmune disease with and without ss. S100A8 was detected in 6(66.7%) patients in the group with SS, compared with 4(25%) healthy subjects, 4(19%) ss patients, and only 1(5%) patient with autoimmune disease without ss. A statistically significant difference among the 4 groups in terms of frequency and levels of detection was calculated.

S100A9 long was detected in 7(77.8%) patients in the group with SS compared with 6(37.5%) healthy subjects and 6(28.6%) ss patients and compared with 5 patients (25%) with autoimmune disease without ss. A statistically significant difference among the 4 groups in terms of frequency and levels was found. S100A9 short was detected in 8 patients (88.9%) in the group with primary SS, in 13 (65%) patients in the group with ss, in 7 patients (33.3%) with autoimmune disease and in 8(50%) healthy controls, with statistically significant differences in terms of frequency and levels of detection.

Conclusions: The higher frequency and the increased level of S100 salivary proteins characterized patients with Sjögren’s syndrome and patients with sicca syndrome and autoimmune disease.
Patterns of Inflammation and Dysfunction in pSS: Identification of Proteomic Salivary Biomarkers Correlated with Different Disease Phenotypes


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Aims of this study were 1) to characterize salivary proteomic biomarkers in pSS by using LC-MS/MS after the removal of the high-abundance proteins in order to broaden the panel of candidate biomarkers for pSS. 2) to search for specific biomarkers associated with pSS phenotypes, defined on the basis of the focus score (FS) and on the variation of the salivary flow rate (USFR).

Methods: USFR was collected from 18 patients with pSS (AECG criteria, 2002). Six patients presented a high focus score (FS≥3) and normal ESFR (group A), six patients presented a FS≥3 and an USFR<1.5 ml/15 (Group B), and 6 patients a low focus score (FS<3) and USFR <1.5 ml/15 (group C). Six healthy volunteers (HV) represented the controls. High-abundance proteins were depleted using affinity and immunodepletion methodologies. A high-throughput liquid chromatography tandem mass spectrometry (LC-MS/MS) was used for the proteomic analysis. Principal component analysis (PCA) was utilized for statistical analysis.

Results: We identified a number of differently expressed low-abundance proteins in the saliva of pSS patients vs controls, including proline-rich proteins, calcium-binding proteins, profilin and other cell motion-related proteins, proteins involved in apoptosis, defence and inflammatory response. More specifically: 1) Group A samples vs HV: 7 proteins decreased and 66 increased 2) Group B samples vs HV: 18 proteins decreased and 159 increased 3) Group C samples vs HV: 16 proteins decreased and 164 increased. When we used PCA, these differentially expressed proteins distinctively discriminate between the four sample groups and especially distinguished HV from Group B and Group C samples.

Conclusions: Depletion techniques of high-abundance proteins increased visualization of low-abundance proteins unveiling novel biomarkers potentially related to specific phenotypes of pSS disease. Once validated, these candidate biomarkers might be useful to better clarify pSS pathogenesis and, ultimately, treatment.
Conclusion: This first high-throughput analysis of metabolic pathways disclosed a specific metabolomic signature of pSS allowing discriminating all patients with pSS from controls. This new and very potent means of metabolic analysis may help to increase our knowledge on the pathogenesis of pSS, identify biomarkers, and new therapeutic targets.

S3.7

Fatigue in Primary Sjögren’s Syndrome; A Proteomic Study of Cerebrospinal Fluid

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Background: Fatigue is a frequent and often disabling phenomenon that occurs in patients with chronic immunological diseases, such as primary Sjögren’s syndrome (pSS). The biological mechanisms that cause fatigue are largely unknown and hypotheses are conflicting. An important task is to uncover the pathophysiology and signaling pathways that lead to fatigue. The aim of this study was to discover cerebrospinal fluid (CSF) proteins potentially involved in the generation and regulation of fatigue using a liquid chromatography mass spectrometry (LC-MS/MS)-based proteomic approach.

Methods: From a cohort of 55 pSS patients that underwent lumbar puncture for research purposes, CSF samples from 10 subjects with high and 10 with low fatigue (measured by a fatigue visual analogue scale, FVAS) were selected for this study. To minimize abundant proteins masking the detection of the less abundant proteins, 14 high abundance proteins (HAPs) were depleted from the CSF samples prior to proteomic analysis. The depleted CSF samples were then proteolytically digested and analyzed by LC-MS/MS (LTQ Orbitrap). The resulting protein profiles from patients with high and low fatigue were compared by multivariate statistical analysis.

Results: A total of 1016 proteins were identified in the CSF samples. Supervised partial least square discrimination analysis (PLS-DA) showed that pSS patients with low and high fatigue can be separated based on their CSF protein profiles, and a set of 7 proteins were selected as the most promising discriminatory proteins. An unsupervised principal component analysis (PCA) was then performed to confirm that the majority of subjects with low versus high fatigue could be separated based on the reduced dataset, which included the 7 selected proteins only.

Conclusion: Seven proteins in the HAP depleted CSF proteome were identified which enables the discrimination between pSS patients with low or high fatigue. These proteins may have central roles in regulating fatigue.
S3.8
Antibodies Against Carbamylated Proteins in Serum from Patients with Primary Sjögren’s Syndrome
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Sjögren’s syndrome (SS) is an autoimmune rheumatic disease with organ specific features, characterized by the infiltration of mononuclear cells in the exocrine glands, primarily the salivary and lacrimal glands. The presence of autoantibodies towards intracellular Ro/SSA and La/SSB antigens is commonly found and serves as diagnostic criteria. In this study, we sought to evaluate the presence of antibodies against carbamylated proteins (anti-CarP) in patients with primary Sjögren’s syndrome (pSS).

Serum from 78 patients with pSS recruited from the Rheumatology Clinic at the Haukeland University Hospital in Bergen, Norway, and 77 gender and age matched healthy controls, recruited from the same geographical area through the Haukeland University hospital Blood Bank were used in this study. All pSS patients fulfilled the American-European Consensus criteria (AEC) for the classification of SS. Samples were analyzed for the presence of IgG antibodies against carbamylated FCS by ELISA. pSS patients had significantly increased level of aCarP antibodies in the serum as compared to healthy controls. Our results show that 27% of the serum of pSS patients and 7% of healthy controls analyzed were positive for IgG anti-carbamylated protein antibodies.

The reactivity against modified protein was over three times more frequent in patients as compared to healthy controls indicating that the presence of anti-carbamylated protein antibodies may serve as an additional predictive or diagnostic marker for Sjögren’s syndrome.

S3.9
Primary Sjögren’s Syndrome Patients Display Inflammasome Activation in the Peripheral Blood and the Salivary Glands that Correlates with Deficient Degradation of DNA
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DNA-sensing by cells leads to the activation of inflammasome complex and interleukin-1β release. We have recently shown that primary SS patients manifest increased serum levels of circulating nucleosomes and cell-free genomic DNA (CF-DNA) owing to impaired DNA degradation. Herein, we assessed whether the inflammasome is activated in the peripheral blood and salivary glands (SG) of SS patients. SS patients and non-SS controls were investigated for mRNA expression of inflammasome-related molecules (ASC, NLRP3, AIM2 and IL-1β) in PBMC and DNasel activity (SRED assay), circulating CF-DNA (RT-PCR), IL-1β and ASC protein (ELISA) in sera. SG biopsies were examined for the presence of CF-DNA (nucleic acids-immunostaining) and ASC protein expression (confocal microscopy). To evaluate CF-DNA capacity for inflammasome activation, healthy PBMC were treated with SS sera-derived CF-DNA and the inflammasome pathway was evaluated.

The PBMC of SS patients expressed significantly high ASC, IL-1β and NLRP-3 (all for \( P < 0.05 \) vs. controls, with ASC strongly correlating with ESSDAI scores; \( P = 0.007 \)). The sera of SS patients displayed increased levels of ASC protein (\( P < 0.0001 \), vs. controls, particularly high among patients with lymphoma, \( P = 0.009 \)) and of CF-DNA (\( P < 0.05 \) vs. controls) that correlated inversely with DNasel activity (\( r = -0.763 \), \( P < 0.01 \)) and positively with ASC levels (\( r = 0.654 \), \( P = 0.015 \)) in serum. In SG specimens, only SS patients manifested ample evidence of CF-DNA, as well as strong ASC protein expression (mainly epithelial cells and macrophages). Serum CF-DNA of SS patients induced high expression of inflammasome-related genes and IL-1β secretion by healthy PBMC.

SS patients display evidence of chronic inflammasome activation in the peripheral blood and the SG, likely due to the presence of lingering non-degraded CF-DNA. The clinical correlations indicate that inflammasome activation in SS patients likely has role in disease pathogenesis and may specify novel disease biomarkers.

S3.10
Two Year Results with IgG, IgA and IgM Antibody Specific to SP-1, PSP and CA-6 early Novel Antigens Compared to Classic Biomarkers in 2306 Dry Eye Patients
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Background/Purpose: Sjögren’s syndrome (SS) is a difficult disease to identify especially in early stages.
S3.11

Serum Free Light Chains are Associated with Clinical Disease Activity of Primary Sjögrens Syndrome in the Cutaneous, Biological and Renal Domains

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Background: Serum free light chains (FLC) are reported to be elevated in primary Sjögrens Syndrome (pSS) patients in comparison with healthy controls (HC). Consequently, FLC may represent potential biomarkers for disease activity in pSS.

Objectives: To investigate the relationship between serum FLC and disease activity in the UK primary Sjögrens syndrome registry (UKPSSR), a cohort of clinically well-characterised pSS patients and HC.

Methods: Sera from pSS patients (N = 599) and HC (N = 287) were analysed using Freelite (κ and λ), BAFF, β2M, and Combylite, a new assay which determines κ and λ FLC in a single test. Patients with an abnormal κ:λ ratio (<0.26/1.65) were excluded (N = 101). Clinical data, including ESSDAI, ESSPRI and immunological markers, were retrieved from the UKPSSR database. Logistic regression analyses using the Poisson distribution were performed to identify independent predictors of ESSDAI and its domains.

Results: κ, λ, FLC and cFLC were significantly higher in pSS patients than in HC. FLC levels correlated significantly with β2M, IgG and average Schirmer’s, but not with BAFF, age or disease duration. Poisson regression indicated that BAFF, β2M and FLC levels were predictive of disease activity, both with and without inclusion of the biological and haematological domains (all P < 0.001). BAFF, β2M and FLC levels were also predictive of cutaneous and biological domains (all P < 0.005), while BAFF and β2M levels were also associated with PNS and respiratory domains (all P < 0.05). β2M alone was predictive of muscular domain (P = 3.76E-03).

Conclusions: Serum FLC levels were increased in pSS patients in comparison with HC. FLC (κ, λ or cFLC) were predictors of clinical disease activity as determined by ESSDAI in the absence of the biological and haematological domains, and of the cutaneous, biological and renal domains individually.

S3.12

Anti–Carbonic Anhydrase II Antibody and Dysfunction of Renal Tubular Acidification in Patients with Primary Sjögren’s Syndrome


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Background: Sjögren’s syndrome is an autoimmune disorder characterized by chronic inflammation of exocrine glands as well as systemic impairments, including kidneys. Clinically, renal involvement often manifests as dysfunction of renal tubular acidification. Carbonic anhydrase II
(CA II), which could be found in the cytosol of renal tubular cells, is an enzyme that catalyzes the reversible hydration of carbon dioxide to generate a proton and a bicarbonate ion. Evidence shows that the autoantibody against CA II is related to the dysfunction of renal tubular acidification.

Objective: To elucidate the relationship between anti-carbonic anhydrase II (anti-Ca II) antibody and dysfunction of renal tubular acidification in patients with primary Sjögren’s syndrome (pSS) and analyze the clinical/laboratory characteristics of pSS patients with high anti-Ca II antibody level.

Methods: We measured the levels of anti-Ca II antibody in serum samples from 62 pSS patients by ELISA assay and 41 healthy controls (HC) and analyzed the relationship between anti-Ca II antibody and pSS patients’ clinical/laboratory data retrospectively, including hydrogen ion and bicarbonate levels in urine to evaluate the renal tubular acidification dysfunction.

Results: pSS patients were first classified into four groups according to the function of renal tubular acidification – hydrogen ion secretion insufficiency group, bicarbonate ions recovery failure group, combined dysfunction group and normal acidification group. It revealed that there was no significant difference of anti-Ca II antibody levels between HC and pSS patients with normal acidification. However, hydrogen ion secretion insufficiency group, bicarbonate ions recovery failure group and combined dysfunction group presented higher levels of anti-Ca II antibody ($P = 0.104, 0.0074$ and <0.0001, respectively). Then, we defined the anti-Ca II antibody (+) and (-) groups in 62 pSS patients by their levels of antibody and correlated them with their clinical and laboratory data. It was shown that compared with antibody (-) group, antibody (+) group patients had lower complement C3 and C4 level ($P = 0.016$ and 0.013, respectively), higher γ-globulin level ($P = 0.007$) and higher incidence of dysfunction of renal tubular acidification (100% vs 25%).

Conclusion: This study infers that anti-Ca II antibody level is related to the type of dysfunction of renal tubular acidification in pSS patients. Furthermore, anti-Ca II antibody may indicate the systemic damage of pSS, which could be a potential biomarker in the future.

S3.13

MiRNA Signature of Minor Salivary Glands Strongly Correlates with Salivary Flow Rate in Patients with Primary Sjögren’s Syndrome

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Aim of this study were 1) to extensively analyze the cellular microRNAome in primary Sjögren’s syndrome (pSS), in order to identify different patterns of microRNA expression able to distinguish control samples from those of patients with pSS 2) to explore whether a miRNA signature might correlate with the dysfunction of saliva production and secretion in pSS.

Methods: Samples: MSGBs were collected at the diagnosis from patients with suspected pSS. The final diagnosis of pSS was made according to the AECG criteria. Controls were represented by those patients with a suspected pSS who did not fulfill the AECG criteria. Unstimulated salivary flow (USFR) was assessed by sialometry. MSG biopsies were classified according to the focus scoring system. Since the presence of lymphocytic infiltrates can span from a wide range and might became a confusing factor in detecting the miRNAs involved in the loss of salivation process, the study was restricted to a subset of pSS patients with high focus score (FS>3) but different USFR. More specifically, MSG specimens were obtained from 3 pSS patients with low USFR, 3 with high USFR and 3 no-SS sicca syndrome. MiRNA gene expression profiling: MicroRNA expression profiles of the MSG specimens were analyzed using the TaqMan low-density Arrays A and B that allow to simultaneously examine the expression of 754 human cellular miRNAs.

Results: Of 754 miRNAs, 285 were significantly up-regulated and 25 were significantly down-regulated in patients compared with the controls. These expression differences vary accordingly to the severity of the USFR decrease, suggesting the existence of a consistent trend and a molecular signature associated with salivary gland hypofunction.

Conclusions: The results of this preliminary study support the amount of data suggesting that miRNAs may represent useful diagnostic biomarkers for pSS able to characterize different disease phenotypes and to shed new light on the mechanisms involved in the pathogenesis of SS.
S3.14

Metagenomic Analysis of Salivary Exosome Transcriptomes from Patients with Primary Sjögren’s Syndrome Using RNA-Seq

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Background: Saliva is a biofluid secreted by the salivary glands (SGs) that is critical for the health of the oral cavity and the commensal microflora. In Sjögren’s Syndrome (SS), an autoimmune exocrinopathy that affects the SG, changes in salivary biomarkers are not only useful as for diagnosis, but may also elucidate the mechanisms underlying SG dysfunction. The RNA content of saliva has been shown to be useful for monitoring the health of oral tissue and the oral microbiome, and next-generation sequencing (NGS) offers a high throughput method for comparing the salivary transcriptomes of SS patients and healthy volunteers (HV).

Methods: Total RNA was extracted from exosomes isolated from saliva of from 4 healthy volunteers and 4 primary Sjögren’s syndrome patients. The amount and the quality of the RNA were assessed using Nanodrop, Qubit and Bioanalyzer. The Ion Torrent Proton sequencer from Life Technologies was used for library preparation and sequencing according to manufacturer protocols. Reconstructed reads were aligned using the TMAP (Torrent Mapper) algorithm to the human hg19 reference genome. Read counts were generated using the HTSeq python module, and differential expression analysis was done in R using the DESeq2 package. Ingenuity Pathway Analysis (IPA) was used to analyze pathway enrichment and visualization. Reads that were unmapped or mapped poorly (MAPQ < 10) to the human genome were aligned to the 16s rRNA Reference Sequence curated by the Human Oral Microbiome Database (HOMD). Metagenomic profiles were analyzed and visualized using VIsualization tool for Taxonomic COMpositions of Microbial Community (VIT-COMIC).

Results: Expression pairing between miRNAs and their target genes using IPA showed significant enrichment for canonical pathways for pancreatic adenocarcinoma signaling, and estrogen-mediated S-phase Entry. However, in both patients and controls, nearly half of the reads (49% HV, 46% pSS) were left unmapped or mapped poorly to the human genome. Metagenomic analysis of these reads showed distinct profiles between patients and controls as well as shared signatures. The phylum Bacteroidetes was underrepresented in both groups, while clusters in the genera Klebsiella, Delftia, Leptotrichia, and Mycoplasma were common between them. Global similarity of the profiles measured as the Yue-Clayton theta for pairwise comparisons, however, showed distinct unsupervised hierarchical clustering of patients and controls.

Conclusions: Although further study is required, the unsupervised clustering of the microbiome profiles shows a potential diagnostic value for patients with SS. Salivary exosomes offer a rich, non-invasive source of biomarkers for studying SS. The RNA content of these exosomes may reflect not only the human pathophysiology, but also the changes in the oral microbiome.

S3.15

A Potential Microbiomic Biomarker for Sjögren’s from Oral Tissues

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Objective: To compare the supra and subgingival microorganisms of Sjögren’s subjects with those of periodontally healthy and periodontitis patients to identify microbiomic differences.

Methods: Separate supra and subgingival plaque and 8 oral soft tissue samples were taken from the mesial aspect of each tooth at baseline in 63 Sjögren’s, 59 periodontally healthy and 53 periodontitis subjects using sterile Gracey curettes and sterile buccal brushes. Samples were individually analyzed for their content of 40 bacterial species using checkerboard DNA-DNA hybridization. Levels and % DNA probe counts of each species were determined for each sampled site and averaged within each subject for supra and subgingival plaque oral soft tissue sites samples separately. Significance of difference for each species among clinical groups was determined using the Kruskal–Wallis test and adjusted for multiple comparisons.

Results: Mean % DNA probe count of 41 taxa in supragingival plaque samples from 57 Sjögren’s subjects and 53 control subjects The significance of differences among groups was sought using the Kruskal–Wallis test [and adjusted for multiple comparisons (Socransky et al. 1990)]. Species were ordered according to the complexes described by Socransky et al. (1998) V. parvula was highly significantly higher in Sjögrens subjects P < 0.001. This was not seen in edentulous, periodontal disease in our study population. Subgingival plaque was similar and did not contain any known periodontal pathogens. The microbial species on the soft tissues and in saliva of Sjögren’s subjects differ in quantity and proportions from that detected in...
control subjects. Reduced levels of the bulk fluid, saliva, have a major impact on biofilm formation on the soft tissues, on the hard tissues also. 

Conclusions: If validated in larger population, V. parvula along with our previously found proteomic biomarkers may help provide the diagnostic alphabet necessary for the early diagnosis.

S3.16
High Throughput Sequencing of Salivary Microbiota in Primary Sjögren’s Syndrome
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Sjögren’s syndrome is characterized by a reduced salivary flow rate. This is probably accompanied by changes in the microbiota. In Sjögren’s syndrome, little is known how the salivary microbiota is affected. Changes in the microbiota associated with reduced salivary flow have been studied by culture. Culture can only recover half of the bacteria in the oral microbiota. More reliable results are achieved with high throughput sequencing. That has not been done with oral bacteria in Sjögren’s syndrome.

We examined the microbiota of whole unstimulated saliva from 9 patients with primary Sjögren’s syndrome and 9 healthy controls using culture-independent 16S rDNA high throughput sequencing. Sequences were analyzed with MOTHUR v.1.34.4 and identified using the Human Oral Microbiome Database. 10 different phyla were detected in Sjögren’s syndrome, with DNA sequences predominantly assigned to Firmicutes (54%), Bacteroidetes (26%), Proteobacteria (9%), Actinobacteria (8%) and Fusobacteria (2%). Compared to control saliva, Sjögren’s syndrome displayed a higher proportion of Firmicutes (35% vs 54%), and lower proportions of 5 other major phyla.

Forty-nine genera were found in Sjögren’s syndrome. Streptococcus, Prevotella and Veillonella were most abundant (29%, 24% and 23%). There was a minor reduction in the numbers of genera identified in Sjögren’s syndrome (56 genera vs 49). Sequence abundance of the three major genera differed between patients and controls. While Prevotella was lower in Sjögren’s syndrome (30% vs 24%) an increase in Streptococcus (21% to 29%) and Veillonella (11% to 23%) was observed.

A reduced number of species was observed in Sjögren’s syndrome compared to controls. While P. melaninogenica\textunderscore oral\_taxon\_469 was the major species in controls (24%), V. atypica\textunderscore oral\_taxon\_524 was most predominant in the patient samples (18%).

Our results suggest a shift in the salivary microbiota of Sjögren’s syndrome compared to controls. High throughput sequencing can be a powerful tool towards better understanding oral health in Sjögren’s patients.

S3.17
Mucosal Dysbiosis in Sjögren’s Syndrome: Lessons Learned from Patients and Mouse Models
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Purpose: To investigate the ocular, oral and gut microbiome in patients with Sjögren syndrome (SS).

Methods: Conjunctival, tongue and fecal samples were obtained from 10 patients with primary SS meeting revised ACR criteria and controls. Severity of oral and ocular surface disease was graded. Conjunctival goblet cell density was counted in impression cytology. 16s ribosomal DNA gene sequencing was performed, and sequences were mapped to microbial databases. Relative abundance of phyla and genera and \( \alpha \) and \( \beta \) diversity of observed OTUs between groups were compared. C57BL/6 mice were subjected to desiccating stress (DS) in the presence of a cocktail of antibiotics.

Results: A low abundance ocular surface microbiome consisting of core phyla Actinobacteria, Bacteroidetes, Proteobacteria and Firmicutes was identified. There were no differences in \( \alpha \) or \( \beta \) diversity between normal and SS eyes; however, there was greater abundance of Firmicutes in the SS group. Compared to control, \( \alpha \) diversity was greater in the tongue and reduced in the stool in the SS group. Between group differences in relative abundance were observed with greater Streptococcus and Hemophilus and reduced Neisseria and Fusobacterium genera in the SS tongue and greater abundance of Blautia, Escherichia/Shigella, and Streptococcus and reduced Akkermansia, Subdoligranulum, Faecalibacterium and Prevotella in the SS stool. Distinct clustering of OTUs was seen in SS stool, and lower conjunctival GC numbers were observed in subjects with distinct clustering of stool genera. Antibiotic treated mice had lower \( \beta \) diversity of gut microflora and worse disease parameters in response to DS.

Conclusions: Minimal differences in abundance and diversity were found in the SS ocular microbiome. In contrast, SS stool showed a less diverse microbiome that
A Pilot Study of HPV Related Sialadenitis

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There is increasing recognition of an association between infectious agents and autoimmunity, with some evidence that Human Papilloma Virus (HPV) may be implicated with systemic lupus erythematosus. The salivary tissue from 14 Sjogrens syndrome patients (classified using the 2002 American-European consensus classification) and 16 controls (non-Sjogren’s patients) was examined using HPV L1 capsid immunohistochemistry, chromogenic in-situ high risk HPV-DNA hybridisation, and PCR typing for both high and low risk HPV strains, to determine the presence of HPV.

The glandular epithelium of seven Sjogren’s patients who has strongly positive evidence of Sjogren’s syndrome on histopathology (using Chisholm’s criteria) was found to have positive HPV L1 capsid staining associated with marked inflammation. The samples of control patients all stained negatively for HPV using HPV L1 capsid staining suggesting HPV may play a role in the aetiopathogenesis of patients with severe Sjogren’s syndrome. The serology, histopathology and clinical findings of these patients have been correlated to explain the possible association.

Adipokines in Primary (pSjS) and Secondary Sjögren’s Syndrome (sSjS)

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The emerging role of Adipokines is growing. This new family of soluble receptors (Leptin, Adiponectin, Resistin, Visfatin, Chemerin, Lipocalin2, SAA3, Vaspin and Omentin) secreted by adipocytes as well as immune cells act through endocrine, paracrine, autocrine, or yuxtacrine cross-talks in a wide range of physiopathological processes.

Method: We compared in non menopausal women suffering from pSjS and sSjS to RA the prevalence of metabolic syndrome(MtS) and adipokines levels. We look for MS and plasma levels of Leptin/Adiponectin and log-transformed. Homeostasis model Assessment for insulin resistance (HOMA-IR) was calculated. For continuous variables: T test and for discrete ones: X2 test. For association of MS and Adiponectin in pSjS and in sSjS we used multivariate regression models (MR), after adjusting the potential confounding variables.

Results: Fifty-one pSS pts and 75 sSS pts were included. The prevalence of MS was slight higher in sSS compared to pSjS not reaching statistical significance (36.5% vs 27.1%, P = 0.056). Adipokines levels were significantly higher in sSS compared to pSjS: adipokines (8.8 ± 5.2 vs 7.4 ± 4.5 log (Ug/ml, P = 0.009) and Leptin (3.1 ± 0.8 vs 2.8 ± 0.8 log(ug/ml P = 0.04). HOMA-IR was also higher in sSS (0.97 ± 0.63 vs 0.68 ± 0.81, P < 0.001). We found an association with MS using MR sSS (P = 0.04) and pSjS, CRP/DAS28 (P = 0.05), anti TNF alpha inhibitors therapy (P = 0.03) and active joint counts (P = 0.00010).

Conclusions: MS and adipokines correlate with increased burden of joint scores (pain and inflammation).

Faecal Levels of Calprotectin are Increased in Patients with Primary Sjögren’s Syndrome and Correlates with Disease Activity

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Objectives: FC is a validated biomarker differentiating inflammatory bowel disease from irritable bowel syndrome (IBS). We have evaluated faecal levels of calprotectin (FC) in patients with primary Sjögren’s syndrome (pSS) in reference to patient-reported indices of gastrointestinal discomfort as well as clinical features of pSS.

Methods: Forty-four consecutive pSS patients (median 62 (IQR 55, 69) years, 43 females), diagnosed according to the American-European Classification Criteria (AECC), were recruited from the open clinic at the Dept of Rheumatology, Malmö, Sweden and included in the study. Patients were evaluated by patient-reported indices of GI disease (the Rome III diagnostic questionnaire) and Sjögren’s syndrome (ESSPRI). Disease activity was evaluated by the ESSDAI. FC was measured in stool samples with a commercially available ELISA. For comparison, FC levels were evaluated in 21 healthy hospital workers (median age 56 (IQR 47, 58) years, 17 females).
pSS patients displayed significantly increased levels of FC in comparison with healthy controls (41 µg/g (20, 118) vs. 20 µg/g (20, 53); \( P = 0.036 \)). Of the pSS patients, 39% fulfilled the Rome III criteria for IBS. FC levels correlated significantly with the ESSDAI total score (\( r_s = 0.40; \ P = 0.008 \)) whilst not with the ESSPRI total score. Furthermore, patients fulfilling Rome III criteria for IBS had significantly increased ESSDAI (10 (7, 10) vs. 5 (0, 9); \( P = 0.028 \)) and ESSPRI total scores (7 (6, 9) vs. 6 (4, 7); \( P = 0.029 \)), but did not show any increased levels of FC (47 µg/g (20, 70) vs. 41 µg/g (20, 128); \( P = 0.415 \)).

Conclusion: FC levels are moderately increased in pSS patients and show a moderate association with disease activity. pSS patients fulfilling the criteria for IBS did not show increased levels of FC but had higher disease activity as well as increased scores in the Sjögren’s syndrome patient reported index. We suggest that FC has potential as an objective biomarker in pSS.
Session 4. Classical Sicca Symptoms – Oral and Eye

S4.1 Oral Manifestations of Sjögren’s Syndrome
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A key feature of Sjögren’s syndrome is its oral manifestations, a malevolent symptom for most Sjögren’s patients. Everyone, at some time in his or her life, suffers from dry mouth. But most of these forms of desiccation are transitory. The sicca symptoms Sjögren’s patients experience are chronic and long lasting. They do not reverse, but are progressive. These sicca symptoms have a typical pattern, being present during the night and at rest at the beginning, and causes more and more problems when salivary dysfunction progresses. As a result of this dysfunction, the mouth becomes sore and sensitive to acid and salty tasting foods. The saliva becomes scanty, thick,ropy and/or foamy. The cheeks dry, rough and/or coated. The tongue dry, fissured, pale or red, tingling, burning and/or sore. The palate dry and/or red. Teeth are prone to dental caries, related to the accumulation of dental plaque on smooth surfaces and cervical areas. The oral mucosa is prone to yeast infections. Salivary glands become swollen, obstructed, non-tender and/or painful. Development of lymphomas is rather frequently observed, particularly in parotid glands. Patients get a thirsty feeling and are in need of frequent sips of water and keep water at bedside at night, notwithstanding the rather inefficacy of water to moist oral surfaces. Taste and smell diminishes. Mastication becomes difficult. Speech is corrupted as the tongue may stick to the palate. Hoarseness may occur. All these problems are in need of prevention and/or effective therapy, which, unfortunately is not yet available in many cases. At the same time, the typical pattern of oral complaints may facilitate dentists and physicians in early detecting Sjögren’s syndrome as well as that features obtained during diagnostic work-up of a Sjögren’s patient may add in deciding what risks Sjögren’s patients are prone to and what therapies might be effective for the individual patient (personalized medicine).

S4.2 Keratoconjunctivitis Sicca in Sjögren’s Syndrome
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Purpose: Review clinical manifestations and advances in diagnosis and therapy of lacrimal keratoconjunctivitis in Sjögren’s Syndrome (SS).

Methods: Disease mechanisms, clinical manifestations and treatment recommendations will be reviewed based on evidence in literature, consensus recommendations and personal experience.

Results: SS causes tear instability, aqueous and mucus deficiency, ocular surface inflammation and epithelial disease, including goblet cell loss and accelerated desquamation resulting in an irregular and poorly lubricated corneal surface. Advances in diagnosis include methods to image the tear film and measure tear composition. Therapeutic advances include broader use of anti-inflammatory therapy, serum/plasma drops and therapeutic contact lenses to create a supportive corneal environment. Research has identified key inflammatory mediators that may lead to more targeted therapy.

Conclusions: Advances in understanding disease pathogenesis, diagnosis and management of SS KCS have improved quality of life and decreased risk of sight-threatening complications.

S4.3 Sutureless Cryopreserved Amniotic Membrane Transplantation Accelerates Ocular Surface Healing and Topographic Stabilization for Dry Eye Patients
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Background/Purpose: Severe dry eye disease (DED) in Sjögren’s syndrome (SS) may require months to stabilize despite rigorous multifaceted treatment and prevention interventions. Sutureless cryopreserved amniotic membrane (ProKera Slim, BioTissue) provides a natural protective bandage, high oxygen permeability, sustained topical applied medication release, wound healing, anti-inflammatory, anti-scarring, and anti-microbial properties with an expedient outpatient office insertion. This adjunctive
strategy may accelerate resolution of signs and symptoms of DED and stabilize topography, thereby rendering more accurate biometry for elective surgical procedures.

Methods: Forty patients receiving a ProKera for DED, accompanied by EBMD (epithelial basement membrane dystrophy) in 14 (35%), were retrospectively reviewed. Symptoms (SPEED test and history), signs (slit lamp exam, staining, TBUT) and corneal topography (OPD-III, Marco, Jacksonville, FL) were compared. ProKera lenses were observed weekly and removed after 5 to 15 days, or sooner if the amniotic membrane was absorbed.

Results: Thirty-eight (95%) showed improved fluorescein staining, 34 (76%) showed improved symptoms, and of 32 with topography data, 30 (94%) showed decreases in higher order aberrations including corneal coma. 16 (40%) noted some discomfort initially after insertion, and 2 (5%) complained of discomfort until the ProKera was removed. There were no significant device related adverse events.

Conclusions: The ProKera sutureless cryopreserved amniotic membrane platform provides a patient friendly office procedure that accelerates ocular surface healing in patients with DED.

S4.4
Permanent Bilateral Inferior Punctal Occlusion Surgery for Severe Dry Eye Disease: Long-Term Safety, Efficacy, Osmolarity and Recanalization Results in 844 Patients

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Background/Purpose: Severe dry eye disease (DED) with aqueous tear deficiency (ATD) responds to careful selection and execution of coordinated treatment interventions including punctal occlusion.

Methods: Eight hundred and forty-four consecutive patients with a previous permanent cautery punctal occlusion (PCPO) presenting for routine examination were assessed for the duration of occlusion, sign and symptom improvement following PCPO, complications including significant irritation, delayed healing, infection or significant lower lid deformity, and recanalization necessitating repeat PCPO. All patients underwent Schirmer’s tear testing, slit lamp biomicroscopy, vital staining, and previous temporary collagen punctal plug occlusion followed by silicone punctal occlusion trials prior to a recommendation for PCPO. The procedure was performed with a 4% lidocaine pledget followed by injection of 0.4 cc of 1% lidocaine into the everted tarsal surface 1 mm inferior to the punctal orifice. The hyfrecator needle tip was buried deep into the horizontal canaliculus and activated until a bubble with sizzling sound and retraction occurred at the punctum, then carefully retracted.

Results: The average follow-up post-surgery was 5.4 years. 829 (99%) noted improvement in signs of DED and 792 (94%) noted improved symptoms 3 months or more after PCPO. 16 (2%) noted significant irritation following the procedure, 7 (1%) had delayed healing in one or both lids, 25 (3%) were prescribed topical antibiotic ointment for a presumed infection, and 2 (<1%) complained of a lid deformity. 36 (2%) of 1688 eyes of 27 (3%) patients required repeat PCPO for recanalization of the punctum. Mean osmolarity was 304 mOsm/L compared to 315 mOsm/L (P < 0.05) in a matched group of DED patients without occlusion or plugs.

Conclusions: Permanent cautery punctal occlusion is a safe, well tolerated, and effective procedure for patients with severe DED which improves tear osmolarity. Complication rates and patient satisfaction are acceptable.

S4.5
Point of Service Antigen Hypersensitivity, MMP-9, Lipid Layer Thickness and Osmolarity Office Diagnostics Characterize Ocular Surface Co-Morbidity in Sjögren’s Syndrome

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Background/Purpose: Sjögren’s syndrome (SS) is characterized by severe forms of dry eye which present therapeutic challenges to eye care providers. Ocular allergy (OA), surface inflammation, lipid deficiency and hyperosmolarity are concomitant morbidities that may adversely impact treatment, all of which can now be quantified within minutes in the office setting. Specific interventions can be guided by the relative severity of each ocular surface disease component.

Methods: Hundred consecutive patients with a SPEED (Standard Patient Evaluation of Eye Dryness) score > 10 underwent diagnostic allergy testing, MMP-9, lipid layer interferometry and tear osmolarity. Slit lamp examination was categorized into one predominant clinical impression: OA, aqueous tear deficiency (ATD), lipid tear deficiency (LTD) or neurotrophic keratitis (NTK). Treatment was chosen by two means: point of service test result or clinical impression.

Results: Allergy testing revealed anergy in 40 patients, mild hyperactivity in 35, and severe hypersensitivity in 25. MMP-9 test was positive bilaterally in 39 patients, positive unilaterally in 3, and negative in 58. Lipid layer deficiency

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(<75 nm) was noted in 72 patients, and tear osmolarity was abnormal (>307 mOsm) in 92. The clinical impression by slit lamp biomicroscopy suggested allergy in 11 patients, ATD in 23, LTD in 59 and neurotropic disease in 7. A treatment plan based upon clinical impression alone was changed in 62 patients when diagnostic testing was also included in treatment selection.

Conclusions: The current data illustrate that point of service diagnostic testing allows more specific therapeutic recommendations for patients with moderately severe ocular surface disease. This algorithm impacts health care favorably: patients receive more accurate diagnosis and build revenues, and practices diversify their services.

S4.6
Interventions for Xerostomia of Sjögren’s Syndrome: A Meta-Analysis
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Background: There remains no meta-analysis focused upon the effectiveness of interventions for lessening dry mouth symptoms in patients with Sjögren’s syndrome (SS).

\textbf{Aim and Methods:} We searched the MEDLINE, Cochrane Central, EMBASE and AMED database for randomized controlled trials (RCTs) comparing any topical or systemic intervention for the treatment of xerostomia of pSS.

\textbf{Results:} Nine RCTs were included in the quantitative analysis. With respect to the primary outcome of lessening dry mouth symptoms, three RCTs on pilocarpine (pooled total of 638 participants) showed that 12-week use of pilocarpine tablets (5 mg four times a day) is associated with reduction in dry mouth symptoms (at least 55 mm difference in the VAS score from baseline) in significantly more patients compared with the placebo group (OR 3.58 [95\% CI 2.55–5.03]). However, the actual effect size remains unknown as mean VAS scores were not reported; also, it remain unclear whether measurements at endpoint were taken pre or post-dose. Two RCTs (n = 180) showed that 6 to 12 weeks use of cevimeline (30 mg three times a day) is more effective than placebo in lessening xerostomia (MD of 10.11 mm [95\% CI 2.52–17.69]). Again, it remains unclear whether the primary outcome was assessed before or shortly after administration of the medication.

With respect to the secondary outcomes of unstimulated whole salivary flow rate (uWSFR), pilocarpine, cevimeline and electrostimulation (two RCTs; pooled total n = 101) are associated with a short-term increase (60 min) in uWSFR with a mean difference of 0.22 ml/min, 0.08 ml/min, and 0.12 ml/min, respectively.

\textbf{Conclusion:} Pilocarpine and cevimeline can be superior to placebo in lessening xerostomia of pSS. It is, however, unclear whether benefits are limited to post-dose peak level time or also include baseline pre-dose trough level. Pilocarpine, cevimeline and electrostimulation all seem to determine a short-term increase in salivary flow compared to placebo.

S4.7
Salivary Electrostimulation in Primary Sjogren’s Syndrome: Results from LEONIDAS–1 Feasibility Study
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\textbf{Background:} None of the available treatments of hyposalivation of primary Sjögren’s syndrome (pSS) is supported by robust evidence, and adverse side effects are common. Neuro-electrostimulation of salivary glands has shown preliminary promising results in increasing salivary flow and reducing symptoms of dry mouth.

\textbf{Objective:} The aim of LEONIDAS–1 was to carry out feasibility work so to estimate feasibility and acceptability as well as to inform important aspects of the design of a substantive clinical trial (main study) that will investigate long-term effectiveness of salivary electrostimulation in pSS.

\textbf{Methods:} We designed a double-blinded feasibility study with central 1:1 randomization into 6 months of active vs sham electrostimulating therapy and a pragmatic samples size of 30 pSS patients. The feasibility parameters that we planned to estimate included ability to recruit, dropout rate, and compliance with the protocol and outcome measures (VAS, XI questionnaire, ESSPRI, uSFR). We also planned to look at changes in sicca symptoms scores and salivary function before and after treatment so to identify an indication that the intervention has indeed potential to be beneficial.

\textbf{Results:} Recruitment, retention rates and availability of usable (non-missing) scores for the outcomes measured at 6-month (endpoint) were high. At endpoint salivary flow increased more in participants receiving electrostimulation with respect to those who had sham stimulation (uWSFR = 1 g/15 min higher). The XI score reduced more in the active group than in the sham group by 3.3 points.

\textbf{Conclusion:} Our results suggest that a substantive RCT of salivary electrostimulation in pSS can be feasible and
Salivary Gland (SG) Fibrosis is Increased in Primary Sjögren’s Syndrome (pSS) and Correlates with Ocular Damage and Focus Score

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Hypothesis: SG fibrosis is a feature of pSS and not only a consequence of aging.

Methods: Two cohorts of European American patients with available hematoxylin/eosin stained SG biopsy slides from the OSSCORT repository were studied. All patients presented with sicca symptoms and were classified by AECG criteria (exclusions applied) as either pSS or not meeting criteria (DNMC). Discovery cohort totaled 66 patients (n = 36 pSS, DNMC n = 30) and validation cohort totaled 66 patients (n = 36 pSS, n = 30 DNMC). SG cross-sections were imaged and digitally reconstructed. Scorer was blinded to classification status. Fibrosis was quantified using a digital grid; sections were scored and reported as average percent area fibrosis per individual. Relationships of degree of fibrosis with age and clinical measures of dryness were evaluated using Spearman correlations. Logistic regression was implemented using average fibrosis scores to assess their predictive value for disease classification.

Results: Age was not different between pSS and DNMC in discovery, validation or meta-cohorts. Fibrosis was greater (Wilcoxon test) in pSS compared to DNMC SGs in discovery (P < 0.0001) and validation (P = 0.006) groups (Pmeta < 0.0001). In pSS, degree of fibrosis correlated (Spearman, unpaired two-tailed) with focus score (r = 0.300, P = 0.012), age (r = 0.24, P = 0.04) and van Bijnerveld score (r = 0.25, P = 0.03). In DNMC, degree of fibrosis correlated with age alone (r = 0.30, P = 0.04). In a predictive model including fibrosis and age, degree of SG fibrosis predicted disease classification irrespective of patient age with 70% accuracy in discovery cohort (fibrosis P = 0.0003, age P = 0.22), 64% accuracy in validation cohort (fibrosis P = 0.02, age P = 0.73) and 68% in meta-analysis (fibrosis P = 8.1x10^-5, age P = 0.482).

Conclusions: Degree of SG fibrosis is part of pSS pathology and not only a consequence of aging. Correlation with focus score implies a relationship between fibrosis and inflammation.
Could the Virus Persistence in Salivary Glands be a Feature of the Preclinical Sjögren's Syndrome?

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Research data continue to implicate viral infection in the cause of Sjögren’s syndrome, but there are no definitive studies incriminating a particular virus.

Objective: To study whether paramyxovirus persist in salivary glands of patients with Sjögren’s syndrome (SS). The main task of this research work was to investigate the prevalence (frequency) of the ribonucleic acid of paramyxovirus in the focus of autoimmune epithelitis in patients with SS.

Patients and Methods: Search for paramyxovirus in saliva collected using non-stimulated whole sialometry and minor salivary glands was made by reverse polymerase chain reaction (PCR) in 15 patients with primary Sjögren’s syndrome, 10 patients with secondary Sjögren’s syndrome due to rheumatoid arthritis and in 20 patients with sicca syndrome without autoimmune disease. All patients with SS met American-European classification criteria for Sjögren’s syndrome. Also, all patients were born before global MMR vaccination. Viral RNA was isolated using QIAamp MinElute Virus Spin kit (QIAGEN), the test was carried out according to the recommendations of the manufacturer. RT-PCR was performed using QIAGEN OneStep RT-PCR Kit (QIAGEN). Samples (15 μl) of the RT-PCR products were analyzed by electrophoresis on a 1% agarose (TopVision LE GQ agarose, Fermentas) gel precasted with ethidium bromide (0.5 μg/ml) in Tris-acetate-EDTA (TAE) buffer. Amplified DNA fragments were evaluated and photographed in BioDocAnalyze (Biometra, Germany).

Results: The paramyxovirus was identified in saliva and/or minor salivary glands of 18 patients with primary Sjögren’s syndrome and of 4 patients with secondary Sjögren’s syndrome due to rheumatoid arthritis. The frequency of the paramyxovirus persistence is 88% in SS. The paramyxovirus was not identified in patients with sicca syndrome without autoimmune disease. Conclusion The paramyxovirus persistence in salivary glands can be a feature of the preclinical Sjögren’s syndrome.

IP3R Deficit in Acinar Cells Underlies Loss of Salivary Gland Fluid Secretion in the Autoimmune Exocrinopathy, Sjögren’s Syndrome

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Sjögren’s syndrome (SS) is an autoimmune disease associated with lymphocytic infiltration and reduced fluid secretion from salivary glands that results in xerostomia. Intriguingly, some patients display substantial loss of saliva secretion despite minimal lymphocytic infiltration or tissue damage in the gland. Saliva secretion is regulated by neurotransmitter stimulated increases in cytosolic [Ca2+] of acinar cells, which are the primary sites of fluid secretion within the gland. Here we have examined agonist-stimulated acinar cell function in minor salivary gland biopsies from SS patients as well as healthy volunteers. Glands from SS patients with low inflammation showed acinar destruction within the small areas of infiltration and acini with relatively normal morphology in large areas where there was no infiltration. However, compared to acinar cells from healthy volunteers, acinar cells in these relatively normal areas of the gland displayed significant attenuation of carbachol (CCh)-stimulated volume decrease, which occurs due to flow of water out of the cells. In addition, both CCh-stimulated intracellular Ca2+ release and Ca2+ entry were also reduced in these cells relative to controls. In contrast to the poor correlation between inflammation and saliva flow, there was significant correlation between CCh-stimulated acinar cell responses and saliva secretion within the patient population. Importantly, IP2R and IP3R3, but not AQP5 or STIM1, were decreased in these areas of the gland. Notably, in IL14α transgenic mice, an animal model of SS, secretagogue-stimulated Ca2+ signaling was also markedly reduced in submandibular glands of 10 month old female mice, a time point where fluid secretion is attenuated in the absence of significant inflammation. This reduction in Ca2+ signals was also accompanied by a selective reduction in IP2R. Finally, Ca2+ signaling was also reduced in human minor salivary glands from healthy volunteers incubated with lymphotoxinα, a cytokine prominently elevated in both SS patients and in IL14α mice and implicated
mechanistically in the progression of the disease. Together, our findings reveal a novel IP$_3$R deficit that can account for the loss of saliva flow and xerostomia in SS patients who display low lymphocytic infiltration and minimal damage of salivary glands.

**S4.12**

**TNF-α and IL-20 Affect the Glandular Secretion by Modulating the Calcium-Signalling in Human Salivary Acinar Cells**

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**Objectives:** Primary Sjögren’s syndrome (pSS) is characterised by an autoimmune-mediated lymphocytic infiltration of the salivary glands and glandular hypofunction. We hypothesise that cytokines, like TNF-α and IL-20, modulate receptor functions and cause degeneration of acinar cells through production of oxygen radicals (ROS).

**Materials and Methods:** Labial salivary gland biopsies were obtained from 10 patients fulfilling the AECG criteria for pSS (mean age 44.4 ± 12.6 years) and 24 age-matched healthy females (48.2 ± 16 years). On collagenase-isolated labial salivary gland cells, the sialogogue-induced changes in the intracellular free calcium concentration ([Ca$^{2+}$]) and the production of ROS were measured using fluorescence microscopy, digital imaging and fluorescent dyes. Quantitative PCR was applied to measure mRNA.

**Results:** Acini from both pSS patients and healthy controls displayed a normal initial rapid rise in [Ca$^{2+}$], upon stimulation with acetylcholine. However, when pre-incubated with TNF-α, the initial peak in [Ca$^{2+}$], decreased markedly and the calcium-signalling in individual acinar cells in acini appeared to be unsynchronized. IL-20 also caused a rapid increase in [Ca$^{2+}$], as well as generation of ROS. We also found mRNA for TNF-αR1 in salivary acini.

**Conclusions:** TNF-α affects calcium-signaling in labial acinar cells diminishing the initial calcium spike and thereby the secretion process. The TNF-α action appears to be mediated through its activation of PKC since a PKC activator (PMA) can mimic the TNF-α response, and an inhibitor of PKC (staurosporine) can block the TNF-α-mediated effects on the peak of [Ca$^{2+}$]. PKC activators such as PMA and IL-20 cause synthesis of ROS, and thereby possibly enhance the stress on the acinar cells. IL-20 causes ROS formation. Overall, TNF-α and IL-20 appear to modulate calcium-signaling in acinar cells through interactions with intracellular pathways and ROS production leading to diminished secretion.
Fatigue

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Fatigue – an overwhelming sense of tiredness, lack of energy, and a feeling of exhaustion - occur in all chronic inflammatory diseases, cancer, and numerous other diseases. In systemic lupus erythematosus and in primary Sjögren’s syndrome, 70–80% of patients suffer from chronic fatigue to such an extent that it affects daily life.

Although social and psychological factors may play a role, there is growing evidence that biological factors are involved in generation and regulation of fatigue. Activation of the innate immune system by invading pathogens, autoimmune diseases, cancer, or other “danger-signals” may be a common denominator for generation of fatigue.

A model for the understanding of this is the “sickness behavior” model in animals. When animals get infections (inflammation) they exhibit a behavior characterized by withdrawal, inactivity, drowsiness, and low intake of food and water. This behavior is signaled by IL-1 in the brain and has many similarities with chronic fatigue in humans.

In patients with primary Sjögren’s syndrome, we have shown that increased activation of the IL-1 system as measured in cerebrospinal fluid is associated with increasing levels of fatigue and that fatigue improves when the patients receive IL-1 inhibitors. A number of therapeutic studies in other chronic inflammatory diseases indicate that biological agents in general exert a beneficial effect on fatigue.

Another possible cause of fatigue in chronic inflammation is oxidative stress. Oxidative stress is a term used to refer to an imbalance with increased production of free radicals compared to the compensatory mechanisms that inhibit or repair the damage that may occur. Mechanisms that protect against cellular stress may be “fatigue generators” that act on the brain through unknown pathways.

Recent studies indicate that fatigue has a genetic basis, and it is therefore possible that genetic polymorphisms and genetic regulation determine the severity of fatigue in the individual subject.

Interstitial Lung Diseases, Current Definitions and Diagnostic Challenges

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The idiopathic interstitial pneumonias (IIPs) are a diverse group of diseases of unknown cause, characterized by increased inflammation and production of interstitial fibrosis. The most common syndromes are idiopathic pulmonary fibrosis (IPF), non-specific interstitial pneumonia (NSIP), cryptogenic organized pneumonia (COP), acute interstitial pneumonia (AIP), desquamative interstitial pneumonia (DIP), respiratory bronchiolitis interstitial lung disease (RB-ILD), and the lymphocytic interstitial pneumonia (LIP). Idiopathic pulmonary fibrosis is characterized by a fibrotic pattern discernible on high-resolution CT scans as “usual interstitial pneumonia” (UIP).

In up to 25% of the cases, the changes typical for IIPs are seen in conjunction with a rheumatological disorder, in which case the interstitial lung disease is often termed connective tissue interstitial lung disease (CT-ILD). Rheumatoid arthritis is most often associated with a UIP pattern of lung disease, whereas for instance systemic lupus erythematosus and systemic sclerosis is more commonly associated with NSIP.

Sjögren’s syndrome is also commonly associated with NSIP; however, the rare LIP is in 25% of cases associated with Sjögren’s syndrome for reasons unknown.

The diagnostic challenges when faced with interstitial lung disease are considerable. However, both the prognosis and treatment strategies are dependent upon an accurate diagnosis. This talk will outline some of the key diagnostic challenges when faced with interstitial lung disease today, and briefly discuss treatment options and future directions.

Fatigue is Closely Associated with Quality of Life in Patients with Primary Sjögren’s Syndrome and Younger Age, Autonomic Dysfunction and Xerostomia are the Major Determinant of Severe Fatigue

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Objective: The aim of this study was to investigate the relationship of fatigue severity and other clinical charac-
teristics in primary Sjogren’s syndrome (pSS) and to determine factors contributing to fatigue.

**Methods:** We analyzed 105 participants from the Korean Initiative of primary Sjogren’s Syndrome (KISS), a prospective pSS cohort. Fatigue was assessed with the fatigue domain of European League Against Rheumatism Sjogren’s Syndrome Patient reported index (ESSPRI). Severe fatigue was defined as an ESSPRI fatigue ≥ 6. Autonomic dysfunction was assessed by heart rate variability test and defined as standard deviation of normal to normal RR interval (SDNN) < 30 ms in age < 50 year-old patients, SDNN < 20 ms in age ≥ 50 year-old patients or root mean square of standard deviation < 10 ms.

**Result:** The median total ESSPRI score was 5 (IQR 4–6). Forty-two percent of patients reported their fatigue score ≥ 6. Younger and premenopausal patients presented more fatigue (P = 0.04 and P = 0.015, respectively). Dysautonomia was more frequently observed in patients with severe fatigue (22 (50%) and 17 (28%), respectively, P = 0.025). Moderate to severe xerophthalmia was observed in 71.4% of patients (n = 30) with severe fatigue, whereas ocular stain score and the presence of meibomian gland dysfunction were not different according to the fatigue severity. Higher xerostomia inventory score (P = 0.011) was observed in patients with severe fatigue. Multivariate analyses identified younger age (Odd ratio (OR) 0.947, 95% confidence interval (CI) 0.904–0.992), dysautonomia (OR 2.84, 95% CI 1.045–7.18) and xerostomia inventory (OR 2.151, 95% CI 1.181–4.247) as being associated with an increased risk of severe fatigue. ESSPRI fatigue score was correlated with EQ-5D by time trade off values and vas score (r = −0.371 [P < 0.001] and r = −0.357 [P < 0.001], respectively).

**Conclusion:** In patients with pSS, younger age, xerostomia and dysautonomia increase the risks for severe fatigue.

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**S5.4  Is Fatigue Immune-Mediated in Primary Sjögren’s Syndrome? Data from the Prospective ASSESS Cohort**


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10Internal Medicine, Avicenne Hospital, 11Internal Medicine, Cochin Hospital, Paris, 12Rheumatology, University Hospital, Montpellier,
13Rheumatology, University Hospital, Rennes, 14Rheumatology, University Hospital, Orléans, 15Internal Medicine, Lariboisière Hospital, Paris,
16Rheumatology, University Hospital, Strasbourg, 17Rheumatology, University Hospital, Rouen, and 18Epidemiology center, Hôtel Dieu Hospital, Paris, France

**Rationale:** Little is known about the pathogenesis of fatigue, a hallmark of primary Sjögren’s syndrome. The respective contribution of depression, disease activity, or auto-immunity itself has never been studied ever since international disease activity scores were established. Due to this uncertainty area regarding the driver(s) of fatigue, some reluctance can be observed about including the improvement of fatigue, a component of the EULAR-Sjögren’s Syndrome patient-related index (ESSPRI), as a primary outcome in clinical trials of biologics. We therefore investigated the association between fatigue and other characteristics of the disease in a large multi-center prospective cohort.

**Methods:** Assessment of Systemic complications and Evolution of primary Sjögren’s Syndrome (ASSESS) is a French multi-center 5-year prospective cohort which included 395 patients. At baseline and every year of the follow-up, systemic disease activity and patient-related outcome are evaluated by the ESSDAI and ESSPRI (the average of patient’s visual analogic scales (VAS) for fatigue, pain and dryness), respectively, and patients fill questionnaires including the Hospital Anxiety and Depression (HAD) scale. Unstimulated salivary flow and Schirmer’s test are also assessed. Serum markers of B-cell activation, BAFF, interferon (IFN)-inducible chemokines, and IL-21 were assessed at baseline.

**Results:** Median (25th–75th) fatigue VAS was 6 (4–8). Fatigue VAS was correlated with dryness VAS (r = 0.46, P < 0.0001) and pain VAS (r = 0.54, P < 0.0001). Fatigue VAS was not correlated with age or disease...
duration, systemic disease activity assessed by the ESSDAI, unstimulated salivary flow or Schirmer’s test. Fatigue VAS was not significantly higher in anti-SSA-positive patients. Fatigue VAS was significantly higher in patients with depression (defined by a HAD subscale for depression (HAD-D) ≥ 8 (among the 341 patients with available data for HAD), median fatigue VAS of 7 (6–9) in the 114 patients with depression and of 6 (4–8) in the 227 patients without depression, P < 0.0001). In order to analyse the potential role of auto-immunity, we subsequently focused our analyses on patients without depression. In patients without depression, fatigue VAS remained not associated with anti-SSA, the ESSDAI, unstimulated salivary flow and Schirmer’s test. Among patients without depression, a high fatigue VAS (≥ 5), the median of fatigue VAS in these patients) was not associated with a higher level of serum IFN-inducible chemokines (CCL2, CXCL10, CCL19), B-cell activating cytokines such as BAFF or IL-21, markers of B-cell activation (total Ig levels, free light chains of immunoglobulins, rheumatoid factor levels). Conversely, some of these markers (serum IgG, kappa and lambda free light chains, beta2-microglobulin, and CCL19) were significantly lower in patients with a high fatigue VAS.

Conclusion: Data from the ASSESS cohort confirm the diversity of factors associated with fatigue in primary Sjögren’s syndrome, including depression and the two other main symptoms, dryness and pain. Even when focusing on patients without depression, no association was observed between fatigue and systemic disease activity, autoantibody profile, serum surrogate markers of the IFN signature and of B-cell activation. Further work is necessary to determine the existence of potential other immunological drivers.

**S5.5**

**A Transcriptional Signature of Fatigue Derived from Patients with Primary Sjögren’s Syndrome**


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**Objectives:** Fatigue is a debilitating condition with a significant impact on patients’ quality of life. Fatigue is frequently reported by patients suffering from primary Sjögren’s Syndrome (pSS), a chronic autoimmune condition characterised by dryness of the eyes and the mouth. However, although fatigue is common in pSS, some patients suffer minimal fatigue, providing an excellent opportunity to explore the potential underpinning biological mechanisms.

**Methods:** Whole blood from 133 pSS patients and 33 healthy controls collected by the UK primary Sjögren’s Syndrome Registry was used for whole genome micro-array. The resulting data were analysed both on a gene-by-gene basis and using pre-defined groups of genes. Finally, gene set enrichment analysis (GSEA) was used as a feature selection technique for input into a support vector machine (SVM) classifier. Classification was assessed using area under curve (AUC) of receiver operator characteristic and standard error of Wilcoxon statistic, SE (W).

**Results:** No genes were found to be individually associated with fatigue. However, GSEA identified 19 pathways enriched in the high fatigue patient group. Analysis revealed that these enrichments arose from the presence of a subset of 55 genes. A radial kernel SVM classifier with this subset of genes as input displayed significantly improved performance over classifiers using all pathway genes as input. The classifiers had AUCs of 0.866 (SE(W), = 0.002) and 0.525 (SE(W) = 0.006), respectively.

**Conclusion:** Systematic analysis of gene expression data from pSS patients discordant for fatigue has identified a 55-gene signature predictive of fatigue level. This list represents the first step in understanding the underlying pathophysiological mechanisms of fatigue in patients with pSS.

**S5.6**

**Machine Learning of Fatigue-Related Clinical Features in Primary Sjögren’s Syndrome**

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**Objectives:** Fatigue is a profound symptom in several autoimmune diseases and can considerably affect patients’ health-related quality of life. Several studies have revealed the complex, multi-dimensional nature of fatigue and have attempted to investigate its biological basis in search for better therapeutic approaches. Here, we investigate fatigue-related clinical features in primary Sjögren’s Syndrome using machine learning.
Methods: Data were collected for 150 patients from the UK primary Sjögren’s Syndrome Registry. These data included ESSDAI sub-domains, SSDDI damage score, current treatments, comorbidities and other objective clinical features. Stepwise linear discriminant analysis was used to select features for the optimal separation of patients with high (n = 88) and low (n = 62) fatigue as measured using a visual analogue scale of 0–100. Support vector machine (SVM) classifiers were then developed using the selected features and a range of kernel functions. Classifiers were assessed using the area under curve (AUC) of receiver-operator characteristic curves and standard error of the Wilcoxon statistic (SE(W)).

Results: The SVM classifier with linear kernel function achieved an AUC of 0.675 with all 57 features as input (SE (W) = 0.0019). SLDA selected nine features comprising the ESSDAI constitutional and respiratory domains, the SSDDI index, methotrexate and NSAIDs treatments, and the serum variables ESR, creatinine, paraprotein and elevated lambda free light chains. Using only the selected features as input, the final classifier achieved a statistically significant improvement with an of AUC of 0.725 (SE(W) = 0.0017).

Conclusion: Fatigue is a prominent and disabling symptom in pSS patient’s lives. However, its study is non-trivial due to confounding factors. Using machine learning nine clinical features have been identified which are collectively predictive of fatigue level.

S5.7
Sicca Symptoms Induce Fatigue, Anxiety, Depression and Decreased Quality of Life, Irrespective to Sjögren’s Syndrome Diagnosis

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Objective: To compare the quality of life, depression, anxiety and fatigue in primary Sjögren’s Syndrome (pSS) versus non-Sjögren’s sicca patients in a prospective cohort.

Methods: Patients addressed to a multidisciplinary visit for suspected pSS were included in the single-center Brittany cohort between November 2006 and December 2013. Patients without sicca symptoms were excluded from this study. All patients benefited of the same standardized investigations. PSS and non-Sjögren’s sicca diagnoses were based on evaluating physician opinion. Three validated questionnaires were sent to the patients in order to evaluate quality of life (SF-36), fatigue (MFI), depression and anxiety (HAD).

Results: Study population consisted in 95 patients, 55 (57.9%) with pSS and 40 (42.1%) with non-Sjögren’s sicca patients. Patient’s characteristics were similar in term of sex, age, disease duration and sicca symptoms. Objective tests for dryness (Schirmer’s test and salivary flow rate) were significantly more often abnormal in patients with pSS. All the patients had impact on SF-36, HAD and MFI score with no differences between both groups. Anxiety was more present than depression in both groups. Vitality was the most affected domain of the SF-36 as was the General/Physical fatigue dimension for the MFI questionnaire.

Conclusions: Sicca symptoms induce severe impact on SF-36, HAD and MFI scores regardless of objective tests and diagnosis of pSS. Anxiety is more frequent than depression and should be taken into account when managing patients with sicca symptoms.

S5.8
Impact of Symptoms and Disease Activity on Quality of Life in Primary Sjögren’s Syndrome

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Objective: To investigate the impact of symptoms and disease activity on HRQOL in primary Sjögren’s syndrome (pSS).

Methods: In a case–control study, 77 women with pSS (AECG) were evaluated through the questionnaires FACIT-Fatigue, ESSPRI, ESSDAI, SF-36 and WHOQOL-BREF. Healthy controls (n = 77) answered HRQOL tools. Mann–Whitney, t-test and Spearman’s test were used.

Results: pSS patients and healthy controls were matched for gender and age (52.3 (±9.03) vs 52.2 (±8.91)) and gender. Patients had lower employment rate (36.4% vs 62.3%, P = 0.00) and higher work disability (10.4% vs 1.3%; P = 0.04). The mean scores of ESSDAI, ESSPRI and FACIT-fatigue were 3.34 (±4.61), 6.58 (±2.29) and 26.17 (±11.02), respectively. SF-36 and WHOQOL-BREF scores were significantly lower in patients with pSS (P < 0.001) except WHOQOL-BREF Environment domain (P < 0.07). There was no correlation between disease activity and HRQOL. FACIT-fatigue and ESSPRI total showed significant correlation with all domains of SF-36 and WHOQOL-BREF, while ESSPRI pain domain showed the highest correlations (table 1).
Conclusion: Fatigue, dryness and mainly pain, but not disease activity, are associated with low quality of life. These findings suggest that management strategies to reduce symptoms, especially pain are important to improve quality of life in pSS.

§5.9

Heat Shock Proteins and Fatigue in Patients with Primary Sjögren’s Syndrome
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Background: Heat shock proteins (Hsps) are important molecules serving protective functions for cellular life in a wide range of cellular stresses. Hsp released into extracellular space may act as signalling molecules that induce cellular defence mechanisms in adjacent or remote cells, both in the central nervous system and peripheral organs. Chronic fatigue occurs frequently in patients with cancer, neurological diseases and inflammatory diseases like primary Sjögren’s syndrome (pSS), but the biological mechanisms leading to fatigue are unclear.

The aim of this study was to investigate whether the degree of fatigue in pSS patients is associated with cellular stress responses as assessed by measures of a selected set of Hsps in blood.

Methods: Fatigue was measured by a fatigue visual analogue scale (fVAS). Twenty pSS patients with low and 20 with high fatigue were selected for this study.

Blood samples were collected in EDTA tubes, immediately placed on ice, and centrifuged at 2500 g at 4°C for 15 minutes. Plasma was thereafter aliquoted in cryotubes and stored at -80°C until analyzed.

The concentrations of Hsp32, -60, -72, and -90α were measured in duplicate using specific ELISA assays according to the manufacturer’s protocols.

Results: Hsp90α plasma concentrations were significantly higher in patients with high fatigue compared to patients with low fatigue (50.3 ± 27.4 ng/ml vs. 32.3 ± 14.6 ng/ml, P = 0.02). There were no statistically differences between concentrations of Hsp32, -60, and -72, although a close to significant association between higher fatigue and higher Hsp72 concentrations was observed (P = 0.07).

Conclusion: pSS patients with severe fatigue demonstrate higher Hsp90α concentrations in plasma compared to pSS patients with less fatigue. This finding supports the hypothesis that fatigue may be generated and signalled by cellular defence mechanisms.

§5.10

Cognitive Symptoms are Common in Primary Sjögren’s Syndrome and are Associated with Anxiety
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Background: Cognitive impairment in primary Sjögren’s syndrome (PSS) has been identified in several small studies using self-reported measures.

Table 1 Correlation between HRQOL and symptoms.

<table>
<thead>
<tr>
<th>SF-36</th>
<th>ESSPRI dryness</th>
<th>ESSPRI fatigue</th>
<th>ESSPRI pain</th>
<th>ESSPRI Total</th>
<th>FACIT-Fatigue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical Function</td>
<td>-0.29**</td>
<td>-0.43*</td>
<td>-0.57*</td>
<td>-0.55*</td>
<td>0.54*</td>
</tr>
<tr>
<td>Role-Physical</td>
<td>-0.08</td>
<td>-0.12</td>
<td>-0.24**</td>
<td>-0.24**</td>
<td>0.35*</td>
</tr>
<tr>
<td>Bodily Pain</td>
<td>-0.22</td>
<td>-0.34*</td>
<td>-0.59*</td>
<td>-0.53*</td>
<td>0.52*</td>
</tr>
<tr>
<td>General Health</td>
<td>-0.16</td>
<td>-0.25**</td>
<td>-0.35*</td>
<td>-0.36*</td>
<td>0.49*</td>
</tr>
<tr>
<td>Vitality</td>
<td>-0.26**</td>
<td>-0.22</td>
<td>-0.37*</td>
<td>-0.37*</td>
<td>0.49*</td>
</tr>
<tr>
<td>Social Functioning</td>
<td>-0.18</td>
<td>-0.31*</td>
<td>-0.22</td>
<td>-0.29**</td>
<td>0.45*</td>
</tr>
<tr>
<td>Role-Emotional</td>
<td>-0.12</td>
<td>-0.28**</td>
<td>-0.23**</td>
<td>-0.28**</td>
<td>0.48*</td>
</tr>
<tr>
<td>Mental Health</td>
<td>-0.23**</td>
<td>-0.21</td>
<td>-0.31*</td>
<td>-0.34*</td>
<td>0.45*</td>
</tr>
<tr>
<td>WHOQOL-BREF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical Health</td>
<td>-0.19</td>
<td>-0.34*</td>
<td>-0.43*</td>
<td>-0.43*</td>
<td>0.66*</td>
</tr>
<tr>
<td>Psychological</td>
<td>-0.19</td>
<td>-0.32*</td>
<td>-0.36*</td>
<td>-0.38*</td>
<td>0.56*</td>
</tr>
<tr>
<td>Social Relationships</td>
<td>-0.12</td>
<td>-0.17</td>
<td>-0.34*</td>
<td>-0.28**</td>
<td>0.35*</td>
</tr>
<tr>
<td>Environment</td>
<td>-0.18</td>
<td>-0.19</td>
<td>-0.49*</td>
<td>-0.39*</td>
<td>0.29**</td>
</tr>
</tbody>
</table>

*P < 0.01; **P < 0.05
Objectives: To quantify cognitive impairment symptoms in a large cohort of 150 PSS patients compared with controls and to explore the relationship between cognitive impairment with fatigue, pain and mood symptoms.

Methods: PSS patients fulfilling the American European Consensus Criteria were recruited from 12 sites in England. They completed the Cognitive Failures Questionnaire (CFQ) as well as measures of mood (Hospital Anxiety and Depression Scale), fatigue (visual analogue scale (VAS)), dryness (VAS) and pain (VAS). CFQ scores were compared with data from controls. Completion of the CFQ yields a possible score between 0 and 100. The higher the score the greater the impairment.

Results: One hundred and fifty PSS patients and 198 controls completed the CFQ. Cognitive symptoms were worse in the PSS group (43.7 ± 17.8 vs 35.9 ± 12.9; P < 0.001). This difference persisted (P < 0.001) following analysis of covariance adjusting for age and gender. There were significant correlations with pain, fatigue, anxiety, depression and subjective dryness scores with CFQ scores. In order to partition the variability in CFQ scores into its component parts, we performed a multiple regression analysis. This confirmed that anxiety was the most important predictor of CFQ scores (P = 0.004).

Conclusion: Cognitive symptoms are common in PSS and independently associate with anxiety. Clinicians should give consideration to cognitive failure and anxiety in the management of PSS patients.

S5.12
Oxygen Uptake, Fatigue and Quality of Life in Primary Sjögren’s Syndrome

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Background: Aerobic capacity is a key biomarker of health and well-being, from risk of secondary disease to quality of life (QOL). Although anecdotally reported to be reduced in primary Sjögren’s syndrome (pSS), aerobic capacity has not been objectively assessed. The lack of data limits the ability to target aerobic capacity as a therapy. This pilot study defines the relationship between aerobic capacity with fatigue, disease activity, depression and QOL in pSS.

Methods: In a preliminary cross-sectional study, 20 pSS (AECG) sedentary women with a mean age of 53 (±7) performed a maximal progressive treadmill exercise test. Expired gas analysis was performed with a computerized metabolic system. They were evaluated through the questionnaires FACIT-Fatigue, ESSPRI, ESSDAI, BDI and SF-36. Pearson’s tests were used to correlate the variables. Multiple linear regressions were used to examine the effect of the variables on VO2max and VO2AT.

Results: ESSDAI and ESSPRI scores mean were 1.55 (±1.43) and 6.13 (±2.75), respectively. Mean of VO2max was 19 (±4) ml/kg/min and VO2AT was 15 (±3) ml/kg/ min–1. VO2max correlated with ESSPRI dryness (r = –0.53; P < 0.05), ESSPRI fatigue (r = –0.59; P < 0.01) and ESSPRI total (r = –0.53; P < 0.05). VO2AT correlated with ESSPRI fatigue (r = –0.47; P < 0.05), ESSPRI total (r = –0.44; P < 0.05), SF-36 General Health (GH) domain (r = 0.49; P < 0.05) and SF-36 Social Functioning (SF) domain (r = 0.59; P < 0.01). Patients reporting higher levels of fatigue, pain and dryness have worse QOL and depression. Multiple linear regression analysis showed that creatine kinase (CK) was independently associated with VO2max (β=0.02; P < 0.05), as well SF-36 GH and SF domains with VO2AT (β=0.07; P < 0.05 and β=0.06; P < 0.01, respectively).
Conclusion: In pSS, low aerobic capacity is associated with worse symptoms, higher levels of CK and lower QOL. The optimization of aerobic capacity should be explored as a way to modify clinical presentations and increase QOL in pSS.

S5.13
Saliva Variations may Make Gastro-Esophageal Reflux Disease in Sjögren Syndrome

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Objectives: The protective role of saliva in the case of esophageal exposition to gastric acid has long been studied but some contradictions still remain. The main end-point of this study was to evaluate whether a qualitative and quantitative alteration in salivary secretion exists in sjögren syndrome patients affected by gastro-esophageal reflux disease (GERD).

Methods: Twenty sjögren syndrome patients with endoscopically diagnosed GERD (LA classification A-D) and 10 sjögren syndrome patients without GERD had been evaluated; salivary tests (basal flow rate, stimulated flow rate, pH, [Na+] and [K+]) were performed, socio-demographical variables and oral GERD-related symptoms were taken into account.

Results: Sjögren syndrome patients with GERD and sjögren syndrome patients without GERD were found to have a similar basal flow rate but different stimulated salivary function [sjögren syndrome with GERD group mean value 0.989 ml/min (±0.48718) vs. sjögren syndrome without GERD group 1.2197 ml/min (±0.6108), pH [sjögren syndrome group mean value 8.935 (±0.471) vs. sjögren syndrome without GERD group 7.879 (±0.526)] and a higher K+ concentration. In sjögren syndrome with GERD patients, we also registered a significant association with severe xerostomia [18/20 vs. 3/10] and severe an oral burning sensation [17/20 vs. 2/10].

Conclusions: Our findings assess that variation of saliva is altered in sjögren syndrome with GERD patients and highlight the need for further investigations in order to define the role of saliva in the etiology of GERD in sjögren syndrome.

<table>
<thead>
<tr>
<th>Outcome variables</th>
<th>Sjögren syndrome with GERD</th>
<th>Sjögren syndrome without GERD</th>
<th>ANOVA (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal flow rate (+S.D.)</td>
<td>0.52 ml/min (±0.39401)</td>
<td>0.5187 ml/min (±0.3185)</td>
<td>0.002 (0.966)</td>
</tr>
<tr>
<td>Stimulated flow rate (+S.D.)</td>
<td>0.989 ml/min (±0.48718)</td>
<td>1.2197 ml/min (±0.6108)</td>
<td>9.577 (0.002)</td>
</tr>
<tr>
<td>PH (+S.D.)</td>
<td>8.935 (±0.471)</td>
<td>7.879 (±0.526)</td>
<td>2.41330 (0.00001)</td>
</tr>
<tr>
<td>Na+ (+S.D.)</td>
<td>14.309 mequiv./l (±3.589)</td>
<td>14.306 mequiv./l (±3.766)</td>
<td>0.218 (0.641)</td>
</tr>
<tr>
<td>K+ (+S.D.)</td>
<td>23.4179 mequiv./l (±3.2505)</td>
<td>21.6866 mequiv./l (±3.500)</td>
<td>14.191 (0.0001)</td>
</tr>
</tbody>
</table>

S5.14
Extraglandular Manifestations of Type 1 Renal Tubular Acidosis and Osteomalacia in a Patient with Sjögren’s Syndrome

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Sjögren’s syndrome is a chronic autoimmune disease characterized by decreased function of lacrimal and salivary glands. In addition, many other organs can be involved in patients with Sjögren’s syndrome. Overt or latent renal tubular acidosis is an uncommon extraglandular manifestation in Sjögren’s syndrome, and osteomalacia is a rare complication of renal tubular acidosis. It has been rarely reported that osteomalacia is associated with distal renal tubular acidosis in patients with Sjögren’s syndrome. We report a case of a 34-year-old female patient who initially presented with muscle weakness in both lower extremities and then diagnosed as Sjögren’s syndrome complicated by osteomalacia and renal tubular acidosis.

S5.15
Analysis of Urinary Manifestations in Patients with Sjögrens Syndrome

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Objective: This study attains to describe the most prevalent lower urinary tract symptoms (LUTS) in a cohort of patients with Sjögren’s Syndrome (SSJ) and its outcomes.

Patients and methods: Observational, retrospective study. Consecutive adult patients with SSJ (American-European Consensus Group Criteria 2002) that consulted due to LUTS were included. Urinary symptoms were
assessed by a direct interview with a medical doctor. Urinary studies were performed according to medical criteria. **Statistical Analysis:** Descriptive analysis. Continuous variables are expressed as median and interquartile range (IQR), while categorical variables as frequencies and percentages.

**Results:** Twenty-six patients were included, all females and with a mean age of 61.15 years (IQR 44–78). Eight patients reported urinary urgency incontinence (UI), 4 urinary urgency (UU), 3 repeatedly inferior urinary tract infection (rUTI), 3 chronic urinary retention (CUR), 3 UI + fecal incontinence, 2 chronic pelvic pain (CPP), 1 UI + CPP, 1 UI + rUTI and 1 patient UU + dyspareunia. In 11 (42.3%) patients, it was feasible to reproduce urodynamically the symptomatology. 12 (46.15%) patients presented neurogenic pathology in electrophysiological tests, and the endoscopic findings most frequently seen were cystitis and chronic cervicotrigonitis.

Filling symptomatology was treated with low doses of anticholinergic drugs, electro stimulation or botulinic toxin, whereas emptying symptomatology was treated with benzodiazepines and alpha blockers. Patients with rUTI received antibiotic prophylaxis and those with CPP, Amitriptyline and/or Baclofen associated with posterior tibial nerve stimulation (PTNS). Out of the 26 patients, 1 did not continue with the treatment, 5 did not respond to it, 3 had a partial response and the remaining 17 patients had a good control of their urinary symptoms.

**Conclusion:** In SSJ, it is frequent to see LUTS. Therapy outcomes depend on the cause and the patients’ compliance to therapy.

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**S5.16**

**Effect of Age at Onset on Disease Activity and Clinical Features in Patients with Primary Sjögren’s Syndrome**

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**Objectives:** To examine relationship between age at onset and clinical features including disease activity in patients with primary Sjögren’s syndrome (pSS).

**Methods:** Clinical data of 75 women with pSS who visited our hospital from August 2010 to December 2014 were collected from medical charts. Of them, ESSPRI and ESSDAI were assessed in 72 and 71 patients, respectively.

**Results:** Average age, age at onset, and duration of disease were 60.9 ± 12.6, 48.3 ± 13.9, and 12.8 ± 7.3 years, respectively. Number of patients with low (ESSDAI < 5), moderate (ESSDAI 5 ~ 13), and high disease activity (ESSDAI ≥14) were 45, 20, and 6, receptively. Corticosteroid was used in 60.8% of patients. Average dose of steroid (equivalent dose of prednisolone) was 3.1 ± 2.1 mg/day and that of high disease activity group (6.3 ± 4.4 mg /day) was significantly higher than that of moderate (1.6 ± 1.4 mg /day) or low disease activity group (1.6 ± 1.6 mg/day). Patients with younger onset (under 40) had significantly high score of ESSDAI (7.8 ± 5.9) compared with middle age or elderly onset patients (3.7 ± 4.3), and there was a marked difference in its domain of lymphadenopathy between younger onset and middle age or elderly onset patients. There was no difference of ESSPRI between them (5.1 ± 2.1 vs. 4.3 ± 2.1); however, its item of fatigue score was significantly higher in younger onset group than in middle age or elderly onset group (5.9 ± 2.9 vs. 4.3 ± 2.9). Positive rate of anti-Ro/SSA and anti-La/SSB antibody in younger onset group was 100% and 36.8, respectively, and it did not differ from that in middle or elderly onset group (89.3% and 33.9%, respectively).

**Conclusion:** Younger onset pSS patients had higher disease activity than middle age or elderly onset patients. Severity of fatigue and lymphadenopathy was the clinical characteristics in younger onset pSS.

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**S5.17**

**Autoimmune Cytopenia in Primary Sjogren’s Syndrome is Associated with Severe Ocular Surface Damage, Tear Film Instability, and Less Articular Involvement**

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**Objective:** To determine the characteristics of patients with primary Sjogren’s syndrome (pSS) who have autoimmune cytopenia.

**Methods:** We analyzed 113 participants from the Korean Initiative of primary Sjogren’s Syndrome (KISS), a prospective pSS cohort. Autoimmune cytopenia was defined as autoimmune origin neutropenia, anemia, and/or thrombocytopenia without vitamin or iron deficiency or drug-induced cytopenia. To identify the association between autoimmune cytopenia and clinical characteristics of pSS, extraglandular manifestations were analyzed according to the ESSDAI definition. Xerophthalmia was assessed with the Ocular Surface Disease Index, Schirmer I test, ocular stain score (OSS), tear film break up time (TBUT).

**Results:** The median total ESSDAI score was 2 (IQR 0.5–6). About a quarter of patients had no systemic activity.
Autoimmune cytopenia was observed in 23.9% of patients (n = 27). Moderate biological features were more frequently observed in patients with autoimmune cytopenia than in patients without [10(37%) and 11(12.8%), respectively, P = 0.016]. Articular involvement was exhibited in one patient with autoimmune cytopenia, but in 22 patients (26.5%) without autoimmune cytopenia (P = 0.039). Higher OSS (P = 0.003 using SICCA method, P = 0.019 using van Bijsterveld’s scoring system) and lower mean Schirmer I test (P = 0.029) were observed in patients with autoimmune cytopenia than in those without. Leukocyte counts correlated with OSS by both methods (van Bijsterveld’s, r = -0.216 [P = 0.045]; SICCA, r = -0.265 [P = 0.007]) and TBUT (r = 0.241 [P = 0.019]).

Conclusions: Autoimmune cytopenia is closely associated with severe ocular surface damage in pSS. Therefore, assessment of xerophthalmia by ophthalmologists may be mandatory, particularly in pSS patients with cytopenia, even if patients do not complain of eye dryness.

S5.18
Podocytopathy Associated to Sjögren’s Syndrome Treated with Rituximab

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We present a case of podocytopathy associated with primary Sjögren’s Syndrome (pSS) with no tubular disease or glomerular lesion detectable on light microscopy. A young Brazilian female, 25 years old, started with cervical lymphadenopathy, livedo reticularis, compensated hypothyroidism, self-limited arthralgia, xerostomia and xerophthalmia. After 6 months, she presented nephrotic syndrome (generalized edema, ascite, pleural effusion, weight gain of 10 pounds in three months, cholesterol level of 416 mg/dL, proteinuria of 7.8 g /24 h and creatinin of 0.8 mg/dL). Urinalysis showed microscopic hematuria, heavy proteinuria and absence of cylindruria. Laboratory tests revealed normal transaminases, negative C-reactive protein, normal C3 (192 mg/dL) and C4 (29 mg/dL) levels, and negative syphilis, HIV, hepatitis B and C serology. The white blood cell count was 3,100/μL, with 1,240 lymphocytes, hemoglobin level was 11.7 g/dL and platelet count was 211,000. Further laboratory studies showed polycional hypergammaglobulinemia (27.5 g/dL), high hemosedimentation velocity (113 mm/h), and positive rheumatoid factor (1:32), antinuclear antibody (1:320, fine speckled nuclear type), anti-Ro-SSA (240 U/ml) and anti-La-SSB (320 U/mL). Other antibodies were negative (anti-ds DNA, anti-Sm, anti-RNP, antcardiolipin, antineutrophil cytoplasmic antibodies). pSS diagnosis was based on AECG criteria and salivary gland biopsy (focus score ≥ 1 and positive germinal centre-like). Renal biopsy showed no abnormalities and absence of deposits by light microscopy and immunofluorescence. Podocytes foot processes effacement was observed by Electron Microscopy. She was not responded to prednisone 1 mg/kg/day, azathioprine 2 mg/kg/day, pulsotherapy with metilprednisolone 1 g for 3 days associated to cyclophosphamide 1 g monthly during 3 months, and mycophenolate mofetil 3 g/day (MMF), by keeping significant levels of proteinuria. The latter association consisted of MMF and rituximab (1 g, 2 weeks) with clinical improvement (edema, ascite, pleural effusion) and proteinuria reduction (1,7 g/day) after 6 months. MCD and pSS have been described previously only in two isolated case reports. We hypothesize that cytokines, like IL-13 produced by T cells can induce podocyte lesion, as it has been rarely and recently described in systemic lupus erythematosus.

S5.19
Anca-Associated Vasculitis in Patients with Primary Sjögren’s Syndrome: Detailed Analysis of 6 New Cases and 15 Cases from the Literature

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1Rhumatologie, 2Néphrologie, CHR U Brest, Brest, 3Médecine Interne, Hôpital Cochin, Paris, 4Médecine Interne, Claude Huriez Hospital, Université Lille Nord-de-France, Lille, 5Néphrologie, Hôpital Européen Georges Pompidou, 6Médecine Interne, Institut Mutualiste Montsouris, Paris, and 7Médecine Interne, Hôpital Avicenne, Bobigny, France

Background: Primary Sjögren’s syndrome (pSS) is a systemic autoimmune disease characterized by B-cell hyperactivation. In some patients, multiple autoantibodies develop, including anti-neutrophil cytoplasmic antibody (ANCA), which could lead to the development of ANCA-associated vasculitis (AAV).

Objectives: To describe clinical presentation, therapy and prognosis of patients diagnosed with both pSS and AAV.

Methods: Nation-wide survey in France collecting information on cases of AAV associated with pSS. The study was completed by a systematic review of the literature.

Results: This work identified 6 new cases of coexisting pSS and AAV: 2 microscopic polyangiitis (MPA), 2 MPO-ANCA renal-limited AAV, 1 granulomatosis with
polyangitis (GPA), and 1 eosinophilic granulomatosis with polyangitis (EGPA). The systematic literature search identified 15 previously published cases. Among the 21 patients, 18 were females. Mean age at diagnosis of AAV was 64 + /-10 years with mean pSS duration of 76 + /-122 months. All individuals with pre-existent pSS experienced at least one extra-glandular manifestation attributable to pSS before AAV diagnosis. p-ANCA with anti-MPO specificity were found in 80% (16/20), c-ANCA with anti-PR3 specificity in 9.5% (2/20) and isolated c-ANCA in 14.3% (3/21). Vasculitis involved kidneys (n = 13), lungs (n = 6), peripheral nerves (n = 5), skin (n = 5), central nervous system (n = 2), small bowel (n = 1), muscle (n = 1), and sinuses (n = 1). The mean follow-up duration of AAV was 20.5 (+/- 31.1) months. A favourable response to treatment was observed in 76.2% of cases (16/21), stabilization in 19% of cases (4/21) and one death was reported.

Conclusions: This work shows that AAV may occur in patients with pSS. These are most commonly p-ANCA associated vasculitis with anti-MPO specificity. The AAV may reveal an underlying pSS or arise during its evolution. In this latter case, its occurrence appears to be correlated with extra-glandular manifestations of pSS.

S5.20
Sjögren’s Syndrome and Metabolic Disorders
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Background/Purpose: Sjögren’s syndrome (SS) is a complex disorder involving both the innate and immune system. The incidence of SS is estimated at a frequency of 0.1–3% of the population. Fatigue is a common feature of the disease. Metabolic disorders of aerobic and anaerobic metabolism occur in 1–8% of the population. We investigated the presence of metabolic disorders in patients with SS and the involvement of SS in patients with metabolic disorders from a University Rheumatology/Immunology practice.

Methods: Clinic records were searched for the diagnoses of metabolic disorders or Sjögren’s syndrome and the charts reviewed for the patients with either or both of these diagnoses.

Results: In the metabolic disease clinics, there were 798 patients of whom 6% had SS. Of 105 patients with primary SS, 48% had a metabolic disorder. Of 32 patients with Sjögren’s syndrome who complained of severe fatigue and exercise intolerance, 93% had a metabolic disorder.

Metabolic disorders included both problems with aerobic as well as anaerobic metabolism.

Conclusions: 1. Sjögren’s syndrome occurs in patients with metabolic disease at a higher frequency than is seen in the general population.
2. In patients with Sjögren’s syndrome, metabolic disease occurs far more frequently than in the general population
3. In patients with Sjögren’s syndrome and complaints of severe fatigue and exercise intolerance, most have a metabolic disorder
4. Treatment of the existing metabolic disorders in Sjögren’s syndrome patients is an important component of their medical care.

S5.21
Identifying Stakeholder Informed Priority Targets for a Non-Pharmacological Intervention Package to Improve Functional Capacity: A Multi-Centre Mixed Methods Study
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Background: Functional difficulties are a problem in 70% of primary Sjögren’s syndrome (PSS) patients.

Objective: To identify the perceived most important barriers to performing daily activities from the viewpoints of patients, their family members and health care providers.

Methods: Group concept mapping (GCM) is a robust, mixed-methods, equitable, and systematic approach which has been successfully utilised to identify priorities in healthcare and research.

We conducted a GCM study and recruited PSS patients fulfilling the American European Consensus Group diagnosis criteria, adult household members (AHM) from 12 sites across England and health care professionals (HCP) from across the UK (total n = 232). First participants completed a statement generation brainstorming activity and identified key barriers to performing daily activities. Next, an individual card sorting activity was completed with the refined set of 94 statements/unique responses. Here, participants each sorted similar meaning statements into groups. Finally, each statement was rated for importance on a 1–5 scale.

Multi-dimensional scaling and hierarchical cluster analysis statistical techniques were applied to generate concept maps - a visual representation of all stakeholders’ ideas and
S5.22

Traditional and Disease-Associated Cardiovascular Risk Factors are Correlated with Carotid Plaque in Sjögren’s Syndrome

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Background: Traditional cardiovascular risk factors are higher in Sjögren’s syndrome (SS) patients, but their relation with carotid atherosclerosis, disease-risk factors and biomarkers are not known.

Objective: To investigate the association of biomarkers, traditional and disease-associated risk factors with presence of carotid atherosclerosis plaque.

Methods: Sixty-three Brazilian patients with primary SS (AECG) and 63-matched healthy controls underwent carotid ultrasound, and clinical and laboratory assessment.

Results: Most traditional cardiovascular and some disease-associated risk factors showed higher risk for plaque (Table 1). Patients with SS showed higher prevalence (13% vs. 2%) and higher risk of carotid atherosclerotic plaque even after adjusting for age, creatinin, cholesterol levels and systolic blood pressure (OR= 15.4, CI= 1.001–236.409). Patients showed higher levels of anti-carbamyalted protein (20% vs. 3%), calprotectin (1878.8 ± 1501.13 vs. 1215 ± 519.99 ng/mL) and higher level of TNF-R2 (8144.32 ± 4106.39 vs. 5914.74 ± 2458.23 pg/ml).

Atherosclerotic plaque was associated with ESSDAI constitutional domain (13% vs. 0%), menopause (100 vs. 49%) and calprotectin (2945.2 ± 1973.4 vs. 14070.09 ± 5996.66 pg/ml), all P ≤ 0.05.

Conclusion: Patients with SS have higher prevalence of carotid atherosclerosis which is modulated by higher traditional cardiovascular risk factor as well as disease itself. Inflammation, protein carbamylation and calprotectin are possible mechanisms involved in atherosclerosis pathogenesis in SS.

Table 1 Cardiovascular risk factors for carotid atherosclerosis in SS.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>OR</th>
<th>P-value</th>
<th>95% CI Limit</th>
<th>95% CI Limit</th>
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</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.78</td>
<td>0.002</td>
<td>0.78</td>
<td>0.95</td>
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<td>Gender (male)</td>
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<td>1.05</td>
<td>39.06</td>
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<tr>
<td>Hypertension</td>
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<td>0.04</td>
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<tr>
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<td>5.8</td>
<td>0.017</td>
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<tr>
<td>Familiar history of myocardial infarct</td>
<td>10.27</td>
<td>0.032</td>
<td>1.22</td>
<td>86.33</td>
</tr>
<tr>
<td>Low HDL</td>
<td>1.06</td>
<td>0.002</td>
<td>1.02</td>
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<tr>
<td>Serum creatinine</td>
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<td>Saliva substitute</td>
<td>4.9</td>
<td>0.045</td>
<td>0.93</td>
<td>25.86</td>
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</table>

ESSDAI= EULAR Sjögren’s syndrome disease activity index, OR= Odds Ratio (Univariate regression analysis), CI= confidence interval.

S5.23

Obstructive Airway Symptoms (OAS) Precede the Onset and Diagnosis of Primary Sjögren’s Syndrome (PSS) by Many Years

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Background: Recently, we showed that COPD is common in pSS, even in the absence of smoking (Nilsson A. et al, Rheumatology, 2015). In a nested case control study, we now investigate the predictive role of obstructive airway symptoms for development of pSS.

Methods: Data were extracted from two population-based surveys, Malmö Preventive Medicine, (N = 33346) and Malmö Diet & Cancer (N = 30447). OAS was defined as either COPD (defined as FEV1/VC <70% at inclusion, part of the Malmö Preventive Medicine survey) or a history of...
Interstitial Lung Disease in Sjögren Syndrome: Risk Factors and Prognosis

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Background: Interstitial lung disease (ILD) has been described in patients with pSS but there are limited data on its prognosis and risk factors.

Objectives: To analyse the clinical, immunological, radiological features, outcome and risk factors of ILD in pSS.

Methods: Retrospective study of all patients diagnosed at Internal Medicine Department between January 1992 and January 2014 with pSS, according to the European-American Consensus criteria, who developed ILD. Immunology, chest-x-ray, high resolution CT (HRCT) scans, and complete pulmonary function tests (PFT) were reviewed.

Results: Two hundred forty-four patients (233 women), mean age at pSS diagnosis 55.6 ± 14.5 y (range 17 to 86), were included. Thirty-two (12.7%) developed ILD. The mean age at ILD diagnosis was 61.8 y (33 to 86). No patient was smoker. Most frequent initial symptoms were cough and dyspnea. Raynaud phenomenon was reported by 50% of patients and fatigue by 66%. Arthritis was present in 66% of cases, bronchiectasis in 40.6%, liver involvement in 28%, and peripheral neuropathy in 19%. The mean interval between pSS diagnosis and ILD development was 6.9 y. All patients showed positive AAN, 90.6% polyclonal hypergammaglobulinemia, 72% RF, 37.5% anaemia, 12.5% leukopenia (<4000), 15.6% lymphopenia (<1000), 9.4% low C3 levels and 15.6% low C4 levels. Anti-Jo1 antibody was negative in all patients. Two patients showed PL12 antibodies. On chest-x-ray bilateral consolidation, reticulonodular infiltrates, or multiple cysts were observed in all cases. The most frequent HRCT patterns were NSIP, UIP and LIP. ILD was significantly associated with Raynaud’s phenomenon (P < 0.000), arthritis (P = 0.002), polynuropathy (P = 0.006), liver involvement (P = 0.002), kidney involvement (P = 0.034), positive anti-Ro/SSA (P = 0.000), anti-La/SSB (P = 0.018) and RF (P = 0.012), hypergammaglobulinemia (P < 0.000) and low C4 levels (P = 0.048).

Twenty-seven (84%) patients received oral prednisone (1 mg/kg/day), 16 (50%) immunosuppressant drugs (7 AZA, 3 CF, 5 MMF, 1 FK-506) and 2 Rituximab. One patient required lung transplantation. Fourteen (43.8%) patients died during the follow-up. Death was directly related to lung involvement in 9 cases. Cox regression analysis showed that Raynaud’s phenomenon, arthritis and hypergammaglobulinemia were independent risk factors for ILD development. ILD was significantly related to death (P < 0.000). The interval between ILD diagnosis and death was 8.6 years.

Conclusions: Raynaud’s phenomenon, arthritis, and hypergammaglobulinemia are associated with ILD development in pSS. NSIP is the commonest radiologic pattern. Because ILD is clearly related with a poor prognosis, improvement of management of ILD may contribute to the reduction of disease burden.
S5.25
Pulmonary Involvement in Sjögren’s Syndrome and its Risk Factors – A Prospective Study

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Background: Pulmonary involvement is common in primary Sjögren’s syndrome (pSS). Clinicopathologic pulmonary manifestations associated with pSS have yet to be reviewed in a large series. However, the pulmonary functional and radiological characteristics of pSS-associated lung disease which are more practical in clinic were less studied.

Objective: To evaluate the prevalence of lung involvement in patients with primary Sjögren’s syndrome (pSS) and analyze its radiological (high-resolution computed tomography, HRCT) characteristics and risk factors for lung impairments.

Methods: A total of 1341 hospitalized SS patients from 2003 to 2012 were retrospectively reviewed. The prevalence of pulmonary involvement was calculated. Among them, 105 pSS patients (2008–2012) with lung complication and 84 without any organ damage were explored further. Demographic data, laboratory tests and arterial blood test (ABG) were obtained from medical documents. The high-resolution computed tomography (HRCT) scans were re-evaluated by two experienced chest radiologists. Case–control study was performed to explore the risk factors of lung involvement in pSS patients.

Results: Among the 1341 hospitalized patients (853 with pSS and 488 with sSS), 165 patients (19.34%) from pSS group and 126 patients (25.82%) from sSS group presented lung involvement. For the 105 pSS associated lung diseases patients who were recruited as case group, 96 (91.40%) were female, with the mean age of 61.3 ± 9.9 years old. The median disease duration was 84.00 months (24.0–156.0 months). Hyperglobulinemia (IgG) and elevated RF were prominent, however, anti-SSA and anti-SSB positive were only 52.5% and 37.8%, respectively. For the 36 patients who took ABG, 20 presented with hypoxemia and 7 with type 1 respiratory failure. There were more frequent and severer of the lower lung lobes involvement. The most common HRCT findings were linear opacities (93.8%), ground-glass attenuation (87.7%), pleural involvement (64.6%) and reticular pattern (63.1%). Compared with the control group, percentages of patients from age ≥60 yr, low C4, elevated RF and ANA positive were significant higher (P < 0.05), whereas, that of anti-SSA positive was significant lower (P < 0.05). Advanced age (≥60 yr) and elevated RF were retained as independent correlates after adjusting for all the factors with significant difference.

Conclusion: Lung involvement is a severe and common complication for pSS patients. Advanced age and RF positive are independent risk factors for pulmonary complication in pSS patients.

S5.26
Peripheral Neuropathy in Primary Sjögren’s Syndrome

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Background: Primary Sjögren’s syndrome (pSS) is a systemic autoimmune disease. Nearly, three-quarters of patients with primary Sjögren’s syndrome manifest signs or symptoms of extraglandular disease, including peripheral neuropathy.

Objective: To analyze the clinical manifestations of primary Sjögren’s syndrome (pSS) with peripheral neuropathies.

Methods: Eighty-six patients who fulfilled the 2002 American-European Consensus Group criteria for pSS were enrolled in the study. For each patient, all medical records, including clinical, laboratory, immunologic and electromyography data were obtained. The clinical manifestations of primary Sjögren’s syndrome were compared between patients with and without peripheral neuropathy. Statistics method used t-test, chi-square test and logistic regression.

Results: Eighty-six patients were analyzed; neurological involvement was noted in 25.6% (22/86) patients. The clinical spectrum of peripheral neuropathies encountered in Sjögren’s syndrome patients is wide, with sensory neuropathies being the most common. Median nerve, peroneal nerve and sural nerve were the most likely involved, and lower limb involvement accounted for 72.7% (16/22). Peripheral neuropathy was diagnosed during the SS course in all of patients, and about 45.5% patients’ neurological involvement were diagnosed early in course of the disease.

The frequency of Raynaud’s phenomenon was significantly higher (31.8% vs 4.7%, P = 0.002) as well as acrocyanesthesia (68.2% vs 4.7%, P < 0.001) in pSS with peripheral neurological involvement than in pSS without peripheral neuropathy. The median values of EULAR Sjögren’s syndrome disease activity index (ESSDAI) were 5.3 (range 2.8–7.8) and 3.4 (range 1.5–5.3) in the PNS and non-PNS groups, respectively (P < 0.001). We found a significant elevation of peripheral neuropathy risk associated with Raynaud’s phenomenon (odd ratio 9.489, 95% CI 2.191–41.093, P = 0.003) and ESSDAI (odd ratio 1.528, 95% CI 1.179–1.979, P = 0.001). Elevated titers of rheumatoid factor (P = 0.023) and ANA (P = 0.003) were more common in patients with peripheral neuropathy.
Conclusion: Peripheral neuropathy is not a rare manifestation of pSS. Elevated titers of rheumatoid factor and ANA were more common in patients with peripheral neuropathy. Neurological involvement was diagnosed early in course of the disease. Raynaud’s phenomenon and high disease activity may be risk factors of peripheral neuropathy.

S5.27
Clinical and Immunological Characteristics of Primary Sjögren’s Syndrome with Thrombocytopenia
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Backgrounds: Primary Sjögren’s Syndrome (pSS) is a chronic systemic autoimmune disease characterized by functional impairment of exocrine glands. The spectrum of the disease extends from an organ-specific autoimmune disease to systemic processes, including hematologic abnormalities. Thrombocytopenia (TP) can be observed in about 13% of pSS patients with hematological involvement. However, studies focusing on this field are scarce, and the mechanisms are still uncertain.

Objective: To study the clinical and immunological features of pSS complicated with thrombocytopenia and to investigate the probable mechanisms through cytokines, autoantibodies and sex hormones.

Methods: 1. The levels of six cytokines: IFN-γ, IL-2, IL-6, IL-10, IL-17a, and B-cell-activating factor (BAFF), which stand for different lymphocyte subsets, were measured using ELISA in sera from 125 pSS patients, of whom 63 were complicated with thrombocytopenia. 2. The levels of thrombopoietin (TPO) and anti-TPO antibody were measured using ELISA in sera from patients described above and 60 healthy controls (HC). 3. Levels of sex hormones, including testosterone, dihydroepiandrosterone-sulfate (DHEA-S), and estradiol were measured using radioimmunoassay in sera from 22 pSS patients with thrombocytopenia and 21 patients without, excluding factors which may affect the results. 4. Medical records of 8 pSS patients with refractory thrombocytopenia treated with danazol were reviewed, and the changes of platelet counts and levels of sex hormones were evaluated.

Results: 1. The mean age at pSS onset with and without thrombocytopenia was 49.74 ± 15.33 years and 42.10 ± 15.79 years, respectively. Compared with normal platelet counts patients, thrombocytopenic patients showed longer duration and higher levels of immunoglobulin (P = 0.011 and P = 0.005, respectively). 2. Levels of IL-6, IL-17a and BAFF were significantly higher in pSS patients with thrombocytopenia (P < 0.001, P = 0.002, and P = 0.003, respectively). 3. Levels of anti-TPO antibody were significantly higher in pSS with thrombocytopenia (P < 0.05), while TPO levels were lower than patients without thrombocytopenia (P < 0.01). 4. Levels of serum IL-6 and IL-17a were significantly correlated with platelet counts (P = 0.005 and P = 0.02, respectively). 5. Levels of testosterone (T) and dihydroepiandrosterone-sulfate (DHEA-S) were lower in the thrombocytopenic patients (P = 0.001 and P < 0.001, respectively). 6. After 3 months’ treatment with danazol in 8 pSS patients with refractory thrombocytopenia, both platelet counts and serum concentrations of sex hormones were elevated.

Conclusion: Thrombocytopenia in pSS is resulted from many factors, including cellular immunity, autoantibodies, and hormones background.
Session 6. Patient Panel

S6.1
Sjögren’s Syndrome Foundation (SSF) Clinical Practice Guidelines for Systemic Management: Treatment of Inflammatory Musculoskeletal (MSK) Pain, Fatigue and Use of Biologics

S. Carsons, A. Parke, B. Segal, N. Carteron, V. Sankar, R. Brasington, R. I. Fox, W. Ehlers, M. Brennan, R. H. Scofield, P. Hurley, K. Hammitt & F. Vivino

For the Sjögren’s Syndrome Foundation Clinical Practice Guidelines Committee (CPGC)

Purpose: To improve quality of care for Sjögren’s (SS) systemic manifestations through consensus guidelines development

Methods: Of 97 clinical management questions for systemic manifestations identified from patient and rheumatologist surveys, 16 were selected for guideline development. The 1st topics — Biologic use, DMARDS for treatment of MSK pain and Management of fatigue are presented. Each clinical question was developed in PICO format and assigned to a topic review group (TRG) which performed a systematic review, data extraction and drafted a guideline. Quality of evidence and strength of recommendation were rated using a modification of GRADE. Guideline recommendations were reviewed by a consensus expert panel comprised of 30–40 clinicians from academia and community practices as well as RNs and patients. A modified Delphi method was used with 75% agreement required. Of 1700 abstracts identified, 68 articles were reviewed.

Results: Consensus was achieved for 20 recommendations; for 11 additional modalities, available data were insufficient to allow a recommendation to be formulated. Of the 20 recommendations, 18 required 1 Delphi round, 1 required 2 and 1 required 3. Key recommendations include a decision tree for the use of oral DMARDs for MSK pain with HCQ as first-line therapy, use of self-care measures and provision of advice regarding exercise to reduce fatigue and the use of HCQ in certain patient subsets (e.g. inflammatory fatigue). The Clinical Practice Guidelines Committee (CPGC) recommended that the use of rituximab may be considered in specific clinical situations including vasculitis, severe parotid swelling, inflammatory arthritis, pulmonary disease and mononeuritis multiplex unresponsive to other therapies. The CPGC strongly discouraged the use of TNFα inhibitors for sicca symptoms and for the majority of clinical contexts in primary SS. The CPGC updates its systematic reviews regularly and recommendations may be revised in the future.

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S6.2
International Sjögren’s Network of Patient Organizations

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The International Sjögren’s Network, led by Sjögren’s Syndrome Foundation and the Association Francaise du Gougerot-Sjögren, conducted an international survey of 14 Sjögren’s Patient Groups representing Finland, Japan, USA, Canada, Portugal, Germany, Republic of Korea (South Korea), France, India, New Zealand, UK, Switzerland and Spain.

Selected survey findings are as follows:

Mission: All 14 groups have similar missions of patient education and awareness. Some focus additionally on research and advancement of therapeutic development but all respondents agree that education and awareness are a primary focus.

Patient and Physician Education: 100% of the groups have a website or webpage that helps educate patients and connect them to the organization as well as provides patient support through peer-to-peer connections or active support groups or conferences. All but 1 organization has educational brochures. Half have specific materials for healthcare professionals.

Research and Drug Development: 42% (6) of the organizations provide research funding while only one (1) country (USA) works directly with pharmaceutical companies to encourage and support the development of new treatments. In contrast, 57% (8) organizations work with medicine approval agencies to assist with the approval of new treatments and issues of reimbursement.

Quality of Life: 11 organizations conduct patient surveys to better understand the disease, quality of life and burden of illness. One challenge continues among the International Sjögren’s Network in how to conduct multi-national surveys and projects. Funding for such projects is limited and thus needs to be reviewed further.

Organizations vary in funding sources—38% have membership dues and 41% accept donations while 54% receive funding from public sources (i.e. government agencies, etc.). However, 100% of the organizations surveyed agreed that more funding in their respective countries would help with awareness, education and research efforts!
S6.3
Sjögren’s Syndrome Foundation (SSF)
Clinical Practice Guidelines for Oral Disease Management: Caries Prevention
For the Sjögren’s Syndrome Foundation Clinical Practice Guidelines Committee (CPGC), Bethesda, MD, USA.

Purpose: The goals for the development of SSF Clinical Practice Guidelines are to improve quality of care in Sjögren’s (SS) by establishing guidelines for the management of oral and other clinical manifestations.

Methods: Clinical questions were developed in a PICO format (Population, Intervention, Comparison and Outcomes) for the first overarching topic in oral management of Caries Prevention and included use of fluoride, antimicrobials, salivary stimulants, and non-fluoride remineralizing agents. A systematic literature search was conducted from January 1991 on articles selected according to pre-established parameters. Data were extracted by at least two members and evidence rated by the full group. Evidence grading and strength of the recommendations were based on a variation of GRADE. Recommendations were finalized following a Delphi consensus panel process involving at least 40 dentists and dental hygienists from academia and community-based practice and other healthcare professionals with a minimum of 75% agreement required.

Results: Final Recommendations include 1) Topical fluoride SHOULD BE USED in all Sjögren’s patients with dry mouth (Moderate). 2) Chlorhexidine administered by varnish, gel or rinse MAY BE CONSIDERED in SS patients with dry mouth and high root caries rate (Weak). 3) While no studies link improved salivary flow to caries prevention, it is generally accepted by the oral health community that increasing saliva may contribute to decreased caries incidence. Therefore, in SS patients with dry mouth, increasing saliva through gustatory, masticatory or pharmaceutical stimulation (e.g. sugar-free lozenges, or chewing gum, pilocarpine or cevimeline) MAY BE CONSIDERED (Weak). 4) Non-fluoride remineralizing agents MAY BE CONSIDERED as an adjunct therapy in Sjögren’s patients with dry mouth and a high root caries rate. (Moderate). Development of guidelines for caries management, restoration and oral mucosal management are currently in progress.

The SSF Clinical Practice Guidelines are fully supported by the Sjögren’s Syndrome Foundation with no pharmaceutical support. No compensation was paid to any author. All participating authors completed Conflict of Interest forms of the American College of Rheumatology.

S6.4
Sjögren’s Syndrome Foundation (SSF)
Clinical Practice Guidelines for Ocular Manifestations of Sjögren’s
For the Sjögren’s Syndrome Foundation Clinical Practice Guidelines Committee (CPGC), Bethesda, MD, USA.

Purpose: To provide a consensus clinical guideline for the management of dry eye disease associated with Sjögren’s disease by evaluating published treatments and recommending management options.

Design: Consensus panel evaluation of reported treatments for dry eye disease.

Methods: Using the 2007 Report of the International Workshop on Dry Eye (DEWS) as a starting point, a panel of eye care providers and consultants evaluated peer-reviewed publications and developed recommendations for evaluation and management of dry eye disease associated with Sjögren’s disease. Publications were graded according to the American Academy of Ophthalmology Preferred Practice Pattern guidelines for level of evidence. Strength of recommendation was according to the Grading of Recommendations Assessment, Development and Evaluation (GRADE) guidelines and recommendations developed using a Delphi process.

Results: Evaluation should include symptoms of both discomfort and visual disturbance as well as determination of the relative contribution of aqueous production deficiency and evaporative loss of tear volume. Objective parameters of tear film stability, tear osmolarity, degree of lid margin disease, and ocular surface damage should be used to stage severity of dry eye disease to assist in selecting appropriate treatment options. Patient education as to the nature of the problem, aggravating factors, and goals of treatment is critical to successful management. Tear supplementation and stabilization, control of inflammation of the lacrimal glands and ocular surface, and possible stimulation of tear production are treatment options that are used according to the character and severity of dry eye disease.

Summary: Management guidelines for dry eye associated with Sjögren’s disease are presented. The SSF Clinical Practice Guidelines are fully supported by the Sjögren’s Syndrome Foundation with no pharmaceutical support. No compensation was paid to any author. All
S6.5

Patients Experiences of Salivary Gland Biopsies for Primary Sjögren’s Syndrome and Barriers to Consenting to Future Procedures


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Lip-biopsies are increasingly used to inform diagnosis, prognosis and treatment efficacy in primary Sjögren’s Syndrome (pSS). To give informed consent, patients should understand the procedure and its potential outcomes. However, little is known about patients’ barriers to consenting to future biopsies.

A patient panel attended a histology workshop and developed consensus statements on patient perspectives of lip-biopsies. This information informed an interview schedule and a questionnaire, which were subsequently administered to newly diagnosed patients with pSS. Eighteen patients were interviewed and 48 completed questionnaires. Participants were aged between 32 and 80 years and 83% were female.

Seventy-seven percent of patients knew what a lip-biopsy was for and described receiving a good level of information before the procedure. However, 12% described that the information given was average and 10% described it as poor. In addition, 12% felt that the side-effects were not properly explained and 38% were anxious before the biopsy. Patients also reported varying information needs; some preferred comprehensive information before the procedure, whereas others preferred restricted details to minimise anxiety. Where the information given was inadequate patients sought information on the internet, which increased anxiety.

Forty-two percent of respondents experienced expected short-term side-effects, including pain, bleeding, infection and lip numbness. Forty-two percent stated that they would agree to a biopsy in the future, while only 25% would participate in a clinical trial involving a biopsy. Those declining a future biopsy were anxious about potential side-effects and were unsure of the procedures necessity.

In most cases, patient experience of lip-biopsies was good; however, the majority of patients would decline a future lip-biopsy. Information on side-effects and necessity could reduce anxiety; however, information must be tailored to patients preferences.
Session 7. Immune Cells in Sjögren’s Syndrome

S7.1
B Cells in Sjögren’s Syndrome: To be or Not to be Depleted

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B cells play a complex role in the development of primary Sjögren syndrome (pSS): B cells produce antibodies in response to stimulation, act as antigen-presenting cells, produce cytokines, predominate in severe infiltrates where they organize in ectopic germinal centers. Consequently, the rationale for targeting B cells in pSS was particularly sound. The TEARS study (Tolerance and Efficacy of Rituximab in primary Sjögren syndrome) in France, including 122 patients, has been recently published in Annals of Internal Medicine. Its primary end point (improvement of at least 30 mm on 2 of 4 visual analogic scales exploring global activity, fatigue, pain and dryness, between weeks 0 and 24) was not met, but several secondary evaluation criteria (single dryness or fatigue scores, salivary flow rate) were significantly improved in patients who received rituximab. Furthermore, a post-hoc analysis defining a core set of outcome measures used in combination suggests that rituximab could be effective in some patients with pSS.

Therefore, if treatments based on B-cell depletion were successful in some patients, it can be assumed that treatment had eliminated B cells providing a beneficial control of disease trends in others. Growing evidence accumulates on the importance of regulatory B lymphocytes (Breg) in the pathogenesis of pSS. This is the reason why we have established in vitro experimental approaches to understand the functioning of Breg cells in patients with pSS. However, no clear phenotype of Breg cells has been described yet. Several specifications of Breg have been claimed, such as production of IL-10 or TGFβ, high expression of CD24 and CD38 (transitional B cell-like phenotype). Interestingly, an increase of several potential Breg populations has been described in the blood of pSS patients.

A great effort has to be made in clinical research in order to recognize the different subsets of patients, to be able to propose the best treatments in each individual situation.

S7.2
In-Depth Characterization of \( \text{CD24}^{\text{high}} \text{CD38}^{\text{high}} \) Transitional Human B Cells Reveals Different Regulatory Profiles and are Abnormally Distributed in Sjögren’s Syndrome

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\( \text{CD24}^{\text{high}} \text{CD38}^{\text{high}} \) transitional B cells represent a key stage in the developmental pathway of B cell peripheral tolerance and functional maturation. These B cells have been widely ascribed regulatory functions. However, the phenotypic and functional overlap between these cells and regulatory B cells remains controversial. In this study, we use multi-color flow cytometry with bioinformatic analyses and functional studies to show that CD24\(^{\text{high}}\)CD38\(^{\text{high}}\) B cells can be differentiated into multiple subsets. The study also reveals for the first time that human transitional B cells encompass transitional type 1 (T1) and T2 B cells but also distinct anergic T3 B cells as well as IL-10-producing CD27\(^+\) transitional B cells. Interestingly, the latter two subsets differentially regulate CD4\(^+\) T cell proliferation and polarization towards Th1 effector cells. Additional analyses show that the percentage of T3 B cells is reduced while the frequency of CD27\(^+\) transitional B cells is increased in patients with Sjögren’s syndrome and systemic lupus erythematosus compared with matched healthy individuals. This study provides evidence for the existence of different transitional B cell subsets each displaying unique phenotypic and regulatory functional profiles. Furthermore, the study may suggested that altered distribution of transitional B cells subsets highlights different regulatory defects in autoimmune diseases.

S7.3
Analysis of Naive, Memory and Granzyme B Expressing Peripheral B Cell Subsets in Sjögren’s Syndrome

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Background: Formerly, we reported elevated proportion of circulating follicular T helper cells and higher soluble IL-21 level in primary Sjögren’s syndrome (pSS). The interaction of invariant NKT (iNKT) cells with B cells and granzyme B (GrzmB) production may also have signifi-
Increased CD38\textsuperscript{high}IgD\textsuperscript{+} B Cells Contribute to Pathogenesis and Activity of Primary Sjögren’s Syndrome

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Background and Objectives: Primary Sjögren syndrome (pSS) is a chronic autoimmune disease characterized by production of autoantibodies and hypergammaglobulinemia. Abnormally activated B cells are considered to play a key role in its pathogenesis. Therefore, elucidation of regulatory mechanism of abnormal activation of B cells may provide a novel therapeutic target for pSS. In this study, we focused on peripheral CD38\textsuperscript{high}IgD\textsuperscript{+} B cells known as activated B cells and also precursor of plasma cells.

Methods: Peripheral blood was collected from pSS patients (n = 18) and age-matched healthy individuals (HI, n = 9). B cell subsets were analyzed by flow cytometry and B cells were purified with CD19 microbeads for stimulation in vitro with anti-IgM antibody, CD40 ligand and IL-4 (combined stimulation) to induce IgG production. ESSDAI (EULAR Primary Sjögren syndrome disease activity index) was measured for activity of pSS.

Results: The percentages of naive B cells were increased, while the ratios of IgM and IgG memory B cells were decreased in pSS, especially in EGM positive patients. The ratios of both transitional and mature B cells were elevated in pSS, mainly in patients with EGMs. CD5 + B cells, but not CD5- B cells showed elevated GrzmB and IL-21R expression in patients. Intracytoplasmic IL-21 expression of iNKT cells was also elevated in pSS.

Conclusion: Disturbance in distribution of peripheral B cell subsets is characteristic in pSS; while levels of circulating IgM and IgG memory B cells decrease, their presence may increase in exocrine glands. Our data suggest that increased IL-21R expression of CD5 + B cells and production of IL-21 by iNKT cells may play an important role in the pathogenesis of pSS by regulating CD5 + B cell functions and increasing GrzmB production.
ligands for TLR-7 and -9. We have previously shown that pSS patients and controls have similar expression pattern of TLR-7 and -9 in various B cell populations. In this study, we further analysed the responsiveness of B cells upon TLR-7 and -9 stimulation.

**Methods:** We negatively isolated B cells from 21 pSS patients and 18 healthy controls, and stimulated with TLR-7 and -9 ligands for 24h before we analysed expression of certain surface markers and intracellular cytokine levels by flow cytometry. Secreted cytokines were also measured by a multiplex cytokine assay.

**Results:** CD80, HLA-DR and CD25 were upregulated after stimulation via TLR-7, and -9 in both, pSS patients and controls. Moreover, TLR-7 and -9 stimulated B cells of pSS patients produced increased amounts of several cytokines.

**Conclusions:** B cells of pSS patients show a different responsiveness upon stimulation of TLR-7 and -9 compared to controls.

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**Background and Objective:** Patients with primary Sjögren’s Syndrome (pSS) have an increased risk of non-Hodgkin’s lymphoma, predominantly of the Mucosa Associated Lymphoid Tissue (MALT) type, which commonly occur in the parotid glands. MALT lymphomas in general express Fc receptor-like 4 (FcRL4/IRTA1/CD307d)\(^1\). Normally, FcRL4 is expressed on a very small subset of mucosa-associated B-cells. FcRL4\(^+\)B-cells might be closely related to the MALT lymphoma cells. Therefore, FcRL4\(^+\)B-cells are a potential source of progenitor cells for MALT lymphoma in primary Sjögren’s Syndrome.

**Methods:** We negatively isolated B cells from 21 pSS patients and 18 healthy controls, and stimulated with TLR-7 and -9 ligands for 24h before we analysed expression of certain surface markers and intracellular cytokine levels by flow cytometry. Secreted cytokines were also measured by a multiplex cytokine assay.

**Results:** CD80, HLA-DR and CD25 were upregulated after stimulation via TLR-7, and -9 in both, pSS patients and controls. Moreover, TLR-7 and -9 stimulated B cells of pSS patients produced increased amounts of several cytokines.

**Conclusions:** B cells of pSS patients show a different responsiveness upon stimulation of TLR-7 and -9 compared to controls.

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**Figure 1.** FcRL4\(^+\) cells in pSS patients: MALT lymphoma, parotid gland and labial gland.

(A) FcRL4\(^+\) MALT lymphoma in the parotid gland of a pSS patient. The FcRL4\(^+\)B-cells cluster in and around the lymphoepithelial lesions and the marginal zone. (B) HE stain of the same MALT lymphoma. (C) FcRL4\(^+\)B-cells in the parotid gland of a pSS patient. FcRL4\(^+\)B-cells are in close association with the ductal epithelium. Less FcRL4\(^+\)B-cells are found in the infiltrate with lower intensity of the FcRL4 stain. (D) Corresponding HE stain of the parotid gland. (E) FcRL4\(^+\)B-cells in the labial gland of a pSS patient. Few FcRL4\(^+\)B-cells with low intensity are found despite inflammation. (F) Corresponding HE stain of the labial gland.
we assessed whether FcRL4⁺ B-cells are present in the inflamed salivary gland tissue of pSS patients, and whether these cells are targeted by biological therapy.

**Methods:** Forty-nine parotid gland MALT lymphomas, 30 parotid gland biopsies, 26 labial gland biopsies and parotid gland biopsies before and after treatment with rituximab (18 patients) or abatacept (15 patients), all obtained from pSS patients, were stained for FcRL4 expression. As control served parotid gland biopsies of 8 non-pSS sicca patients and 5 non-sicca patients.

**Results:** Nearly all (96%) parotid gland MALT lymphomas expressed FcRL4 (Fig. 1). Low numbers of FcRL4⁺ B-cells were detectable in parotid glands of most (90%) pSS patients. Intensely stained FcRL4⁺ B-cells were in close relation to lymphoepithelial lesions with some FcRL4⁺ B-cells within the surrounding infiltrate. Even lower numbers of FcRL4⁺ B-cells were discernible in the labial glands. Treatment with rituximab strongly reduced the number of FcRL4⁺ B-cells in the parotid glands, whereas abatacept treatment did not affect FcRL4⁺ B-cells in the glandular tissue.

**Conclusion:** FcRL4⁺ B-cells are found in the salivary glands of pSS patients and are likely the cells from which MALT lymphomas arise. The observation that FcRL4⁺ B-cells are enriched in the parotid glands may explain why MALT lymphomas preferentially develop in these glands. Our treatment studies reveal that FcRL4⁺ B-cells can be targeted by anti-CD20 therapy and that these cells are maintained in a CD28-independent manner.

**Reference:**

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**S7.7**

**Autoantigen-Specific B Cells are Prominent in Areas of Fatty Infiltration in Salivary Glands of Patients with Primary Sjögren’s Syndrome**

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**Backgrounds and Purpose:** Primary Sjögren’s syndrome (pSS) is characterised by the production of Ro/SSA and La/SSB autoantibodies, and mononuclear cell infiltration of lacrimal and salivary glands (SG). Adipocytes can also occupy a large percentage of the SG area, although little is known about their role in the autoimmune process. We have previously characterised the general and SSA-specific glandular B cell pattern through double and single immunohistochemical staining of paraffin-embedded SG tissue from 10 well-characterised pSS patients ¹,². This helped distinguish the CD27⁺/CD20⁺ memory B cells, the CD138⁺ plasma cells and the CD20⁺ B cell zones (BCZ).³ Moreover, Ro52 and Ro60-specific cells detected in SG tissue were found to be CD19⁺/CD27⁺ differentiating plasma cells located outside the BCZ and also interstitially². In the present study, we wished to examine adipose tissue infiltration in the SG of these 10 pSS patients, in relation to both the general and SSA-specific B cell pattern. The stained SG sections were evaluated for fatty replacement, acinar atrophy and focal infiltration. Nine out of the ten patients displayed adipose tissue replacement in the glandular tissue, where three showed prominent fatty infiltration. In all instances, scattered CD138⁺ cells were evenly distributed within the adipose tissue, both in the periphery and also interstitially. Interestingly, the Ro52- and Ro60-specific cells were observed both within and in close proximity to the adipose tissue. No evident relation was observed between the BCZ, the focal infiltrates and the fatty infiltration. Together our observations suggest that the detection of these novel CD138⁺/SSA-specific antibody-secreting cells in close proximity to the adipocytes indicates a possible relationship between fat disposition and the autoimmune process in glandular tissue. Functional studies are needed to explore the potential association between B cell subsets, SSA-specific cells and fatty infiltration in the SG microenvironment.

**References:**

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**S7.8**

**BAFF-Stimulated Monocytes Accelerates IgG Production by B Cells in Patients with Primary Sjögren’s Syndrome**

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**Backgrounds and Purpose:** We found that B cell activating factor (BAFF) drastically enhances IL-6 produc-
tion by peripheral monocytes of patients with primary Sjögren’s syndrome (pSS) in vitro and the expression level of a BAFF receptor (BR3) was significantly elevated in pSS monocytes. In this study, we investigated the possible involvement of monocytes in the development of hypergammaglobulinemia (H\textsubscript{\gamma}G) which is often accompanied by pSS.

**Methods:** Peripheral pSS B cells were cultured in vitro with peripheral pSS monocytes and stimulated with soluble BAFF (sBAFF). The cells in a well were either allowed to contact to each other or separated with a transwell insert and were cultured with or without an anti-human soluble IL-6 receptor (sIL-6R) antibody. The amounts of IL-6 and IgG in the culture supernatants were measured by ELISA. FACS analysis of whole blood samples was employed to analyze the expression of BR3.

**Results and Discussion:** The proportion of BR3-positive monocytes to total monocytes (BR3/CD14\textsuperscript{+} ratio) was positively and significantly correlated with sBAFF-induced IL-6 production by monocytes among pSS patients. In addition, the BR3/CD14\textsuperscript{+} ratio also has a significant and positive correlation with the serum IgG levels of pSS patients. These data suggest that sBAFF-triggered overproduction of IL-6 by monocytes is involved in IgG overproduction by B cells in pSS patients. Concordantly, stimulation of a co-culture of pSS B cells and pSS monocytes with sBAFF drastically enhanced IgG production in vitro. It should be noted that separation of these cells by a transwell insert in a well did not suppress the IgG production. Moreover, addition of an anti-human sIL-6R antibody to the co-culture significantly inhibited the IgG production. These data strongly suggest that monocyte-derived humoral factors, such as IL-6, triggered by sBAFF play a pivotal role in the IgG overproduction by pSS B cells and that the abnormalities of monocytes may underlie pSS-associated H\textsubscript{\gamma}G.

**S7.9**

**B Cells Regulate Humoral Responses through the Control of Follicular Helper T Cells in Healthy Individuals and Sjögren’s Patients but are Defective in SLE Patients**

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Follicular helper T (T\textsubscript{fh}) cells are instrumental in the development of humoral responses, bringing helper signals to B cells for their terminal differentiation. However, abnormal functions are deleterious and trigger autoimmune reactions. Regulatory B (Breg) cells modulate inflammatory reactions through the control of Th1 polarization. We developed in vitro models to induce the polarization of human T cells into T\textsubscript{fh} cells to assess the control of human Breg on T\textsubscript{fh}-dependent humoral responses. The T\textsubscript{fh} polarization was obtained by polyclonal stimulation with IL-12 and IL-21. T\textsubscript{fh} cells induced the maturation of naive B cells into CD138\textsuperscript{+} plasma and IgD-CD27\textsuperscript{+} memory B cells and triggered the secretion of IgGs. Breg cells restrained the expression of Bcl-6, IL-21, ICOS, CXC5 and PD-1, all characteristics of T\textsubscript{fh} cells. They inhibited the induction of CD138\textsuperscript{+} and IgD-CD27\textsuperscript{+} B cells, and the secretion of IgGs. Interestingly, we demonstrated that SLE Breg cells were defective while Sjögren Breg cells were highly efficient in the regulation of T\textsubscript{fh} cell maturation and function. Our results suggest differential impairment of the autoimmune humoral responses in SLE and Sjögren patients.

**S7.10**

**BAFF is a Risk Factor and Potentially Synergize with T-Dependent Pathogenic Pathway in Sjögren’s Syndrome**

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**Background:** Primary Sjögren’s syndrome (pSS) is a systemic autoimmune disease. It has been proposed that increased levels of B cell activation factor (BAFF) breach B cell tolerance in SS by enhancing survival of self-reactive B cells, thus leading to a lot of inflammatory lesions in pSS. However, it is unclear if BAFF is sufficient to produce these abnormalities in pSS, so it is important to explore the pathogenesis of BAFF in pSS. This project will reveal them through two aspects of animal and patient, and a special emphasis was placed on follicular helper T cells (T\textsubscript{fh}) in BAFF reaction in SS.

**Objective:** To analyze the relationship between BAFF (including soluble BAFF (sBAFF) in serum and membrane BAFF (mBAFF) in labial glands of pSS patients) and clinical characters. Using BAFF Tg mice immunized with submandibular gland (SG) to study the role of BAFF in autoimmunization-induced experimental SS model. To identify T cell subpopulations participating in the autoreactive B cell develop in Sjögren’s syndrome.

**Methods:** 1. Soluble BAFF was evaluated by ELISA, mBAFF was detected through immunohistochemistry by
Enhanced Expression of TECK (CCL25) to Facilitate Increased Numbers of CCR9-Expressing Tfh-like Cells in Salivary Glands of pSS Patients


Introduction: Tfh cells play a critical role in the formation of germinal centers and B cell activation, which are hallmark immunopathological features in pSS. Recently, a novel subset of CCR9+ T cells with Tfh-like characteristics was found to be attracted to mucosal sites by TECK (thymus expressed chemokine, CCL25). Promising results have been shown in recent trials targeting CCR9 in Crohn’s disease.

Purpose: To investigate the presence of TECK and CCR9-expressing Th subsets in pSS and non-Sjögren's sicca (nSS) patients.

Methods: Levels of TECK and 103 other soluble targets were measured in serum and washouts from labial salivary gland (LSG) biopsies by Luminex. TECK mRNA in LSG was quantified by qPCR. Circulating CCR9-expressing cells were assessed by flow cytometry.

Results: Increased TECK levels were observed in serum of pSS and nSS patients as compared to HC (P = 0.06 and P = 0.08 resp all sicca patients vs HC P = 0.04, nSS vs pSS ns). TECK serum levels in pSS correlated with the presence of chemokines involved in formation of ectopic lymphoid structures BLC and MIP3β, and pro-inflammatory cytokines IL-12 and TWEAK. In addition, pSS patients displayed an increase in TECK mRNA levels in LSG (P = 0.02). Preliminary IHC data point towards enhanced CCR9+ cells in pSS. A trend towards an increase was observed in TECK protein levels in LSG washouts compared to nSS patients (P = 0.08), correlating with BLC (r = 0.59, P = 0.02). pSS patients showed enhanced numbers of circulating CCR9-expressing Th2 and Th17 cells but not Th1 cells as compared to HC.

Conclusion: Our results suggest that enhanced expression of TECK in the labial salivary gland might promote elevation of CCR9-expressing Tfh-like cells at the site of inflammation. Considering the Tfh-like characteristics and the capacity of TECK to induce pro-inflammatory cytokine secretion, this suggests that the CCL25/CCR9-axis might play a role in the immunopathology of pSS, representing a novel therapeutic target in this disease.
analyzing immune-competent cells and serological markers. We enrolled 50 pSS patients and 16 healthy controls in the study. Patients had elevated ratio of peripheral T<sub>FH</sub> cells, however, when dividing patients into two groups defined by the presence of extraglandular manifestations (EGMs), only patients with EGMs differed from controls significantly. Moreover, T<sub>FH</sub> cell percentages correlated positively with both activated T cell and Tr1 cell values, but T<sub>FH</sub> cell percentages showed negative correlation with both IgM and IgG memory B cell proportions. Elevated T<sub>FH</sub> percentages were observed in the anti-SSA/SSB positive patients. In the second part, we concentrated on the site of the inflammation and determined the composition of lymphocyte infiltration in labial salivary gland (LSG) biopsies with special emphasis on T<sub>FH</sub> cells. We selected tissue blocks obtained from 10 patients at the time of disease onset. LSGs were graded based on the organizational level of periductal lymphocytic infiltrates. T<sub>FH</sub> cell markers occurred predominantly in more organized structures with higher focus scores. The co-expression of CD3 and Bcl-6 markers identified T<sub>FH</sub> cells close to Bcl-6<sup>+</sup> B cells with the typical formation of germinal centers. Systemic features were developed later in the disease course only in patients with more structured infiltrates.

Our results indicate that the presence of T<sub>FH</sub> cells in LSGs at the disease onset may predict a more pronounced clinical course of pSS. We expect that the further understanding of the regulation of T<sub>FH</sub> cells will provide new potential therapeutic targets in the treatment of pSS patients with EGMs.

S7.13
Circulating Precursor CCR7<sup>lo</sup>PD-1<sup>hi</sup>CXCR5<sup>+</sup>CD4<sup>+</sup> T Cells Indicate Disease Activity in Sjögren’s Syndrome

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Central to the pathogenesis of primary Sjögren’s syndrome is the dysregulation of T cells and B cells. Follicular helper T (Th<sub>f</sub>) cells select mutated B cells in germinal centres, which can then differentiate into long-lived high affinity memory B cells and plasma cells. Growing evidence of the important role of Th<sub>f</sub> cells in maintaining germinal centre tolerance and demonstration that autoimmunity can arise when Th<sub>f</sub> cells are dysregulated have placed this helper T cell subset in the limelight of the pathogenesis of autoantibody-driven autoimmune diseases. Indeed, aberrant accumulation of Th<sub>f</sub> cells has been linked with Sjögren’s syndrome, systemic lupus erythematosus and autoimmune arthritis. Here we demonstrate two major subsets within circulating CXCR5<sup>+</sup> CD4<sup>+</sup> T cells in both humans and mice: the CCR7<sup>lo</sup>PD-1<sup>hi</sup> subset with a partial Th<sub>f</sub> effector phenotype and the CCR7<sup>hi</sup>PD-1<sup>lo</sup> subset with a resting phenotype. CCR7<sup>lo</sup>PD-1<sup>hi</sup>CXCR5<sup>+</sup>CD4<sup>+</sup> T cells in blood was indicative of active Th<sub>f</sub> differentiation and correlated with clinical indices in patients with Sjögren’s syndrome, thus establishing the CCR7<sup>lo</sup>PD-1<sup>hi</sup> subset as an important cellular biomarker. Differentiation of both CCR7<sup>hi</sup>PD-1<sup>lo</sup> and CCR7<sup>lo</sup>PD-1<sup>hi</sup> subsets is dependent on ICOS and BCL6, but not SAP, suggesting that circulating CXCR5<sup>+</sup> helper T cells are primarily generated before germinal centres. Upon antigen re-encounter, CCR7<sup>lo</sup>PD-1<sup>hi</sup> CXCR5<sup>+</sup> precursors rapidly differentiate into mature Th<sub>f</sub> cells, accelerate germinal centre formation and promote antibody production.

S7.14
Expansions of Salivary Gland CD4<sup>+</sup> T Cells from Sjögren’s Syndrome Patients: Single-Cell Repertoire Analysis and Correlation with Clinical Measures of Disease

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Purpose: To compare the T cell receptor (TCR) repertoire of clonally expanded CD4 memory T cells in salivary gland (SG) and peripheral blood (PB) of primary Sjögren’s syndrome (pSS) patients and determine whether the frequencies of clonally expanded cells correlate with specific measures of disease.

Method: Multiplex single cell RT-PCR was used to amplify both the α and β TCR sequences from individual memory CD4 T cells sorted from SG lip biopsy tissue and PB of 10 pSS patients. Percentages of T cells that were part of clonal expansions were calculated separately for SG and PB and correlated with various measures of disease.

Results: Over 3000 TCR sequences were obtained from 50–115 (median 91) individual SG and 75–121 (median 104) individual PB CD4 memory T cells per patient. The percentages of cells that were part of clonal expansions were significantly higher in SG (median 11%, range 0–28%)
compared to PB (median 0.9%, range 0–7%, \( P = 0.003 \)). The TCR sequences of expanded memory CD4 T cells in SG were largely distinct from those in PB. Sequence analysis revealed: 1) highly homologous CDR3s among different expanded SG T cell clones within single patients, suggesting antigen-driven expansion and 2) unique cases of convergent recombination among unrelated patients, where different V segments/additions/deletions were utilized to make identical CDR3 amino acid sequences. The percentages of clonally expanded CD4 memory T cells in SG (but not in PB) correlated significantly with the degree of SG fibrosis and reduced whole unstimulated salivary flow but not with systemic features of disease.

Conclusion: SG clonal expansions detected in this study likely identify T cells involved in recognition of common antigen(s) and glandular dysfunction. More sensitive methods will be needed to detect clonally expanded pathogenic T cells in PB.

S7.15
Limited TH17 Involvement in Primary Sjögren’s Syndrome

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Background: Primary Sjögren’s syndrome (pSS) is a chronic autoimmune rheumatic disease characterised by focal mononuclear cell infiltration in salivary and lacrimal glands. Patients suffer from xerostomia and/or keratoconjunctivitis sicca, and many present with extraglandular disease manifestations like hypergammaglobulinemia, Raynaud’s phenomenon and arthralgia. Th17 cells are a pro-inflammatory subset of T cells known to be pivotal in tissue destruction and regulation of B-cell activity, which produce the autoantibodies typifying pSS. Classical dendritic cells (cDCs) are very potent antigen presenting cells known to induce strong T-cell proliferation and cytokine production. In pSS patients, cDC numbers are lower in the peripheral blood while an increased number of these cells is present in the salivary glands, suggesting migration of these cells towards the exocrine glands. Since micro-RNAs (miRNAs) are master switches of cellular functions, potential dysregulation of miRNAs in isolated cDCs of pSS patients was investigated.

Material and Method: Serum samples from pSS patients (\( n = 141 \)) from the Haukeland University Hospital in Bergen were analysed for IL-17 and autoantibody levels. Clinical features such as extraglandular manifestations, serological data (rheumatoid factor [RF], C-reactive protein [CRP], erythrocyte sedimentation rate [ESR] and SS-associated autoantibodies [anti-Ro/SSA and anti-La/SSB]), and minor salivary gland (MSG) inflammatory focus score were obtained from patients’ charts. MSG tissue samples (\( n = 24 \)) were analysed for the presence of IL-17, IL-23 and CD56 by immunohistochemistry. Genetic variances in genes associated with Th17 cells were assessed by SNP analysis in patients (\( n = 540 \)) and controls (\( n = 532 \)) from a Swedish-Norwegian patient cohort.

Results: Serum levels of IL-17 correlated with levels of anti-Ro/SSA and anti-La/SSB autoantibodies but not with clinical features. The Th17 related cytokines IL-17 and IL-23 were both expressed in minor salivary glands. IL-17 was mainly detected interstitially in the gland epithelium and in adipose tissue, whereas IL-23 was distributed both interstitially and in focal mononuclear cell infiltrates. Most CD56 positive cells were IL-17 negative, but IL-17 expressing cells were often associated with CD56 positive cells. No genetic associations were found between II-17, II-23 and Il-10 and pSS.

Conclusions: Elevated serum levels and minor salivary gland expression of Th17 associated cytokines indicate a role for Th17 cells in pSS, but the clinical implication of Th17 cells remains unclear in the cohort investigated.

S7.16
Dysregulation of miRNAs in Classical Dendritic Cells of Patients with Primary Sjögren’s Syndrome

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Introduction: In primary Sjögren’s syndrome (pSS), T-cells play a central role in tissue destruction and regulation of B-cell activity, which produce the autoantibodies typifying pSS. Classical dendritic cells (cDCs) are very potent antigen presenting cells known to induce strong T-cell proliferation and cytokine production. In pSS patients, cDC numbers are lower in the peripheral blood while an increased number of these cells is present in the salivary glands, suggesting migration of these cells towards the exocrine glands. Since micro-RNAs (miRNAs) are master switches of cellular functions, potential dysregulation of miRNAs in isolated cDCs of pSS patients was investigated.

Methods: All patients were diagnosed according to American-European consensus group criteria. CD1c-expressing cDCs were isolated from peripheral blood of 15 pSS patients and 7 healthy donors. Total RNA was isolated and the expression of 750 miRNAs was analyzed by RT-qPCR on the OpenArray platform. Differences of \( P < 0.05 \) were considered statistically significant and expression differences between patients and controls of <0.5 or >2.0 biologically relevant.

Results: Six miRNAs were significantly downregulated in pSS patients compared to healthy donors at biologically relevant levels. None of the targets have previously been
described as differentially expressed in any cell type or biological fluid in pSS. These miRNAs are involved in the regulation of molecular pathways associated with interferon signaling, immunity to viruses and MTOR signaling pathways. The miRNAs are currently being tested in validation cohorts and functional assays.

Conclusions: Though classical dendritic cells are pivotal immune regulatory cells, they are rarely studied in pSS patients. We here show clear dysregulation of several miRNAs associated with signaling pathways involved in major inflammatory processes. Further analysis of dysregulated miRNAs and their pathways will reveal the role of cDCs in the pathogenesis pSS.

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S7.17
IL-33 Secreted by M2 Macrophage Promotes the Pathogenesis of IgG4-Related Disease

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Objectives: Interleukin (IL)-33 is a unique IL-1 family cytokine produced from damaged epithelial cells, macrophages and dendritic cells (DCs), and stimulates helper T type 2 (Th2) inflammatory responses via the IL-33 receptor (ST2). IgG4-related disease (IgG4-RD) is a novel clinical disease entity characterized by elevated serum IgG4 and infiltration of IgG4-positive plasma cells in the multiple tissues and now is considered to be a Th2-dependent disease. In this study, we thus investigated the expression of IL-33 and ST2 in salivary glands (SGs) from IgG4-RD patients.

Patients and Methods: SGs from patients with IgG4-RD (n = 7), Sjögren’s syndrome (n = 30), and healthy subjects (n = 30) were screened for 1) expression of IL-33, ST2, and Th2 cytokines (IL-4 and IL-13), 2) producing antigen presenting cells (APCs) (macrophages (M1 + M2; CD68, M2; CD163), DCs (mDC; CD11c, pDC; CD123)) by immunohistochemical staining; 2) relationship between mRNA expression of IL-33 and Th2 cytokines; 3) co-localization of IL-33 and IL-33 producing cells by double immunofluorescence staining; 4) detection of IL-33 and the producing cell by flow cytometry.

Results: mRNA expression of IL-33, ST2, and Th2 cytokines in IgG4-RD was significantly higher than that in the other groups. Moreover, mRNA expression of IL-33 was positively correlated with that of Th2 cytokines only in IgG4-RD. IL-33 was detected in/around epithelial cells in all the groups, while it was strongly detected in infiltrating lymphocytes around ectopic germinal centers only in IgG4-RD. CD68 and CD163-positive cells almost merged with IL-33. In addition, the number of CD163-positive cells in IgG4-RD was significantly higher than that in the other groups. Additionally a large fraction of the CD68+CD163+ cells produced IL-33 only in IgG4-RD.

Conclusion: These results suggest that IL-33 secreted by M2 macrophages plays a key role in Th2 cytokine production and then be involved in the pathogenesis of IgG4-RD.

S7.18
Analysis of IL-6 Secretion by Salivary Cells in Sjögren’s syndrome

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Sjögren’s Syndrome (SS) is an autoimmune disease in which the immune system attacks its own salivary and lacrimal glands. Toll-like receptors (TLRs) are upregulated in salivary tissue from SS patients; however, the functional effects of receptor ligation are poorly understood and the ligand(s) that activate TLRs in the context of SS remain unknown.

Objective: The objective of this study is to determine whether IL-6 secretion in salivary cells occurs in a TLR4-dependent manner in both human and murine salivary cells.

Methods: We used real time PCR (RT-PCR) to examine TLR4 and associated signaling molecules in the human submandibular salivary gland (SMG) cell line A253. We then stimulated A253 cells with lipopolysaccharide (LPS) and performed ELISAs to quantify the IL-6 secreted. We also assayed SMG tissue from the SS mouse model NOD.B10Sn-H2b/J (NOD.B10) with clinical disease and age and gender matched C57BL/Sn10j (BL/10) controls for TLR4 and related signaling molecules by RT-PCR. To examine IL-6 production by primary culture salivary gland cells, we harvested SMG tissue from NOD.B10 animals with clinical disease and BL/10 controls. Cells were treated with two types of LPS (10 µg/ml) for 24 hours, supernatant was harvested, and IL-6 quantified by ELISA.

Results: A253 cells express TLR4, CD14, LY96, and MyD88. Whole SMG from NOD.B10 and BL/10 also
Expression of Indoleamine 2,3 Dioxygenase-1 and -2 (IDO1 and IDO2) in Focal Sialoadenitis of Patients with Sjögren’s Syndrome (SS)

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Background: IDO function in autoimmunity is controversial. In collagen-induced arthritis IDO1 inhibition limits the arthritis phenotype. Conversely, IDO2 is critical for arthritis development in K/BxN transgenic mice. Serum tryptophan/kynurenines ratio, regulated by IDO, is increased in patients with SS, suggesting its implication in disease pathogenesis.

Aims: To investigate whether IDO1 and IDO2 are expressed in focal sialoadenitis of patients with SS and identify the cellular localization of these enzymes in the inflammatory infiltrates.

Patients and Methods: Tonsils and minor salivary gland (SG) biopsies from 7 patients with SS and non-inflamed control were examined for IDO and IDO2 expression using immunohistochemistry (IH) and immunofluorescence (IF). Double staining was used to identify the expression of IDO in T-cells, B-cells, plasmacytoid and myeloid dendritic cells (pDC, mDC), plasma cells (PCs) and macrophages.

Results: In control SG we could not detect significant expression of IDO. In inflamed tonsils limited expression of IDO2 was observed both in the follicular and in the interfollicular area, respectively, by B cells and PCs. SG sections showed variable levels of IDO and IDO2 expression within the lymphoid aggregates. In segregated foci we detected both IDO and 2 within the B cell area. Similar pattern of expression of IDO2 was found in the B cell population in close proximity to the ducts and on PCs. Double staining showed prevalent co-localization of IDO with pDCs, and a less consistent expression of the enzymes in the mDC. A small percentage of macrophages was found to express IDO2, while no expression of IDO1 and IDO2 was detected on T cells. IDO1 and 2 were detected in vascular structures and ductal epithelial cells in all SG evaluated.

Conclusions: Increased and diffuse expression of IDO1 and 2 was detected in SS SG, in specific cell populations within the inflammatory foci, suggesting an important role for this molecule in disease pathogenesis.
Conclusions: Our data show that, in pSS, T cells in SG present high levels of autophagy, which may up-regulate the expression of pro-inflammatory cytokines, providing evidence for a role of this process in the pathogenesis of pSS.

S7.21
B and T Cells Count in Minor Salivary Glands of Primary Sjögren’s Syndrome: Development and Validation of a Digital Procedure

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Background: Quantification of B and T-cell infiltrate in minor salivary glands (MSG) of primary Sjögren’s syndrome (pSS) patients is an on-going challenge. It might be a powerful tool to predict pSS prognosis. Manual counting on serial images is the most widely used method, but is very time consuming.

Objectives: Our objectives were to develop and evaluate an automated digital method to quantitatively assess B and T-cell infiltrate in a whole gland section.

Methods: Sixty-two complete sections of MSG from well characterized pSS patients were studied. MSG histology was analysed according to the classical Chisholm’s classification, the Tarpley’s score, the presence of germinal centres and the focus score. B and T-cells were, respectively, immunostained with CD-20 and CD-3 antibodies using red and brown chromogenes. Slides were digitized and spliced into a mosaic of smaller JPEG-format images. The digital procedure was based on colour isolation of red and brown signal for each pixel. Results were obtained as a number of pixels per JPEG format image. The digital procedure was compared to a manual cell counting method by two pathologists, on a panel of 31 JPEG-format images. For each MSG of the cohort, digital B and T-cell counts were performed for one whole gland section. Levels of agreement between manual and digital methods were evaluated using Bland-Altman plots and ICC.

Results: We found a good correlation between the number of pixels and the manual cell count. Intraclass coefficients between digital and manual quantification on the 31 JPEG-format images was excellent with ICC = 0.92 (95% 0.85 –0.96) for B and T-cells. We observed a significant positive correlation between B-cell proportion (B cells/total lymphocytic infiltrate) and the focus score (Spearman coefficient 0.463, P < 0.0001). Median B-cell proportion was 2.5% (0.2–13.9) vs 30.0% (15.5–45.2) in MSG with Chisholm score 1–2 (n = 24) vs 3–4 (n = 38), respectively; 2.2% (0.2–6.6), 27.2% (13.0–38.9) and 48.5% (29.4–56.4) in MSG with Tarpley score of 1 (n = 23), 2 (n = 23), and 3–4 (n = 16), respectively; and 12.3% (1.9–30.6) vs 51.4% (36.6–58.9) in MSG without germinal centres (n = 50) versus MSG with germinal centres (n = 12), respectively (P < 0.001 for all comparisons). The time needed to analyse a whole MSG section was less than 3 minutes on average using this digital count.

Conclusions: This digital procedure exhibited good levels of accuracy compared to the gold standard. Software detection produced reliable, reproductive and fast results.

S7.22
Patients with Primary Sjögren’s Syndrome have Distinct Profiles of Leukocyte Populations in Peripheral Blood Revealed by TruCount Technology

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Objectives: We analyzed cellular profiles of peripheral blood leukocytes from patients with primary Sjögren’s syndrome (pSS) and healthy controls to determine if this could be used to differentiate between sub-groups of patients.

Methods: Peripheral blood from patients with pSS (n = 84) and age- and gender- matched healthy controls (n = 74) was collected into heparin tubes, and 50 µl were transferred to a TruCount tube (BD Bioscience) containing titrated amounts of antibodies, to determine the absolute number of different leukocyte populations. The cells were analysed on a BDFortessa flow cytometer (BD Bioscience). Analyses were performed using FlowJo software (Treestar) with leukocytes subpopulations identified by light scatter properties and expression of CD3, CD4, CD8, CD14, CD16, CD20, CD38, CD45 and CD56.

Results: Cellular profiles of peripheral blood from pSS patients and healthy controls varied significantly. Patients with pSS displayed significant decreases in CD56+ NK cells, CD3+ T cells, CD3+CD4+ T helper cells, and CD3+CD56+ NKT cells. Additionally, patients displaying extraglandular manifestations showed decreases in CD3+ T cells, CD3+CD4+ T helper cells, and CD3+CD8+ cytotoxic T cells compared to patients without extraglandular manifestations.
manifestations. Patients positive for SSA and SSB had significantly decreased number of CD3−CD56hiCD16− NK cells compared to SSA and SSB negative patients.

Conclusion: TruCount analyses indicate significant alterations in the cellular profiles of peripheral blood leukocytes in pSS patients and may help to subgroup the patients according to disease severity.

S7.23

CyTOF Analysis of Peripheral Blood from Sjögren’s Subjects Identifies Dysregulated Immune Subsets

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Background: Blood lymphopenia is a common feature in patients with pSS, even considered as a marker of activity. But the characterization of the subsets of lymphocytes decreased in blood has been more rarely studied and has relied only so far on the detection of selected antigens using cytomeric methods with low dimensionality.

Purpose: In order to identify and quantify cellular variations associated with disease pathogenesis, we used here highly multiparametric cytometry by time-of-flight (CyTOF) to analyze PBMCs from patients with pSS and healthy controls.

Methods: The CyTOF instrument is based on the recently-developed mass cytometry technology, which combines single cell detection used in flow cytometry with mass spectrometry detection of metal-conjugated antibodies. Using a panel of 36 antibodies, cell populations present in heterogeneous cell suspensions from patient blood were characterized and correlated with clinical parameters and disease activity.

Results: We studied 51 pSS patients and 47 non-pSS control subjects. Among pSS patients, 69% were anti-SSA+ and 33% were anti-SSB+. Using this new approach, we confirmed the previously-reported CD4+ T cell lymphopenia in Sjögren’s patients. However, we extended it to B cells and showed that the reported decrease in the frequency of memory B cells in fact results from a more general B cell lymphopenia affecting both naive and memory B cells, but sparing plasmablasts. Lymphopenia was present in a large number of patients (50%) and correlated with autoantibody positivity and ESSDAI scores, thus pointing to its potential relevance to disease pathogenesis. Finally, several other cell subsets, such as plasmacytoid dendritic cells and subsets of T cells were found dysregulated in Sjögren’s patients.

Conclusion: The CyTOF instrument is a new tool allowing precise determination of PBMC subsets, which may generate new mechanistic hypotheses and potential biomarkers.

†*Equal contribution.

S7.24

CyTOF Analysis of Lip Biopsies from Sjögren’s Subjects Identifies Dysregulated Immune and Non–Immune Cell Subsets

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Background: The cellular characterization of the immune infiltrate in exocrine glands from patients with primary Sjögren’s syndrome (pSS) has relied so far on methods with low dimensionality. As a result, our knowledge of cellular events taking place in the exocrine glands of these patients is limited.

Purpose: In order to quantify cellular variations associated with pathogenesis, we used here highly multiparametric cytometry by time-of-flight (CyTOF) to analyze diagnostic biopsies from patients with pSS and non-pSS sicca controls.

Methods: The CyTOF instrument combines single cell analysis used in flow cytometry with mass spectrometry detection of metal-conjugated antibodies. Using a panel of 36 antibodies, cell populations present in heterogeneous cell suspensions were characterized and correlated with clinical parameters. In parallel, paired formalin-fixed, paraffin embedded sections of salibial glands from 15 individuals from this cohort were used to examine anatomic relationships between cell subsets identified by CyTOF.

Results: We studied 16 pSS patients and 13 non-pSS sicca controls, among which 81% were anti-SSA+, 44% were anti-SSB+, and 65% had a focus score of 1 or more. In addition to confirming the well-characterized presence of CD4+ T cells and B cells in Sjögren’s biopsies, this experimental approach revealed several additional pathologic features, among which are: (a) a dramatic accumulation of CD38+/CD27− HLA-DRlow cells (up to 50% of glandular B cells); (b) an unexpected abundance of CD8+ T cells showing marks of activation; (c) the upregulation of HLA-DR on epithelial cells in pSS patients with grades ≥2, which constitutes the first direct evidence of a potential pathological contribution of the epithelium using unmanipulated, ex vivo, primary human cells.

Conclusion: The CyTOF instrument is a new tool allowing precise determination of salivary gland subsets, which may generate new mechanistic hypotheses on pathogenesis of the disease.

*Equal contribution.
Session 8. Type I Interferons in Sjögren’s Syndrome

S8.1
Interferon Activation in Primary Sjögren’s Syndrome: A Biomarker and Pathogenic Factor?

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Primary Sjögren’s syndrome (pSS) is characterized by a heterogeneous clinical manifestation ranging from ocular and oral dryness to vasculitis and severe fatigue. Recently, a key role for Interferon (IFN) type I has been implicated in the pathogenesis of pSS and other systemic autoimmune diseases.

Interestingly, development of pSS has been described in hepatitis C patients upon treatment with IFN type I supporting a role for IFN type I in the pathogenesis of systemic autoimmune diseases.

Type I IFN production is induced by viruses, bacteria or microbial nucleic acids when sensed by pattern recognition receptors. In addition to inhibiting viral replication IFN type I activates natural killer cells, dendritic cells and enhances antibody production. As type I IFN consists of 17 different subtypes, it cannot be easily assessed using conventional techniques. Therefore, the expression of type I IFN inducible genes—the so-called type I IFN signature—is assessed as a readout for type I IFN activity.

Several groups detected the IFN signature salivary gland tissue and blood from patients with pSS.

We described the prevalence of the IFN signature in monocytic pSS patients and its correlation to disease activity. In two different cohorts pSS patients 57% had a positive monocytic IFN type I signature. This IFN signature identifies a subgroup of pSS patients with a higher disease activity. Study of the downstream effects of IFN activation has revealed BAFF and the immune regulatory enzyme IDO as genes correlating to IFN positivity in pSS.

Investigation of pathways leading to IFN activation in IFN positive pSS revealed a role for Toll-like receptor signaling in maintaining a pathogenic loop. Receptor-mediated endocytosis of DNA/RNA containing immune complexes is supposed to trigger TLR induced IFN type I production. Recently also a contribution of IFN type II and III to the observed IFN-induced activation in pSS salivary glands was found. If IFN type II also contributes to systemic IFN activity in pSS is presently unclear.

IFN activation patterns in pSS might potentially be useful as biomarker for disease activity, subclassification, prediction of therapy response and possibly as targets for therapeutic intervention.

S8.2
Interferon Signature in Primary Sjögren’s Syndrome

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Ten years ago, Roland Jonsson’s group demonstrated the existence of the interferon signature in primary Sjögren’s syndrome in minor salivary glands, followed by other teams in salivary glands and peripheral blood. This resulted in a great improvement in the understanding of the pathogenesis of the disease, and in very interesting results on the potential relevance of interferon-induced genes as disease activity biomarkers. Many issues remain to be addressed with regards to the relative contribution of plasmacytoid dendritic cells, myeloid dendritic cells and NK cells to this interferon signature, the prevalence of type I with regards to type II interferon-induced genes, and the association between the interferon signature and fatigue.

From a therapeutic perspective, all the consistent data from different groups have paved the way to the evaluation of new biologics targeting the interferon pathway in primary Sjögren’s syndrome.

S8.3
Toll-Like Receptor 7-Driven Loss of Tolerance in Primary Sjögren’s Syndrome: A RIGged Perpetuation in the Toll of Interferon-Related Autoimmunity

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Primary Sjögren’s syndrome (pSS) is a systemic autoimmune disease characterized by pathogenic autoantibodies, anti-SSA/SSB, targeting RNA-associated antigens. Endosomal Toll-like receptor (TLR)-7 recognizes single-stranded RNA, and its expression in B cells is required for anti-RNA antibody generation. A two-fold TLR7 overexpression induces autoimmunity in susceptible mouse strains and influences the stringency of the autoreactive B cell repertoire as well as spontaneous germinal center-formation in these mice, pointing towards the importance of tight TLR7-regulation in maintaining immune tolerance.

We found a 2 to 3-fold TLR7 overexpression in IFN-positive plasmacytoid dendritic cells (P = 0.013), confirmed...
Type I and II Interferon Signatures in Sjögren’s Syndrome: Contributions in Distinct Clinical Phenotypes and Sjögren’s Related Lymphomagenesis

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Background: Both type I and II interferons (IFNs) have been implicated in the pathogenesis of Sjögren’s syndrome (SS). We aimed to explore the contribution of type I and II IFN signatures in the generation of distinct SS clinical phenotypes including non-Hodgkin’s lymphoma (NHL) development, a major SS complication.

Methods: Peripheral blood from SS patients (n = 31), SS patients complicated by lymphoma (SSL, n = 13) and healthy donors (HD, n = 30) were subjected to real-time polymerase chain reaction for three interferon inducible genes (IFIGs) preferentially induced by type I IFN, two IFIGs preferentially induced by IFNγ as well as for IFNα and IFNγ genes. The same analysis was performed in minor salivary gland tissues (MSG) derived from 31 SS patients, 10 SSL patients and 17 sicca controls (SC). Results: In peripheral blood and MSG tissues, overexpression of both type I and type II IFIGs was observed in SS patients versus HD and SC, respectively, with a predominance of type I IFN signature in peripheral blood and a type II IFN signature in MSG tissues. SS patients with salivary gland enlargement, lymphopenia, anti-Ro/SSA antibodies and hypergammaglobulinemia exhibited higher type I IFN scores in peripheral blood compared to their counterparts without those features. Hypergammaglobulinemia was also associated with increased type II IFN scores in peripheral blood. In MSG tissues derived from SSL patients we observed lower IFNα, but higher IFNγ and type II IFIG transcripts compared to both SS and SC. In ROC curve analysis, IFNγ/IFNα ratio in MSG tissues showed the best discrimination for lymphoma development, with an area under the curve of 0.88 (95% CI: 0.72–1.00, P-value: 0.001).

Conclusion: Discrete expression patterns of type I and II IFN signatures might be related to distinct clinical SS features and SS related lymphomagenesis.

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Expression of IFNαs in Salivary Glands of Patients with Sjögren’s Syndrome (SS)


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Type I and II interferons (IFN) have been implicated in the pathophysiology of SS. Recently, a new family of IFNs, namely type III IFNs or IFNλs, has been identified. IFNλs consist of three distinct members, IFNλ1/IL29, IFNλ2/IL28A and IFNλ3/IL28B and share a common signaling receptor, the IFNλR1/IL10R. Despite their function and regulation has not been delineated, they possess significant antiviral activity. Initial studies implicate them in several human disorders, including cancer and autoimmune diseases. Herein, we sought to investigate their expression in the salivary glands (SG) of SS patients.
Methods: The expression of all IFNαs and their common IFNAR1 receptor was investigated immunohistochemically in SG biopsies from 21 patients with primary SS that had variable degree of infiltration (mild, intermediate or severe; n = 7 each) and 7 non-SS sicca complaining controls.

Results: Expression of all IFNαs and their common IFNAR1 receptor was detected in SGs of both patients and controls. Low, but definite, expression of IFNα1/IL29 was detected in ductal epithelia, displaying higher intensity in SGs from SS patients and particularly those with intermediate lesions. IFNα2/IL28A was expressed in ductal and acinar epithelia, as well as infiltrating mononuclear cells (MNC). Again, higher intensity was observed in the epithelia of SS patients compared to controls. IFNα3/IL28B was also detected in ductal/acinar epithelia and infiltrating MNC, with similar expression between SS patients and controls. The common IFNAR1 receptor was expressed in all types of cells, except fibroblasts. Its epithelial expression was higher in tissues from SS patients compared to controls, whereas strong expression was detected in macrophage-like infiltrating cells.

Conclusion: Our findings implicate IFNαs in SS pathogenesis. Further studies are needed to elucidate its role in disease and the significance of its expression in controls.

S8.6 Possible Involvement of Toll-like Receptor 7–Interferon α Axis Modified with Interleukin-21 and Thymic Stromal Lymphopoietin in IgG4-Related Disease


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Objective: IgG4-related disease (IgG4-RD) is a chronic inflammatory disease that causes multiple organ dysfunctions. Histological findings include the abundant infiltration of IgG4 positive plasma cells, storiform fibrosis, obstructive phlebitis and the formation of ectopic germinal centers (GCs). GCs provide a location for antigen presentations and B cell maturation. We analyze the effect of the GCs in IgG4-RD on innate and acquired immunity.

Methods: GCs were collected from the Formalin-Fixed Paraffin Embedded specimens by using laser capture microdissection and the expression of mRNAs was compared between IgG4-RD and multicentric Castleman’s disease (MCD) with atrophied GCs. To clarify the regulation of interferon α (IFNα) by TLRs and the effect of Th2 cytokines such as thymic stromal lymphopoietin (TSLP) on GCs in IgG4-RD, immunohistochemical analysis of these factors was conducted and their expressions were compared with that in Sjögren’s syndrome (SS).

Results: The expression of Bcl6 and IL-21 was higher in GCs in IgG4-RD than MCD patients. TLR7 expression was higher in IgG4-RD than in SS. IFNα and TSLP were highly expressed in the GCs of IgG4-RD.

Conclusion: Bcl6 and IL-21 have important roles in generating dilated GCs in IgG4-RD. A TLR7-IFNα axis as well as IL-21 and TSLP stimulation has an effect on germinal center activity in IgG4-RD.

S8.7 Occurrence of Glucocorticoid-Induced Avascular Necrosis of the Femoral Heads in IgG4-Related Disease and the Roles of Interferon α to the Pathogenesis


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Objective: We often experience the occurrence of glucocorticoid-induced avascular necrosis of femoral heads (G-AVN) in the patients with IgG4-related disease (IgG4-RD) in daily practice. Recently, it is known that interferon (IFN) α plays a role in the pathogenesis of systemic lupus erythematosus (SLE), in which complicated to G-AVN. On the other hand, the signals inducing IFNα in the liver is concerned with the occurrence of G-AVN (Okazaki S. Rheumatol Int. 2013). We aimed to clarify the incidence of G-AVN in IgG4-RD and to analyze the pathogenesis, mainly the role of IFNα in IgG4-RD.

Methods: The subjects were the 144 rheumatic patients (including 25 patients with IgG4-RD), who were treated with intravenous methyl-prednisolone therapy or high does administration of glucocorticoid (initial doses of prednisolone >35 mg/day), and were evaluated G-AVN by magnetic resonance imaging at the 3 months after initiation of the treatment. We evaluated the incidence of G-AVN in IgG4-RD and other rheumatic disorders. We histopathologically analyzed the IFNα/TLR7/9 immunostaining for the salivary gland specimens of IgG4-RD to disclose the roles of innate immunity to G-AVN.

Results: G-AVN was observed in 55 cases. The incidence of G-AVN in SLE, ANCA-related vasculitis and IgG4-RD were 61.0%, 37.5% and 28.0%, respectively. The germinal centers in the specimens from IgG4-RD were strongly stained by IFNα and TLR7.

Conclusion: The incidence of G-AVN in IgG4-RD was very high as well as that in SLE. It is considered that IFNα
Fatigue or Tolerance-Induction in Primary Sjögren’s Syndrome: A Detrimental or Beneficial Role for IDO and the Tryptophan-Kynurenine Pathway?

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Primary Sjögren’s syndrome (pSS) patients often experience disabling fatigue and depressed mood as their most severe symptom. Indoleamine-2,3-dioxygenase (IDO) – the rate-limiting enzyme in tryptophan (TRP)-catabolism – is driven in part by type I/II IFNs. Interestingly enhanced TRP-catabolism is associated with mood disturbances, possibly contributing to neuroinflammation in pSS. IDO, however, has bi-facial properties, also inducing immune tolerance through regulatory T-cells (Tregs), hence hanging in the balance between inflammation and tolerance.

In 65 Healthy controls (HC), 30 IFNnegative and 32 IFNpositive pSS patients, analysis of TRP and Kynurenine (KYN) were performed simultaneously in serum using HPLC. CD14+ monocyte mRNA-expression of IDO1 and downstream enzymes were assessed. In a sub-cohort CD4+CD45RO+ T-helper memory populations were analyzed by flow cytometry. Furthermore pSS patients where characterized for disease activity (ESSDAI), general fatigue (MFI-20) and depressive symptoms (CES-D).

IDO-activity (KYN/TRP-ratio; P = 0.0054) and CD25hi-FoxP3+ Tregs (P = 0.039) were significantly increased in IFNpositive pSS. CD25hi-FoxP3+ Tregs significantly correlated with the KYN/TRP-ratio (P = 0.002; r = 0.509) and the IFNscore (P = 0.011; r = 0.375). MFI-20 highly correlated with CES-D (P < 0.0001; r = 0.668), however, a negative correlation between total MFI-scores and the KYN/TRP-ratio (P = 0.0046; r = −0.419) was observed. Here we find enhanced IDO-activity in coherence with increased Tregs, and a shift towards more neurotoxic metabolites in IFNpositive pSS. Whether this shift reflects immune-rescue or increased tolerance-to-self remains unknown. A direct link between the TRP-KYN-pathway and symptoms of fatigue and depression was not found, in fact preliminary data showed a negative correlation between fatigue-assessment and IDO-activity. An important question remains whether and how the integrity of the blood brain barrier is compromised, potentially contributing to neuroinflammation in pSS.
Session 9. Autoantibodies and Autoantigens

S9.1 High-Resolution Mass Spectrometric Sequencing of Secreted Immunoglobulin Proteomes in Primary Sjögren’s Syndrome Reveals Clonotypic Anti-Ro/La Autoantibody Sharing and Heavy-Chain Repertoire Expansion

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B-cell hyperactivity, serum hypergammaglobulinaemia and anti-Ro/La autoantibody production are cardinal features of primary Sjögren’s syndrome (pSS) but their molecular basis remains poorly understood. We have developed mass spectrometry-based proteomic technologies to investigate the molecular composition of antibody repertoires at the level of the serum proteome in pSS and related systemic autoimmune diseases. Near full-length sequencing of Ro52/Ro60/La epitope-specific IgGs have revealed marked restriction of systemic autoantibody repertoires in pSS with sharing of public clonotypes across unrelated populations. These findings point to identical molecular pathways of autoantibody production from patient to patient, and suggest new therapeutic approaches for clonal removal of pathogenic autoantibodies. Furthermore, longitudinal studies have revealed a periodic (3-monthly) turnover of serum clonotypes as the underlying basis of long-lived humoral autoimmunity in pSS, challenging models of long-lived autoreactive plasma cells. More recently, we have developed high-end proteomic technologies combined with custom databases to probe secreted heavy (H)-chain variable (V)-region repertoires comprising the hypergammaglobulinaemia of pSS. While high-resolution 2-dimensional gel electrophoresis reveals similar H-chain profiles in pSS patients versus healthy controls, de novo and database-driven variable (V)-region protein sequencing has uncovered a marked increase in V-region repertoire diversity in pSS patients (unpublished data). This discovery argues against a model of non-specific B-cell hyperactivity and points to a major intrinsic distortion of the Ig repertoire in pSS and/or multiple unique antigen-driven clonal proliferations. The unique V-region peptides identified in these studies may serve as novel diagnostic biomarkers in pSS via the use of multiplexed targeted mass spectrometric platforms.

S9.2 Recombinant Monoclonal Antibodies Derived from Salivary Gland Infiltrating Antibody Secreting Cells

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Sjögren’s syndrome is characterized by lymphocytic infiltration of exocrine glands and autoantibodies. B cells present in the salivary gland infiltrates make autoantibody. In particular, anti-Ro (or SSA) and anti-La (or SSB) are produced by these cells. We have produced human recombinant monoclonal antibodies from antibody secreting cells from minor salivary gland (MSG) biopsies of Sjögren’s and control subjects, who were evaluated in a clinic where complete ophthalmological, dental, and medical evaluations were performed. All participants were classified according to the AECG and ACR Sjögren’s criteria. Plasmablasts were single cell purified by flow cytometry. Heavy and light chain Ig genes were expanded by rtPCR and mature Ig genes cloned into human IgG expression vectors and transfected into HEK 293T cells. The human recombinant monoclonal antibodies (hrMab) were then purified from the supernatant. We produced 47 IgG hrMab from pSS subjects and 20 from sicca controls who did not met AECG criteria (DNMC). The rhMab showed diverse gene usage, somatic hypermutation with clonally related antibody in some individuals. pSS rhMab were significantly more likely to bind Ro or La (55 versus 15%, P = 0.0012 by Fisher’s), and the specificity reflected the serological profiles of the subjects. rhMab binding the muscarinic 3 receptor (MR3), either at the 2nd (n = 22) and/or 3rd (n = 16) extracellular loop were also more common among pSS than DMNC. Anti-MR3 activity coincided with anti-Ro in that 11 of 27 rhMabs with anti-Ro activity also had anti-MR3, while only 13 rhMabs bound MR3 ECL without also binding the Ro antigen (χ² = 5.07, P = 0.02). An in vitro assay using cells transfected with MR3 demonstrated receptor antagonistic activity by the rhMab. Thus, these data indicate that rhMab can be produced from plasmablasts infiltrating minor salivary glands and that these autoantibodies have the potential to influence salivary and lacrimal secretion by interaction with MR3.
Ro Negative, La Positive Subset of Primary Sjögren’s Syndrome is a Reality

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**Purpose:** Twenty-nine sera from 503 primary Sjögren’s syndrome patients were identified as anti-Ro60 (anti-SSA) negative and anti-La (anti-SSB) positive by immunodiffusion, line immunoassays and multiplex bead assays. We hypothesize that a significant portion of these are falsely negative for anti-Ro60.

**Materials:** Twenty-nine sera from primary Sjögren’s syndrome patients, fulfilling four AECG criteria, were tested for the presence of antibodies directed against La and Ro60 autoantigen. Anti-La was detected on bovine La treated with or without DNAase and RNAase (to check for false positivity, since anti-La can bind DNA and RNA). Anti-Ro60 antibodies in the sera were detected using HEp-2000 substrate (in which cells are transfected with human Ro60) and HEp-2 substrate. Anti-Ro60 and Ro-52 were also tested by \textit{in vitro} transcription/translation/immunoprecipitation assay.

**Results:** Out of the 29 sera, 25 were unequivocally negative on HEp-2000 (1:40 dilution). Four samples were clearly found to be Ro60 positive with a speckled pattern and three of the four continued to be positive up to 1:320 dilution, as against only two positive samples on HEp-2 at 1:40 dilution. This finding suggests false negativity for Ro60 exists in a small fraction (14 percent) of primary Sjögren’s syndrome patients. However, all the samples were negative for Ro60 and Ro52 by \textit{in vitro} transcription/translation/immunoprecipitation assay.

**Conclusions:** Contrary to our hypothesis, we found only a small fraction of Ro negative, La positive sera to show positive HEp-2000 pattern. This suggests that a subset of primary Sjögren’s syndrome is probably a true entity with Ro60 negativity and La positivity. Thus, we have confirmed that about 6% of our primary Sjögren’s syndrome cohort were anti-Ro negative and anti-La positive.

Correlation of Autoantigen mRNA and Autoantibody Levels with Disease Activity in Sjögren’s Syndrome

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**Background/Purpose:** Deletion of autoreactive cells occurs in both the thymus and the spleen through the actions of the autoimmune regulator gene. Previous studies had shown up regulation of mRNA in the spleen for salivary gland antigens that were targets of autoantibodies. In the current study we investigated whether salivary gland protein 1 (SP1) mRNA levels in the spleen and serum anti-SP1 antibodies correlated with activity of disease in an animal model of Sjögren’s syndrome, the IL14a transgenic mouse (IL14aTG).

**Methods:** IL14aTG mice being evaluated for responses to lymphotoxin inhibitors were evaluated for serum levels of anti-SP1 by a previously described Western blot assay (\textit{Clin. Immunol.} 145:251, 2012) and splenic mRNA expression of SP1 by qPCR, as previously described (\textit{Clin. Immunol.} 130:304, 2009) during the course of their disease and treatment.

**Results:** IL14aTG mice treated with either anti-LTa3 or LTBR-Ig during the early stages of their disease maintained normal salivary gland function. LTBr-Ig treated IL14aTG mice did not develop lymphocytic infiltration of their submandibular and lacrimal glands and failed to produce type 1 interferon in the second wave of disease. IL14aTG mice treated with anti-LTa3 did get lymphocytic infiltration of their salivary and lacrimal glands and reduced but detectable amounts of type 1 interferon production. Normal IL14aTG mice demonstrated elevations in splenic SP1 mRNA that increased with age but decreased again at 14 months of age. Anti-SP1 was expressed in all IL14aTG mice in the early stages of the disease but in fewer IL14aTG mice at 14 months of age, late in the course of disease. Expression of SP1 mRNA and anti-SP1 antibodies was suppressed in IL14aTG mice receiving either anti-LTa3 or LTBR-Ig.

**Conclusions:**
1. Expression of splenic mRNA occurs simultaneously with autoantibodies directed towards SP1 in IL14aTG mice.
2. Levels of splenic mRNA correlate with disease activity in IL14aTG mice.
3. Splenic mRNA of salivary gland antigens may be an attempt of the body to eliminate autoreactive cells in the periphery.
4. LTa and LTb have distinct but overlapping roles in the pathophysiology of Sjogren’s syndrome.

S9.5

A Novel Comprehensive Serology for Detection of Sjögren’s Syndrome

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Purpose: Sjögren’s syndrome (SS) is a complex autoimmune disease involving the salivary and lacrimal glands along with various systemic manifestations. It is a difficult disease to identify especially in its early stages. Average time from disease onset to diagnosis is 4.7 years. The serological markers suggested by the American College of Rheumatology for diagnosis of SS are antinuclear antibodies (ANA), rheumatoid factor (RF), anti-Ro, and anti-La. Novel SS autoantibodies, anti-salivary gland protein 1 (SP1), anti-carbonic anhydrase 6 (CA6) and antiparotid secretory protein (PSP), are expressed earlier in the course of the disease than anti-Ro and anti-La in animal studies. Here, we evaluated a newly approved diagnostic blood panel (Sjö™) that incorporates the classic and novel early biomarkers for SS.

Methods: Serum samples from patients with idiopathic dry eyes, suspected of having SS were analyzed with the Sjö™ panel from September 2013 to December 2014. These patients had not previously been diagnosed with SS. Antibodies to RF, anti-Ro, anti-La, anti-SP1, anti-CA6 and anti-PSP were determined by ELISAs and ANA by indirect immunofluorescence using Hep-2 substrate.

Results: Of the 6300 (83% women, 17% men) dry eye patients analyzed, 1544 (24.5%) turned out to be positive for early and/or late SS markers. Of the positive patients, 1121 (72.6%) were identified solely by early SS markers and 422 (27.3%) expressed both early and late SS markers.

Conclusions: The current data illustrate many patients with “idiopathic” dry eyes have autoantibodies consistent with early SS, at a frequency higher than that currently reported in the literature. The majority of patients express novel autoantibodies (against SP1, CA6 and PSP) associated with an early stage of SS and without anti-Ro or anti-La. Early diagnosis of SS in these patients may lead to better management of their dry eye and other systemic manifestations via referral/co-management with rheumatologists.

S9.6

Combination Autoantibody Testing Improves the Diagnostic Yield in Suspected Sjögren's Syndrome

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Purpose: Without a lip biopsy, positive SSA/SSB serology is essential for Sjögren’s syndrome (SS) diagnosis. However, the prevalence of SSA antibodies (ab) in SS may be as low as 37%. We sought to determine the sensitivity (sens) and specificity (spec) of routinely-tested ab and their combinations for SS diagnosis.

Methods: We studied 3297 SICCA participants (pts), each with suspected or established SS, for whom there were data for each of the key phenotypic features, including lip biopsy. All had ANA, rheumatoid factor (RF), SSA/SSB, and centromere (CENT) ab testing by a central commercial laboratory. These included 1490 with SS (ACR criteria) and 98 (5%) non-SS pts (sens 100%, spec 95%); 1068 with early (≤1:320) SSA/SSB, RF, and CENT (sens 97%, spec 55%); 301 with late (≥1:320) SSA/SSB, RF, and CENT (sens 98%, spec 95%); and 802 with non-SS (sens 100%, spec 76%). Combination testing does not add discriminating value in this decision making.

Results: SSA/SSB, RF, and CENT had a high predictive value in this decision making.

Conclusion: The combination test [SSA/SSB, (ANA ≥ 1:320 + RF), or CENT] had the best sensitivity and specificity for SS diagnosis. In suspected SS (e.g. pre-test probability = 50%), negative tests for each of these substantially reduces the likelihood of SS (e.g. to 10%) and should influence a clinician’s decision to perform an invasive lip biopsy. CCP ab testing does not add discriminative value in this decision making.

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S9.7

Comparison between Multiplex and Routine Clinical Immunology Analysis of Antibodies Against SSA and SSB


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Background: Anti-SSA/SSB positivity in primary Sjögren’s syndrome (pSS) is associated with a higher prevalence of extraglandular manifestations, a more distinct type I interferon signature and certain HLA haplotypes. Subdividing patients according to antibody status can be of interest in genetic and immunological studies. Data on anti-SSA/SSB collected from medical records include analyses at different clinical immunology laboratories using different methods over time. The aim of this study was to perform a multiplex antibody analysis and compare the anti-SSA/SSB results with the results obtained from routine clinical immunology.

Methods: Sera from 367 Swedish patients with pSS from four University hospitals were analyzed with a multiplex fluorescent immunoassay. Data on anti-SSA/SSB analyzed in clinical care was retrieved from the patient records. Multiplex and clinical immunology results were compared with Spearman rank correlation test. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) with the multiplex analysis as golden standard were calculated.

Results: Multiplex analysis detected antibodies against Ro60 in 62.1%, Ro52 in 61.3%, any of Ro60/Ro52 (SSA) in 66.8%, La48/SSB in 39.2%, RNP in 4.9%, Sm in 0.5%, Ribosome P in 0.5%, Chromatin in 6.5%, Scl-70 and Jo-1 both in 0%. The frequency of anti-SSA/SSB in clinical immunology was 72.8% and 45.2%. Concordant results between multiplex and clinical immunology were seen in 88.0% of sera for SSA (r = 0.72) and 78.2% for SSB (r = 0.56), both P < 0.0001. Combined clinical immunology data showed sensitivity, specificity, PPV and NPV for SSA (95.6%, 73.0%, 87.6% and 89.0%) and SSB (79.9%, 77.1%, 69.3% and 85.6%) although the results varied between the four hospitals.

Conclusion: Analysis in routine clinical care can in most cases be used for dividing pSS patients according to anti-SSA/SSB status. However, in genetic and immunological studies a uniform antibody analysis might be advantageous.

S9.8

Anti-Centromere Antibodies are Associated With More Severe Exocrine Glandular Dysfunction in Primary Sjögren’s Syndrome: An Analysis of the Sjögren’s International Collaborative Clinical Alliance (SICCA) Cohort

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Purpose: Anti-centromere antibodies (ACA) define a subgroup of Sjögren’s syndrome (SS) patients who are older, have more frequent Raynaud’s phenomenon (RP), and a lower frequency of anti-SSA/SSB, hyperglobulinemia, and rheumatoid factor (RF). We sought to test whether ACA are associated with more severe exocrine gland dysfunction.

Methods: We obtained data on 1361 SICCA participants (pts) with SS (ACR criteria) who did not have an underlying connective tissue disease by criteria extant at the time of registration. ACA were assayed by immunofluorescence.

Results: ACA were present in 82 (6%) of the 1361 SS pts and associated with an older median age (59 versus 52; P < 0.0001), female sex (99 versus 93%; P = 0.0379), lower frequencies of anti-SSA/SSB (29 versus 82%, P < 0.0001), RF (39 versus 60%, P = 0.0003), and IgG > 1445 mg/dl (30 versus 58%, P < 0.0001), and higher median focus score (FS) (2.8 versus 2.5, P = 0.0433). ACA (+) pts had higher frequencies of RP (62 versus 28%, P < 0.0001), sclerodactyly (16 versus 1%, P < 0.0001) and dilated capillary loops (20 versus 5%, P < 0.0001). Systemic Sclerosis (Scs) classification criteria (2013) were fulfilled by 14/82 (17%) of the ACA (+) pts. Exocrine gland function was worse in the ACA (+) pts: Schirmer: median 4.5 versus 6.5 mm/5 min, P = 0.0002; unstimulated whole saliva flow (UWSF): median 0.077 versus 0.367 ml/5 min, P < 0.0001. In univariate analyses, duration of dry eye and mouth symptoms was not associated with ACA. ACA (+) pts had an increased risk of UWSF < 0.1 ml/min [OR = 12.1 (95% CI, 4.9–40.7)] and Schirmer<5 mm/5 min [OR = 2.5 (95% CI, 1.5–4.4)] after correcting for age, sex, anti-SSA/SSB, and FS.

Conclusions: ACA are uncommon in primary SS but define a subset characterized by more severe exocrine glandular dysfunction, in addition to older age and a lower frequency of anti-SSA/SSB, RF and hyperglobulinemia. This glandular dysfunction is independent of age, sex, FS,
and SSA/SSB. Although the majority have RP, only a minority have sufficient features to satisfy 2013 SSc classification criteria.

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S9.9
Evaluation of Salivary Gland Protein 1 Antibodies in Chinese Patients with Primary Sjögren’s Syndrome

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Background/Purpose: Sjögren’s syndrome (SS) is currently identified serologically by ANA, RF, anti-Ro and anti-La. Novel autoantibodies have been identified in animal models and patients with SS including anti-salivary gland protein 1 (SP1). Previous studies utilizing SS patients from the United States and Greece demonstrated anti-SP1 antibodies in patients lacking anti-Ro/anti-La and patients with low focus scores on lip biopsies (Clin. Immunol. 155: 42, 2014). The current studies were undertaken to examine the expression of anti-SP1 antibodies from a population of patients with SS in China.

Methods: Patients with SS were identified from the Rheumatology clinics at the First Affiliated Hospital of Xiamen University, Xiamen, China. Sera were evaluated using commercial kits (EUROIMMUN AG) for ANA, RF, anti-Ro and anti-La. Anti-SP1 was determined by a Western blot assay as outlined in a previous publication (Clin. Immunol. 145:251, 2012).

Results: Of 49 patients with both anti-Ro and anti-La, 29% also expressed anti-SP1. Of 57 patients with only anti-Ro, 51% also expressed anti-SP1. Of 21 patients with neither anti-Ro nor anti-La, 38% expressed anti-SP1. At the same time, 19 normal controls were evaluated, of whom 10% expressed anti-SP1. In comparison, data from previous SICCA study indicate that among 26 patients with ethnic background of Asia, 16 patients with mild SS, consisting of minimal dry eyes and dry mouth and no systemic symptoms, 44% expressed anti-SP1. In 10 SS patients with more systemic symptoms, 30% expressed anti-SP1.

Conclusions:
1. Expression of anti-SP1 antibodies in patients with SS is similar in American, Greek and Chinese patients.
2. Anti-SP1 identified many patients with SS who lack anti-Ro and/or anti-La antibodies (US – 45%, Greek – 35% and Chinese – 42%).
3. Anti-SP1 is more common in SS patients with mild (? Early) manifestations than patients with more systemic symptoms.

S9.10
Characterization of Anti-Centromere Antibody Positive Sjögren’s Syndrome in a Hungarian Center

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Anti-centromere antibody positive Sjögren’s syndrome (SS) represents a special subset: patients’ symptoms are transitional between the symptoms of SS and the limited form of systemic sclerosis.

Among the 800 patients followed-up in our clinic with SS, 19 proved to be anti-centromere antibody (ACA) positive. Their demographic and clinical data were compared to 20 age and gender-matched ACA-negative SS patients. Current age, age upon diagnosis and follow-up time of patients did not differ. Since each ACA-positive patient was female, no male patients were enrolled into the ACA-negative group, either.

Unsurprisingly, prevalence of anti-Ro/SS-A and anti-La/SS-B antibodies was significantly higher among ACA-negative patients (P < 0.001). No significant difference was found regarding complement levels, prevalence of antiphospholipid antibodies or hypergammaglobulinemia. Prevalence of gastro-esophageal reflux, sclerodactyly, pulmonary fibrosis and Raynaud’s syndrome did not differ among the subsets. However, dyspnea and teleangiectasia were significantly more frequent among ACA-positive patients (P = 0.02 and 0.03, respectively). ACA-negative patients required immunosuppressive treatment significantly more often (P < 0.05). Co-existence of Hashimoto-thyroiditis was more frequent among ACA-positive SS patients, although the difference was not statistically significant (P = 0.051).

According to the latest classification criteria for systemic sclerosis, five out of our ACA-positive patients could be classified as having systemic sclerosis. Each of the aforementioned patients had teleangiectasia.

Based on our findings, ACA-positive SS patients differ from ACA-negative SS patients regarding some clinical symptoms (dyspnea, teleangiectasia), and disease course (need for immunosuppressive treatment).
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S9.11

Anti-CCP Antibody–Positive Articular Symptom in Sjögren’s Syndrome: Case Series of Nine Cases

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Objective: It is sometimes difficult to definitively differentiate between articular involvement in primary Sjögren’s syndrome (SS) and an overlap of SS and rheumatoid arthritis (RA), despite a positive anti-CCA antibody (ACPA) test. We analyzed long-term follow-up data on ACPA-positive arthralgia and arthritis in SS.

Method: We retrospectively studied nine patients with SS diagnosed with the Japanese criteria and/or ACR criteria who showed articular symptoms with ACPA positivity. RA was diagnosed by rheumatologists, and all cases met the ACR/EULAR criteria.

Results: The patients were eight women and one man (mean age, 44 ± 12 years). The mean follow-up period was 69 ± 47 months. The final diagnosis was primary SS in five cases, osteoarthritis in 1, and an overlap of SS and RA in 3. The five cases of primary SS showed arthralgia or temporary arthritis, and no patients showed joint destruction on radiographs during follow-up. Three cases with SS and RA overlap showed small joint arthritis and no significant difference in clinical data compared with five SS cases. Two of three cases were treated with biologics.

Conclusion: Articular symptoms in SS patients cannot be diagnosed as an overlap with RA at a single time point despite ACPA positivity. ACPA-positive SS needs close follow-up instead of immediately concluding a diagnosis of RA overlap.

S9.12

Novel Autoantibodies in Pediatric Sjögren’s Patients

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Purpose: Sjögren’s syndrome (SS) is an autoimmune disease involving the exocrine glands and other organs. SS normally prevalent in elderly females is considered ‘rare’ in children. The current study assesses the prevalence of recently reported novel SS markers: anti-SP1 (salivary protein 1), anti-CA6 (carbonic anhydrase-VI) and anti-PSP (parotid specific protein) antibodies along with classic parameters in pediatric patients.

Methods: Sera from 25 patients aged between three and 22 years (mean of 13.1 years) who fulfilled the Japanese Criteria (13 were primary, 12 were secondary) and 11 who were suspected SS from their symptoms and laboratory data were analyzed. Fifty age and gender matched pediatric controls were included in the analysis. Comprehensive serological panel consisting of RF, anti-Ro, anti-La, anti-SP1, anti-CA6 and anti-PSP ELISAs and ANAs by HEp2 were used for autoantibody determination.

Results: Thirty (83.3%) of the analyzed patients were female. Twenty seven (75.0%) patients were positive for anti-Ro/La and 6 (16.7%) were positive for early SS markers. One patient expressing early marker was negative for both anti-Ro and anti-La. Three out of six patients expressing early markers expressed relatively lower levels of anti-Ro and anti-La compared to the study group. 100% specificity for ANA, anti-Ro, anti-La, anti-CA6 and 98% for anti-PSP and 1 anti-SP1 was obtained for the pediatric control panel.

Conclusions: Pediatric patients with SS analyzed here were frequently ANA and anti-Ro positive. One patient expressed novel markers in the absence of Ro or La and another sialography negative patient expressed anti-SP1 IgA in high titer. Results indicate that novel SS antibodies may be useful for early diagnosis of SS before the onset of systemic immune response in the form of anti-Ro/La. Additional studies are essential to further characterize the autoantibody profiles in pediatric SS.

S9.13

Development of Dryness in Children with Positive Anti-Ro with or Without Treatment by Immunosuppressants

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Objectives: Anti-Ro is found in children with either Sjögren’s syndrome (SS), Systemic lupus erythematosus (SLE), or sometimes with chronic non-specific complaints such as fatigue and low grade fever. We have reported that about 10% of children with chronic nonspecific complaints and with positive antinuclear antibodies were positive for anti-Ro. Although a few of them showed positive lip biopsy findings equivalent to SS (subclinical SS), majority of such children had no evidence for SS. To clarify the pathogenic role of anti-Ro in various conditions and future development of dryness, those children have been followed
for more than 10 years both clinically and immunologically.

**Methods:** Patients included in this study were, 1) 27 children with chronic non-specific complaints, with anti-Ro, and with negative lip biopsies, 2) 10 children with chronic non-specific complaints, with anti-Ro, and with positive biopsies, 3) Nine children with SLE and with positive anti-Ro, 4) Three children with MCTD and with positive anti-Ro. They have been followed for more than 10 years. Anti-Ro were measured by either Ouchterlony, ELISA, Western Immunoblot or in some cases RNA-immunoprecipitation methods.

**Results:** Twenty-three out of 27 patients in the group 1) had no evidence for SS even after more than 10 years. Most of the patients in the group 2) who had not been treated by immunosuppressant have gradually developed dryness. On the other hand, patients who had been treated have developed no or little dryness. Patients in the groups 3) and 4) have developed no dryness.

**Discussion:** All patients in the groups 3) and 4) had been treated by immunosuppressants against their basic conditions and developed no dryness. In the same way, most patients with only non-specific complaints treated with immunosuppressant did not develop sicca. These results suggest that there may be chance to prevent SS with immunosuppressants for children with positive anti-Ro as long as it starts before they have dryness.

**S9.14**

**Association of Novel Early Sjögren Syndrome (SS) Markers in Diffuse Cystic Lung Diseases**

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**Purpose:** Sjögren syndrome (SS) is a systemic, autoimmune exocrinopathy characterized in a majority of the patients by the presence of sicca symptoms. Pulmonary involvement from SS is a known disease manifestation, and can rarely present in the form of diffuse cystic lung disease (DCLD). In such cases, distinguishing SS from other DCLDs, such as lymphangioleiomyomatosis (LAM), Birt-Hogg-Dubé syndrome (BHD), pulmonary Langerhans cell histiocytosis (PLCH) and cystic emphysema (CPE) is particularly challenging. The current study aims to determine the frequency of recently reported novel early SS markers: anti-SP1 (salivary protein 1), anti-CA6 (carbonic anhydrase-VI) and anti-PSP (parotid specific protein) antibodies along with classic markers such as anti-Ro and anti-La in patients with DCLDs.

**Methods:** Serum specimens from 19 patients with DCLD (1 PlCH, 7 BHD, 5 LAM, and 6 cystic emphysema) along with five SS patients with DCLD and seven healthy controls were part of a blinded study to assess for anti-Ro, anti-La, anti-SP1, anti-CA6 and anti-PSP by ELISAs.

**Results:** All five SS cases were positive for classical SS markers (Ro/La) and had sicca symptoms. Two SS cases were positive for the novel markers; Sp1 IgA and Sp1 IgM, respectively. Of the 19 patients with DCLD, 3 (15.8%) low positive for Ro, 5 (26.3%) were positive for the novel SS markers but had no sicca symptoms. Interestingly, one LAM patient with high titer for PSP IgA and Sp1 IgM but negative for the classic SS markers (Ro/La) exhibited sicca symptoms. All Healthy controls (7) were negative for both classic and novel markers.

**Conclusions:** Addition of novel SS autoantibodies to the currently available SS markers (Ro/La) was not sensitive or specific to differentiate DCLD secondary to SS from other etiologies of DCLD. The analysis was limited by selection of SS patients with advanced disease course. Analysis of these markers earlier in the disease course might improve detection rates. Further studies are needed to establish the significance of novel SS markers in DCLDs.

**S9.15**

**Inhibitory Antibodies to Type-3 Muscarinic Acetylcholine Receptor (M3R) are Highly Prevalent in Rabbits Immunized with 60-kDa Ro**

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**Purpose:** In Sjögren’s syndrome (SS), an autoimmune disease, the lacrimal and salivary glands are affected. Autoantibodies that target type-3 muscarinic acetylcholine receptors (M3R) may be a SS marker involved in the dysfunction of these exocrine glands. We hypothesized that rabbits immunized with 4-hydroxy-2-noneal (HNE)-modified or unmodified 60-kDa Ro have anti-M3R and anti-Ro. Two rabbits were immunized with 500 µg of 10 mM of HNE-modified Ro60 and two rabbits were immunized with 500 µg of unmodified Ro60. ELISA was used to detect antibodies binding Ro60, HNE-modified Ro60, as well as the second extracellular loop (2ECL) or the third extracellular loop (3ECL) of M3R using the serial bleed from the immunized rabbits. GeneBLAZer™ M3-Nfat-Bla CHO-K1 cells were used to perform M3R functional assays. We
found immunization with HNE-modified Ro60 or unmodified Ro60 abrogates tolerance. Rabbits immunized with unmodified Ro60 showed anti-Ro60 and anti-2ECL, but no anti-3ECL. Conversely, rabbits immunized with HNE-modified Ro60 had no antibody to 2ECL or 3ECL of M3R, but did have increased levels of anti-Ro60 and anti-HNE Ro60. Additionally, using the M3R-transfected cells and an M3R agonist, Carbachol, we showed significant inhibition of M3R activation by immunized rabbit IgG, whereas pre- and post-immune sera did not inhibit M3R activation. Thus, rabbits immunized with unmodified or HNE-Ro60 develop anti-Ro antibodies, as commonly seen in SS sera. We now show anti-M3R antibodies in rabbits immunized with Ro60, which functionally inhibit M3R activation. Taken together these data suggest that the second extracellular loop of M3R is a target of SS autoimmunity, and these autoreactive-M3R antibodies may inhibit lacrimal and salivary flow in rabbits.

S9.16
Single-Cell Production and Characterization of Fully-Human Monoclonal Antibodies from the Minor Salivary Glands of Sjögren’s Patients

Sjögren’s Syndrome (SS) is mediated by inflammatory and lymphoproliferative components primarily affecting the exocrine glands. Serum antibodies to Ro and La as well as antigen-experienced CD4+T and antibody secreting (ASC) B cell glandular infiltrates are hallmarks of SS. The purpose of our study was to interrogate the glandular ASC humoral immune response of SS patients and controls by producing fully human, monoclonal antibodies (hmAbs) from single cell-sorted minor salivary gland (SG) ASC infiltrates by comparing their specificities by various immuno-assays. Samples and data were obtained from nine subjects in the OMRF Sjögren’s Research Clinic. Four subjects met AECG criteria for SS. One of these also met the ACR criteria for SLE. Five subjects that did not meet disease criteria served as sicca controls. ASCs were isolated from labial SGs by single-cell-sorting for hmAb production by the OMRF Human Antibody Core Facility. Serum Ab and hmAb profiles of patients and controls were evaluated by various immuno-assays to determine specificities. From the 72 hmAbs analyzed to date (52/patient; 20/control), we found diverse antigen specificities and found correlations between patient serum and SG hmAb specificities, with hmAbs from SS patients more often binding self-antigens (P = 0.04). While 55% of the hmAbs from SS patients bound Ro and/or La, only 15% from sicca controls did (P = 0.003). We found hmAbs from SS patients to be more often polyreactive than the controls (30% versus 10%, respectively). In addition, many hmAbs were reactive to muscarinic receptor 3 (M3R) extracellular loop 2 or extracellular loop 3 ELISAs, indicating a potential mechanism for dryness in these patients. Functional testing for the anti-M3R mAbs is currently underway. Finally, these are the first fully human, antigen-specific monoclonal antibodies produced from SS salivary gland ASCs and therefore may have clinical or diagnostic importance.

S9.17
Next-Generation Sequencing Demonstrates Dynamic Recirculation of B Cell Clones in Ectopic Lymphoid Structures of Sjögren’s Syndrome
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Background: B cells play a central role in Sjögren’s syndrome (SS) pathogenesis whereby autoreactive B-cells populate ectopic germinal centres (EGC) in SS-salivary glands (SG) and undergo somatic hypermutation (SHM) and class-switch recombination of the immunoglobulin (Ig) genes. However, the capacity of specific B cell clones to seed ECG in different SG and undergo clonal diversification is unclear.

Objectives: To unravel the dynamics of B cell recirculation among adjacent minor SG (mSG) biopsies, we investigated immunoglobulin heavy chain (IgH) gene rearrangements and SHM using a high-throughput next-generation sequencing approach.

Methods: IgH gene usage and SHM were investigated in four pairs of mSG biopsies from 4 pSS patients with high B cell infiltration and EGC. Retro-transcription of 100 ng total RNA using constant domain Ig-μ, -γ and -κ specific primers yielded Ig-specific cDNA for use as template in producing libraries representing B cell Ig-gene rearrangements in SS mSGs. Sequencing was performed using the Roche GS-FLX titanium platform.

Results: We generated ~166,000 reads >350 bp in total, and detected 1631 clonotypes (defined as reads with the
same IgHV and IgHJ gene usage and equal CDR3 length) across all eight samples. Between 5 and 9 shared clonotypes were observed among paired SG biopsies in all four patients, demonstrating the same B cell can recirculate between different glands. Lineage tree analysis revealed three different patterns of B cell circulation across the biopsies: a) unidirectional circulation of B cell clones from one biopsy to another; b) circulation between the two biopsies – circulating in both directions; c) an undefined pattern with a less-mutated and unidentified precursor migrating from one site to the other.

Conclusions: We show that B cells recirculate between mSG in SS and undergo further rounds of SHM in adjacent glands. These findings demonstrate the dynamic nature of B cell affinity maturation in SS within ECGs.

**S9.18 Characteristics of Germinal Center–Like Structures in Patients with Sjögren’s Syndrome**


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**Background:** Sjögren’s syndrome (SS) is an autoimmune disease characterized by progressive lymphocytic infiltration and destruction of exocrine glands. Histopathologically, the disease manifests as infiltration of focal mononuclear cells in the salivary and lacrimal glands, which is the golden standard for its diagnosis. Several articles have reported the presence of GC-like structures in SS patients, although there is considerable heterogeneity in these findings.

**Objective:** We aimed to analyze the relationship between ectopic germinal centers (GCs) in the salivary glands of patients with Sjögren’s syndrome (SS) and the clinical/laboratory characteristics of SS, and furthermore, to determine whether ectopic GCs are associated with autoantibody production and disease activity.

**Methods:** In this retrospective study, minor salivary gland biopsies were evaluated for the presence of GC-like structures in SS patients, although there is considerable heterogeneity in these findings.

**Results:** We analyzed the data of 126 and 16 patients with primary and secondary SS (pSS and sSS), respectively, who had undergone salivary gland biopsy. GC-like structures were observed in 36 (28.6%) pSS patients and 4 (25%) sSS patients. The mean inflammatory focus score of the gland was significantly higher in GC-positive samples than in GC-negative ones both in pSS and sSS patients (P = 0.007 and 0.024, respectively). In pSS patients, high levels of rheumatoid factor (P = 0.023), ANA (P = 0.036), IgG (P = 0.017), and IgA (P = 0.0118) were more commonly encountered in GC-positive patients than in GC-negative patients. In sSS patients with ectopic GC formation, the anti-CCP, RF-IgM and ANA titers, and the prevalence of AKA, APF, anti-SSA and anti-SSB were higher, although this was not statistically significant.

**Conclusion:** There were no differences in the incidence of GC-like structures between the salivary glands of pSS and sSS patients. GC structures were particularly noted in patients with higher focus scores. Ectopic GC might play an essential role in sustaining antibody production; however, no association with clinical phenotypes and disease activity was found.

**S9.19 Expression of miRNAs that are Predicted to Target Ro/SSA and La/SSB Autoantigens in Primary Sjögren’s Syndrome (SS)**

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miRNAs, the post-transcriptional regulators of gene expression, have been implicated in SS pathogenesis and autoantigen expression. We had identified 11 miRNAs that are predicted to target Ro52/TRIM21, Ro60/TROVE2 and La/SSB mRNAs. Herein, we investigated their expression in salivary gland (SG) tissues, peripheral blood mononuclear cells (PBMC) and long-term cultured salivary gland epithelial cells (SGEC) from SS patients and sicca-controls.

**Methods:** The expression of let7b, miR16, miR129-5p, miR153, miR181a, miR200b, miR200b*, miR223, miR483-5p, miR573 and miR583 miRNAs, as well as Ro52/TRIM21, Ro60/TROVE2 and La/SSB mRNAs was investigated by qPCR in samples from 29 SS patients and 23 sicca-controls. Non-parametric Mann-Whitney and Spearman’s rank correlation tests were employed for the analysis of significant different expression between SS patients and sicca-controls and possible associations, respectively.

**Results:** The miRNAs 129-5p, 153, 573 and 583 were not expressed in any of the samples, whereas miR-200b-5p in PBMCs. miR16, miR200b-3p and miRs 223 and 483-5p were differentially expressed in SGs, SGECs and PBMCs of SS patients and sicca-controls, respectively (Mean ± SD: 7.74 ± 3.05–2.82 ± 0.88/P = 0.05, 2771 ± 634–1182 ± 215/P = 0.04, 1028000 ± 544700–63700 ± 11130/P = 0.03 and 96.4 ± 92.8–1.19 ± 0.96/P = 0.003). In
SGs, the levels of let7b, miR16, miR223 and miR483-5p expression were positively correlated with Ro52/TRIM21 mRNA ($r = 0.4194/\text{P} = 0.002$, $r = 0.6256/\text{P} < 0.0001$, $r = 0.4056/\text{P} = 0.002$, $r = 0.4167/\text{P} = 0.002$, respectively). miR200b-5p expression was associated with La/SSB mRNA ($r = 0.4208/\text{P} = 0.002$) in SGECs, whereas let7b, miR16, miR181a, miR483-5p with Ro52/TRIM21 ($r = 0.4249/\text{P} = 0.002$, $r = 0.413/\text{P} = 0.002$, $0.4243/\text{P} = 0.002$, $r = 0.4225/\text{P} = 0.002$, respectively) and miR-181a with La/SSB mRNA ($r = 0.388/\text{P} = 0.004$) in PBMCs.

Conclusion: These findings indicate that miR16, miR200b, miR223 and miR483-5p are deregulated in SS. Further studies are needed to elucidate their role in SS pathogenesis and autoantigen expression.

S9.20 Expression and Association of RO52, RO60 and LA48: Implications for their Antigenicity in Sjögren’s Syndrome

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Autoantibodies against Ro52, Ro60 and La48 are often present in the serum of patients diagnosed with primary Sjögren’s syndrome (pSS). Anti-Ro52 and anti-Ro60 (also collectively denominated SSA), and anti-La48 (also called SSB) can be measured contemporarily in individual pSS patients. It is not known why the three proteins are autoantigens in pSS, or why patients can have the different reactive autoantibodies at the same time.

The aim of this study was to investigate the expression of Ro52, Ro60 and La48 in pSS and non-pSS salivary glands, as well as in A-253 and HeLa cells; and whether Ro52 was associated to Ro60 or La48 in the two cell lines.

In confocal microscopy after staining with commercially available antibodies, Ro52 was expressed in epithelial cells, hematopoietic cells, and fibroblasts. Ro60 and La48 stained epithelial and hematopoietic cells, and especially La48, also endothelial cells. Epithelial and hematopoietic cells of pSS tissues displayed a more intense fluorescence signal compared to non-pSS samples, but no difference in subcellular localisation could be seen. The immunofluorescence signal from the three proteins was both nuclear and cytoplasmatic with partial colocalisation. Differences in signal intensity and localisation were seen in the two cell lines. A-253 or Hela cell lysates were immunoprecipitated with commercially available anti-Ro60 or anti-La antibodies, and no convincing band for Ro52 could be seen when the precipitates were tested against Ro52 in Western Blot.

In conclusion, the suggested association of the autoantigen Ro52 to Ro60 and La48 might not explain the contemporary presence of autoantibodies measurable in body fluids in many patients. A higher availability of the autoantigens in diseased tissues might be implicated in the selection of Ro52, Ro60 and La48 in pSS.

S9.21 Sjögren’s Syndrome: Another Facet of the Autoimmune/Inflammatory Syndrome Induced by Adjuvants (ASIA)

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Recently, a new syndrome, namely the “Autoimmune/inflammatory syndrome induced by adjuvants” (ASIA) has been defined. In this syndrome different conditions characterized by common signs and symptoms and induced by the presence of an adjuvant are included. The adjuvant is a substance capable of boosting the immune response and of acting as a trigger in the development of autoimmune diseases.

Post-vaccination autoimmune phenomena represent a major issue of ASIA. Indeed, despite vaccines represent a mainstay in the improvement of human health, several of these have been implicated as a potential trigger for autoimmune diseases. Sjögren’s Syndrome (SjS) is a systemic chronic autoimmune inflammatory disease characterized by the presence of an inflammatory involvement of exocrine glands accompanied by systemic manifestations. Own to the straight association between infectious agents exposure (mainly viruses) and sicca syndrome development, the possible link between vaccine and SjS is not surprising. Indeed, a few cases of SjS following vaccine delivery have been reported. At the same extent, the induction of SjS following silicone exposure has been described too. Thus, the aim of this review was to focus on SjS and its possible development following vaccine or silicone exposure in order to define another possible facet of the ASIA syndrome.
Session 10. Lymphoma in Sjögren’s Syndrome

S10.1 Transition to Lymphoma in Sjögren’s Syndrome
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Primary Sjögren’s Syndrome (pSS) is the autoimmune disease associated with the higher risk of lymphoma. Lymphomas complicating pSS have specific features. They are mostly low grade B cell non-Hodgkin lymphomas, with predominance of marginal zone histological type. Mucosal localization is predominant and notably MALT lymphomas. Lymphomas often develop in organs where pSS is active such as salivary glands. Germinal center (GC) like structures, high BAFF, high Flt3-ligand and high CXCL13 levels and genetic impairment of TNFAIP3 are new predictors of lymphoma development. These new findings allow a better understanding of the pathogenic mechanisms leading to lymphoma.

We propose the following scenario: auto-immune B cells with rheumatoid factor (RF) activity are continuously stimulated by immune complexes containing antibodies against more specific auto-antigens like SSA/Ro, SSB/La or others. Germline abnormality of TNFAIP3, or other genetic or acquired abnormalities of genes controlling NF-kB activation, lead to a decreased control of the NF-kB pathway and thus promotes survival of B cells and oncogenic mutations especially in GC structure. Moreover, B cells are stimulated by BAFF, increased in pSS, and acquire the capacity of autocrine BAFF production leading to a positive loop of activation.

Thus, lymphomagenesis associated with pSS exemplifies the development of antigen driven B-cell lymphoma. The control of disease activity by a well-targeted immunosuppressor is the primary objective of the management of the patient in order to repress chronic B cell stimulation.

S10.2 Germline Variant of TNFAIP3/A20 Promotes B Cell Malignant Transformation in Primary Sjögren’s Syndrome Patients: Meta-Analysis of Data from France and UK
Gaetane Nocturne, Jessica Tarn, Saida Boudaoud, James Locke, Raphael Carapito, Corinne Miceli-Richard, Simon Bowman, Jacques-Éric Gottenberg, Lindsey Criswell, Siamak Barahm, Wan-Fai Ng & Xavier Mariette
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Background: Lymphomagenesis in autoimmune disease (AID) involves persistent inflammation and activation of autoimmune B cells leading to NF-kB activation. The TNFAIP3 gene encodes the A20 protein, a central gatekeeper of NF-kB activation. We have demonstrated the role of germlinal and somatic abnormalities of TNFAIP3 in pSS associated lymphoma [1]. Among these variants, the rs2230926 has been shown to be functional and leads to an impaired control of the NF-kB pathway.

Objective: To confirm the association between pSS-associated lymphoma in an independent cohort of European pSS patients from the UK and perform a meta-analysis with our French cohort.

Patients and methods: The UK cohort included 263 controls and 586 pSS patients. Among pSS patients, 26 had a history of lymphoma. The French cohort consists of 448 controls and 589 pSS patients, 47 of them with lymphoma. The rs2230926 polymorphism was genotyped in all cases and controls. All subjects were of European ancestry. Case control association tests were performed (Fisher’s test) in the two cohorts (UK and France), followed by a meta-analysis of the two cohorts.

Results: In the UK cohort, the rs2230926 was not associated with pSS alone. However, there was a clear trend for an association between the rs2230926G allele and lymphoma when compared with controls and pSS patients.

<table>
<thead>
<tr>
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<th>Controls versus all pSS</th>
<th>Controls versus pSS with lymphoma</th>
<th>pSS without versus with lymphoma</th>
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<tbody>
<tr>
<td>% G in cases</td>
<td>% G in controls</td>
<td>P-value</td>
<td>% G in cases</td>
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<tr>
<td>UKPSSR</td>
<td>8.53</td>
<td>7.22</td>
<td>0.588</td>
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<tr>
<td>French cohort</td>
<td>12.90</td>
<td>12.05</td>
<td>0.7058</td>
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<tr>
<td>Meta-analysis</td>
<td>10.72</td>
<td>10.27</td>
<td>0.8166</td>
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Scandinavian Journal of Immunology, 2015, 81, 329–450
without lymphoma \((P = 0.051\) and \(P = 0.0613\) respectively, see Table 1). In the French cohort, we confirm the absence of association between the polymorphism and pSS. However, the rs2230926 minor allele was significantly associated with lymphoma \((P = 0.0391\) versus controls and \(P = 0.0381\) versus pSS without lymphoma). The meta-analysis of the two cohorts confirms these results: OR versus controls = 2.453, \(P = 0.0058\); OR versus pSS without lymphoma = 2.46, \(P = 0.0077\).

**Conclusion:** This study based on 711 controls and 1175 pSS patients (73 cases of lymphoma) confirms the role of A20 impairment in pSS-associated lymphoma. Subtle germline abnormalities of genes leading to impaired control of NF-kB activation in B cells continuously stimulated by autoimmunity enhance the risk of lymphoma.


### S10.3 Predicting the Outcome of Sjögren’s Syndrome-Associated non-Hodgkin’s Lymphoma Patients

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Non-Hodgkin’s lymphoma (NHL) development in Sjögren’s syndrome (SS) remains a potentially lethal complication and efforts should focus on the identification of predictors that could aid in appropriate therapeutic decisions. In order to identify potential prognostic factors for outcome in SS-associated NHL, we retrospectively analyzed a cohort of 77 SS patients, diagnosed with NHL and examined the effect of SS-activity (defined as the EULAR SS disease activity index-ESSDAI) in the prognosis of SS-associated NHLs, as defined in terms of overall and event-free survivals (OS and EFS). During the follow-up (median = 57.93 months), the 5-year OS was 90.91% (95% CI: 82.14–95.80%) and the EFS was 77.92% (95% CI: 67.37–85.82%). Patients with high ESSDAI score at lymphoma diagnosis had a greater risk for death (OR = 5.241, 95% CI: 1.034–26.568) or for event (OR = 4.317, 95% CI: 1.146–9.699, \(P = 0.008\)). These patients had also significantly worse EFS (HR = 4.541, 95% CI: 1.772–11.637) and OS (HR = 5.946, 95% CI: 1.259–28.077). An unfavorable 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). After adjustment for identified risk factors, IPI score retained a significant prognostic role following by a strong effect of ESSDAI in survival outcomes. At the point of NHL diagnosis, IPI and ESSDAI might be proved useful predictive tools in SS-associated lymphoma prognosis, directing to a more patient-tailored approach.

**Reference:**


### S10.4 Characteristics of the Major Salivary Gland’s MALT Lymphomas Arising in the Background of Sjögren’s Syndrome (SS)


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**Background:** Presumably only 30% of all MALT lymphomas in oncohaematological practice arise in the background of the primary or secondary SS (pSS or sSS).

**Aim:** To characterize major salivary gland’s MALT lymphomas, diagnosed in four Russian research centers.

**Materials and Methods:** During 2004–2014 132 lymphomas of major salivary glands were diagnosed. All cases fulfilled ACR and Russian criteria for SS and were proved by immunohistochemical examination of the major salivary gland biopsy and molecular examination of B-cell clonality in the tissue. Trepanobiopsy was performed as well.

**Results:** The majority of MALT lymphomas arose in the background of pSS – 95 (72%) and sSS – 15 (11.4%). Primary MALT lymphoma had 5 (3.8%) patients.

MALT lymphomas had 110 SS patients (105 female, five male) with mean age 55 years (range 18–75) and mean duration of the disease 7 years. The main manifestations were massive enlargement of parotid (90%), submandibular (15%), lacrimal (10%), labial salivary (4%) glands, regional (26%) or generalized (9%) lymphadenopathy, bone marrow infiltration (4%), Vasculitis (22%), interstitial lung disease (6%) and glomerulonephritis (5%) were observed. Manifestations of articular syndrome, systemic sclerosis, chronic hepatobiliary disease persisted during the MALT lymphoma course. In 80% MALT lymphoma was
localized. ANA, elevated rheumatoid factor and Ro/La antibodies were detected in 92–96%, C4 hypocomplementemia in 40% and high cryoglobulinemia in 24% of patients. Immunochemical examination revealed monoclonal secretion in the serum of 26% (IgMx 14%, IgMx+BxJk 8%, IgGx+BxJk 2%, IgAx 1%, IgGx+IgGα 1%). Polyclonal immunoglobulin deficiency had 8%. B-cell clonality was detected in PCR analysis of Ig heavy chain genes rearrangements (on frozen tissue section) in 90% of patients.

Conclusion: In our research 95% of MALT-lymphomas of major salivary autoimmune disease.

S10.5
The Main Causes of the Massive Enlargement of Major Salivary Glands in Rheumatological Practice
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Aim: To characterize the spectrum of the diseases in rheumatological practice that manifest with massive enlargement of the parotid, submandibular salivary glands or both of them.

Materials and Methods: During 2004–2014 years 298 patients (female 258, male 40) with massive enlargement of the major salivary glands were examined in Institute of Rheumatology, Moscow, Russia. Patients had the full ophthalmologic, stomatological and oncohaematological examination. Minimally invasive incision biopsy of major salivary glands was used for pathomorphological, immuno-histochemical and molecular evaluation.

Results: Nonneoplastic conditions (the 1st group) were diagnosed in 164 (55%) patients and included: lymphoepithelial sialadenitis in primary Sjogren Syndrome (pSS) – 50 (30.5%), granulomatous diseases (sarcoidosis – 37, Wegener’s granulomatosis – 1) – 38 (23.2%), IgG4-related sialadenitis – 32 (19.5%), dysmetabolic disturbances (diabetes mellitus-21, hypoprenusatal syndrome-10, gout-4) – 35 (21.3%). Rare diseases with major salivary gland enlargement were diagnosed in 9 (5.5%) patients. Oncohaematological diseases (the 2d group) were diagnosed in 134 (45%) patients that included: MALT lymphomas in pSS – 95 (71%) and secondary SS (SSS) – 15 (11%), primary MALT lymphoma – 5 (4%). Rather common were diffuse large B-cell lymphomas – 7 (5%) in pSS – 5, in sSS – 1, primary-1 and AL-amyloidosis – 6 (4.5%). Rare oncohaematologic disorders with salivary gland enlargement were follicular lymphoma -1, mantle cell lymphoma -1, T-cell lymphoblastic leukemia -1, Hodgkin disease -1, oncocytoma of parotid salivary gland -1, primary squamous cell carcinoma in patient with SLE and sSS -1. Conclusion: Nonneoplastic diseases as a cause of major salivary glands enlargement dominate in rheumatological practice (55%). Accurate differential diagnosis with oncohaematologic diseases is crucial.

S10.6
Identification of Whole Blood Gene Expression Signature in Primary Sjögren’s Syndrome Associated Lymphoma
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Background: Five decades now since Primary Sjögren’s syndrome associated lymphoma was reported, many attempts to identify potential risks factors of lymphoma development in pSS patients were carried out through this time mainly on the level of clinical features and few on the molecular level. The aim of our study is to identify a whole blood gene expression signature of pSS-associated lymphoma and to explore the potential biological significance of such signature using pathway and network analysis.

Methods: A total of 144 Whole blood RNA samples from pSS patients and healthy controls were used in the study, categorized as five clinical subsets (pSS = 61, pSS with lymphoma = 16, pSS with other cancers = 21, pSS with paraproteinemia = 23 and healthy controls = 23), all samples taken part in the UK primary Sjögren’s syndrome registry (UKPSSR) and fulfilled the AECG criteria RNA was extracted and globin mRNA were removed using GLOBINclear kit. Whole genome microarray (illumina, HumanHT-12 v4 BeadChips) was used for gene expression profiling. Microarray data were analyzed by R Bioconductor. The differentially expressed genes between the pSS-associated lymphoma group and pSS patients without lymphoma were validated using RT-PCR.
**Results:** Sixty-eight differentially expressed genes were observed when comparing the “Lymphoma” group and those without lymphoma (25 upregulated genes and 43 downregulated genes), 25 genes of them were validated in RT-PCR (14 upregulated and 11 downregulated). When compared with other cancers, only one gene was differentially expressed and it was downregulated in the ‘lymphoma’ group. No differentially expressed gene were found when comparisons were made between other clinical subsets. Go terms and KEGG pathway analysis revealed 14 biological processes and 23 different biological pathways that might be important which included (e.g. mismatch Repair, T-cell receptor signaling pathway and pathways in cancer). Comparison between each clinical subset with healthy controls reveals distinct gene expression profiles and similar differentially expressed genes but generally the IFN genes were dominated.

**Conclusion:** A potential gene expression signature for pSS-associated lymphoma was identified. Further experiments to validate the biosignature with another cohort and to evaluate the sensitivity and specificity of such signature are in progress.

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**S10.7**

**Sporadic Occurrence of Non-Diagnosed IgG4-Related Disease in Lymphoma Patients with a Previous Sjögren’s Syndrome Diagnosis**

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**Background:** Patients with sicca symptoms can be misdiagnosed as primary Sjögren’s syndrome (pSS) instead of IgG4-related disease (IgG4-RD) because of clinical and histopathological similarities. An increased risk for lymphoma in pSS is reported but the relation between IgG4-RD and lymphoma is largely unknown. The aim of this study was to investigate signs of IgG4-RD in a population-based cohort of patients diagnosed with SS complicated by lymphoma.

**Methods:** Patients with SS and lymphoma diagnoses and available lymphoma specimens were identified after linkage with the Swedish patient registry 1964–2007 and the Cancer registry 1990–2007 (n = 72). Clinical data and lymphomas were reviewed and the diagnoses evaluated. Lymphoma tissues and available biopsies from minor salivary glands (MSGs) (n = 11) were immunostained for IgG4⁺ plasma cells (PCs). In positive cases (IgG4⁺ PCs > 10 per high power field (HPF)) immunostaining of available tissue specimens with inflammation was performed for IgG⁺ PCs and IgG4⁺/IgG⁺ ratio.

**Results:** We identified a positive staining for IgG4 in lymphoma tissue from one patient. None of the MSG biopsies showed any IgG4⁺ PC. The patient with findings compatible with IgG4-RD was a 64-year-old man with idiopathic pulmonary fibrosis and suspected pSS, based on subjective and objective sicca findings, a positive ANA but negativity for anti-SSA and anti-SSB antibodies. MSG biopsy was not performed. Unspecified low-grade B-cell lymphoma was diagnosed in the submandibular gland, which showed also features of storiform fibrosis. Immunostaining showed 60 IgG4⁺ PCs/HPF in the lymphoma tissue and 153 IgG4⁺ PCs/HPF in the lung tissue, biopsied 5 years before lymphoma diagnosis, and the IgG4⁺/IgG ratio was >0.4, findings indicative of IgG4-RD.

**Conclusion:** Sporadic patients with a previous SS diagnosis complicated by lymphoma may have unrecognized IgG4-RD. This case suggests an association between IgG4-RD inflammation and the development of a lymphoma.

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**S10.8**

**Persistent Rather than Fluctuating Serum Cryoglobulins are Associated with Cryoglobulinemic Vasculitis in Primary Sjögren’s Syndrome, and Relate to the Risk of Lymphoma**

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Cryoglobulinemia is a known risk factor lymphoma in primary Sjögren’s syndrome (pSS) and it is a hallmark of a more aggressive systemic disease. Whether this is related to the sole presence of serum cryoglobulins (SC) or also to an associated cryoglobulinemic vasculitis (CV) is unknown, since criteria for the classification of the latter were developed and validated only very recently (1, 2). The aim of this study is to describe the characteristics of cryoglobulinemia in the course of pSS and to evaluate the association with CV, classified according to the validated criteria (1, 2), and with B-cell lymphoma. One hundred and fourteen consecutive patients with pSS were repeatedly evaluated for SC according to the standard methods (3). The 2011 classification criteria for CV and the presence of lymphoma were investigated in positive cases. SC were repeatedly present in 28/144 patients (19.4%). In 14/28 cases (50%), the presence of SC was fluctuating, while in 14/28 cases (50%) SC were persistently positive in all the tests performed. SC were type II in 11/28 (39.3%) patients (8/11 persistently positive, 3/11 fluctuating), and
type III in 7/28 patients (2/7 persistently positive, 5/7 fluctuating); in 10/28 cases, SC typing was not performed for the insufficient cryocrit (4/10 persistent, 6/10 fluctuating). Patients with positive SC fulfilled the CV classification criteria in 16/28 cases (57.1%). CV was more frequent in pSS with persistent SC (11/14, 78.6%) than pSS with fluctuating SC (5/14, 35.7%) (P = 0.02, Pearson). Lymphoma was observed in 4/12 (33.3%) pSS patients with SC without CV, and in 6/16 (54.5%) pSS patients with CV. Persistently detectable, rather than fluctuating SC, are more associated with CV and with malignant lymphoma in pSS. The repetition of SC testing is then recommended in pSS. (1) De Vita S, et al. Ann Rheum Dis. 2011. (2) Quartuccio L, et al. Rheumatology (Oxford). 2014. (3) Brouet JC, et al. Am J Med 1974.

S10.9
Cryoglobulinemic Vasculitis and Primary Sjögren’s Syndrome are Independent Risk Factors for Lymphoma in a Large Worldwide Population of Patients with Serum Cryoglobulins


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Serum cryoglobulins (SC) (1) may be found in many diseases, and SC are a known risk factor for lymphoma evolution in some non-malignant diseases. The aim of this study was to distinguish the role of cryoglobulinemic vasculitis (CV), classified according to the validated criteria (2, 3), and primary Sjögren’s syndrome (pSS) as risk factors of lymphoma in patients with SC. Importantly, SC, CV and pSS may occur together. 950 charts from consecutive patients with positive SC were evaluated. Patients carrying both pSS and HCV infection, as well as incomplete cases, were excluded. 657 patients with SC were selected, 374 with CV and 283 without CV (2.3). PSS, classified according to the AECG criteria was present in 96 patients (44 with CV, 52 without).

Lymphoma was reported in 61/657 (9.8%) patients with SC. Among them, CV was present in 44/61 (72.1%); 14 also with pSS, and pSS in 17/61 (27.9%); and 14/17 had CV. Patients with SC with CV showed an higher prevalence of lymphoma than patients with SC without CV (44/374, 11.5% versus 17/283, 6.3%; P = 0.02, OR = 1.93 [95% CI: 1.1–3.4]). Patients with pSS, SC and CV also showed a higher prevalence of lymphoma than patients with pSS, SC but without CV (14/44, 31.8% versus 3/52, 7.4%; P = 0.001, OR = 7.6 [95% CI 2.0–28.7]). CV and pSS were confirmed as independent risk factors for lymphoma by multivariate analyses (OR 2.2 95% CI 1.2–3.8, P = 0.01; OR 2.6 95% CI 1.1–6.8, P = 0.04, respectively).

Hepatitis C virus (HCV) infection was detected in 467/561 (83.2%) patients with SC without pSS, and did not statistically predispose to lymphoma when associated with CV in this subset (P = 1.0). CV and pSS are independent risk factors for lymphoma in patients with SC. Patients with both the conditions (CV and pSS) have the highest risk. In SC positive patients, a very high attention should be deserved to pSS, in particular when CV is present.

References:

S10.10
Activation of NFkB Pathways in Sjögren’s Syndrome Related Lymphomagenesis—Role of the His159Tyr Mutation of the BAFF-R Receptor

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Background: Sjögren’s Syndrome (SS) bears the highest risk for lymphoma development among all autoimmune diseases. A growing body of evidence suggests activation of NFκB pathways as a critical step in the pathogenesis of both SS and B-cell non Hodgkin’s lymphomas (NHL), the major type of SS-related lymphomas. The mutation His159Tyr of the BAFF-R receptor has been found to confer increased risk in patients with NHL through activation of the NF-κB pathway. The aim of the current study was to evaluate the contribution of NFκB pathways activation in SS related lymphomagenesis and explore the potential role...
of the His159Tyr BAFF-R mutation. Methods: Quantitative gene expression of both NFKB1 and NFKB2 transcripts was measured by real-time PCR in peripheral blood (PB) derived from 31 SS, 13 SS-lymphoma and 30 healthy controls (HC), in isolated B cells from 2 SS, 6 SS-lymphoma and 5 HC as well as in minor salivary gland tissues (MSG) tissues from 31 SS, 10 SS-lymphoma and 17 sicca controls (SC). The BAFF-R His159Tyr mutation was evaluated in 247 SS patients (177 non lymphoma and 70 SS-lymphoma patients), 145 systemic lupus erythematosus (SLE) patients, 101 rheumatoid arthritis (RA) patients and 180 healthy controls (HC), by PCR-RFLP and PCR-sequencing.

Results: NFKB2 transcripts were significantly upregulated in the PB, MSG tissues and isolated B cells derived from SS patients complicated by lymphoma compared to HC in PB and B-cells, and to both SS-non lymphoma patients and SC in MSG tissues. At PB level, an opposite pattern was observed in regard to NFKB1 transcripts; they were found to be significantly reduced in SS patients complicated by lymphoma compared to the HC group. As a result, NFKB2/NFKB1 ratio was significantly increased in the peripheral blood from SS patients complicated by lymphoma compared to both SS and SC with an area under the receiver operating characteristic (ROC) curve of the NFKB2/ NFKB1 being 0.804, \( P = 0.002, 95\% \text{ CI} (0.670–0.938) \). In regard to His159Tyr BAFF-R mutation, we observed an increased prevalence in SS patients complicated by lymphoma compared to HC [17 out of 247 (6.9%) versus three out of 180 (1.7%), \( P = 0.01 \)]. No such statistically significant difference was found among SS, SLE and RA groups, (6.9% versus 3.5% and 3%, respectively, \( P \)-values >0.05 in all comparisons). Both SS subgroups exhibited significantly higher frequencies of the His159Tyr BAFF-R mutation compared to HC (SS-lymphoma: 6.6% and SS-non lymphoma: 6.2% versus 1.7% in HC). When we stratified the SS-lymphoma subgroup according to the lymphoma setting of SS, with mutation of the BAFF-R receptor being a main contributor particularly in MALT patients with a SS onset at the fourth decade of life, though other concomitantly operating mechanisms cannot be excluded.

Table S10.11

| Activity of the Disease are Predictive of Lymphoma Development in Primary Sjögren Syndrome: A Case Control Study of 64 Cases |
|-------------------|------------------|-----------------|-----------------|-----------------|-----------------|
| Objective: To identify predictors of lymphoma development in pSS patients. |
| Methods: Eighty-two pSS patients who developed lymphoma were included in this retrospective study. A case-controls study was performed including 64 patients who developed lymphoma after pSS diagnosis and 128 pSS patients without lymphoma randomly selected from the | | | | | |
| Univariate analysis | Multivariate analysis |
| **cases** | **controls** | **cases** | **controls** | **OR** | **95CI** |
| history of salivary gland enlargement | 37/62 (59.7) | 21/37 (56.8) | 0.804 | 0.804 | 1.606 |
| purpura | 17/63 (27) | 17/126 (13.5) | 0.0096 | 0.0096 | 0.0096 |
| arthritis | 15/63 (23.8) | 54/127 (42.5) | 0.01 | 0.01 | 0.0055 |
| Positivity of anti SSA | 48/62 (77.4) | 73/126 (57.9) | 1.604E-04 | 1.604E-04 | 1.895 to 11.132 |
| Positivity of RF | 32/54 (59.2) | 33/118 (28) | 0.0057 | 0.0057 | 2.982 |
| Presence of cryoglobulinemia | 20/53 (37.7) | 19/111 (17.1) | 0.0434 | 0.0434 | 1.126 to 7.900 |
| Presence of monoclonal component | 17/61 (27.9) | 15/103 (14.6) | 0.0021 | 0.0021 | 0.0021 |
| low C4 | 25/52 (48.1) | 28/119 (23.5) | 4.330E-05 | 4.330E-05 | 1.780 to 11.309 |
| lymphopenia | 32/57 (56.1) | 31/128 (24.2) | 0.0015 | 0.0015 | 0.0015 |
| ESSDAI ≥ 5 | 42/63 (66.7) | 50/127 (39.37) | 6.343E-04 | 6.343E-04 | 6.343E-04 |
| ClinESSDAI ≥ 5 | 43/63 (68.2) | 54/127 (42.5) | 0.0011 | 0.0011 | 0.0011 |
CXCL13 and CCL11 Serum Levels are Associated with Lymphoma Occurrence and Disease Activity in Primary Sjögren Syndrome – Data from the French Prospective Cohort Assess

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Objectives: Development of non-Hodgkin lymphoma (NHL) is one of the most severe complications associated with primary Sjögren’s syndrome (pSS). Presence of ectopic germinal center (GC) in salivary glands biopsy is a predictor of NHL occurrence in pSS patients. Given the association between CCL11 and CXCL13 and ectopic GC, we decided to assess the link between these chemokines and B cell lymphoma in pSS patients.

Methods: CCL11 and CXCL13 serum levels at inclusion were evaluated in 385 patients from the ASSESS cohort by multiplex assay. The association between serum chemokine levels, B cell biomarkers and subsets of patients was assessed using Spearman’s test for continuous data and nonparametric Wilcoxon test for categorical data. Multivariate analyses were performed to identify parameters associated with lymphoma and disease activity.

Results: Twenty-two patients of the ASSESS cohort had a NHL (history of NHL [n = 17] or future [n = 5]). The median [IQR] serum levels of CCL11 and CXCL13 were 106.48 [69.33–149.85] pg/ml and 108.31 [58.88–200.13] pg/ml, respectively. Patients with lymphoma presented higher levels of CXCL13 compared to patients without lymphoma (P = 0.006; trend for an association for CCL11 (P = 0.056)). If we focus on the five patients who developed lymphoma subsequently after the dosage, serum CXCL13 levels was even higher: 584.76 [262.99–756.19] pg/ml and statistically different from patients without any lymphoma (108.31 [59.95–197.25], P = 0.0014). Low C4 and BAFF levels were associated with NHL in the multivariate analysis (P = 0.01 and P = 0.0002, respectively). CCL11 and CXCL13 levels positively correlated with three B-cell biomarkers: rheumatoid factor, κ/λ free light chain ratio and β2-microglobulin. CXCL13 level was the only parameter associated with disease activity in the multivariate analysis.

Conclusion: This study demonstrates a link between serum levels of CXCL13 and CCL11 and disease activity and lymphoma. This highlights the continuum between chronic B-cell activation, disease activity and lymphomagenesis in pSS patients.
Session 11. Genetic Aspects of Sjögren's Syndrome

S11.1

Insights into Sjögren’s Syndrome Etiology through Discovery of Genetic Risk Variants

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Genetic susceptibility to Sjögren’s Syndrome (SS) involves multiple risk loci that influence innate and adaptive components of the immune system. In large scale case-control studies performed by the international Sjögren’s Genetics Network (SGENE), we found strong association spanning the HLA Class I, III, and II regions, with peak association observed 5' of HLA-DQB1 ($P_{meta} = 7.65 \times 10^{-114}$). Six non-HLA loci surpassed the genome-wide significance threshold ($P < 5 \times 10^{-8}$), including IRF5, STAT4, IL12A, FAM167A-BLK, DDX6-CXCR5 and TNIP1. SS risk variants that influence RNA expression levels included five HLA Class I and II loci (A, C, DRB6, DPB1, and DQA1), IL12A, BLK, and TNIP1. Identification of precise causal variants in these loci and characterization of their contribution to disease mechanisms in the specific pathways and cell subsets in which these genetic variants operate are ongoing. Integrating genotypes with RNA expression levels to identify disease-associated eQTLs (expression quantitative trait loci) can also increase statistical power for novel gene discovery. Through eQTL analysis, we have established associated eQTLs (expression quantitative trait loci) can also increase statistical power for novel gene discovery. Through eQTL analysis, we have established

S11.2

Epigenetic Signatures of Salivary Gland Inflammation in Sjögren's Syndrome

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Sjögren’s Syndrome (SS) is a chronic, multi-system autoimmune disease characterized by progressive destruction of the exocrine glands, with subsequent mucosal and conjunctival dryness. A growing body of evidence indicates that many epigenetic changes are associated with disease status and that epigenetic marks can provide unique insights into complex disease mechanisms. We report results of a case-control study of DNA methylation differences within labial salivary gland tissue, using biopsies sampled from 13 severe SS cases and 13 controls in the Sjögren’s International Collaborative Clinical Alliance registry (HHSN268201300057C). These subjects are part of a 28-subject study group for which blood, gland tissue, and sorted PBMCs have been methylyotyped using the Illumina HumanMethylation450 BeadChip. Principal Component Analysis applied to the 404,353 CpG sites passing strict QC criteria visibly separates glands of primary SS cases from controls. We find >10,000 significant differentially methylated positions (DMPs) across the 450K array, and a hypergeometric test demonstrates significant overlap with the set of genes differentially expressed in salivary glands of SS patients (Hjelmervik et al. A&R 2005). Although we do not detect overall enrichment for GWAS-genic CpGs in our set of DMPs, we report DMPs within 10 candidates, including COL11A2, CXCR5, and IRF5. Gene-wide DMP enrichment is observed in 19 genes not previously associated with SS in GWAS, including LTA, TNF, KALRN, and several homeobox genes, while promoter-specific enrichment is seen in another set of 12 genes, including CD6, CD79B, and LTA. We derive DNA methylation signatures from sorted PBMCs (CD19+, CD4+, CD14+) to show that disease-associated changes in methylation are linearly correlated with cell-type specific patterns, resolving differential lymphocyte proportions. Our results emphasize the utility of CpG methylation as a biomarker of disease status, severity, and target tissue cell composition.
S11.3

**Genome-Wide Analysis of DNA Methylation Profiles in Multiple Tissues in Primary Sjögren’s Syndrome**

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**Background:** Increasing evidence suggests an epigenetic contribution to the pathogenesis of autoimmune diseases, including primary Sjögren’s Syndrome (pSS). The aim of this study was to comprehensively investigate genome-wide DNA methylation profiles in whole blood, CD19⁺ B cells and minor salivary gland biopsies from pSS patients and controls.

**Methods:** Whole blood samples were obtained from 100 pSS patients and 400 control individuals. All patients fulfilled the AECG criteria for pSS. Genome-wide DNA methylation profiles were generated on the Illumina HumanMethylation450K BeadChip array. After quality control 383,258 CpG sites remained for further analysis. Differential cell count estimations as well as age and sex were included as covariates in the association model, and a P-value of $<1.3 \times 10^{-7}$ was considered significant (Bonferroni correction). In addition we included primary CD19⁺ B cells from 24 patients and 47 controls, and minor salivary gland biopsies from 15 patients and 13 controls.

**Results:** We identified 12,147 differentially methylated CpG sites in whole blood (6071 hypo- and 6076 hypermethylated) annotated to 9205 genes. The top associated site was annotated to the interferon induced gene IFI44L with an average decrease in methylation of 20% in cases compared to controls. Pathway analysis of the most significantly associated genes annotated to the hypomethylated sites resulted in enrichment of antigen presentation and interferon signaling pathways. Differentially methylated sites were also enriched in genes with disease association to cancer and viral infections as well as B cell lymphoma. We further identified disease-associated DNA methylation patterns in CD19⁺ B cells and in salivary gland biopsies.

**Conclusion:** Our results indicate a role for DNA methylation in the pathogenesis of pSS. The importance of genes in the interferon system is highlighted, and the enrichment of genes involved in B cell lymphoma is intriguing and warrants further investigation.

S11.4

**DNA Methylation is Altered During Primary Sjögren’s Syndrome and Particularly in Those Patients with Severe Disease and Positive for Anti-SSB Antibodies**

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**Introduction:** New data support a role for epigenetic factors and DNA methylation in the etiology of Sjögren’s syndrome (SS). Accordingly, the present study was conducted to evaluate 5-methyl cytosine (5meCyt) staining as a new biomarker in SS.

**Material and Methods:** Twenty-five pSS patients were subdivided according to the clinical data (age, sex, disease duration), biological features (anti-nuclear antibodies, anti-SSA Ab, anti-SSB Ab, C3 and C4 complement levels, and rheumatoid factor) and the class of minor salivary gland (SG) histopathological lesion (Tarpley) in mild ($n = 10$), intermediate ($n = 7$) and severe ($n = 8$) disease. SG serial sections were stained with anti-3meCyt (global DNA methylation), anti-CK19 (epithelial cells), anti-CD20 (B cells) and anti-SSB antibodies (Ab). Ab staining was analyzed by confocal microscopy, and the intensity of 5meCyt was quantified by ImageJ software.

**Results:** When analyzing global DNA methylation in SG from SS patients, it was observed that anti-5meCyt staining was reduced in CK19-positive salivary gland epithelial cells from SS patients and among them anti-5meCyt staining was inversely correlated with the severity of the disease (5meCyt: 2.0 ± 2.9 in mild; 1.3 ± 1.9 in intermediate; 0.3 ± 0.5* in severe, *$P < 0.05$). Furthermore, low levels of DNA methylation were associated with anti-SSB positivity ($P < 0.05$) and C4 complement activation ($P < 0.05$) that may result from SG SSB overexpression and B cell infiltration as observed ($P < 0.05$).

**Conclusions:** Our data suggest that 5meCyt staining can be used as a new biomarker in SS to characterize patients with active disease and suggest a role for DNA methylation in the appearance of anti-SSB Ab.
S11.5

Genome-Wide DNA Methylation Patterns Associated with Fatigue in Primary Sjögren’s Syndrome

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Background: Epigenetic modifications can influence immune responses and are related to disease development. Recent studies point to a potential role for DNA methylation in the development of chronic fatigue. The aim of this study was to investigate genome-wide DNA methylation patterns in whole blood from patients with primary Sjögren’s syndrome in relation to fatigue.

Methods: Whole blood samples were obtained from 48 pSS patients with high (n = 24) and low (n = 24) fatigue. All patients fulfilled the AECG criteria for pSS. Genomewide DNA methylation was investigated using Illumina HumanMethylation450 K BeadChip array. After quality control 383,358 CpG sites remained for further analysis. Age, sex and differential cell count estimations were included as covariates in the association model, and a P-value of <0.001 was considered significant. In addition, a cut off of 5% average difference in methylation levels between high fatigue and low fatigue patients was applied.

Results: We identified 492 differentially methylated CpG sites in whole blood (224 hypo- and 268 hypermethylated) annotated to 387 genes. The top associated site was annotated to the gene MAP3K5, implicated in innate immune responses and apoptotic signaling. Pathway analysis of the most significantly associated genes annotated to the hypomethylated sites in subjects with fatigue resulted in enrichment of regulation of macrophage activation pathway, B cell activation and kinase activity.

Conclusion: Genes involved in activation of the innate immune system and B-cells are differently methylated in pSS patients with high and low fatigue. These findings suggest that fatigue in pSS may be influenced by DNA methylation.

S11.6

Defective Regulation of L1 Endogenous Retroelements in Primary Sjögren’s Syndrome and Systemic Lupus Erythematosus: Role of Methylating Enzymes

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Background: To investigate whether deranged methylating mechanisms are involved in the inappropriate expression of LINE-1 (L1) retroelements in primary Sjögren’s syndrome (SS) and systemic lupus erythematosus (SLE).

Methods: Minor salivary glands (MSG) were obtained from 35 patients with primary SS [23 without adverse predictors for lymphoma development (SS-low risk) and 12 complicated by B-cell lymphoma (SS-lymphoma)] and 17 sicca controls (SC). Additionally, kidney biopsy specimens and PBMCs were obtained from 23 and 73 lupus patients, respectively. Relative mRNA expression was quantified for full-length L1 transcripts, along with mediators of methylation. In an independent set of 22 MSG samples (8 SS-low risk, 11 SS-lymphoma and 3 SC), methylation levels of the L1 promoter were determined by bisulphite pyrosequencing.

Results: A strong positive correlation was demonstrated between L1 transcripts and gene products that mediate de novo and constitutive DNA methylation, DNA methyltransferase (DNMT) 3B, DNMT1, and methyl CpG binding protein 2 (MeCP2), in both SS MSG and lupus renal tissues. A significantly negative correlation was observed between expression of L1 and lymphoid-specific helicase (LSH, encoded by HELLS) in both SS MSG and SLE kidney tissues, as well as between DNMT3A transcripts and L1 expression in SLE kidney tissues and PBMCs. Reduced levels of L1 promoter methylation along with increased DNMT3B, DNMT1, and MeCP2, but reduced LSH levels were detected in SS-low risk patients compared to both SS-lymphoma and SC. The SS-lymphoma group was also characterized by a profound decrease of MeCP2 and DNMT3B compared to SC.

Conclusion: Our data support a contributory role of altered methylation mechanisms in the pathogenesis of systemic autoimmune disorders and related lymphoproliferative processes and suggest that LSH and DNMT3A should be investigated as candidate upstream mediators of
decreased L1 promoter methylation and increased L1 expression.

Funding: This study was supported by a Stavros Niarchos Fellowship grant through the Arthritis Foundation, New York Chapter to CPM and a Stavros Niarchos Foundation Research Grant to Department of Physiology, University of Athens; and NIH R01AI059893, a Novel Research Grant from the Lupus Research Institute, a Target Identification in Lupus grant from the Alliance for Lupus Research, and the Mary Kirkland Center for Lupus Research to MKC.

Contribution of MTHFR Gene Polymorphisms in Sjögren’s Syndrome Related Lymphomagenesis

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Background: Sjögren’s syndrome (SS) exhibits the highest susceptibility, among systemic autoimmune diseases, to non-Hodgkin lymphoma (NHL) development. Genetic instability and DNA methylation have been previously implicated in the pathogenesis of lymphoproliferative disorders. Methylene tetrahydrofolate reductase (MTHFR) is an enzyme essential in DNA synthesis and methylation pathways. Two common polymorphisms of the MTHFR gene, C677T and A1298C, have been implicated in the development of NHL, as they reduce the MTHFR enzyme activity and may affect DNA methylation and stability. The aim of this study was to investigate the possible contribution of the MTHFR C677T and A1298C polymorphisms in SS-related lymphomagenesis.

Methods: One hundred and eighty-nine SS patients without NHL, 72 SS patients with NHL (57 with MALT and 15 with non-MALT lymphoma), 160 healthy controls (HC) and 124 rheumatoid arthritis (RA) patients were genotyped for the detection of the MTHFR gene polymorphisms (C677T and A1298C) using polymerase chain reaction followed by restriction fragment length polymorphism (PCR-RFLP). Methylation levels of CpG islands of the promoter of the long interspersed nuclear element 1 (LINE-1) -a marker of global methylation status- were also evaluated by pyrosequencing in genomic DNA derived from 23 salivary gland tissues from SS (10 with NHL and 13 without) patients.

Results: Non NHL SS patients had significantly reduced rates of the A1298C AC heterozygous genotype compared to both RA patients and HC (OR = 0.57, 95% CI = 0.36–0.91, P = 0.02 and OR = 0.57, 95% CI = 0.37–0.88, P = 0.01, respectively). In contrast, the prevalence of both MTHFR C677T and A1298C polymorphisms did not significantly differ between SS patients complicated by NHL compared to uncomplicated SS, RA and HC groups. Further analysis according to the lymphoma subtype revealed 677 T as a risk allele and the 1298 C as a protective allele for non-MALT NHL development in patients with SS (OR = 2.1, 95% CI = 0.99–4.45, P = 0.05 and OR = 0.19, 95% CI = 0.04–0.80, P = 0.01). The concomitant presence of 1298 AA and 677 TT genotype conferred an increased risk for non-MALT NHL development among SS patients (OR: 3.4, 95% CI: 1.1–10.9, P = 0.04). Of interest, an association was observed between the presence of the MTHFR 677 T -but not MTHFR 1298 C allele- with lower methylation levels (TT versus CT versus CC: 67.9 ± 2.2 versus 69.7 ± 2.9 versus 72.3 ± 2.1, P = 0.027, by Kruskal–Wallis test), implying methylation defects as potential underlying mechanisms in the pathogenesis of SS related non-MALT lymphoma.

Conclusion: In the current study, we identified novel associations of MTHFR polymorphisms with non NHL SS as well as with SS complicated by non-MALT lymphoma. Preliminary data suggest that alterations of global methylation related to the presence of MTHFR 677 T variants may contribute to the pathogenesis of non-MALT lymphoma among SS patients.

Funding: This study was supported by a Stavros Niarchos Fellowship grant through the Arthritis Foundation, New York Chapter and a Greek Rheumatology Society Grant to CPM and a Stavros Niarchos Foundation Research Grant to Department of Physiology, University of Athens; and by NIH R01AI059893, a Novel Research Grant from the Lupus Research Institute, a Target Identification in Lupus grant from the Alliance for Lupus Research, and the Mary Kirkland Center for Lupus Research to MKC.

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A B-Cell Activating Factor Receptor (BAFF-R) His159Tyr Mutation in Sjögren’s Syndrome Related Lymphoproliferation

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Objective: To study the prevalence, clinical associations and functional implications of the His159Tyr mutation of the BAFF receptor (BAFF-R) in Sjögren’s syndrome (SS).

Methods: The BAFF-R His159Tyr mutation was evaluated by PCR-based assays in 247 SS patients (70 complicated by lymphoma, SSL), 145 systemic lupus erythematosus (SLE) and 101 rheumatoid arthritis (RA) patients, as well as 180 healthy controls (HC). Real time PCR and Western Blotting were performed for the quantification of both NF-κB1 and NF-κB2 mRNA transcript and protein levels in isolated B-cells from SSL patients carrying the mutation (SSL-BAFF-RHis159Tyr) compared to SSL patients without the mutation and HC. Results: Both SSL and SS subgroups exhibited significantly higher frequencies of the His159Tyr BAFF-R mutation compared to HC (8.6% versus 6.2% versus 1.7%, P-values: 0.03 and 0.04, respectively). The corresponding frequencies for SLE and RA patients were 3.5% and 3%, respectively. Of interest, 71.4% of SSL patients with mucosa-associated lymphoid tissue (MALT) lymphoma between 31 and 40 years old were mutation carriers. The generalized odds ratios for SS-related MALT development in the younger onset group (<40 years) in the presence of the BAFF-R mutation were 6.1 [95% (CI) 2.0–18.7, P < 0.01]. NF-κB2 at both mRNA and protein level were up-regulated in SSL-BAFF-RHis159Tyr derived B-cells. Conclusion: We identified an increased prevalence of BAFF-R His159Tyr mutation in SS patients, particularly in the younger onset subgroup complicated by MALT lymphoma. BAFF-RHis159Tyr-mediated activation of the alternate NF-κB2 pathway might contribute to the pathogenesis of SS-related lymphoproliferative disease.

*Equally contributed to this study.

Association of Genetic Ancestry with Sjögren’s Syndrome Phenotypes

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Background: Little is known about the extent to which genetic ancestry influences the manifestation of Sjögren’s syndrome (SS). Although few studies have addressed this question in SS, recent studies focusing on systemic lupus erythematosus demonstrate an important association between genetic ancestry and disease phenotype.

Methods: Subjects were derived from the Sjögren’s International Collaborative Clinical Alliance (SICCA) registry (HHSN 268201300057C). Study subjects (n = 3334) were genotyped with the Illumina HumanOmni2.5–4v1 or Illumina HumanOmni25M-8v1-1 using a 2.5 million genome-wide SNP dataset. Eigenvectors (EV) were generated by principal components analysis from a set of 144,003 SNPs for genetic ancestry assessment. Genetic ancestry was defined based on EV using cluster analysis. SS phenotypes studied included focus score (FS) (transformed to log scale), maximum ocular staining score (OSS), and SSA/SSB autoantibody production. Associations between each disease phenotype and genetic ancestry, represented by the EVs, were assessed using meta-analysis in Stata v13. Each model was stratified and weighted by recruitment site using random-effects analysis and adjusting for sex and smoking history. Multivariate linear and logistic regression modeling was also performed.

Results: EV1 distinguished Europeans from East Asians (P < 2.2e−16). EV3 distinguished Hispanics from other ethnic groups (P < 2.2e−16). Higher FS was associated with Hispanic (Amerindian) ancestry (P = 7.6e−4; I2 = 0%), and SSA/SSB autoantibody production was associated with East Asian (P = 4.9e−7; I2 = 26%) and Hispanic (Amerindian) ancestry (P = 1.1e−8; I2 = 0%) in weighted stratified models. These associations were also statistically significant in regression models. OSS showed no statistically significant association with any EV in meta-analysis models.

Conclusions: These results indicate that, similar to recent work in SLE, genetic ancestry influences SS phenotype and severity.

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S11.10

Early B Cell Factor 1 is Associated to Clinical Manifestations in Primary Sjögren’s Syndrome and SLE


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Background: Early B cell factor 1 (EBF1) is a transcription factor important for B cell development. Genetic variations in EBF1 have been associated with B cell leukemias, multiple sclerosis and primary Sjögren’s syndrome (pSS). Because both pSS and SLE are characterized by increased activation of B cells, we asked if EBF1 variants associate to disease susceptibility and clinical phenotype.

Methods: We included 633 patients with pSS from Sweden (n = 463) and Norway (n = 170), 852 Swedish patients with SLE and 2941 healthy controls from Sweden (n = 2760) and Norway (n = 181). All patients fulfilled AECG criteria for pSS or the 1982 ACR classification criteria for SLE. Samples were genotyped on the Illumina® ImmunoChip with 200 K SNPs. SNPs annotated in or surrounding EBF1 spanning over 1.1 MB were selected where 109 SNPs (pSS) and 145 SNPs (SLE) remained after quality control. Allele frequencies were compared between patients and controls and between patients with or without different clinical manifestations. Benjamini and Hochberg step-up FDR was used for multiple-test correction, and \( P_{\text{corr}} < 0.05 \) was considered significant.

Results: There were no associations to either pSS or SLE in the case–control analyses. In the pSS case-only analyses, one SNP was associated to major salivary gland swelling (\( n_{\text{pos}} = 183, n_{\text{neg}} = 312 \)) with \( P_{\text{corr}} = 0.03, \text{OR} = 1.6 \) (1.2–2.1). In the SLE case-only analyses, there was an association to nephritis (\( n_{\text{pos}} = 293, n_{\text{neg}} = 559 \)) for 51 SNPs with the top SNP rs116278653 located 8 KB upstream of EBF1 (\( P = 0.03, \text{OR} = 3.0 \) (1.4–6.5)). After adjusting for rs116278653, no significant signals remained suggesting high linkage disequilibrium within the region.

Conclusion: Genetic variation in EBF1 might have implications for the clinical manifestations of pSS and SLE, both diseases characterized by B cell activation. The functional consequences of the identified variations remain to be elucidated.

S11.11

The genetic Basis of Sjögren’s Syndrome in Asian versus European populations from Genome-Wide Association Analysis of an International Cohort

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Objectives: Our goal was to define the contribution of genetic factors to Sjögren’s Syndrome (SS) susceptibility among different populations in an international cohort.

Methods: We studied 3334 participants in the Sjögren’s International Collaborative Clinical Alliance (SICCA; contract # HHSN 268201300057C) genotyped on the Illumina HumanOmni 2.5-Quad marker set. Participants were enrolled at nine international sites, including Argentina, China, Denmark, India, Japan, the UK and the US. QC included filters based on SNP and sample missingness, unexpected relatedness, non-Mendelian inheritance, and chromosomal regions of anomaly. We also utilized external controls from dbGap. Principal components analysis was used to characterize each participant for both continental and intra-continental genetic ancestry. We analyzed the entire group and the two largest strata by ethnicity, European and Asian. In regions of interest, additional genotypes were imputed from common SNPs using the 1000 Genomes reference panel.

Results: A total of 302,689 genotyped SNPs common to the SICCA and external control platforms and passing all QC filters were used for this analysis. We observed striking differences between the Asian (402 cases, 1125 controls) and European (585 cases, 2674 controls) subgroups. While the MHC region was the most significantly associated in both populations (top \( P = 3e-8, P = 1e-29 \), respectively), it was much less so in Asians, even when analyzing a random subset of the European subgroup of the same sample size. Outside of the MHC, the most strongly associated peak in Asians was in the \( KLRG1 \) gene (top \( P = 9e-7 \) imputed, \( P = 3e-6 \) genotyped); this region was not suggestive of association in Europeans.

Conclusions: These results demonstrate that the genetic profile for SS susceptibility differs substantially according to ancestry, in particular when comparing European and...
Asian subjects. Thus studies across ethnicities have potential to elucidate new disease mechanisms.

**S11.12**

*X Chromosome Aneuploidies in Sjögren’s Syndrome: Comparison to Systemic Lupus and Rheumatoid Arthritis*

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Primary Sjögren’s syndrome (pSS) has a strong female bias that has not been explained. We evaluated the hypothesis of an X chromosome dose effect by analyzing the presence of 47XXX (Klinefelter’s syndrome, 1 in 500 live male births) and 47, XXX (1 in 1000 live female births) among a large cohort of subjects with pSS. The presence of extranumerary X chromosomes was determined by examination of fluorescence intensity of single nucleotide polymorphisms from the X chromosome from either Illumina genome-wide association study platform or the ImmunoChip. Karyotype or fluorescent in situ hybridization confirmed some of these results. Among 126 pSS men, there were 4 with 47XXX. This was significantly different from healthy controls (1 of 1254 had 47XXX, P = 0.0012 by Fisher’s exact test) as well as when compared to men with rheumatoid arthritis (RA, 0 of 363 with 47,XXY). pSS men were not statistically different when compared to men the systemic lupus erythematosus (SLE), however (4 of 136 versus 8 of 306, Fisher’s exact test P = NS). We found parallel results when examining women for 47XXX. Among pSS and SLE women, 3 of 1033 and 7 of 2826, respectively, had X chromosomes (χ² = 0.05, P = 0.82). Meanwhile, 47XXX was present in two of 7074 healthy controls and one of 2671 women with RA (χ² = 10.1, P = 0.0015 for pSS versus controls, χ² = 4.4, P = 0.035 for pSS versus RA). These results are consistent with the hypothesis that the number of X chromosomes is critical for the female bias of pSS. The mechanism is likely shared with SLE, where we found very similar increases in extranumerary X chromosomes, but not with RA, a disease in which we found no increase in X chromosome aneuploidies.

**S11.13**

*Characterization of a Sjögren’s Syndrome-Associated Long Non-Coding RNA at 2p25.1*

John A. Ice¹, He Li¹,², Kathy L. Sivils¹,², Christopher J. Lessard¹,² submitted on behalf of the Oklahoma Sjögren’s Syndrome Center of Research Translation (OSSCORT)

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SS is a common autoimmune disorder characterized by immune-mediated exocrine gland destruction and systemic inflammatory responses. We performed an RNA-sequencing (RNA-seq) study using whole blood RNA from 57 SS patients and 27 healthy controls in which we identified 2632 statistically significant differentially expressed (DE) transcripts, including 969 long non-coding RNAs (lncRNAs). Significant upregulation of SSINCR1, a lncRNA at chromosome 2p25.1, was noted in SS patients versus controls (P = 3.69 x 10⁻⁵; Fold Change = 2.4). Here, we sought to validate, replicate, and functionally characterize, SSINCR1, to better understand its role in SS pathogenesis. Technical validation by qRT-PCR using the RNA-seq cDNA library confirmed SSINCR1 upregulation (P = 0.0096) and correlation with RNA-seq results (r = 0.869). Upregulation of the SSINCR1 transcript was replicated in an independent sample set of 36 SS patients and 21 controls (P = 0.0183). Bioinformatic analyses using GAMMA-seq identified co-expression patterns of SSINCR1 with other coding transcripts involved in T, NK, and dendritic cell activation, development, and proliferation. Employing fluorescence-assisted cell sorting staining for nine distinct immune cell subsets followed by RNA isolation and qRT-PCR, we noted highest SSINCR1 expression levels in the CD8⁺ T (RU = 3.34), followed by CD56int NK (RU = 2.08), CD56hi NK (RU = 0.83), and CD4⁺ T cells (RU = 0.81). Expression was not detected in CD141⁺ and CD1c⁺CD11c⁺ myeloid DCs, monocytes, B cells, or pDCs. Ongoing studies are assessing protein binding partners and SSINCR1 expression in refined T and NK subsets and in expanded patient groups to determine subset-specific DE. This study establishes SSINCR1 as the first lncRNA associated with SS and lays the groundwork for further functional characterization in the pathogenesis of this complex disorder.
Gene Set Enrichment Analysis Across Multiple Expression Studies Reveals a Core Subset of Primary Sjögren’s Syndrome–Related Genes

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Objectives: Gene expression studies of primary Sjögren’s Syndrome (pSS) have low overlap of genes due to differing protocols, experimental designs and sample handling. Previous data indicate that there is an overlap between these studies in terms of the underlying biology. Gene set enrichment analysis (GSEA) is a technique developed to address such situations by detecting pathway-level biological changes. Here, we apply this technique to microarray data from pSS patients and healthy controls.

Methods: Whole blood from 133 pSS patients and 29 age-matched healthy controls (HC) collected by the UK primary Sjögren’s Syndrome Registry was used for whole genome microarray analysis (HumanHT-12 v4 BeadChip). Raw data were processed using the R lumi package, before GSEA analysis was carried out. Data were first compared to previously reported pSS transcriptomic datasets and modules. Then, the data were analysed to identify enrichments of metabolic pathways from the Molecular Signature Database. Gene sets were considered significant at an FDR cut-off of 25%. Finally, leading edge analysis was carried out on the gene sets. Gene ontology enrichments were calculated using the GStats R package.

Results: Significant enrichment of all previously-reported pSS datasets was observed in the data (all FDR < 25% and nominal P-value < 0.05). Four transcriptional modules—three cell cycle and one interferon—were enriched in the pSS group. Sixteen Reactome, 41 KEGG and 55 BioCarta pathways were also enriched. Three subsets of genes were identified in the leading edge overlap. GO analysis indicated that these genes are related to immune processes, including “response to type I interferon” (P = 1.76E – 29) and “defence response to virus” (P = 2.55E – 29).

Conclusion: Although transcriptomic studies of pSS have low overlap of individual genes, GSEA indicates that these studies have high overlap at the pathway level. In particular, the type I interferon signalling pathway is implicated in pSS pathogenesis.

Identification of a Sjögren’s Syndrome–Associated Variant that Influences OAS1 Isoform Switching and Protein Expression

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Gene expression profiling (GEP) studies have demonstrated overexpression of transcripts induced by type I interferons (IFNs) in SS. Notably, OAS1, an IFN-inducible gene involved in inhibition of viral replication, was overexpressed in SS patients. Through integration of gene expression data and genotype data from our genome-wide association study (GWAS), we identified multiple cis-expression quantitative trait loci (eQTL) in OAS1 with the most significant peaking at rs10774671 (P = 6.05 × 10−14), strengthening evidence of this variant for disease association (P = 8.47 × 10−5) obtained in the GWAS. We further replicated this genetic association in an independent set of 514 cases and 3466 controls followed by meta-analysis using a weighted Z score (P = 2.59 × 10−19; OR = 0.75). The risk allele A of rs10774671 is a splice site consensus variant located at the junction between intron-5 and exon-6 of OAS1 and thus may switch the primary isoform, p46, to various alternatives. To characterize functional impact of this variant, we evaluated alternative splicing events in OAS1 using RNA-sequencing (RNA-seq) performed in 57 SS cases and 27 controls on the Illumina platform. Transcripts measured by RNA-seq were reconstructed using Cufflinks and the abundance of isoforms was compared across samples according to the genotype of rs10774671. The risk allele A, which demotes the splicing consensus sequence, was correlated with higher expression of p42, p48, and p44 isoforms, but a lower expression of the normally spliced OAS1 isoform, p46. Functional characterization of different OAS1 isoforms indicated that the alternatively spliced isoforms resulted in impaired protein expression and lack of response to type I IFNs. Interestingly, the risk A allele has been reported to result in decreased Oas1 enzyme activity. These results suggest a mechanism that reduced capability of viral clearance by OAS1 due to the alternative spliced transcripts may involve in type I IFN signaling perpetuation in SS.
Analysis of Sjögren’s Syndrome Gene-based Concept Profiles Generated by Text Mining

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Purpose: The goals were as follows (i) to establish gene-based biological concept profiles associated with Sjögren’s Syndrome (SS); (ii) to determine the extent to which these profiles can integrate the knowledge acquired from human gene polymorphism and gene expression studies of SS and related autoimmune diseases.

Methods: Anni 2.1 biomedical text mining program was used to build gene-based concepts profiles associated with SS and related autoimmune diseases (i.e., systemic lupus erythematosus [SLE], rheumatoid arthritis [RA], and multiple sclerosis [MS]). Each disease, defined as a concept, was queried against the human genome in Anni 2.1. Biological concepts relevant to SS pathophysiology were also queried. Lists of genes ranked by scores were obtained, providing a theoretical strength of association of each gene with a concept. We determined whether intersections between gene sets for the queried concepts included genes previously shown to be differentially expressed in SS or genes with single nucleotide polymorphisms (SNPs) associated with SS.

Results: Genes mostly associated with SS compared to SLE, RA, and MS included AQP5, CHRM3, CXCL13, CXCR5, ICA1, and TRIM21. Additionally, a query of the concepts autoimmunity, cytokine, epigenetics, estrogen, histone modification, methylation, ubiquitin activity, virus, and X-linked heredity yielded 118 genes within the intersection of all concepts. Four of these genes were previously shown to be differentially expressed (DNMT1, II-6, IL-10, and MECP2) and two to contain SNPs (II-10 and STAT4) in SS human studies. These results suggest that understanding of differences between SS and related autoimmune diseases and the association of II-10 and STAT4 with all queried concepts could potentially expand the knowledge brought by recent genome-wide association studies. To determine the relevance of these associations to etiological processes in SS, further investigation using pathway analysis tools is required.

DNA Microarray Analysis of Submandibular Glands in IgG4-Related Dacryoadenitis and Sialoadenitis

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Objectives: IgG4-related dacryoadenitis and sialoadenitis (IgG4-DS) is a novel clinical disease entity characterized by elevated serum IgG4 and infiltration of IgG4-positive plasma cells in glandular tissues. Although recent studies demonstrated that abnormal innate immune responses might promote IgG4 production, the pathological mechanism of IgG4-DS is still unclear. In this study, we thus addressed to identify the disease-associated genes, especially innate immune molecules, by using exhaustive analysis.

Methods: Gene expression was analyzed by using DNA microarray in submandibular gland (SMG) from patients with IgG4-DS (n = 3) and controls (n = 3). Differentially, expressed genes (DEGs) between the two groups were identified, and gene-annotation enrichment analysis was performed by using Gene Ontology (GO) annotation. Validation of the results was performed by using quantitative PCR and immunohistochemistry from salivary glands in IgG4-DS (n = 7), Sjögren’s syndrome (n = 10), and controls (n = 10).

Results: In IgG4-DS, 450 up-regulated genes and 732 down-regulated genes were identified as DEGs (adj P-value <0.01). GO term analysis indicated that the up-regulated genes of DEGs in IgG4-DS encoded proteins that function in T/B cell activation and chemotaxis. PCR validated significantly higher expression of “macrophage receptor with collagenous structure (MARCO)” in IgG4-DS (P < 0.001). MARCO belongs to Class A scavenger receptor and expressed on “alternatively activated (M2) macrophages” which is activated by Th2 cytokines. Immunohistochemical analysis confirmed that the number and frequency of M2 macrophages in IgG4-DS were significantly higher than those in the other groups.

Conclusion: MARCO was identified as a disease-associated gene and might be critically important in elucidating the relationship between innate immunity and IgG4-DS.
DNA Microarray Analysis of Labial Salivary Glands in Patients with Sjögren’s Syndrome: Comparison with IgG4-Related Disease

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Objective: To compare gene expression in labial salivary glands (LSGs) of Sjögren’s syndrome (SS) with IgG4-related disease (IgG4-RD) and to identify genes involved in the pathogenesis of SS.

Methods: (1) Gene expression was analyzed by DNA microarray in LSGs of SS (n = 5), IgG4-RD (n = 5) and healthy controls (n = 3). Differentially expressed genes (DEGs) in SS were identified. (2) Validation of the result was performed by quantitative PCR using LSGs from SS (n = 11), IgG4-RD (n = 11) and healthy controls (HC) (n = 3). (3) The protein production of validated genes in LSGs from SS (n = 3) and IgG4-RD (n = 1) was examined by indirect immunofluorescence assay. (4) The expression of validated gene in CD4⁺ T cells isolated from peripheral blood mononuclear cells (PBMCs) in patients with SS (n = 4) was compared with those in HC (n = 3) by quantitative PCR.

Results: (1) In SS, 1320 up-regulated genes were identified as DEGs (false discovery rate <0.05) in comparison with IgG4-RD. (2) CXCL9, NR4A2, DPP4, SGK1, and PDK1 were selected as candidate genes for validation, according to rank <150, high expression levels, small variance, relation to T cell functions. PCR validated significantly higher expression of NR4A2 and DPP4 in SS than in IgG4-RD (P < 0.05). (3) Immunofluorescence staining in LSGs revealed higher production of NR4A2 in SS than in IgG4-RD and localization of NR4A2 dominantly in CD4⁺ T cells of SS. (4) The mRNA expression level of NR4A2 in CD4⁺ T cells isolated from PBMCs of patients with SS was significantly higher than in CD4⁺ T cells isolated from PBMCs of HC (P < 0.05).

Conclusion: The results suggest that NR4A2 might be a novel molecule involved in the pathogenesis of SS via CD4⁺ T cell activation.
S12.1
Th17 Cells: The Culprit for Sexual Dimorphism in Sjögren's Syndrome

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Sjögren’s syndrome (SjS) is a complex autoimmune disease with an array of diverse immunological, genetic and environmental etiologies, making identification of the precise autoimmune mechanism difficult to define. One of the most distinctive aspects of SjS is the high sexual dimorphism with women affected 10–20 times more than men. It is nearly impossible to study the sexual dimorphic development of SjS in human patients due to the inability to examine the disease process prior to the onset of clinical disease and the insufficient number of male patient counterpart. Therefore, it is pertinent to develop an appropriate animal model, which resembles human disease.

Using the spontaneous or naturally-occurring SjS-susceptible C57BL/6.NOD-Aec1Aec2 mouse animal, we have determined that C57BL/6.NOD-Aec1Aec2 mice developed an earlier onset of sialadenitis with a higher composition of B220⁺ B cells and CD3⁺ T cells with a 10-fold increase in glandular infiltration of CD4⁺IL17⁺ T helper-17 (Th17) cells at onset of clinical disease in female compared to male mice. Inflammatory lymphocytes specifically Th17 cells of female salivary glands exhibited a stronger proliferative potential than their male counterpart. At the clinical disease stage, altered autoantibody patterns can be detected in females whereas they are seldom observed in male C57BL/6.NOD-Aec1Aec2 mice. Interestingly, male C57BL/6.NOD-Aec1Aec2 mice developed an earlier loss of secretory function, despite the fact that female C57BL/6.NOD-Aec1Aec2 mice exhibited a more rapid secretory loss. These data indicate the strong sexual dimorphism in the SjS-susceptible C57BL/6.NOD-Aec1Aec2 animal model, making it an ideal animal model to examine human disease.

S12.2
Local IL-17 Sequestration in the Salivary Gland with Non-Viral Gene Therapy Reveals Suppression Of The Putative Autoantigen Klk1b22 in the Aec1/Aec2 Mouse Model of Sjogren's Syndrome

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Introduction: IL-17 producing cells have been observed in the salivary glands of primary Sjogren’s syndrome patients, and a role for IL-17 in the initiation and/or progression of primary SS has been suggested. IL-17 overexpression in the salivary has been implicated as a key contributor to the pathological cascade of Sjogrens-like disease in the Aec1/Aec2 mouse. An earlier study utilized an IL-17R:Fc fusion protein to sequester IL-17 through gene therapy in the Aec1/Aec2 mouse, leading to improvement in symptoms. Our goal was to characterize the proteome of salivary glands treated with IL-17R:Fc gene therapy and gene transfer with Luciferase (Luc, negative control), relative to the proteome of the salivary glands of the healthy background strain C57/BL6.

Methods: Ultrasound-assisted gene transfer (UAGT) was utilized to express IL-17R:Fc or Luc in the salivary glands of the Aec1/Aec2 mice at 7 weeks of age and transgene expression, as well as sequestration of IL-17 protein was confirmed 48 h after gene transfer. Thereafter, another cohort of Aec1/Aec2 mice was treated with UAGT/IL:17R:Fc or UAGT/Luc and allowed to survive 4 weeks before being sacrificed for proteomic profiling of the salivary gland, using the salivary glands of the health C57/BL6 background strain as controls.

Results: IL-17R:Fc gene therapy broadly suppressed the expression of Kallikrein1-related peptidases, including the putative autoantigen Klk1b22 which was associated with disease in this model and has been shown to be sufficient to induce keratoconjunctivitis sicca in otherwise healthy rats. Conclusions: Non-viral IL-17R:Fc gene therapy is feasible and potently suppresses IL-17 expression within the salivary gland. Expression of Klk1b22 appears to be downstream of IL-17 expression and thus suggests that IL-17 blockade early in the disease may prevent expression of autoantigens. Category: Personalized and cell-based treatments.
Differing Roles of CD8 T Cells in Lacrimal and Salivary Gland Autoimmunity in the Nonobese Diabetic Mouse Model of Sjögren Syndrome

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Early focal lymphocytic infiltrates that characterize Sjögren syndrome are dominated by T cells. Both CD4 and CD8 T cells are invariably present within lacrimal and salivary gland biopsy specimens from Sjögren syndrome patients and mouse models. Despite their presence, contributions of CD8 T cells to the development of dacrooadenitis and sialadenitis are not well characterized, and both pathogenic and regulatory roles have been suggested. Here we utilize our recently described nonobese diabetic (NOD) mouse-based adoptive transfer model of Sjögren syndrome to evaluate the role of CD8 T cells in the development of dacrooadenitis and sialadenitis. Transfer of a highly enriched CD8 T cell population from cervical lymph nodes (LN) induced marked dacrooadenitis in NOD-SCID recipients but failed to induce any sialadenitis. Surprisingly, depletion of CD8 T cells from bulk cervical LN cells prior to transfer did not abrogate dacrooadenitis development, and recipients of CD8-depleted cells developed comparable dacrooadenitis to those receiving bulk cervical LN cells. Conversely, sialadenitis was decreased in recipients of CD8-depleted cervical LN cells compared to those receiving bulk cervical LN cells. Together, these data suggest that CD8 T cells may play pathogenic roles in both lacrimal and salivary gland autoimmunity. However, CD8 T cells are sufficient but not necessary for dacrooadenitis and necessary but not sufficient for sialadenitis. Whether a lack of CD8 T cells has an effect on the development of antibodies or exocrine gland dysfunction is currently under investigation using CD8 knockout NOD mice. Ultimately, understanding the role of CD8 T cells in Sjögren syndrome-like autoimmunity in NOD mice may provide novel targets for diagnostic and therapeutic modalities.

The anergy induction of M3R reactive CD4+ T cells suppresses experimental sialadenitis like Sjögren’s syndrome in vivo

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Objective: Rag1−/− mice transferred with splenocytes of M3 muscarinic acetylcholine receptor (M3R)−/− mice immunized with M3R peptides mixture (N-terminal regions; N1, N2, N3, and three extracellular loops; 1st, 2nd, 3rd) developed sialadenitis like Sjögren’s syndrome (SS) (M3R induced sialadenitis; MIS). In MIS, M3R reactive CD4+ T cells were dispensable and the T cell epitopes were both N1 and 1st regions. Besides, altered peptide ligands (APLs), substituted in amino acid residues at TCR contact sites, can regulate the T cell activation. The present study was designed to establish the antigen specific therapy on the experimental SS and to clarify the therapeutic mechanism.

Methods: 1) APLs of N1 and 1st peptide (N1-APLs, 1st-APLs) were synthesized and antagonistic APLs were selected by in vitro pre-pulse assay. 2) 100 μg of antagonistic APLs was administered to MIS (APLs) intravenously on day 7 and 10 after the cell transfer. 3) The expression of immunologically relevant molecules of CD4+ T cells at the cervical lymph nodes was evaluated by quantitative PCR. Also the proliferative ability was evaluated. 4) The molecules related to the state of anergy were assessed by in vitro co-culture assay.

Results: 1) Seven N1 APLs (N1-APL 1-7) and eight 1st APLs (1st-APL 1-8) were designed, and of those, N1-APL5 (AA15 N→T), APL6 (AA15 N→C) and APL7 (AA15 N→S) significantly suppressed IFN-g (Inhibition ratio: N1-APL5 89.3%, N1-APL6 84.9%, N1-APL7 89.5%) (P < 0.05). 1st-APL8 (AA140 A→M) significantly suppressed IL-17 (Inhibition ratio: 86.5%) (P < 0.05). 2) N1-APL7 significantly suppressed sialadenitis in vivo (Focus score: N1-APL7: 0.11 ± 0.12, control: 1.44 ± 0.51) (P < 0.05). 3) Early Growth Response-2 (Egr-2), known as a transcription factor that regulates the anergic state, was significantly higher in the CD4+ T cells of [N1-APL7] than those of [PBS]. The T cell proliferation in [N1-APL7] was reversed by exogenous IL-2 administration. 4) Egr-2 and downstream anergy related E3 ubiquitin ligases such as Itch, Cbl-b, and GRAIL were significantly higher in the CD4+ T cells when cultured with N1-APL7 than with N1 peptide. Conclusion: N1-APL7, selected as one of the antagonistic APLs in vitro, significantly suppressed the induction of MIS.
cells that express TNFR1.

Results: The sg of infected mice on a population of activated stromal with adenovirus ICOSL is significantly upregulated within lymphocytes/stroma were used.

Methods: Salivary glands (sg) of wild-type (wt), ICOSL, CD3ε and p55/75 (TNFR1) knockout (KO) mice were intra-dually cannulated with luciferase-encoding replication-deficient adenovirus to induce TLO formation as previously described (1). Immunofluorescence, quantitative RT-PCR, flow cytometry on digested sg and in vitro culture of lymphocytes/stroma were used.

Results: We demonstrated that early post cannulation with adenovirus ICOSL is significantly upregulated within the sg of infected mice on a population of activated stromal cells that express TNFR1. In vivo experiments in ICOSLKO, CD3εKO and p55/75KO revealed a dramatic defect in TLO formation and reduced levels of lymphoid chemokines. ICOSL+ stromal cell stimulated effector T cells to produce LTα that in turn stimulates lymphoid chemokine production by TNFR1+ stromal cells. This was further confirmed in vitro, in mixed co-cultures of lymphocytes and stromal cells from wt and ICOSLKO and in vivo on wt/ICOSLKO bone marrow chimeras.

Conclusions: These data demonstrate that ICOSL expression by stromal cells supports the up-regulation of LTα on T cells that in turn is required for chemokine expression via TNFR1 stimulation. These data provide a novel mechanism of action between ICOSL on stromal cells, ICOS on T cells and LTα pathways in the context of TLO formation. Our work highlights an important role for stroma-derived ICOSL in TLO formation providing a biological rational for ICOS-ICOSL blocking in SS.


ICOS-ICOSL Modulates Tertiary Lymphoid Organs Formation, Regulating the Lymphotoxin Pathway in an Animal Model of Sjögren’s Syndrome

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Background: Costimulation via ICOS-ICOSL interaction is critical for germinal center reaction in secondary lymphoid organs. In autoimmunity, this signal has been extensively investigated and believed to contribute to the development of follicular T helper cells, autoreactive B cells and autoantibody production. In this work, we aim to dissect the role of ICOS-ICOSL pathway in a animal model of tertiary lymphoid organ (TLO) formation that mimics Sjögren’s syndrome.

Methods: Weights glands (sg) of wild-type (wt), ICOSL, CD3ε and p55/75 (TNFR1) knockout (KO) mice were intra-dually cannulated with luciferase-encoding replication-deficient adenovirus to induce TLO formation as previously described (1). Immunofluorescence, quantitative RT-PCR, flow cytometry on digested sg and in vitro culture of lymphocytes/stroma were used.

Results: We demonstrated that early post cannulation with adenovirus ICOSL is significantly upregulated within the sg of infected mice on a population of activated stromal cells that express TNFR1. In vivo experiments in ICOSLKO, CD3εKO and p55/75KO revealed a dramatic defect in TLO formation and reduced levels of lymphoid chemokines. ICOSL+ stromal cell stimulated effector T cells to produce LTα that in turn stimulates lymphoid chemokine production by TNFR1+ stromal cells. This was further confirmed in vitro, in mixed co-cultures of lymphocytes and stromal cells from wt and ICOSLKO and in vivo on wt/ICOSLKO bone marrow chimeras.

Conclusions: These data demonstrate that ICOSL expression by stromal cells supports the up-regulation of LTα on T cells that in turn is required for chemokine expression via TNFR1 stimulation. These data provide a novel mechanism of action between ICOSL on stromal cells, ICOS on T cells and LTα pathways in the context of TLO formation. Our work highlights an important role for stroma-derived

Aquaporin Gene Therapy Corrects Exocrine Gland Dysfunction and Inflammation in Mouse Model of Sjögren’s Syndrome

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Loss of secretory epithelial function is a hallmark of Sjögren’s syndrome; however, the physiological changes in the gland associated with this alteration are poorly understood. The present work revealed that altered expression of the cytokine Bone Morphogenetic protein 6 (BMP6) is strongly associated with the most common symptom of primary Sjögren’s syndrome, the loss of salivary gland function. We report that BMP6 signaling inhibits the expression of aquaporin 5, a water channel critical in salivary gland fluid secretion. This finding led us to develop a novel therapy in the treatment of Sjögren’s; increasing the water permeability of the gland to restore saliva flow. Indeed, we found that exogenous expression of a water channel in BMP6 treated human cell lines or mouse models of Sjögren’s syndrome restored gland activity. Most surprisingly, our study demonstrates that targeted increase in gland membrane water permeability resolved the salivary glands and associated systemic inflammation in the disease. Corresponding with the decrease in inflammation, an increase in secretory function was observed in other epithelia, such as lacrimal gland, often associated with primary Sjögren’s syndrome, suggesting this localize therapy could treat the systemic symptoms associated with primary Sjögren’s syndrome.
Integrative Network-Based Analysis of the Primary Sjögren’s Syndrome Transcriptome between Human and Mouse

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Objectives: Primary Sjögren’s Syndrome (pSS) is highly heterogeneous in nature, and the pathogenesis of the disease remains poorly understood. Several gene expression studies have been applied to the study of pSS in humans and in mouse models of pSS. However, the overlap between these studies in terms of individual genes is low. Here, we apply probabilistic functional integrated network (PFIN) analysis using gene orthology in order to better understand the relationships between these studies on the interactome level.

Methods: Two PFINs, one constructed from human interaction data, and the other from mouse interaction data, were produced using a two-stage integration method against a Gold Standard dataset derived from BioSystems metabolic pathways. The networks were mapped to one another using orthology from the Mouse Genome Database. Differentially expressed genes (DEGs) were identified from existing human and mouse studies of pSS. The combined human-mouse PFIN was filtered to extract a network of DEG interactions, before network-based visualisation and analyses using Cytoscape.

Results: Mapping of the DEGs to the network produced a pSS-specific sub-network of 2580 nodes and 3403 edges. Key orthologous genes linking the human and mouse networks were identified using the network topology. These genes included the pSS-associated gene beta-2-microglobulin (B2M). Several biological processes were enriched in the gene overlap, in particular the immune and inflammatory responses.

Conclusion: Despite the available gene expression datasets for pSS having low overlap, the areas of differential gene expression within the networks are highly similar. In particular, several immune-related processes are implicated in pSS pathogenesis in both the human and mouse networks. An integrated approach such as the one presented allows all available information to be assessed as a whole, revealing patterns in expression changes and allowing the comparison of human and mouse models.

Ultrasoluble Curcumin/Turmeric Ameliorates Lesions and Increases Survival in a Mouse Model of Sjögren’s Syndrome and Lupus

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Purpose: Commercial curcumin (CU), derived from food spice turmeric (TU), has been widely studied as a potential therapeutic for a variety of oncological and inflammatory conditions. However, lack of solubility and bioavailability has hindered CU’s therapeutic efficacy in human diseases. We solubilized CU with heat/pressure (ultrasoluble CU). Solubilized CU had anti-oxidant effects and inhibited binding of anti-Ro60 [from Sjogren’s syndrome (SS) and systemic lupus erythematosus (SLE) subjects] to Ro60 autoantigen in vitro. We hypothesized that ultrasoluble CU/TU will ameliorate SS and SLE like disease in MRL-lpr/lpr mice.

Materials: Eighteen female MRL-MpJ and 18 female MRL-MpJ MRL-lpr/lpr mice (6 week old) were used. Six mice of each strain received autoclaved water only, water with ultrasoluble CU or water with ultrasoluble TU.

Results: Salivary gland histopathology studies showed significantly reduced cellular infiltration in TU/CU treated MRL-lpr/lpr mice, compared to controls. 2/5 CU mice had focus score (FS) <1, 4/4 TU treated mice had FS <1, while 3/3 control mice had FS >1. CU/TU treated mice had significantly reduced proteinuria and urinary cell casts until week 14. There was delayed onset of autoantibodies in CU/TU treated mice, compared to controls. CU treated mice had a 20% survival advantage over control mice. However, TU-treated animals lived an average of 16 days shorter than control mice due to complications unrelated to SS or lupus-like illness. CU/TU treated MRL-MpJ controls did not have problems with CU/TU treatment and were sacrificed at 36 weeks of age. CU or TU treatment inhibited lymphadenopathy significantly compared to controls (P = 0.03 and P = 0.02, respectively). TUNEL assay showed that lymphocytes in lymph nodes of TU/CU treated mice underwent apoptosis.

Conclusion: Heat solubilized CU/TU could prove useful as a therapeutic intervention in SS and SLE S12.7.
A Pathological Role of RORγt in the Development of Sialadenitis like Sjögren’s Syndrome

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Objective: The nuclear receptor retinoic acid-related orphan receptor (ROR) γt is required for the generation of Th17 cells, which are involved in various autoimmune diseases, including Sjögren’s syndrome (SS). However, the pathological role of RORγt in SS remains to be elucidated. This study was designed to clarify the role of RORγt in the pathogenesis of sialadenitis-like SS.

Methods: (1) Histological analysis of salivary glands from T cell specific RORγt transgenic (Tg) mice was performed. (2) Splenic CD4+ T cells from Tg mice were transferred into Rag2-/- (CD4+→Rag2-/-) mice, and histological analysis was examined. (3) The histological examination was performed in salivary glands isolated from IL-17-/-/RORγt Tg mice. (4) Compartment of Treg cells was analyzed. (6) CD4+CD25-Foxp3- (GFP+) T cells (Treg cells) and CD4+CD25-Foxp3+ (GFP) T cells (Teff cells) from Tg mice were co-transferred into Rag2-/- mice, and histological analysis was examined.

Results: (1) Tg mice developed the severe sialadenitis like SS. (2) In CD4+→Rag2-/- mice, sialadenitis was observed. (3) Development of sialadenitis was noticed in the IL-17-/-/RORγt Tg mice. (4) Foxp3 expression in CD4+CD25+ T cells and the number of CD4+CD25-Foxp3- cells was significantly decreased in Tg mice. (5) STAT5 phosphorylation was inhibited in IL-2 stimulated Treg cells of Tg mice. (6) Co-transfer of Teff cells with sufficient number of Treg cells from Tg mice could not develop any sialadenitis in Rag2-/- mice, whereas co-transfer with reduced number of Treg cells from Tg mice did not inhibit the development of sialadenitis.

Conclusion: These results suggested both RORγt overexpressed CD4+ T cells and reduced Treg cells might contribute to the development of sialadenitis like SS.

IL22 Regulates Autoantibody Production by Inducing Lymphoid Chemokine Expression in Tertiary Lymphoid Organs

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Tertiary lymphoid organs (TLOs) are organized clusters of immune cells that preferentially form in the salivary glands (sg) of patients with Sjögren’s syndrome (SS). Recent observational studies have proposed a relationship between IL22 expression, B cell infiltration and humoral autoimmunity in SS but failed to provide a molecular mechanism for this relationship (1,2,3).

Objective: To investigate the functional role of IL22 in TLO formation within the sg.

Methods: We used a previously described mouse model of SS (4) to assess the role of IL22 in vivo. Sg of wildtype (WT) and IL22-/- mice were infected with a replication deficient adenovirus and sg was studied by immunofluorescence, flow cytometry and RT-PCR on sorted cells.

Results: Increased expression of IL22 was found to occur within hours of wt sg cannulation. IL22-/- and WT mice treated therapeutically with anti-IL22 antibody showed profound defects in TLO maturation and autoantibody production. IL22 exerts its role regulating B cell recruiting chemokine expression. In particular IL22 induces up regulation of CXCL13 on gp38+IL22Rα+stromal cells in synergy with TNFα and LTβ. Conversely, IL22Rα engagement increases CXCL12 production (but not CXCL13) on epithelial cells. This effect is direct, as treatment of isolated stromal and epithelial cells with recombinant IL22 in vitro was sufficient to induce CXCL13 and CXCL12 production by gp38+stromal cells and epithelial cells, respectively.

Conclusions: The dual impairment of CXCL13 and CXCL12 expression is responsible for the reduced B cell accumulation, aberrant follicle maturation and lack of autoantibody production observed in the IL22-/- and anti-IL22 treated mice. The functional connection that we have established between the expression of IL22, the aberrant chemokine production and autoantibody response support a role for IL22 as potential therapeutic target in SS.

References:
S12.11

Downregulation of MicroRNA-183 in Sjögren’s Syndrome Minor Salivary Glands; Implications in the Control of ezrin Expression and Salivary Gland Function


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Sjögren’s syndrome (SS) is mainly characterized by salivary and lachrymal gland (SG) hypofunction. SGs from SS patients show loss of microvilli, accumulation of secretory granules in the acinar cells and an overexpression and aberrant localization of ezrin. Ezrin regulates the microvilli organization and secretion of exocrine cells. The mechanisms for gene expression control and function of ezrin in SG are unknown. We showed that hsa-miR-183 is downregulated in SG from SS patients; and that modifies the expression of ezrin in cell culture. Here we further confirm the function of hsa-miR-183 in vivo and explore a mechanism by which ezrin mislocalization may produce saliva hyposecretion.

LNA-antimir-183 was transfected in mice parotid SG. After transfection we evaluated the saliva secretion and ezrin protein levels. Proximity ligation assay was performed to determine the interaction of ezrin with three possible targets in SG: Sodium-hydrogen antiporter 3-regulator1 (SLC9A3R1), Anotacmin1 (ANO1) and Rab27A. The in vivo transfection of the LNA-antimir increased ezrin protein and decreased saliva secretion in mice. ANO1 did not appear to interact directly with ezrin. The complex ezrin/SLC9A3R1 was decreased in apical pole of acinar cells of SS patients. A similar change was observed for ezrin/Rab27.

These experiments suggest that in SS patients the overexpression of ezrin is caused by the downregulation of hsa-miR-183 and propose a role of ezrin in the mechanism of SG hyposecretion. In this mechanism the mislocalization of ezrin affects the localization of the SLC9A3R1, altering the activation of the SLC9A3 and the electrolyte balance that regulates water secretion. Also, based on the function of Rab27a, the change in the interaction of ezrin/Rab27a could affect the fusion of the secretory granules with apical plasma membrane. This mechanism of hyposecretion presents new molecular target for SS treatment.

S12.12

Recruitment and Activation of NK Cells in the Salivary Glands Regulates Early Viral Control but is Dependable for Autoimmunity and Focal Lymphocytic Sialoadenitis in an Inducible Murine Model of Sjögren–Like Disease

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Background and Aim: Dysregulation of Natural Killer (NK) cells has been shown to influence the development of autoimmune diseases, but their role in Sjögren’s syndrome (SS) is unclear. The main goal of our study was to investigate the recruitment and functional relevance of NK cells in the early phases of salivary gland (SG) inflammation in an inducible murine model of sialoadenitis displaying some features of SS.

Results and Methods: Sialoadenitis was induced in WT-C57BL/6 mice via SG retro-cannulation of a luciferase-encoding adenovirus-5 (AdV). Flow cytometric analysis of CD45pos/NK1.1pos/CD3neg NK cells in digested SG showed an early NK recruitment peaking at 4 days post-cannulation (dpc). These results were also confirmed by cell transfer experiments showing active NK migration to the SG at 1dpc followed by their intra-SG proliferation at 3–5 dpc. Immunofluorescence (IF) staining for NK1.1pos/CD3neg demonstrated preferential accumulation of NK cells before B/T cell aggregates organization. Activating markers NKp46 and CD69 were up-regulated from 5 dpc onwards when an increased degranulation potential was observed, using a CD107a functional assay. Accordingly, cytotoxic activity, as measured by granzyme-Bpos expression, peaked at 4/5 dpc. NK depletion (with anti-NK1.1 antibody treatment) significantly impaired viral control (as assessed by luciferase activity) at 3 dpc but not at later time-points. Conversely, NK depletion did not affect the formation of SS-like inflammatory foci nor the production of ANA or anti-AdV antibodies.

Conclusions: Here we show that NK cells are actively recruited to the inflammatory site of the SG and are critically involved in the early immune controlling AdV infection in this organ but are dispensable for the development of SS-like inflammatory foci and autoimmunity.
Hepatitis Delta Virus Detected in Salivary Glands of Sjögren’s Syndrome Patients and Induces a Sjögren’s Syndrome Phenotype in vivo

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Objective: Viral-mediated mechanisms have been suspect in the development of primary Sjögren’s syndrome (pSS). Prior studies have detected the presence of viruses in salivary glands of pSS patients but a clear cause and effect relationship had yet to be established. The objective of this study was to identify potential viral triggers in the pathogenesis of pSS and to evaluate the capacity of differential viral profiles to trigger a pSS-like phenotype in vivo.

Methods and Findings: A custom viral microarray was developed to globally identify potential viral triggers in the pathogenesis of pSS. Analysis was performed using RNA isolated from minor salivary gland tissue from 15 pSS patients and 14 healthy controls. A significant increase in signal was identified for hepatitis delta virus (HDV) in minor salivary glands of over 50% of pSS patients compared to healthy controls. The presence of HDV sequence and antigens were confirmed in salivary gland tissue in pSS patients tested. HDV sequence was confirmed in a second pSS cohort in an independent lab with similar results. Patients testing positive for HDV were negative for detectible hepatitis B virus (HBV) antigen and antibody in serum and presented with normal transaminase levels. Animal models were developed to evaluate pSS-like pathology induced by HDV antigens HDV expression in salivary gland tissue. Expression of HDV antigens in the salivary glands of healthy female mice triggered the development of the three hallmarks of pSS: reduction of saliva flow, an increase in lymphocytic focal infiltrates, and development of autoantibodies associated with pSS.

Conclusion: Identification of HDV antigens and sequence in Sjögren’s syndrome patients and induction of a pSS-like phenotype in vivo provide further support for a viral-mediated mechanism in Sjögren’s syndrome development. Detection of HDV in salivary glands in absence of a detectible HBV past or current infection suggests a novel biodistribution and persistence of HDV.
Session 13. Treatment

S13.1
Treatment of Extraglandular Manifestations of Sjögren’s Syndrome: The View from San Diego
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This audience contains the authors of numerous controlled studies and case report series on systemic therapy. As we have not obtained formal FDA approval for systemic SS therapy, our approach is based on expert opinion. We can now move forward with our new SS criteria and Delphi method to provide algorithms. Current issues to be discussed:

1. Hydroxychloroquine—when to use and how to monitor toxicity
2. DMARDs—methotrexate dosage and indications
3. Other DMARDs such as azathioprine, sulfasalazine and leflunomide
4. Immune suppressants such as mycophenolic acid and sirolimus (rapamycin)
5. Medications for “fibromyalgia” such as neurontin, duloxetine, and pregabalin
6. Biologic agents such as rituximab, abatacept, and belimumab
7. Other agents under consideration: tofacitinib, as well as CD22, PI3K, Syk kinase and mTor targets.

Particularly difficult decisions revolve around lymphoproliferation, as well as central and peripheral nerve involvement. This includes fatigue and cognitive impairment. New approaches to these problems from Scripps Research and Salk Institute in San Diego will be presented, based on murine demyelinating and viral flu models of fatigue.

S13.2
New systemic treatment options in primary Sjögren’s syndrome
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The last years, significant progress has been made in the evaluation and treatment of primary Sjögren’s syndrome (pSS). 40–50% of the pSS patients will develop extraglandular manifestations like purpura, polyneuropathy and arthritis. Lymphomas develop in about 7.5% of pSS patients, mostly marginal zone B-cell lymphomas. Furthermore, pSS has a very substantial impact on the patients’ quality of life and their daily activities. Despite these facts systemic treatment options are limited. The most commonly applied traditional DMARD is hydroxychloroquine. In a randomized controlled trial it is concluded that hydroxychloroquine is of limited value in the treatment of pSS. The most recent biological agents evaluated for safety and efficacy in clinical trials in pSS are: (1) Cytokine blocking agents as B-cell activating factor (BAFF) antagonists, (2) agents blocking B-cells (anti-CD20) and (3) agents blocking co-stimulation. Cytokine blocking agents: BAFF levels were found elevated in serum and saliva in pSS patients and circulating levels of BAFF in pSS patients were shown to be a marker for disease activity. Promising results of a study with the BAFF blocker belimumab, a human antibody that binds to soluble BAFF, will be discussed. B-cell blocking agents: Several open label and controlled studies are now available using the B-cell blocker rituximab, a chimeric humanized monoclonal antibody specific for the B-cell surface molecule CD20, which is expressed on the surface of normal and malignant pre-B and mature B lymphocytes, but not on plasma cells. Rituximab seems to be effective in most studies for at least 6–9 months in pSS patients with active disease, improving both subjective and objective complaints. Co-stimulation blocking agents: Abatacept is a construct of the CTLA4 molecule and the Fc-portion of human IgG. Abatacept selectively targets the CD80/CD86:CD28 co-stimulatory signal required for full T-cell activation and T-cell dependent activation of B-cells. The promising results of two open label phase II studies indicate that abatacept treatment is well tolerated, safe and presents a clinically meaningful improvement of disease activity. The efficacy and safety of abatacept treatment in pSS patients will be further explored in a RCT. Finally, the recently developed EULAR Sjögren’s syndrome disease activity index (ESS-DAI) and EULAR Sjögren’s syndrome patient reported index (ESSPRI) will be discussed including their usefulness for evaluating the effect of biologicals in the treatment of pSS.

S13.3
Investigation of Repeat BCG Vaccinations in Autoimmunity: Rationale and Phase I Trial in Sjögren’s Syndrome
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The bacillus Calmette-Guerin (BCG) vaccine, long used in tuberculosis prevention, has gained new attention in recent...
years for potential immunomodulatory effects in multiple types of human autoimmunity, including type 1 diabetes, multiple sclerosis and now Sjögren’s syndrome. This is due in part to mechanistic studies that suggest that multiple BCG vaccinations and/or the related and more pathologic tuberculosis organism modulate the immune system in beneficial ways in autoimmunity. Similar to tuberculosis infection, BCG induces tumour necrosis factor (TNF), a primary ligand for regulatory T cell (Treg) expansion and creation of potent Treg cells. In diverse human autoimmune diseases, TNF is also able to selectively kill autoreactive CD8 T cells. Positive human clinical trial data related to repeat BCG vaccination have recently been published in advanced type 1 diabetes (Phase I) and in new-onset multiple sclerosis (Phase II). The worldwide data now document both the beneficial induction of Tregs after BCG vaccination as well as the halt of these two autoimmune diseases, including temporary restoration of insulin secretion in type 1 diabetes and possible reversal of brain demyelination in multiple sclerosis. The rationale for investigating repeat BCG vaccination in Sjögren’s syndrome is two-fold. First, published NOD mouse studies show that multi-dose BCG halts Sjögren’s-like syndrome and functionally restores salivary gland secretion, even when the therapy is applied in advanced disease. Secondly, the NOD mouse and patients with Sjögren’s syndrome both lack the same protein in the proteasome that prepares self-peptides for T cell education: the LMP2 subunit. In culture, administration of low-dose TNF, which resembles the effect of BCG vaccination in humans, overcomes the poor T cell selection known to be induced by the LMP2 defect. In the USA, Phase I trials are being planned to bring the BCG vaccine forward in patients with Sjögren’s syndrome. These trials will be discussed, with a focus on trial endpoints and on biomarkers that might provide insight into the proper dosing in patients with established Sjögren’s syndrome.

**S13.4**

**Biologics, the Next Generation of Approved Topical Anti-Inflammatory Interventions for Dry Eye Disease: LFA-ICAM Binding Inhibition, IL-1 Receptor Antagonism and Recombinant Human Serum Albumin**

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**Background/Purpose:** Topical anti-inflammatory therapy is the centerpiece of dry eye disease (DED) therapy. The only currently FDA approved drug is cyclosporine 5% emulsion (Restasis, Allergan, Irvine, CA). Although extremely successful in the marketplace, drawbacks include delayed onset of action and burning upon instillation. Biologics enable highly specific targeting and precise replication of product through hybridoma biotechnology, creating molecules specific to DED applications.

**Methods:** Targeting strategy and Phase II and Phase III clinical trial progress for Lifitegrast (Shire PLC, Jersey), EBI-005 (Eleven Biopharmaceuticals, Cambridge, MA) and RU-101 (R-Tech Ueno, Tokyo, Japan) fortell promising pathways to approval.

**Results:** Lifitegrast is a bioengineered protein created by 3-D molecular modeling guided biosynthesis, with an extremely high binding affinity to the LFA (lymphocyte function antigen) on T lymphocytes, acting as an ICAM decoy, blocking signaling. This molecule is stable in unpreserved single dose unit (SDU) aqueous solution at physiologic pH (7.0). 2 confirmatory Phase III multicenter clinical trials support the May NDA submission, making this molecule closest to market. EBI-005 is a recombinant protein designed on a proprietary yeast display system (AMP-Rx platform). EBI-005 binds the IL-1 receptor antagonizing IL-1α and IL-1β receptor signaling. The blocked receptor cannot transmit biological signals responsible for pain, discomfort, itching and inflammation in DED. Eleven has completed the first Phase III trial for DED with promising results. EBI-005 is the first IL-1 signaling inhibitor designed for topical ophthalmic administration. Autologous human serum tears have shown promise despite significant contamination issues. Human serum albumin (HSA) displays intrinsic anti-inflammatory and growth factor properties. RU-101 is a sterile topical unpreserved SDU recombinant HSA protein. Phase I/II clinical study results show improved corneal staining scores without safety issues.

**Conclusions:** Bioengineering technology has created intriguing new molecules specifically intended for topical application in DED, potentially bringing safer and more effective therapy to patients.

**S13.5**

**Sustained Release Hydroxypropyl Cellulose Ophthalmic Inserts Decrease Tear Film Osmolarity in Severe Keratitis Sicca: A Same Day Contralateral Eye Comparison in 40 Patients**

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**Background/Purpose:** Moderate to severe dry eye disease (DED) in Sjögren’s syndrome (SS) presents therapeutic...
challenges to ophthalmologists and optometrists and compliance difficulties to patients. Although safe and somewhat effective, topical artificial tears moisten the ocular surface only momentarily, yet create potential preservative toxicity issues. The Lacrisert™ (Bausch & Lomb) sustained release hydroxypropyl cellulose (HPC) ophthalmic insert provides a safe and effective platform for continuous day long moisture distribution with minimal skill required for insertion. The insert has shown improved DED symptoms, ability to perform daily activities of living, and quality of life in a 418 patient registry study. Ocular surface improvement may be a function of increased blink rate, overall reduction of concomitant medications, or improved osmolarity.

Methods: Fifty volunteers with a SPEED (Standard Patient Evaluation of Eye Disease) score >10 and punctate keratopathy were randomized, then given an insert by a technician in either the right or left eye in the morning after a baseline osmolarity reading OU. A repeat osmolarity reading was taken approximately 8 h later. Questionnaires were administered.

Results: The mean treated eye morning pre-insert osmolarity reading was 314 mOsm/L and 298 mOsm/L post-insert in the afternoon. The mean untreated eye morning osmolarity reading was 316 mOsm/L and 310 mOsm/L in the afternoon ($P < 0.05$). Patients noted minor initial irritation in the insert eye, but only 2 (5%) said they would prefer not to use it again. 28 (70%) stated they wanted to try the insert in both eyes while 10 (25%) were indifferent. 23 (57.5%) stated that the treated eye felt better during the day. No untoward side effects were noted.

Conclusions: The HPC ophthalmic insert is a safe and effective treatment for more advanced DED and improves tear osmolarity.

S13.6
Polyethylene Glycol Biodegradable Punctal Plug Impregnated with Sustained Release Dexamethasone for Severe Keratitis Sicca: Phase II Preliminary Results
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Background/Purpose: Sjögren’s syndrome (SS) is characterized by moderate to severe dry eye disease (DED). Therapeutic challenges include tear conservation, inflammation control, and minimization of medication toxicity. Polyethylene glycol (PEG) is a well-tolerated insipient. Investigational product OTX-DP is PEG hydrogel impregnated with fluorescein for ready localization, and provides sustained release properties as a carrier for low dose dexamethasone (0.4 mg). The plug remains in the canaliculus, then hydrolyzes and dissipates into the nasolacrimal duct. A previous 4 week cataract surgery trial showed ocular inflammation control with ideal linear pharmacokinetics while mitigating intraocular pressure (IOP) complications. This is the first human trial to evaluate plug based drug release technology for DED.

Methods: This is a 6 week long Phase II prospective, multicenter, randomized, parallel-arm, bilateral, double-masked, vehicle controlled study to evaluate OTX-DP safety and efficacy for DED treatment in 120 eyes of 60 patients. Parameters include OSDI, VAS (visual analog scale), SPEED questionnaire, osmolarity, MMP-9, IOP, slit lamp examination, NEI scale vital staining, Schirmer’s, TBUT and plug integrity. Plugs remaining after 6 weeks are removed by irrigation distally or manual expression on to the conjunctiva.

Results: The first 20 patients, 10 in the treatment group and 10 in the vehicle group have shown no evidence of plug loss or IOP elevation. No patients have shown deterioration of ocular surface vital staining, discomfort, or device related difficulties. Punctate keratopathy has trended towards improvement, but no statistically significant improvements have been noted in this small cohort proof of concept trial.

Conclusions: OTX-DP is a new technology that appears to be well tolerated and very safe while preventing ocular surface deterioration in patients with moderate to severe DED.

S13.7
Recommendations of Brazilian Society of Rheumatology for the Treatment of Sjögren’s Syndrome
Sjögren’s syndrome committee of Brazilian Society of Rheumatology (SBR), Brazil

Objective: Recommendations of Brazilian Society of Rheumatology for the treatment of Sjögren’s syndrome (SS) were developed to guide management of SS considering the Brazilian social and economic context.

Methods: It was based on specialist’s opinion and systematic review on MEDLINE (PubMed) and Cochrane...
database until October 2014, including 127 articles classified according Oxford & Grade.

**Results:** Part 1. General recommendations and patient education: Systemic treatment should be according disease severity measured by ESSDAI. Patients should avoid caffeine, tobacco, alcohol, toothpaste with abrasive, and mouthrinses with alcohol. Patient should be educated about preventive measures for oral health and hydration and conducted by multidisciplinary team. Aerobic exercise improves fatigue and depression. Immunization to influenza and pneumococcus are indicated. Serum levels of vitamin D should be evaluated and supplemented if it is necessary. Part 2. Symptomatic treatment of dryness: Topical treatment for dry mouth includes saliva substitutes, sugar-free candies and gums. Topical treatments for dry eye are lubricants (glucanes or carboximethylcellulose), cyclosporine 0.05%, and punctual occlusion. Topical glucocorticoids may be used for severe dry eye for short time. Pilocarpine and cevimeline should be used for dry mouth and for severe dry eye. N-acetylcysteine may be used for dryness symptoms. Omega-3 supplementation may be used to dry eye. Part 3. Systemic treatment. Immunosuppressant and/or biological therapy are not indicated to dryness treatment. Hydroxychloroquine, glucocorticoid, and immunosuppressants (azathioprine, mycophenolate mofetil, cyclophosphamide, cyclosporine) should be indicated according severity of systemic involvement. Rituximab is indicated to treat systemic manifestations without improvement with immunosuppressive therapy. Abatacept and belimumab may be considered in patients not responding to rituximab and with high level of disease activity.

**S13.8**

**Calcium–Calcineurin–NFAT Signaling Pathway Regulates AQP5 Expression in Primary Salivary Gland Acinar Cells**

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Aquaporin (AQP) five belongs to a family of small integral membrane proteins which function as a water channels in cells. AQP5 plays a critical role in mediating the secretion of fluid in salivary gland. Decrease or no saliva flow is one of the key symptoms in Sjögren’s syndrome patients. Towards understanding the molecular basis of the loss of salivary secretion, here we have studied the regulation of AQP5 expression in primary human salivary gland epithelial cells (phSG). We observed that the phenotype of the cells depended largely on the calcium levels in the culture conditions. AQP5 transcript showed more than 3-fold increase in phSG cells grown in high calcium (0.8 mM) medium compared to that in low calcium (0.05 mM) condition. This was confirmed by both immunofluorescence staining and Western blot studies. Evaluation of expression transcripts of calcium signaling proteins in phSG cells revealed that NFAT1 expression was dependent on calcium in a dose-response manner. Furthermore, when cells were switched to a high calcium medium phGFP-NFAT1 was translocated from the cytoplasm into the nucleus. Expression of AQP5 was also reduced when phSG cells were treated with cyclosporine A confirming the involvement of calcineurin-NFAT1 signaling pathway in regulation of AQP5 expression. Since NFAT activation has been linked to calcium influx, we examined the role of the calcium entry regulatory proteins STIM1 and STIM2 as well as the channel protein Orai1. Knockdown of NFAT1 and STIM1, but not STIM2 or Orai1, resulted in 70% decrease of AQP5 expression. Further analysis revealed that several NFAT binding motifs were identified in the AQP5 promoter and these were validated using AQP5-promoter-luciferase assays. Mutagenesis of putative NFAT-binding regions as well as chromatin immunoprecipitation analyses revealed the presence of functional NFAT binding sites within the proximal AQP5 promoter region. Taken together, these data demonstrate that AQP5 expression in phSG cells is regulated by a calcineurin-NFAT-dependent signaling pathway. Importantly, STIM1, but not Orai1 is involved in this mechanism. We propose that external Ca\(^{2+}\)-dependent Ca\(^{2+}\) entry triggers activation of AQP5 expression in phSG cells. Thus alterations in calcium signaling could lead to alterations in AQP5 expression and function in pSS. Further studies are being directed towards determining the status of calcium signaling in pSS salivary glands.
Session 14. Personalized and Cell-Based Treatment

S14.1

Monitoring of Therapeutic Effects of Biological Therapeutics and Small Molecule Inhibitors by Single Cell Signaling Profiling of Blood Cells

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Personalized therapy defined as therapy adapted to individuals for optimal therapeutic effect and absence of adverse effects is in its infancy. Examples from cancer therapy often depend on detection of a particular mutation or detection of protein expression. In some exceptional cases, like in chronic myeloid leukemia, presence of the fusion gene BCR-ABL is highly predictive of therapy response to small molecular inhibitors of ABL tyrosine kinase. Furthermore, the level of BCR-ABL transcripts, as well as mutational analyses of the ATP-binding domain of ABL is used to monitor therapy response. However, direct effects on signal responses are not employed for diagnostics or therapy. Furthermore, biological therapeutics are often without target measurement upfront, and with few or no disease related biomarkers for therapeutic effect.

We have employed leukocyte single cell analysis of the basal phosphorylation signaling state of key molecules in intracellular signaling cascades like PI3K/AKT/mTOR, ERK/MAPK and JNK/STAT pathways in chronic myeloid leukemia and multiple sclerosis. Patients have been sampled before, hours after therapy and at follow up. Patients with incomplete therapy response are profiled with distinct signaling features.

By combining intracellular signaling with extended immunophenotyping of peripheral blood in extensive panels of probes and analyzed by mass cytometry, an extensive profiling of inflammatory diseases and hematological malignancies is possible. This open a new avenue for monitoring of personalized therapy of inflammatory diseases and hematological malignancies through functional signaling responses in blood cells.

References:

S14.2

A Dendritic Cell-Based Therapy for Rheumatoid Arthritis


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Dendritic cells (DC) are antigen-presenting cells that play a critical role in maintaining immune tolerance to self-antigens by controlling the pathogenicity of auto-reactive T cells. DC can be modified ex vivo to induce stable tolerogenic function and have become a promising immunotherapeutic tool for autoimmune diseases such as rheumatoid arthritis (RA). Our goal is to develop and test DC-based therapy for RA. We have generated human DC with potent immunoregulatory activity (termed tolerogenic DC or tolDC) by treating monocyte-derived DC with dexamethasone, vitamin D3 and a TLR4 ligand. These tolDC express high levels of MHC class II and intermediate levels of the co-stimulatory molecules CD80/CD86. Importantly, tolDC display a stable anti-inflammatory cytokine profile with high levels of interleukin (IL)-10 and membrane-bound transforming growth factor (TGF)-β, and low levels of tumour necrosis factor (TNF)-α, IL-12p70 and IL-23. TolDC have excellent ability to process and present the RA auto-antigen type II collagen on MHC II, but at the same time modulate T cells in a protolerogenic manner: tolDC polarise naïve T cells towards high IL-10, low interferon (IFN)-γ and low IL-17 production, whereas memory T cells are rendered hyporesponsive. The immunosuppressive action of tolDC is partly mediated through TGF-β. Furthermore, tolDC with similar phenotype and function can be generated from the peripheral blood of RA patients. Equivalent murine tolDC successfully suppress established arthritis in the collagen-induced arthritis model. This therapeutic effect of tolDC is associated with reduced T helper type 17 (Th17) responses in vivo. We have also set up protocols to generate...
Development of the Sjögren’s Syndrome Responder Index (SSRI), a Data-Driven Composite Endpoint for Assessing Treatment Efficacy


Objectives: To determine which outcome measures detected rituximab efficacy in the TEARS trial and to create a composite endpoint for future trials in pSS.

Methods: Post-hoc analysis of the multicenter randomized placebo-controlled double-blind TEARS trial. The results were validated using data from two other randomized controlled trials in pSS, assessing rituximab (single center trial in the Netherlands) and infliximab, respectively.

Results: Five outcome measures were improved by rituximab in the TEARS trial: patient-assessed visual analog scale scores for fatigue, oral dryness, and ocular dryness; unstimulated whole salivary flow; and erythrocyte sedimentation rate. We combined these measures into a composite endpoint.

Conclusions: A core set of outcome measures used in combination suggests that rituximab could be effective and infliximab ineffective in pSS. The SSRI might prove useful as the primary outcome measure for future therapeutic trials in pSS.

Which and How Many Patients Should Be Included in Randomised Controlled Trials to Demonstrate the Efficacy of Biologics in Primary Sjögren’s Syndrome?


Objective: The goal of this study was to determine how the choice of the primary endpoint influenced sample size estimates in randomised controlled trials (RCTs) of treatments for primary Sjögren’s syndrome (pSS).

Methods: We reviewed all studies evaluating biological therapies in pSS to identify their inclusion criteria and primary endpoints. Then, in a large cohort (ASSESS), we determined the proportion of patients who would be included in RCTs using various inclusion criteria sets. Finally, we used the population of a large randomised therapeutic trial in pSS (TEARS) to assess the impact of various primary objectives and endpoints on estimated sample sizes. These analyses were performed only for the endpoints indicating greater efficacy of rituximab compared to the placebo.

Results: We identified 16 studies. The most common inclusion criteria were short disease duration; systemic involvement; high mean visual analogue scale (VAS) scores...
for dryness, pain, and fatigue; and biological evidence of activity. In the ASSESS cohort, 35% of patients had recent-onset disease (<4 years), 65% systemic manifestations, 68% high scores on two of three VASs, and 52% biological evidence of activity. The primary endpoints associated with the smallest sample sizes ($n < 200$) were a VAS dryness score improvement $>20$ mm by week 24 or variable improvements (10, 20, or 30 mm) in fatigue VAS by week 6 or 16. For patients with systemic manifestations, the ESSDAI change may be the most logical endpoint, as it reflects all domains of disease activity. However, the ESSDAI did not improve significantly with rituximab therapy in the TEARS study. Ultrasound score improvement produced the smallest sample size estimate in the TEARS study.

Conclusions: This study provides valuable information for designing future RCTs on the basis of previously published studies. Researchers should strive to develop a composite primary endpoint and to determine its best cut-off and assessment time point.

S14.5

High Grade Salivary Gland Involvement, Assessed by Histology or Ultrasonography, is Associated with a Poor Response to Rituximab in Primary Sjögren’s Syndrome Patients

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Objectives: To determine whether the extent of salivary gland involvement, assessed using unstimulated whole salivary flow (UWSF), histological focus score or salivary gland ultrasonography (SGUS), is associated with the response to rituximab in patients with primary Sjögren’s syndrome (pSS).

Methods: Thirty-five pSS patients form the randomized TEARS study, which compared rituximab to placebo, had either centralized salivary gland biopsy or SGUS, at inclusion. Among rituximab-treated patients, 50% were considered responders according to the Sjögren’s Syndrome Responder Index (SSRI)-30 definition of the response at week 24.

Results: SGUS score was positively correlated to the focus score ($r = 0.61$) and inversely correlated to UWSF ($r = -0.68$). Conversely, the focus score was not correlated with UWSF. No patients with grade 4 SGUS score responded to rituximab, compared to 88% of patients with SGUS score ≤3. The median focus score at inclusion was 0.3 (0.0–1.3) in the responders versus 4.0 (2.7–5.3) in the non-responders ($P = 0.02$). Conversely, baseline UWSF was not associated with the response.

Conclusions: pSS patients with the highest histologic or morphologic salivary gland involvement do not respond to rituximab after 6 months, suggesting that a single 1gX2 rituximab course could be an unsufficient treatment for patients with the highest salivary gland B cell activity.

S14.6

Baseline Characteristics of Parotid Gland Histopathology Predict Responsiveness of Patients with Primary Sjögren’s Syndrome to Rituximab Treatment

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Objectives: To assess the prognostic value of parotid gland immunopathology with regard to responsiveness of patients with primary Sjögren’s syndrome (pSS) to rituximab (anti-CD20; RTX) treatment.

Methods: In a double-blinded, placebo-controlled trial sequential parotid gland biopsies were taken from 20 RTX and 10 placebo treated pSS patients, i.e., before and 12 weeks after RTX or placebo treatment. The relative amount of lymphocytic infiltrate (stained for CD45), absolute number of T-cells/mm$^2$ and B-cells/mm$^2$ (stained for CD3 and CD20, respectively), and the quantity of germinal centers and lymphoepithelial lesions in parotid gland parenchyma were assessed. RTX-treated patients were divided into responders (change in ESSDAI 12 weeks after treatment ≥3) and non-responders (change in ESSDAI <3).

Results: In RTX treated patients, a significant reduction in the number of CD20$^+$ B-cells/mm$^2$ parenchyma was observed, while no reduction was observed in placebo-treated patients. The levels of CD3$^+$ T-cells/mm$^2$ in parenchyma did not change in both groups. Furthermore, the number of lymphoepithelial lesions and germinal centres was significantly reduced in RTX-treated patients and did not change in placebo-treated patients. When
Comparing the baseline characteristics of responders with non-responders, number of CD20+ B-cells/mm² parotid parenchyma was significantly higher in responders (1871 cells/mm² versus 353 cells/mm², \( P < 0.05 \)).

**Conclusion:** RTX treatment leads to major reduction of lymphocytic infiltration, germinal centres, lymphoepithelial lesions and B cell component in parotid glands. The number of CD20+ B-cells/mm² parotid gland parenchyma predicts the responsiveness of pSS patients to RTX treatment. Herewith baseline histopathological characteristics may contribute to a more personalized treatment approach of pSS patients.

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**S14.7**

**Rituximab in Primary Sjögren’s Syndrome: A Single Center Experience**

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**Objective:** To evaluate Rituximab (RTX) efficacy in induction therapy and long term maintenance therapy in primary Sjögren’s syndrome (pSS) complicated with systemic manifestations and MALT lymphomas at Nasonova Research Institute of Rheumatology.

**Methods:** Eighty-four pSS patients (24 with systemic manifestations, 46 with MALT lymphomas and 14 with hypergammaglobulinemia) fulfilled ACR criteria for SS (2012). Mean age and median pSS duration were 49 ± 14.4 years and 7 (4–12) years, respectively.

Fifty pts underwent RTX induction therapy (cumulative dose 2.5 gr (2.0–2.8)), 34 – RTX in combination with cyclophosphamide (cumulative dose 5 gr (4.0–5.0)). 34.5% pts (29/84): 7 pts with systemic manifestations, 15 with MALT lymphoma, seven with hypergammaglobulinemia) were under RTX maintenance therapy for median 48 (33–60) months. Median RTX cumulative dose of during it was 2.5 gr (2–4).

**Results:** At month three after RTX induction therapy, there was significant improvement in pSS (see Table 1). During RTX maintenance therapy median ESSDAI score gradually decreased (1.5 (0–2) before and 0.5 (0–1) after treatment, \( P = 0.001 \)). Schirmer test and SWS flow rate improved a little (\( P > 0.005 \)). \( \gamma \)-globulins and IgG lowered significantly, while RF, IgA, IgM didn’t.

Infusion reactions and infections (not severe) were in 10/84 (11.9%) cases and 7/84 (8.3%), respectively.

**Conclusion:** RTX is effective and safety option in induction and long term maintenance therapy in pSS patients complicated with vasculitis, lymphomas.

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**S14.8**

**Efficacy and Safety of Belimumab Given for 12 Months in Primary Sjögren’s Syndrome: The Beliss Open-Label Phase II Study**

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Belimumab, a monoclonal antibody against BAFF registered for the treatment of systemic lupus erythematosus (SLE), was recently employed in primary SS in the BELISS trial (1), with encouraging results reported after 6 months.

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<table>
<thead>
<tr>
<th>Parameter (median(ICR) or mean ± SD)</th>
<th>Before treatment</th>
<th>After treatment</th>
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<tr>
<td>ESSDAI 7 (5–8)</td>
<td>1 (0–2)</td>
<td>( P &lt; 0.001 )</td>
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<tr>
<td>Schirmer test, mm 7 (3–15)</td>
<td>9 (5–15)</td>
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<tr>
<td>Stimulated whole saliva (SWS) flow rate, ml 0 (0–0.5)</td>
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<td>Gammaglobulins, (N = 8, 5–15.1%) 19.7 ± 6.7</td>
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<td>RF, IU/ml 147 (63–350)</td>
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<td>IgM, (60–450 IU/ml) 239 (163–378)</td>
<td>129 (86–183)</td>
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<td>CD19+, (N = 6-19%) 10.7 (5.2–15.4)</td>
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of treatment. We report the efficacy and safety of 1-year treatment of Sjögren’s syndrome (SS) with belimumab, targeting the B-cell activating factor. Nineteen out of 30 patients terminated the 52-week study, 15 of them being responders while four non-responders at W28. Thirty of the 15 responders at W28 also responded at W52 (86.7%). In the 15 responders at W28, the ESSDAI was 7.5 ± 4.0 at baseline, 3.9 ± 3.1 at W28 (P < 0.0001 versus baseline), with a trend of further improvement at W52, i.e., 3.1 ± 3.2 (P < 0.0001 versus baseline). In the same 15 responders, the ESSPRI was 6.0 ± 1.0 at baseline, 4.5 ± 1.8 at W28 (P = 0.003 versus baseline), and 4.3 ± 2.3 at W52 (P = 0.01 versus baseline) (P = 0.2; and P = 0.7, between W28 and W52, for ESSDAI and ESSPRI scores, respectively). A moderate disease activity (i.e., an ESSDAI score ≥5) was observed in 5/15 (33.3%) patients at W52 versus 6/15 (40%) at W28 and versus 10/15 (66.7%) at baseline. Persistence in the response or further improvement was observed in the ESSDAI domains, which mainly contributed to the ESSDAI score (i.e., glandular, lymphadenopathy and articular). The decrease in B-cell biomarkers observed at W28 persisted unchanged until W52, with the exception of the rheumatoid factor, which appeared to further decrease (64.8 ± 78.7 IU/mL at baseline, 51.1 ± 56.8 IU/mL at W28 (P = 0.028, W28 versus baseline) and 42.6 ± 47.3 IU/mL at W52 (P = 0.048, W52 versus baseline)]. Salivary flow, Schirmer’s test and the focus score of salivary biopsy did not change. The safety of treatment was good at W52. Long-term treatment with belimumab may be beneficial in SS. Randomised, controlled studies in larger populations are encouraged.


S14.9
Worsening of Systemic Activity and Increase of B-Cell Biomarkers in Sjögren’s Syndrome after Suspension of Belimumab Treatment: Long Term Follow-Up After the End of the Beliss Study

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Belimumab, a monoclonal anti-B lymphocyte Stimulator (BLyS) antibody is preliminary found to be effective in Sjögren’s syndrome (SS) (1). The ESSDAI score and the serum levels of rheumatoid factor (RF) and IgM immunoglobulins were significantly affected by this treatment in the long term.

The aim of this study is to report the 1 year follow-up after the end of the BELISS study in the Italian cohort of patients in order to further support the clinical and biological benefits of belimumab in SS. Clinical and laboratory data of 13 SS patients were collected at 1 year after the end of the belimumab treatment in the BELISS study. Statistical comparisons by t-test or Wilcoxon test were performed.

The ESSDAI score was 8.8 ± 6.9 at baseline, 3.5 ± 3.7 at week 52 (end of the trial) and 7.0 ± 5.7 at month 12 after the end of the trial (baseline versus month +12, P = 0.2; week 52 versus month +12, P = 0.003). RF level was 93.7 ± 101.2 IU/ml at baseline, 74.7 ± 74.5 IU/ml at week 52, 174.1 ± 220.3 IU/ml at month 12 after the end of the trial (baseline versus month +12, P = 0.5; week 52 versus month +12, P = 0.008). IgM level was 180.7 ± 105.9 mg/dl at baseline, 136.7 ± 83.2 mg/dl at week 52, 165 ± 84.6 mg/dl at month 12 after the end of the trial (baseline versus month +12, P = 0.3; week 52 versus month +12, P = 0.04). BLyS level was 1211 ± 429 pg/ml at baseline, 1704 ± 1044 pg/ml at week 52 and 1896 ± 950 pg/ml 12 months after belimumab suspension. Interestingly, BLyS levels were significantly higher even 4 weeks after the last belimumab infusion (week 48) if compared to baseline levels (P = 0.04), and increased 12 months later (baseline versus month +12 after the end of the trial, P = 0.01; week 52 versus month +12 after the end of the trial, P = 0.2). Two patients developed lymphoma 2 years after the end of the trial.

Worsening in systemic activity and increases of B-cell biomarkers in SS patients after the suspension of belimumab further supports the role of targeting BLyS in SS.


S14.10
Low Numbers of Blood and Salivary Natural Killer Cells are Associated with a Better Response to Belimumab in Primary Sjögren’s Syndrome: Results of the BELISS Study

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Purpose: To address changes in blood lymphocyte sub-populations and labial salivary gland (LSG) inflammation after belimumab in patients with primary Sjögren’s
syndrome (pSS) and identify predictors of response to treatment.

**Methods:** Sequential blood lymphocyte subsets and LSG biopsies were analysed between week 0 (W0) and W28 in 15 pSS patients (all females, median age = 44 [36.5–63.5] years) treated with belimumab. Systemic response to treatment was defined as a decrease of the ESSDAI ≥3 points at W28.

**Results:** After belimumab, we observed a decrease in blood B lymphocytes primarily involving CD27-IgD+ naive B-cells (151 [24–186] at W0 versus 10 [6–40] at W28, P = 0.008, n = 9). No significant change in the total lymphocyte, total T-cell, CD4 or CD8 T-cell counts was observed. By contrast, there was a significant increase in the number of NK cells (P = 0.032).

Regarding histological pattern, lymphocytic sialadenitis (focus score > 1) present in 12 (80.0%) patients before belimumab, became negative in 5 after treatment (P = 0.03). The median LSG B-cell /T-cell ratio decreased from 0.58 [0.5–0.67] to 0.50 [0.5–0.5] (P = 0.06). BAFF staining was detected in 11/14 (78.6%) patients, before, compared to 7/14 (50.0%) after belimumab (P = 0.10). The median percentage of BAFF positive cells in foci significantly decreased from 27.5% [10–40] to 5% [0–20], after belimumab (P = 0.03).

Systemic response was obtained in 6 (40%) patients. The only predictor of response was the presence of a low number of NK cells both in blood (8.5% [7–10] versus 11% [9–21], P = 0.04) and in LSG (20.6/mm3 [20.0–21.4] versus 30.0/mm3 [25.0–100.0], P = 0.003). Serum BAFF levels did not influence response to treatment.

**Conclusion:** Low blood and salivary NK cells numbers are associated with a better response to belimumab. This suggests that 2 distinct subsets of pSS may exist: one with predominant type-I INF/BAFF/B-cell axis, good responders to belimumab, and one with predominant type-II IFN/NK-cell axis, non-responders.

*The 2 first authors contributed equally to the study.*

**Figure 1** Changes in NK-cell count and proportion in responders and non responders Number (upper plot) and percentage (lower plot) of NK-cells in responders and non-responders. *P < 0.05

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**S14.11**

**Germinial Centers Disappear in Parotid Gland Tissue after Treatment of Primary Sjögren’s Syndrome with Abatacept**

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**Background and Objective:** Treatment of early and active primary Sjögren’s Syndrome (pSS) with abatacept decreases disease activity.1 Abatacept inhibits the costimulatory interaction of T-cells and antigen-presenting cells. How abatacept influences the pathogenesis in pSS patients is yet unknown. The aim of this study is to assess the histopathological changes in parotid gland tissue after treatment of early and active pSS patients with abatacept.

**Methods:** Fifteen pSS patients (12 women and 3 men) with disease duration ≤5 years were included in the open-label Active Sjögren Abatacept Pilot (ASAP) study.1 On days 1, 15, 29 and every 4 weeks thereafter patients received abatacept infusions. Before treatment and after 25 weeks of follow up a parotid gland biopsy was performed and analysed for histopathological features: number of foci/4 mm² (focus score, FS), area of lymphocytic infiltrate, germainal centers (GC), lymphoepithelial lesions (LELs), numbers of B-cells, T-cells and plasma cells.

**Results:** Abatacept treatment does not affect FS, area of lymphocytic infiltrate, number of LELs and number of infiltrating B- and T-cells, but clearly influences the presence of GC. At baseline, GC are present in the parotid glands of 5/15 patients. Abatacept treatment resulted in both a disappearance of these GC as well as clinical improvement. The number of GC/mm² at baseline is associated with an improvement in the glandular domain (reduction in swelling) of EULAR Sjögren’s Syndrome Disease Activity Index (ESSDAI). Numbers of IgA and
IgG plasma cells are not affected by abatacept treatment, while IgM plasma cells/mm³ are slightly increased.

**Conclusion:** Abatacept inhibits local T-cell dependent B-cell activation in parotid gland tissue of pSS patients as witnessed by the decline in GC/mm³ after treatment. Presence of GC at baseline predicts response in ESSDAI glandular domain after abatacept treatment.

**Acknowledgements:** This study was supported by Bristol-Myers Squibb, France.

**References:**

**S14.12**

**Abatacept Treatment Targets TFH-Cells in Patients with Primary Sjögren’s Syndrome**

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**Background:** T-cell dependent B-cell hyperactivity is a characteristic feature of primary Sjögren’s syndrome (pSS). Treatment of early and active pSS patients with abatacept, a human fusion molecule of IgG-Fc and CTLA-4, improves disease activity [1]. However, the mode of action of abatacept in pSS remains elusive. The current study was designed to assess the impact of abatacept treatment on effector T-cell subsets in pSS patients.

**Methods:** Fifteen pSS patients, diagnosed according to the 2002 AECG criteria, were treated with abatacept for 24 weeks [1]. Percentages of effector CD4⁺ T-cell subsets were assessed in peripheral blood mononuclear cells by flow cytometry at baseline, and 4, 12, 24, 36 and 48 weeks after the first dose. Expression patterns of CD45RA, CXCR3, CCR6, CCR4, CXCR5, programmed death-1 (PD-1), inducible costimulator (ICOS) and FoxP3 were used for distinction of Th1, Th2, Th17, Tfh and Treg subsets. Serum levels of effector T-cell related cytokines were measured at all time points using Luminex. Generalized estimating equations were used to analyze the presence of different subsets over time within patients, viz. on treatment (week 0–24) and off treatment (week 24–48).

**Results:** Percentages of Tfh-cells (CXCR5⁺PD-1⁺ within total CD4⁺ T-cells) decreased on abatacept treatment (week 0–24; P = 0.001). Furthermore, expression levels of ICOS by Tfh-cells became concomitantly lower (P < 0.001). Treg-cells (FoxP3⁺ within total CD4⁺ T-cells) were also reduced on treatment (P < 0.001), whereas levels of Th1, Th2 and Th17-cells were unaffected. Analysis of effector T-cell related cytokines in serum revealed that IL-21 concentrations decreased on treatment (P < 0.001). All these changes reversed off treatment (week 24–48).

**Conclusion:** Treatment of pSS patients with abatacept primarily affects Tfh-cells. The lower numbers of Tfh-cells may lead to reduced T-cell-dependent B-cell hyperactivity and contribute to the clinical effects of abatacept treatment in pSS patients.


**S14.13**

**New Therapeutic Strategy for Sjögren’s Syndrome Associated with Rheumatoid Arthritis Targeted on T cells**

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**Objective:** To clarify the efficacy and safety of abatacept for secondary Sjögren’s syndrome (SS) associated with rheumatoid arthritis (RA).

**Methods:** We designed open-labeled, prospective, observational, and multicenter study (ROSE trial; Rheumatoid Arthritis with Orencia Trial Toward Sjögren’s syndrome Endocrinopathy) for secondary SS (diagnosed by 1999 revised Japanese diagnostic criteria) associated with RA (diagnosed by 1987 ACR or 2010 ACR/EULAR criteria), who were over 20 years old and consented to this study.

1. Primary endpoint was frequency of Simplified Disease Activity Index (SDAI) remission at 52 weeks after initiation of abatacept.
2. Secondary endpoints included Saxson’s test and Schirmer’s test.
3. Adverse events during observational periods were also analyzed.

**Results:** Thirty two patients (all females) had been enrolled in this study. Interim analysis for 24 weeks included assessment for effectiveness in 31 patients and safety in 32 patients.
1) The mean SDAI significantly decreased from 19.8 ± 11.0 (0 week, baseline) to 9.9 ± 9.9 (24 weeks) (P < 0.05) after initiation of abatacept. Patients with clinical remission by SDAI increased from 0 patient (0 week) to 8 patients (25.8%) (24 weeks).  
2) Saliva volume by Saxson’s test increased slightly from 2232 ± 1908 (0 week) to 2424 ± 2004 (24 weeks) mg/2 min in 29 patients. In 11 patients with Greenspan grading 1 and 2 of labial salivary glands biopsy, saliva volume significantly increased from 2945 ± 2090 (0 week) to 3419 ± 2121 (24 weeks) mg/2 min (P < 0.05). Tear volume by Schirmer’s test significantly increased from 3.6 ± 4.6 (0 week) to 5.5 ± 7.1 (24 weeks) mm/5 min (P < 0.05).
3) Five adverse events occurred in five patients out of 32 patients (15.6%), and three of them were infections. Although abatacept was interrupted in 3 patients, it has been restarted after recovery of the adverse events.
Conclusion: These results indicated that abatacept might be effective for both SS and RA involvements in secondary SS with RA.

S14.14

Antigen/TLR9-Triggered Secretion of IFN-α and IFN-γ is Robustly and Additively Inhibited by a Combination of Leflunomide and Hydroxychloroquine

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Introduction: T and B cell-driven immunity is critically involved in immunopathology of pSS. Recently we demonstrated synergistic T- and B-cell activation upon T cell triggering and TLR7/9-driven B cell activation in pSS patients, accompanied by synergistic induction of immunoglobulins and IFNγ- and IL-17- producing T cells. In addition, TLR7/9-expressing activated pDCs associated with increased type I IFNs and IFN-inducible genes are increased pSS patients. Several studies have shown that the DMARDs leflunomide and hydroxychloroquine inhibit immune activation in pSS but only show moderate efficacy. However, LEF and HCQ target different pathways with overlapping, but also potentially additive mechanisms, where LEF primarily targets T and B cells and HCQ TLR7/9-driven B cell and pDC activation.

Objective: To assess the additive effects of LEF and HCQ on CD4 T- and B-cell activation and production of IFN-α and IFN-γ in vitro employing SEB/TLR9-triggered PBMC.

Methods: PBMCs were cultured with antigen (SEB), TLR9 and their combination (n = 9), in presence or absence of LEF, HCQ and their combination (n = 4). Proliferation of T and B cells and release of IFN-α, IFN-γ, IL-17 and IL-4 was measured.

Results: In line with robust T and B cell activation IFNγ and IL-17 production and synergistic IFNα production, indicative of pDC activity, was achieved by a combination of SEB and TLR9 (all P < 0.001). Both LEF and HCQ very potently and dose dependently inhibited proliferation and IFNα production (mean IFNa: 85% and 97% at 100 μM and 10 μM, resp. P < 0.05). At the latter concentrations LEF and HCQ additively inhibited IFNg production (mean: LEF 45%, HCQ 43 and combi 75%, all P < 0.05). At these concentrations IL-17 production was completely inhibited.

Conclusion: LEF and HCQ robustly inhibited cytokine production with clear additive efficacy, which could indicate the potential surplus value of combination therapy.

S14.15

A Multicenter Phase II Prospective Clinical Trial of Glucocorticoid Treatment for Patients with Untreated IgG4-Related Disease

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Although glucocorticoid treatment is effective for patients with IgG4-related disease (IgG4-RD), (including Mikulicz’s disease) treatment regimens have not yet been standardized. The starting dose, method of tapering, and maintenance dose and duration must therefore be established. We performed a prospective phase II trial to establish a standard glucocorticoid treatment regimen for patients with IgG4-RD (UMIN: 000002311).

Patients and Method: In this trial, the initial dose of prednisolone was 0.6 mg/kg body weight per day. Every 2 weeks, the dose was reduced by 10%. The maintenance dose was 10 mg/day for at least three months. The subsequent maintenance dose and the need for continued prednisolone treatment were determined for each individual patient, based on the results of physical examination, laboratory data, and imaging modalities. The trial we performed enrolled patients who fulfilled the comprehensive diagnostic criteria for IgG4-RD, as established by the Japanese IgG4-RD research group. The primary study endpoint was the complete remission rate at 1 year. Secondary endpoints included the maintenance dose of prednisolone, the relapse rate and adverse events. We had assumed an enrollment of 57 patients over 5 years, but
enrollment rates were higher, with 61 patients registered over 4 years. Registration was subsequently closed.

Results: The complete remission rate was 67.2%, and response rate 91.8%. The most major adverse event was glucose intolerance, furthermore various infections, hyperlipidemia, hypertension, and so on were observed. The median maintenance dose of prednisolone was 7 mg daily. The relapse rate after maintenance therapy was 17.9%.

Conclusion: Although the good remission and response rate were observed, adverse effects of glucocorticoid and recurrence after maintenance treatment are still problematic. Second line treatment for relapsed patients with IgG4-RD should be examined by prospective international multicenter study.
Session 15. Biobanks, Registries and International Networking

S15.1 Integrated International Collaborations in Sjögren’s Syndrome: The Essential (EULAR Sjögren’s Syndrome Experimental and Translational Investigative Alliance) Study Group

Francesca Barone1, Fai Ng2, Tim Radstake3,4 in representation of the Essential Study group

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There is an excellent track record in collaborative Sjögren’s research in the EU, exemplified by the EULAR Sjögren’s syndrome (SS) task force. Multiple leading researchers and research programs on SS exist in the EU, with overlapping and complementary research themes. One year ago EULAR discussed with members of the SS community the possibility to emulate these successes in clinical research and create a novel study group with a specific focus on experimental and translational research in SS. A working group was established and a preliminary draft of the eSSential (EULAR Sjögren’s Syndrome Experimental aNd Translational Investigative Alliance) study group was presented at the EULAR meeting in Paris in June 2015 to a group of SS experts. Feedback and suggestions were integrated in the original proposal and a revised organogram and program of work was presented to the EULAR Standing Committee on Investigative Rheumatology to obtain EULAR endorsement in March 2015.

The eSSential study group will be built as a network of researchers and clinicians interested in SS aimed to promote research initiatives and facilitate interaction at European level and international collaboration. The aim of eSSential is to better understand SS pathogenesis and optimize the identification of novel therapeutic pathways:

1. Promoting the sharing of knowledge, tools and expertise in experimental and translational research among scientists and clinicians with an interest/expertise in SS.
2. Facilitating top quality synergistic collaborative research initiatives that will harness the existing strengths of SS researchers/institutions in Europe.
3. Consolidating and expand the experimental and translational research network in SS in Europe and beyond.
4. Identifying research priorities and contributing to strategy development to address key research questions

The steering committee that includes representatives of several SS research groups will coordinate the operational matters of eSSential including reporting and meeting organization. A series of facilitators will coordinate individual research initiatives. The group will meet face-to-face at EULAR, EWRR and ISSS. The advisory board will coordinate the integration with the ongoing clinical initiatives. A series of research projects will be promoted by eSSential, among which the development of standardized measurements to use salivary gland biopsies as measure of outcome in clinical trials.

S15.2 Clinical and Immunological Characterization at Diagnosis of 5041 Patients with Primary Sjögren Syndrome Fulfilling the 2002 AE Classification Criteria: The Big Data International Sjögren Project


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Objective: To analyse the epidemiological, clinical and immunological characteristics of the largest international cohort of patients diagnosed with primary Sjögren syndrome (SS) according to the 2002 AE classification criteria.
Patients: The Big Data International Sjögren Project (BIGDISP) is an international, multicentre registry formed in 2014 to take a “high-definition” picture of the main features of primary SS at diagnosis by merging international SS databases. International experts participating in the EULAR-SS Task Force were invited to participate. By February 2015, the database included 5041 consecutive patients fulfilling the 2002 classification criteria for primary SS (including probable SS, defined as fulfilment of three criteria pending the results of some diagnostic tests) from nine European and four American countries. The main clinical features at diagnosis (time of criteria fulfilment) or at recruitment were collected and analysed.

Results: The cohort included 4730 (94%) women (female: male ratio, 15:1), with a mean age at diagnosis of primary SS of 54.17 years (range, 10–97), of which 94.1% were Caucasian and 88.6% lived in European countries. The frequency of fulfilment of the 2002 criteria was: 94.4% for dry eye, 92.8% for dry mouth, 88.2% for positive salivary gland biopsy, 85.8% for positive oculor tests, 74.8% for positive oral tests and 70.8% for positive Ro/La autoantibodies. As a minimum of four of the six criteria are required for fulfilment, the percentage of diagnostic tests performed varied: Ro/La autoantibodies were tested in 99.5% of patients, ocular diagnostic tests (Schirmer’s test and/or corneal stainings) were made in 90.1%, oral tests in 77.1% and salivary gland biopsy in 72.5% of patients. With respect to criteria fulfilled, 7.6% fulfilled three criteria, 41.7% four criteria, 36.1% five criteria and 14.5% all six criteria. Systemic involvement at diagnosis was retrospectively measured using ESSDAI definitions in 3314 patients and included articular involvement (35.2%), glandular involvement (19.3%), lymphadenopathy (10.7%), cutaneous involvement (9%), constitutional involvement (8.4%), respiratory involvement (7.3%), peripheral nervous system involvement (5.2%), renal involvement (2.2%), central nervous system involvement (1.7%) and muscular involvement (1.2%). With respect to laboratory abnormalities, 43.5% of patients showed biological abnormalities and 24.7% haematological abnormalities according to ESSDAI definitions. Ro/La positivity was detailed in 3401 cases: 1291 (38%) were Ro+La+, 1040 (31%) had isolated Ro+ and only 136 (4%) patients showed isolated La+.

Conclusion: In the largest cohort of primary SS patients diagnosed homogeneously according to 2002 AE criteria, the broad heterogeneity of clinical and analytical features observed emphasize that SS should be considered a systemic disease rather than a sicca-limited disease (even at diagnosis). Most clinical features and laboratory abnormalities present at diagnosis in primary SS patients are not included in the current criteria, and their inclusion in future proposed classification criteria should be evaluated.

Tobacco Smoking and Sjögren’s Syndrome


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Purpose: Tobacco smoking is implicated in several inflammatory conditions such as uveitis and rheumatoid arthritis. There is little data reported on the association of smoking with the diagnosis of Sjögren’s syndrome (SS).

Methods: Cross-sectional, observational study of 424 patients with Sjögren’s syndrome.

Results: There was a significant negative association with current smoking status and SS classification. 55% of SS patients reported previous smoking at any point; this was similar to the non-SS sicca controls. Among primary SS patients there was a 3% current or recent smoking rate. Non-SS patients with keratoconjunctivitis sicca had an 11% current smoking rate. This is in contrast to the 18% community smoking rate among females in Oklahoma age 45–65 and an age-matched systemic lupus erythematosus cohort from the same institution which was 18.1%.

Conclusion: Compared to the normal and SLE control populations, there is a significantly lower prevalence of smoking among the primary SS and non-SS sicca cohort. This was contrary to our expectations, that the pro-inflammatory effects of tobacco smoking may increase the risk of SS. We propose that the mucosal drying and pro-inflammatory effects of smoking discourage the use of cigarettes, resulting in a profoundly lower prevalence of current tobacco smoking among patients with sicca syndrome compared to controls.
S15.4
Smokers Have Reduced Risk of Primary Sjögren’s Syndrome (pSS), and Smoke Cessation May Trigger Development of the Disease
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Background: Smokers have increased risk of anti-CCP positive RA. In some diseases, such as Ulcerative colitis, Sarcoidosis and Parkinson’s disease smoking seems beneficial or protective. In 2000 we described an inverse association between smoking and focal sialadenitis and anti-Ro/anti-La in pSS (Manthorpe et al, ARD, 2000). In a nested case control study, we now investigate the effects of smoking on development of pSS.

Methods: Among participants in two population-based surveys (Malmö Preventive Medicine (N = 33346) and Malmö Diet & Cancer (N = 30447)), incident cases of pSS were identified and matched with four controls. Chi-square-test and conditional logistic regression analysis were applied.

Results: Sixty-three individuals (8% male, mean age at inclusion 51 (29–72) years) were diagnosed with pSS a median of 98 months (3–329) after inclusion and fulfilled the 2002 AECC-criteria for pSS. ANA, RF, anti-Ro and anti-La were positive in 73%, 57%, 59% and 41%, respectively. Focal sialadenitis with focus score ≥1 was found in 85%.

Findings: Current smoking at inclusion was associated with a reduced risk of subsequent diagnosis of pSS (OR 0.26; 95% CI 0.11–0.60). The pattern of present/former/never smokers was different in cases and controls (Figure), independent of anti-Ro/anti-La positivity. The ratio present/former smoker was 0.3 among cases and 1.5 among controls (P < 0.001). Being a former smoker was associated with an increased risk of pSS: OR 8.1; 95% CI 3.2–21 compared to current smokers; OR 4.1; 95% CI 1.8–10 compared to never smokers. Stopping smoking was not related to pSS symptoms (information available in 22 of 32 former smokers), as these appeared >5 years after smoking cessation in 91%.

Conclusion: Smoke cessation increases the risk of being diagnosed with pSS, while being a current smoker seems to lower it, confirming our former retrospective study.

S15.5
Prevalence and Burden of Damage in Patients with Primary Sjögren’s Syndrome: A Multicenter Study
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Objectives: (1) To describe prevalence/type of organ damage in a large series of patients with primary Sjögren’s syndrome (pSS) assessing the relationship between damage, patient demographics and disease characteristics (duration, activity, lab features) (2) to evaluate the impact of damage on patient-reported symptoms assessed using the recently-proposed ESSPRI index.

Methods: Three hundred and sixty pSS patients (AECG criteria), seen consecutively, were included in this multicenter cross-sectional study. Demographic, clinical and serological features were collected prospectively. All patients completed the ESSPRI questionnaire. The ESSDAI was used to assess disease activity at study inclusion and the SSDDI to evaluate organ damage. Category of damage (oral, ocular and systemic) was analyzed separately.

Results: We included 360 pSS patients: age at study inclusion = 58 ± 15 years, disease duration = 6 ± 7 years. Of 360 pSS patients, 90 (25%) presented a moderate/high disease activity (ESSDAI ≥ 5). The ESSPRI was 6.4 ± 2.3. The vast majority of the patients presented a SSDDI ≥ 1, while only 148/360

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patients did not present any organ damage. The domains mainly contributing to the total damage were the oral/ocular items: teeth loss (24.4%), salivary flow (40%), cataract (14.3%), corneal ulcers (7.2%), tear flow (38.6%). Systemic damage manifestations were observed in 50 (14%) patients and included peripheral neuropathy (5%), lung (2.7%), renal (2.5%) and CNS involvement (0.6%). SSDDI significantly correlated with both the age at diagnosis and the disease duration. Systemic damage was higher in male and in subjects presenting cryoglobulins and low C4 levels. We detected a positive correlation between the SSDDI (systemic items) and the ESSDAI and between the SSDDI and the oral/ocular dryness (ESSPRI).

Conclusions: This study documented that damage manifestations are common in pSS. Disease activity is a modifiable risk factor for damage and should be targeted to optimize patients’ perception of the disease burden.

S13.6
Characterization of Baseline Systemic Activity Using the New ClinESSDAI in the Big Data Sjögren Project Cohort: Analysis in 3316 Patients


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Objective: To characterize and quantify systemic involvement at diagnosis in a large international cohort of patients diagnosed with primary Sjögren syndrome (SS).

Patients: The Big Data Sjögren Project is an international, multicenter registry formed in 2014 with the aim of taking a “high-definition” picture of the main features of primary SS at diagnosis by merging international SS databases. By February 2015, the database included 5041 consecutive patients fulfilling the 2002 classification criteria for primary SS from 13 countries (nine European, four American). Systemic involvement was defined according to the ESSDAI and retrospectively calculated. The new ClinESSDAI, recently developed for retrospective studies in which immunological tests were not carried out at diagnosis, was applied and compared with the classical ESSDAI.

Results: Baseline ESSDAI data were collected in 3316 patients (94% female, mean age at diagnosis 54.24 years). The main features of systemic involvement at diagnosis included biological activity in 43% of patients, articular involvement in 35%, hematological activity in 25% and glandular involvement in 19%. The mean ESSDAI score at diagnosis of the entire cohort was 5.63 ± 6.80 (range, 0–62). Low DAS was reported in 1267 (38%) patients, moderate DAS in 943 (28%) and high DAS in 378 (11%) patients; in the remaining 726 patients (22%), the ESSDAI score at diagnosis was 0. The mean baseline ESSDAI was higher in males (7.33 versus 5.52, P < 0.001), patients diagnosed ≤35 years (6.71 versus 5.63, P = 0.001), those with positive ocular tests (5.75 versus 4.98, P = 0.023) and those with positive immunological markers including ANA (6.71 versus 4.63, P < 0.001), anti-Ro/SSA (6.77 versus 5.48, P < 0.001), anti-Ro/La (6.77 versus 5.48, P < 0.001) and anti-La/SSB (6.91 versus 6.0, P < 0.001). According to the number of criteria fulfilled at diagnosis, a higher mean baseline ESSDAI was found in patients fulfilling the six criteria (7.67 versus 5.65 in those fulfilling five criteria and 5.23 in those fulfilling 3–4 criteria, P < 0.001). With respect to Ro/La combination, the highest mean score was for patients with Ro and La positivity, followed by isolated Ro, isolated La and immunonegative results (6.94 versus 6.52 versus 6.05 versus 5.44, P = 0.001). The mean ClinESSDAI score at diagnosis was slightly higher than the classical ESSDAI (6.01 ± 7.63, range 0–67). All the reported statistical differences found with the ESSDAI were also confirmed using the ClinESSDAI, except for anti-La antibodies (7.12 versus 6.54, P = 0.101).

Conclusion: Epidemiological features (male sex, younger age at diagnosis) and positive autoantibodies at diagnosis were closely related to greater systemic activity retrospectively measured using the ESSDAI and the ClinESSDAI. Scoring systemic activity using the ESSDAI and ClinESSDAI in real-life situations may help identify epidemiological and immunological subsets at high risk of suffering a complicated clinical course.
Sjögren’s Syndrome is the Predominant Autoimmune Disease in Ro/La+ Mothers with Babies Affected with Autoimmune Congenital Heart Block: Results from the Spanish registry (REBACC-GEAS-SEMI)


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Objective: To analyze the main maternal-fetal characteristics of pregnancies affected by autoimmune congenital heart block (CHB) associated with maternal anti-Ro/La antibodies, the outcomes and management of affected pregnancies with CHB.

Methods: The REBACC Spanish Multicenter Registry was created in March 2014. It is integrated by 11 centers with substantial experience in the management of systemic autoimmune diseases. Autoimmune CHB was defined as: a) CHB of any type (I, II or III), fetal endocardial fibroelastosis (EFE) and/or cardiomyopathy, b) cardiac block diagnosed in utero or in the first postpartum month, and c) mothers carrying anti-Ro52, Ro60 and/or La autoantibodies.

RESULTS. By January the 15th, 2015, the REBACC Registry included a total of 40 anti-Ro and/or anti-La+ mothers with 45 single pregnancies with CHB. Mean maternal age at the time of first affected pregnancy with CHB was 31.77 years (range: 22–44). All mothers were anti-Ro60 (+), 13/13 anti-Ro52 (+) and 29/39 (74%) anti-La (+). The mean gestational age at diagnosis of CHB was 23 weeks (range 16–37). AV blocks were of type I in two pregnancies (5%), type II in 13 (32.5%) and type III in 24 (60%); one had an isolated EFE (2.5%). Therapies used in 26/40 (65%) pregnancies included dexamethasone or betamethasone (n = 24), intravenous immunoglobulins (n = 5), plasmapheresis (n = 3), beta2-adrenergic agonists (n = 2), isoproterenol (n = 1). Nine pregnancies were interrupted due to bad fetal prognosis (22.5%), and 31 (77.5%) were successfully carried to term. Pacemaker implantation was required in 16 babies (55%), 14 after birth, 1 at 5 years and another at 12 years of age. After the index pregnancy, 12 mothers had another pregnancy with 3 (25%) babies affected with CHB, 2 type III AV block and 1 type I AV block diagnosed. Four mothers subsequently had a third pregnancy, of which, 2 (50%) were affected by type III CHB diagnosed at 23.5 and 20 weeks. One mother had a fourth and fifth pregnancy, none affected by CHB. At diagnosis of the first affected pregnancy, 28 (70%) mothers did not have any autoimmune disease and the remaining 30% had Sjögren syndrome (n = 5); SLE (n = 5) and undifferentiated disease (n = 2). At the last visit, of the 30 women who initially did not have any autoimmune diagnosis, 19 (63%) developed a systemic autoimmune disease (SS in 13, SLE in 5 and SS+ SLE in 1). Also, 1 mother previously diagnosed with SLE was subsequently diagnosed with Sjögren syndrome.

Conclusions: At the end of the follow-up, nearly half the mothers with affected babies with autoimmune congenital heart block are diagnosed with Sjögren’s syndrome. More than half the affected mothers might have been asymptomatic at the first affected pregnancy, as anti-Ro and anti-La antibodies can be detected several years before SS is diagnosed. Autoimmune CHB is one of the first ‘indirect’ signs of SS in women of childbearing age.
Abstract

S13.8  
**Baseline ESSDAI Disease Activity States (DAS) in 1216 Patients with Primary Sjögren Syndrome: an Score at Diagnosis >13 Predicts Poor Outcome**


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**Objective:** To score systemic activity at diagnosis and correlate baseline activity classified according to the ESSDAI disease activity states with poor outcomes in a large cohort of patients with primary Sjögren syndrome (SS).

**Patients and Methods:** We include 1216 consecutive patients who fulfilled the 2002 classification criteria for primary SS. The clinical and immunological characteristics and level of systemic activity were assessed at diagnosis as predictors of poor outcomes (lymphoma and death) using Cox proportional-hazards regression analysis adjusted for age at diagnosis. European systemic activity index (ESSDAI) scores were retrospectively calculated at diagnosis. Disease activity states (DAS) were defined according to the baseline ESSDAI score (low DAS for an ESSDAI <4, moderate DAS for an ESSDAI between 5 and 13, and high DAS for an ESSDAI higher than 13).

**Results:** After a mean follow-up of 114 months, 52 (4%) hematological neoplasia and 139 (11%) died. The most frequent systemic activity reported at the time of SS diagnosis included articular (37%), biological (35%), hematology (30%) and glandular (24%) involvements. At diagnosis, 138 (11%) patients showed a high DAS, 385 (32%) a moderate DAS, 422 (35%) a low DAS and 266 (22%) had no systemic activity (ESSDAI = 0). No significant differences in the main baseline features were found according to the DAS except for the age at SS diagnosis, which was higher in patients with a high DAS (57 years versus 53 years for moderate DAS, 54 for low DAS and 56 for no activity, P = 0.019). Patients who showed a high DAS at diagnosis of SS developed more frequently hematological neoplasia and had a poor survival in comparison with patients who showed no systemic activity (ESSDAI = 0) (19% versus 2%, P < 0.001, and 19% versus 11%, P = 0.032, respectively). Patients with a high-DAS at diagnosis developed more frequently cancer in comparison with those without high baseline activity (21% versus 10%, P < 0.001); however, a high DAS was related to a higher frequency of hematological neoplasia but to a lower frequency of solid neoplasia (18% versus 2.5%, 2.9% versus 7.5%, P < 0.001).

**Conclusion:** Categorization of baseline systemic activity using the ESSDAI disease activity states at diagnosis of primary Sjögren syndrome is useful to identify patients with a high risk of developing poor outcomes; patients who present at diagnosis with ESSDAI ≥14 (high DAS) should be considered a high-risk population and should be followed more closely than those with no systemic activity.
Systemic and Biological Activity at Diagnosis is Closely Related to the Development of Lymphoma in Primary Sjögren Syndrome, but not with Development of Solid Neoplasias: Analysis in 1230 Patients (GEAS-SS Registry)


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Objective: To analyse the epidemiological, clinical and analytical features at diagnosis and the use of the European systemic activity index (ESSDAI) to predict the development of cancer in a large cohort of Spanish patients with primary Sjögren syndrome (SS).

Patients: The GEAS-SS multicenter registry was formed in 2005 with the aim of collecting a large series of Spanish patients with primary SS and included 13 Spanish reference centers with substantial experience in the management of SS patients. By January 2015, the database included 1216 consecutive patients fulfilling the 2002 classification criteria for primary SS. ESSDAI scores were retrospectively calculated at diagnosis. Disease activity states (DAS) were defined according to the baseline ESSDAI score (low DAS for an ESSDAI <4, moderate DAS for an ESSDAI between 5 and 13, and high DAS for an ESSDAI higher than 13). Cancer was categorized as hematological or solid neoplasia.

Results: After a mean follow-up of 11.4 months, 84 (7%) patients developed solid cancer and 57 (5%) hematological neoplasia. The following baseline features at diagnosis were associated with the development of hematological neoplasia: male gender (18% versus 6%, P = 0.002), age at diagnosis (59 versus 54 years, P < 0.001), baseline systemic activity (75% versus 50%, P < 0.001), C3 values < 0.82 g/L (19% versus 10%, P = 0.048), C4 values < 0.07 g/L (22% versus 11%, P = 0.044), monoclonal serum band (25% versus 8%, P = 0.001) and cryoglobulins (27% versus 9%, P < 0.001). In contrast, age (59 versus 54 years, P < 0.001), monoclonal serum band (20% versus 8%, P = 0.005) and neutropenia (21% versus 10%, P = 0.001) were related to the development of solid neoplasia. High systemic activity (high-DAS) was found in a higher frequency in patients who developed hematological in comparison with those who developed solid neoplasia or those without neoplasia (79% versus 24%, P < 0.001).

Conclusion: Systemic and biological activity is closely related to the development of lymphoma in primary SS. Etiopathogenic factors such as B-cell hyperactivity and cryoglobulinemic-driven immunological responses have a dual effect, enhancing the risk of development of both systemic involvement and hematological neoplasia, but not the risk of non-hematological neoplasia, whose development is independent of systemic Sjögren.
Prevalence and Clinical Significance of AMA and anti-M2 Autoantibodies in 737 Patients with Primary Sjögren Syndrome (GEAS-SEMI Registry): Closely Associated with Ro/SSA Autoantibodies but not with Systemic Disease


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Objective: To analyse the prevalence of AMA and M2 autoantibodies in a large cohort of Spanish patients with primary Sjögren syndrome (SS) and to correlate positive results with the epidemiological, clinical and immunological expression of the disease.

Methods: The GEAS-SS multicenter registry was formed in 2005 with the aim of collecting a large series of Spanish patients with primary SS and included 13 Spanish reference centers with substantial experience in the management of SS patients. The cumulated ESSDAI index was retrospectively calculated at diagnosis. AMA was detected by indirect immunofluorescence in triple rat tissue (liver, stomach and kidney), acetone-fixed cryosections and FITC-conjugated rabbit anti-human immunoglobulins, and M2 autoantibodies by ELISA against M2 proteins (PDC-E2, BCOADC-E2, OGDC-E2).

Results: AMA were tested consecutively in 737 patients with primary SS (692 women, mean age at diagnosis of 55.51 years), of whom 63 (8.5%) showed positive results. No significant differences were found in the main epidemiological, clinical and immunological features according to the presence or absence of AMA, except for a higher frequency of anti-Ro/SSA antibodies (83% versus 68%, P = 0.015) and leucopenia (33% versus 21%, P = 0.038) in AMA+ patients in comparison with those with negative AMA. M2 autoantibodies were tested in 210 patients, of whom 19 (9%) were positive. Comparison of the main epidemiological, clinical and immunological features according to the presence or absence of anti-M2 antibodies showed a higher frequency of anti-Ro/SSA antibodies (95% versus 74%, P = 0.049), leucopenia (53% versus 19%, P = 0.002), neutropenia (48% versus 22%, P = 0.025), low C4 levels (26% versus 6%, P = 0.008) and biological activity measured by the ESSDAI (84% versus 50%, P = 0.007). We have the paired determination of AMA and anti-M2 in 23 patients with positive results: Twelve have concordant positive results and 11 discordant (seven had negative AMA with positive M2, and four positive AMA with negative M2). The mean value of M2 autoantibodies in AMA+ patients was twice than in those with negative AMA, although the difference was not statistically significant (85.67 ± 62.73 versus 41.51 ± 47.31, P = 0.126).

Conclusion: The study showed three key messages: i) the prevalence of AMA/M2 in primary SS was of 9%; ii) there was a close association between AMA/M2 and the coexistence of autoantibodies against the Ro antigen but not with systemic Sjögren; and iii) nearly half the patients showed discordant results between AMA and M2 positivities, with patients carrying M2 autoantibodies and negative AMA determination showing lower mean levels than those with positive AMA. We recommend the use of M2-ELISA to test for AMA in patients with primary SS.
S15.11

Real-Life Therapeutic Management of Systemic Sjögren: Drug-Based Therapeutic Approaches in 1120 Patients (GEAS-SS Spanish Registry)


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Objective: To describe how systemic disease is treated in a large cohort of Spanish patients with primary Sjögren syndrome in daily practice, focusing on the adequacy of drug therapies for the level of systemic activity measured by the ESSDAI score.

Patients and Methods: By December 2014, our database included 1120 consecutive patients who fulfilled the 2002 classification criteria for primary SS. Therapeutic schedules were classified into four categories: no systemic therapies, hydroxychloroquine (HCQ) and/or low dose of glucocorticoids (GCS) (≤20 mg/d), high dose of GCS (>20 mg/d) and use of second-line therapies (immunosuppressive agents, intravenous immunoglobulins -IVIG- and/or rituximab -RTX-).

Results: The cohort consisted of 1120 patients, including 1048 (94%) females and 72 (6%) males, with a mean age at diagnosis of 54 years. The main drug-based therapeutic approaches for systemic Sjögren ever used during the follow-up were HCQ in 282 (25%) patients, GCS in 475 (42%), used at doses >20 mg/d in 255 (23%), immunosuppressive agents in 148 (13%), IVIG in 25 (2%) and RTX in 35 (3%) patients. The use of systemic therapies was more frequent in males (P = 0.036) and was associated with the presence at diagnosis of anemia (P < 0.001), thrombocytopenia (P = 0.001), neutropenia (P = 0.002), rheumatoid factor (P < 0.001), anti-Ro/SS-A (P = 0.033), monoclonal band (P = 0.002), cryoglobulins (P = 0.003), low C3 (P = 0.003) and low C4 (P = 0.014). The ESSDAI score at diagnosis was higher in patients who received systemic therapy than in those who did not (8.12 versus 4.20, P < 0.0001). Multivariate analysis identified neutropenia, rheumatoid factor and the ESSDAI score as independent variables. HCQ was associated with a lower risk of death (adjusted HR of 0.57, 95% CI 0.34–0.95). We classified 16 (7%) of the 255 patients treated with >20 mg of GCS and 21/148 (14%) of those treated with immunosuppressive agents patients as inadequately treated, mainly associated with articular involvement of low/moderate activity.

Conclusion: The management of systemic Sjögren should be organ-specific, using low doses of GCS in patients with moderate systemic activity, limiting the use of high doses of GCS and second-line therapies to refractory or potentially severe scenarios. The use of systemic therapies for dryness, chronic pain or fatigue is not warranted.

S15.12

Histopathological Diagnosis of Salivary Glands Among 1855 Registry Participants Performed at a Single Centre in Bergen, Norway

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This is a large-scale prospective cohort study with the aim to analyse the histopathological diagnosis of minor salivary glands from 1855 participants with suspected Sjögren’s syndrome (SS). All biopsies have been performed at the Department of Otolaryngology/Head and Neck Surgery at Haukeland University Hospital in Bergen between 1991 and 2014. 3–5 salivary glands were excised from the lower lip following standard procedures. Samples were fixed in formalin, processed and embedded in paraffin according to standardized laboratory procedures. All haematoxylin and eosin stained tissue sections were evaluated by two oral pathologists at Department of Pathology, Haukeland University Hospital according to the standards (Daniels T.E., 1984). Among the 1855 participants, 340 patients (18%) fulfilled the criteria of positive lip biopsy, i.e. with focus score of ≥1 per 4 mm². Within this group, 155 (46%) had focus score of 1 and 185 (54%) were evaluated with focus score of ≥2, respectively. Among the cohort 380
(20%) exhibited unspecific chronic inflammation and 874 (47%) with chronic inflammation and atrophy of glandular tissue. Notably, 261 (14%) specimens had normal gland morphology.

Currently, labial salivary gland biopsy remains the best method for diagnosing the glandular component and to explore autoimmune disease activity within the target organ. The specificity of prominent focal lymphocytic infiltrates as compared to other morphological patterns in the glandular tissue i.e. atrophy, fat infiltration and nonspecific chronic inflammation has not been fully established. By using data from this large cohort, we have a unique opportunity to improve diagnostic applications and to delineate weaknesses and strengths in the traditional methods of diagnosing SS.

S15.13

The Prevalence of Sjögren’s Syndrome in United States Health Insurance Claims

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Introduction: In the U.S., the epidemiology of Sjögren’s Syndrome (SjS) is poorly understood. To increase the evidence base, we conducted a study of SjS prevalence and patient characteristics in a large database of health insurance claims, the Clininformatics Data Mart Multi-plan™

Methods: The data resource contains approximately 100 million persons with health insurance coverage in the U.S. from nine Census regions, primarily from commercial insurance plans. Data years 2004 to 2012 Quarter 1 were used in this study. SjS was defined as ≥ 2 ICD-9 medical service dates with codes of 710.2 (sicca syndrome). The first occurrence of 710.2 was assigned as the index date. Pharmacy medication dispensings were described using Uniform System of Classification Level 2 National Drug Code groupings in a subset of 23,235 SjS cases with pharmacy benefits during 12 months post index date. To better understand the epidemiology of primary SjS (pSjS): in a secondary case definition, we excluded patients with a prior service date for any ICD-9 code for systemic lupus erythematosus (710.0), rheumatoid arthritis (714.X), or scleroderma (710.1) that occurred on or before the SjS index date.

Results: Fifty three thousand and three hundred and eighty-one persons met the SjS case definition. 90.3% were female, and the median and mean age at index date were 53.0 and 51.3 (standard deviation: 12.1), respectively. The crude prevalence of SjS was 0.06%. Among medications dispensed to SjS cases, anti-infectives (broad/medium spectrum) were most frequently noted (61.3%), followed by hormone/corticoids (plain) (42.4%) and narcotic analgesics (39.2%). 23.9% were dispensed ophthalmologic anti-inflammatories. 64.6% (N = 34,473) of SjS cases met the pSjS case definition, resulting in a crude prevalence of 0.04% for pSjS.

Conclusion: Our prevalence estimates for SjS and pSjS were in the range of previous studies that used ICD-9 codes to define disease state (0.016% to 0.147%). To our knowledge, this is the first study of SjS prevalence in U.S. health claims.
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