P-1
Utility of testing for murine tissue specific autoantibodies for the diagnosis of Sjögren’s syndrome
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Background. A group of murine parotid tissue specific autoantibodies (TSAs) which includes anti-SP1 (salivary protein 1), anti-PSP (parotid specific protein) and anti-CA6 (carbonic anhydrase) are markers for early disease in the IL-14t transgenic mouse model of SS. These TSAs are also found in NOD mice and patients (pts) classified with SSA+ or - Sjögren’s according to the American European Consensus Group criteria.

Methods. We tested serum for TSAs from 6 pts groups followed in a rheumatology clinic for >1 year at a university medical center including: 1) SS who met published classification criteria (n=145), 2) non-autoimmune controls (n=32), 3) SLE (n=18), 4) RA (n=14), 5) scleroderma (n=4) and 6) chronic nonspecific sialadenitis (n=15). Saliva samples were also obtained from groups 1.2 & 6. Electronic medical records were reviewed to verify diagnoses & all pts were questioned re: the presence & duration of dry eyes/mouth & medical history. Volunteers with history of dry eyes/mouth, any autoimmune diseases or family history of dry eyes were excluded as controls. Serum samples were anonymously coded & assayed by ELISA (Trinity Biotech, Inc., Buffalo, NY). All laboratory personnel were blinded to pts diagnoses. Analyses was performed to determine the sensitivity, specificity & discriminative ability of the presence of ≥1 TSAs to differentiate SS from other groups.

Results. Of the 145 SS pts, 133 (92%) had disease duration > 3 years. TSAs were detected in both the serum and saliva of pts with SS. Most frequently detected TSA in SS was anti-CA6 IgM (14.5%) (Table I). The presence of ≥1 TSA was not significantly different in each group: SS (43%), controls (59%), chronic sialadenitis (40%) and other connective tissues diseases (33%). No particular TSA or isotype was specific for SS. Results suggested a sensitivity and specificity of 44% and 41% respectively for the presence of ≥1 TSAs in SS. Prevalence of ≥1 ANAs/RF IgM in each group were as follows: SS (70%/45%), controls (30%/41%), chronic sialadenitis (43%/33%) and other connective tissue diseases (69%/47%) (Table II). Among controls the presence ≥1 TSAs did not significantly vary between ANA+ vs. ANA- individuals.

Conclusions. The presence of ≥1 TSAs in the serum does not distinguish between established SS and other patient groups. The value of this assay for confirmation of early or undiagnosed SS (<3 years) remains unclear. Further studies in larger pts groups including a prospective study of TSAs in pts with early sicca symptoms are needed. Assay of TSAs in saliva or calculation of a saliva/serum TSA ratio may prove to be a more valuable diagnostic test.

P-2
Utility of novel autoantibodies in the diagnosis of Sjögren’s syndrome among patients with dry eye
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Background. About one in 10 patients with clinically significant dry eye have underlying Sjögren’s syndrome (SS). Unfortunately, SS is greatly underdiagnosed in clinical practice, not only due to its diverse symptomatic expression but also the difficulty with serological testing. Salivary protein 1 (SP1), carbonic anhydrase 6 (CA6), and parotid secretory protein (PSP) have been reported as useful markers to detect SS at an early stage. We aimed to investigate the diagnostic value of these novel autoantibodies in comparison with the traditional serological markers to detect SS among a small sample of dry eye patients.

Methods. Forty-six dry eye patients with SS (SS-dry eye), 14 dry eye patients without SS (non-SS dry eye), and 25 controls over the age of 18 were included. The 2012 American College of Rheumatology classification criteria were used for SS diagnosis. After a detailed review of systems, Ocular Surface Disease Index questionnaire, Schirmer’s test without anesthesia, tear film break-up time, and ocular surface staining were performed to assess dry eye. All participants underwent serological testing using a commercially available finger prick kit.

Results. Thirty-seven patients with SS (80.4%) had a positive traditional antibody and 28 (60.9%) had a positive novel autoantibody. Traditional autoantibodies were absent in all non-SS dry eye patients and controls. Novel antibodies were present in 7/14 (50%) non-SS dry eye patients and 4/25 (16%) controls. Among three novel autoantibodies, anti-CA6 was significantly more prevalent in the SS and non-SS dry eye groups compared to controls (52.2% vs. 42.9% vs. 8.0%, p<0.001). Dry eye patients with positive anti-CA6 alone were significantly younger than patients with only traditional autoantibodies (43.3 vs 57.9 years, p=0.02). Anti-CA6 was associated with worse dry eye signs and symptoms.

Conclusions. Diagnostic criteria for SS are continually being updated as we learn more about the pathophysiology. It is still not clear whether dry eye patients with significant signs and symptoms but no positive serology or biopsy represent a separate entity or early stages of SS. We suggest that adding novel antibodies to the serologic testing, particularly anti-CA6, can improve our understanding to determine who needs close monitoring during the disease process.

P-3
Tissue specific autoantibodies significantly improve diagnosis of primary Sjögren’s syndrome and show a mild clinical course
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Background. The presence of autoantibodies is one of several hallmarks of Sjögren’s Syndrome, the detection of serum autoantibodies has a central role in the diagnosis and classification of Sjögren’s Syndrome. Research on correlations between serum autoantibodies and systemic damages of pSS would be beneficial to the clinical assessment and mechanism study. A group of tissue specific autoantibodies (TSAs, which includes anti-CA6, anti-SP1 and anti-PSP autoantibodies), was first identified in mouse models for SS, then confirmed in the pSS Patients. Previous studies have showed these TSAs may be linked directly to local tissue injury during the early stages of SS development. In current study, a large cohort of SS patients was recruited to address the clinical correlations of these TSAs with clinical symptoms and systemic damage during the disease progression.

Methods. Serum samples were collected from 316 pSS patients (28 male and 288 female) who meet the 2012 ACR Classification Criteria for Sjögren’s Syndrome. SSA and TSAs autoantibodies level was examined by ELISA assays (Trinity Biotech, Buffalo, NY). All clinical and laboratory data were reviewed following protocol approved by Peking University People’s Hospital IRB committee. Change of the autoantibody profiles/titers was further evaluated in 61 pSS patients between onset of the disease and 2 years follow up. Chi-square test was used to compare prevalence rate between different groups.

Results. 1. In general, 70% of pSS patients were positive for SSA autoantibodies and 46% of them were positive for TSAs. When combined both SSA and TSAs, the sensitivity for detecting serum autoantibodies in pSS patients was increased from 70% to 93% (p<0.0001).
2. The prevalence of SSA autoantibodies in pSS patients with different disease duration was 68.4% (<5 years), 80% (5-10 years, p=0.421) and 90.9% (>10 years, p=0.073) whereas the prevalence of TSAs positive (and SSA-) was 26.4% (<5 years), 10% (5-10 years, p=0.194) and 4.5% (>10 years, p=0.051).
3. The prevalence of SSA autoantibodies in pSS patients with different disease activities (as measured by ESSDAI score) was 55% (score 1-3), 92.6% (score 4-6, p=0.002) and 100% (score 7-10, p=0.002) whereas the prevalence of TSAs positive (and SSA-) was 30% (score 1-3), 7.4% (score 4-6, p=0.032) and 0.00% (score 7-10, p=0.019).

Conclusions. 1. In SS patients whose SSA antibody was negative, tissue specific autoantibodies can significantly improve sensitivity, which may help to recognize atypical pSS patients.
2. There appears to be a milder clinical course in patients presenting with tissue specific autoantibodies and SSA negative patients. TSAs might be related with the pathogenesis during the onset of the disease.
Prevalence of novel candidate Sjögren’s syndrome autoantibodies in the Dry Eye Assessment and Management (DREAM™) study

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The novel autoantibodies salivary protein 1 (SP-1), carbonic anhydrase 6 (CA-6), and parotid secretory protein (PSP) have been described in a mouse model for Sjögren’s syndrome (SS), but have not been well-characterized in dry eye disease (DED) patients, with or without SS. We evaluated the prevalence of these novel candidate SS autoantibodies at baseline among patients in the Dry Eye Assessment and Management (DREAM™) Study, a clinical trial designed to evaluate the effectiveness of omega-3 fatty acid supplements for the treatment of DED.

Methods. Baseline medical history questionnaire responses were used to categorize DREAM™ participants into three groups: 1) no history of SS or other autoimmune disease (n=375); 2) no history of SS but with a history of another autoimmune disease (n=66); and 3) a history of SS (n=53). Ocular surface exams and serological testing for traditional and novel SS autoantibodies was performed. The chi-square test or Fisher exact test (count <5) was used to compare the antibody prevalence rate among the 3 groups and between Groups 1 and 3.

Results. Among 494 DREAM™ participants, 53 (10.7%) had a history of SS and had a significantly higher prevalence of the traditional SS autoantibodies compared to the other two groups (Table I). The prevalence of each of the novel antibodies was similar between Group 1 (no SS) and Group 3 (SS) in that in each group approximately 20% had SP-1 antibodies (p=0.85); approximately 16% had anti-CA-6 antibodies (p=0.84), and 10% had anti-PSP antibodies (p=1.00). Participants positive for both traditional and novel antibodies (n=91) had significantly worse corneal fluorescein staining (mean±2) than those who were positive for the traditional antibodies alone (mean±4.7), for the novel antibodies alone (mean±4.4), or were negative for both traditional and novel antibodies (mean±4.1; p=0.03).

Table I. Baseline antibody testing results of DREAM™ study patients by history of Sjögren’s Syndrome (SS) and other autoimmune disease.

<table>
<thead>
<tr>
<th>Baseline antibodies</th>
<th>No history of SS or autoimmune disease (n=375)</th>
<th>History of only non-SS autoimmune disease (n=66)</th>
<th>History of SS (n=53)</th>
<th>Overall P-value</th>
<th>P-value for SS vs. no autoimmune disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Traditional SS antibodies: SS-A(Ro) ≥25 EU/ml</td>
<td>20 (5%)</td>
<td>6 (9%)</td>
<td>31 (60%)</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SS-B(La) ≥25 EU/ml</td>
<td>2 (1%)</td>
<td>3 (5%)</td>
<td>13 (25%)</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Positive tests for SS-A(Ro) and SS-B(La)</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>353 (94%)</td>
<td>59 (89%)</td>
<td>22 (42%)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>22 (6%)</td>
<td>5 (8%)</td>
<td>18 (34%)</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Anti-nucleosome antibody ≥1.40</td>
<td>67 (18%)</td>
<td>20 (30%)</td>
<td>32 (62%)</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Rheumatoid factor positive</td>
<td>91 (24%)</td>
<td>20 (30%)</td>
<td>29 (56%)</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Number of positive tests for traditional antibodies</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>226 (60%)</td>
<td>32 (48%)</td>
<td>14 (26%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>122 (33%)</td>
<td>24 (36%)</td>
<td>6 (11%)</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>23 (6%)</td>
<td>7 (11%)</td>
<td>11 (21%)</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4 (1%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Novel SS antibodies: Salivary protein 1 antibodies positive</td>
<td>73 (20%)</td>
<td>15 (23%)</td>
<td>11 (21%)</td>
<td>0.79</td>
<td>0.85</td>
</tr>
<tr>
<td>Carbonic anhydrase VI antibodies positive</td>
<td>60 (16%)</td>
<td>17 (25%)</td>
<td>9 (17%)</td>
<td>0.16</td>
<td>0.84</td>
</tr>
<tr>
<td>Parotid specific protein antibodies positive</td>
<td>39 (10%)</td>
<td>12 (18%)</td>
<td>5 (10%)</td>
<td>0.20</td>
<td>1.00</td>
</tr>
<tr>
<td>Number of positive tests for novel antibodies</td>
<td>0.1†</td>
<td>0.85</td>
<td>1.0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>256 (60%)</td>
<td>39 (59%)</td>
<td>30 (58%)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>70 (19%)</td>
<td>13 (20%)</td>
<td>19 (37%)</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>42 (11%)</td>
<td>11 (17%)</td>
<td>3 (6%)</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>6 (2%)</td>
<td>3 (5%)</td>
<td>0 (0%)</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

†Missing data in 2 patients (one in SS group, one in group without SS and autoimmune disease).

Conclusions. In this large cross-sectional study, there was no difference in the prevalence rate of novel candidate SS antibodies in DED patients with or without SS. The novel antibodies may define a new class of DED patients with more severe ocular surface disease but not meeting the criteria for SS, but further studies are needed. Longitudinal changes in these antibodies in this cohort will be evaluated in the future and may yield useful insights into the patterns of prevalence in SS and non-SS DED patients.

Evaluation of tissue specific autoantibodies in fibromyalgia patients with sicca and/or xerostomia

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Background. A significant proportion of patients with fibromyalgia complain of dry eyes and mouth. The 2010 preliminary criteria for fibromyalgia even includes dry eyes and mouth as part of the somatic symptoms for the disease. Only a few markers have been described in Sjögren’s syndrome (SS) patients also complain of fibromyalgia symptoms, and there is literature that suggests that there is interplay between these two disorders. Recent evidence suggests that novel tissue specific autoantibodies (TSAs), SP-1, CA6, and PSP, has been observed in the early stages of SS. These early markers present themselves before the classic autoantibodies, such as SS-A/Ro, SS-B/La, ANA, and RF.

Objectives. This study aims to examine the relationship between SS and fibromyalgia by testing patients with fibromyalgia, who also complain of xerostomia and sicca symptoms, for SS related biomarkers SS-A/Ro, SS-B/La, SP-1, CA6, and PSP.

Methods. Over the past 5 years, we identified a cohort of 310 patients that presented with symptoms of fibromyalgia and fulfilled both the 1990 and 2010 preliminary diagnostic criteria. These patients were further questioned about xerostomia and sicca symptoms. Patients that admitted to using artificial tears at least biweekly, drinking water excessively to relieve dry mouth, or have previously experienced a blocked tear duct, but did not meet the strict diagnostic criteria for SS and did not have elevated inflammatory markers ESR or CRP, were selected for this study. Serum from study patients was sent to a tertiary lab, Immco Diagnostics, for testing of the classic autoantibodies (SS-A/Ro, SS-B/La, ANA and RF) and TSAs (SP-1, CA6, PSP).

Results. As of November 2017, 310 patients were selected for this study and tested for the SS markers. 91.0% of the patients were female and 8.6% of the patients were male. The average patient age was 56.5 years. Of the study patients, 271 were tested for both the TSAs and classic autoantibodies, while 39 were tested for only TSAs. Of the patients that were evaluated for both the TSAs and classic Sjögren’s autoantibodies, 29.8% (81) tested positive for SS, 22.8% (71) of the patients were positive for TSAs, 9.4% (29) were positive for the classic Sjögren’s markers, and 2.3% (7) were positive for both the TSA and classic Sjögren’s autoantibodies. Further analysis of all the patients that tested positive for the TSAs (n=71), found 74.5% (53) were positive for SP-1, 8.5% (6) were positive for CA6 and 44.7% (32) were positive for PSP. Of these patients, 68.1% (48) were positive for only one of the TSA and 23 (31.9%) were positive for more than one TSA.

Conclusion. In this cohort of 310 fibromyalgia patients, about 1/3 of patients that were tested for both the TSAs and classic Sjögren’s markers tested positive for SS, with the majority of those patients being positive for only one of the TSAs. This suggests that autoimmunity, specifically early- stage Sjögren’s syndrome, may be a confounding variable in the pathophysiology of fibromyalgia.

References

P-6

Clinicalopathological analysis of labial salivary gland tissues from patients with IgG4-related disease
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Objectives. For the definitive diagnosis of IgG4-related disease (IgG4-RD), biopsies of local lesions are recommended so as to exclude other diseases, including lymphoma and cancer. However, performing biopsies of underlying organs is technically difficult and invasive. In this study, we examined the diagnostic utility of labial salivary gland (LSG) biopsy as a less invasive procedure.

Methods. Eighty-seven patients with suspected IgG4-RD by clinical findings or high serum IgG4 underwent LSG biopsy. We examined the relationship between the number of IgG4-positive plasma cells in LSG and clinical findings. The disease activity and damage of IgG4-RD patients were assessed by clinical findings including the site and number of affected organs, the resistance of steroid treatment, the prevalence of recurrence, and the salivary flow rate.

Results. The final diagnosis was 48 patients with IgG4-RD, 23 with Sjögren’s syndrome (SS), 6 with suspected SS, 3 with malignant lymphoma, 6 with systemic lupus erythematosus, and 1 with Warthin’s tumor. The sensitivity, specificity, and accuracy of LSG biopsy were 58.3%, 94.9%, and 74.7%, respectively. Moreover, Forty-eight IgG4-RD patients were divided into two groups: 1) 28 patients with positive LSG biopsy (IgG4-RD B+), and 2) 20 patients with negative IgG4-RD B-). In IgG4-RD B+ patients, the presence of salivary gland lesions, the number of affected organs, serum IgG4 concentration, and the number and ratio of IgG4-positive plasma cells in LSGs were significantly higher than in those in IgG4-RD B- patients.

Conclusion. These results suggest that LSG biopsy may reflect the disease activity of IgG4-RD. On the other hand, LSG biopsy alone is insufficient for the diagnosis of IgG4-RD because of its low sensitivity. However, combined with clinical findings, including serum IgG4 and number of affected organs, LSG biopsy was indicated to contribute to the diagnosis of IgG4-RD patients with affected underlying organs.

P-7

How single-cell analysis can help diagnose borderline sicca patients with chronic sialadenitis?
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Background. Sjögren’s syndrome (SS) is best characterized by chronic progressive immune attacks primarily against the salivary and lacrimal glands. The essential diagnostic biomarkers of SS are the formation of lymphocytic foci in the glands and the presence of serum autoantibodies. These two inclusive criteria preclude sicca patients with chronic sialadenitis who are borderline from fulfilling the criteria. The objective of this study was to examine labial salivary gland (LSG) biopsies of patients with chronic sialadenitis for infiltrating B cells as autobody production using Single-Cell Autobody Nanochip (SCAN).

Methods. LSG biopsies were obtained from non-primary SS (non-pSS) patients who did not meet the ACR/EULAR classification criteria for pSS. Paraffin-embedded sections were histologically examined for chronic sialadenitis and stained for infiltrating lymphocytes. Subsequently, single-cell analysis of LSG biopsies were isolated and fluorescently conjugated for cell surface markers. Cells were added onto fabricated nanowells and imaged using a high-speed epifluorescence microscope. The microengraved slides were coated with immunoglobulins to capture all secreted antibodies and hybridized with fluorescently-conjugated IgG, SSA/Ro60, SSA/Ro52, and SSB/La antibodies. Microarray spots were analyzed for nanowells with single, live B cells that produced antigen-specific autobody.

Results. Our results indicate that non-pSS patients with chronic sialadenitis exhibited significant infiltration of CD20+ B cells, CD3+ CD4+ and CD3+ CD8+ T cells. Ex vivo analysis of glandular lymphocytes using SCAN demonstrated that individual B cells of these subjects produced high levels of IgG-specific anti-SSA/Ro60 in comparison to IgG-specific anti-SSA/Ro52 or IgG-specific anti-SSA/Ro antibodies.

Conclusions. Single-cell analysis of LSG biopsies revealed the presence of B cells producing pSS autobody in non-pSS patients with chronic sialadenitis. This finding raises a critical issue regarding the appropriate and correct diagnosis of borderline subjects based on the latest classification criteria using focus score and serum anti-SSA/Ro.
Novel radiographic features in Sjögren’s syndrome related Sialo-CBCT

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Objective. Sjögren’s syndrome (SS) is manifested primarily by ocular and or oral dryness. Parotid Sialo-Cone-Beam Computerized Tomography (Sialo-CBCT) demonstrates the ductal architecture and gland’s function. We characterized novel features in SS suspected patients and correlated them with clinical findings and known SS criteria.

Methods. The clinical and radiographic data of SS suspected patients; referred for Sialo-CBCT in 2011-2014, were retrospectively reviewed. Scans were evaluated using a pre-formed questionnaire by two observers for various radiographic features including duct morphology, level of branching, ductopenia and sialectasia. These features were compared between themselves, with clinical data and to 3 well established sets of SS criteria; The American-European Consensus Group (AECG) and the 2 latest American College of Rheumatology (ACR) Group.

Results. Sialo-CBCT scans of 67 suspected SS patients (115 parotid glands) were included. Intra-radiographic association was found between ductopenia and all other radiographic parameters. Minimal, yet important, radiographic differences were found between left and right parotid pair glands. AECG-confirmed-SS patients showed strong correlation with radiographic features, whereas ACR2012-confirmed-SS patients did not. Clinical data was insufficient for ACR2017 assessment.

Conclusion. Sialo-CBCT provides novel radiographic features for SS diagnosis, which may improve SS patient’s diagnosis monitoring and treatment. Further studies are needed to understand their role.
pain in the area of the parotid gland biopsy than in the area of the labial gland biopsy at one week and 6 months post-operatively. At 12 months post-operatively, the change in sensitivity of the biopsied areas and pain level was very low in most patients and comparable for both biopsied areas

**Conclusions.** Patient-reported post-operative change of sensibility and pain in the area of the parotid and labial gland biopsy are comparable after one year. Parotid gland biopsy is a diagnostic technique well-tolerated by patients suspected with pSS.

**References.**

**P-12**

**Assessment of Focus score and additional histopathological parameters for the diagnosis of primary Sjögren’s syndrome in daily practice: possible bias and the need of standardisation**

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**Background.** Recently, a standardization of labial salivary gland histopathology has been proposed for clinical trials in primary Sjögren’s syndrome (pSS). Focus score (FS) remains the key element for the histological diagnosis of pSS; nonetheless additional parameters were proposed. Relatively few information are available regarding the feasibility of the assessment of these parameters in daily clinical practice, including focal lymphocytic sialoadenitis (FLS), focus score (FS) and germinal centre (GC)-like structures.

**Aim of this study.** Was to evaluate the impact of different bias including the areas of glandular tissue in the assessment of FS and additional histopathological parameters in daily routine pSS diagnosis.

**Methods.** Consecutive MSGBs performed in 4 different University and Hospital centers (i.e two ENT Units, one Maxillofacial Unit and one Dentistry Unit) were collected and centralized to the same Pathology Unit from January 1st 2017 to October 31st 2017. The biopsies had been performed by different operators within the same unit. An expert pathologist evaluated the samples focusing on: glandular tissue areas, assessment of FS versus non-specific chronic sialoadenitis (NSCS), number of foci, FS and presence of GCs.

**Results.** MSGBs from 85 subjects (66F:19M) were included in the study. Out of them, 27(31.8%) were diagnosed as having a FLS with a FS≥1, 14 (16.4%) with a 0<FS<1 and 44 (51.8%) with a NSCS. The mean (SD) value of 7.12 (6.19). A great variability in the areas of the MSGB with clear clinical implications.

**Conclusions.** Patient-reported post-operative change of sensibility and pain in the area of the parotid and labial gland biopsy are comparable after one year. Parotid gland biopsy is a diagnostic technique well-tolerated by patients suspected with pSS.

**References.**
and DLCO (r=−0.834, p=0.000). Similarly, total score and partial postero-inferior PI-US score correlated inversely with FVC (r=0.849, p=0.000 and r=−0.836, p=0.000), TLC (r=−0.895, p=0.000 and r=0.829, p=0.000), and DLCO (r=0.953, p=0.000 and r=−0.883, p=0.001). Finally, both PI-US score and PI-US of the infero-posterior field directly correlated with FEV1/SVC (r=0.701, p=0.004 and r=0.619, p=0.01) and with FEV1/FVC (r=0.609, p=0.02 and r=0.901, p=0.05).

Conclusions. This study demonstrated a high correlation between PI-US, HRCT findings and pulmonary function tests, supporting the use of lung ultrasound in clinical practice for the assessment of pSS-associated ILD. The specific correlation between PI-US scores and both FEV1/SVC and FEV1/FVC (not observed with the Warrick HRCT score) seemed to indicate a higher sensitivity of PI-US respect to HRCT in ILD assessment. Further studies are warranted to clarify the role of PI-US for the early diagnosis of ILD.

P-15
Early respiratory disease activity is risk for damage in primary Sjögren’s syndrome
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Background. We studied clinical and epidemiological variables that could be related to greater severity and damage in primary Sjögren’s syndrome (pSS).

Methods. Cross-Sectional study including patients with pSS (AECG 2002 or ACR/EULAR 2017). EULAR Sjögren’s syndrome Disease Index (ESSDAI) was calculated at diagnosis and currently. Patients have performed EULAR Sjögren’s syndrome Disease Damage Index (SSDDI). Non-parametric statistics and logistic regression were applied with p-value of 0.05.

Results. 104 patients with pSS were included, 51.49±12.13 years, 95.2% women. Disease duration was 65.44±41.89 months. Anti-RO was positive in 68% (n=70/103), FAN in 82% (n=82/100), FR in 37.4% (n=37/99) and anti-La in 30.3% (n=30/99), focal lymphocytic salioadenitis in 73% (71/91). The ESSPRI (n=37/99) and anti-La in 30.3% (n=99/104) were women. Disease duration was 65, 44±41.89 months. Anti-Ro/52 and anti-La were significantly increased in induced sputum from pSS patients compared to population-based controls (Figure 2). However, cytokine levels in induced sputum were not associated to pulmonary function tests, disease activity, respiratory symptoms, nor laboratory or serological features of pSS.

Conclusions. The increase in BAFF, IL-6, IL-8 and lymphocytes in induced sputum suggests a specific ongoing inflammatory disease process in the airways in pSS patients. Its association to pSS associated airway disease has to be further examined in future larger studies.

P-16
Increased B-cell activating factor, interleukin-6, interleukin-8 and lymphocytes in induced sputum from patients with primary Sjögren’s syndrome

Background. Small airway disease and chronic obstructive pulmonary disease are common in primary Sjögren’s syndrome (pSS). However, the underlying inflammatory mechanisms behind pSS associated airway disease have not been studied in detail. We therefore wanted to study cytokine and leucocyte levels in induced sputum in never-smoking patients with pSS.

Methods. Induced sputum cytokines and leucocytes were assessed in 20 never-smoking patients with pSS and 19 age and sex-matched population-based controls. In addition, pulmonary function disease, respiratory symptoms, as well as inflammatory and serological features of pSS were assessed.

Results. B-cell activating factor (BAFF), interleukin (IL)-6 and IL-8 were significantly increased in induced sputum in pSS patients compared to population-based controls, whilst IL-1β, interferon-α, and tumor necrosis factor-α levels were not (Figure 1). In addition, lymphocytes were significantly increased in induced sputum from pSS patients compared to population-based controls (Figure 2). However, cytokine levels in induced sputum were not associated to pulmonary function tests, disease activity, respiratory symptoms, nor laboratory or serological features of pSS.

Conclusions. The increase in BAFF, IL-6, IL-8 and lymphocytes in induced sputum suggests a specific ongoing inflammatory disease process in the airways in pSS patients. Its association to pSS associated airway disease has to be further examined in future larger studies.

P-17
A case series of neurological abnormalities associated with Sjögren syndrome – Removing inevitable confounders in complex presentations to make accurate diagnoses and provide effective treatments
Shu F.1
1University of California, Los Angeles, Medical Center, Los Angeles, California, USA.

Background. Sjögren Syndrome (SS) is an autoimmune disorder of exocrine glands classically resulting in xerophthalmia and xerostomia; occasionally it can be associated with a complex array of neurologic abnormalities. When the latter precedes the sicca complex or occurs in isolation, an accurate diagnosis is often impeded. We present a case series of 24 neurologic patients who fulfilled the serological and/or histopathological criteria for SS.
SS and may show variable clinical courses, including acute optic neuritis, chronic optic atrophy, and myelopathy in the context of NMOSD. Longitudinally extensive myelitis and NMO, known as a rare relapsing autoimmune disease of the central nervous system (CNS), are sometimes found as the initial manifestation of primary Sjögren’s syndrome. NMOSD manifestation without apparent Sicca symptoms makes the diagnosis more challenging due to an unknown frequency of association between both conditions. The combination of specific antibodies anti-SSA, anti-aquaporin-4, and biopsy abnormality of salivary gland are supportive for the diagnosis and treatment in atypical cases. Future studies are necessary to clarify the elements implicated in the NMOSD and SS association.

P-19
How phenotype of the small fibre neuropathy (SFN) in primary Sjögren syndrome (pSS) differs from others causes of small fibre neuropathy?
Elise Descamps1, Julien Henry1, Celine Laberge1, David Adams1, Davide Aiello1, Xavier Mariette1 and Raphaelle Seror2.
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Background. Small fibre neuropathy (SFN) is a peripheral neuropathy characterized by neuropathic pain associated with normal routine nerve conduction study but rarefaction of intraepidermal nerve fibres (IENF). Primary Sjögren Syndrome (pSS) is one of the many aetiology of SFN.

The objective was to compare phenotype of SFN in pSS, transhyretin (TTR) familial amyloidosis and idiopathic SFN.

Methods. All patients referred to neurology or rheumatology departments of our hospital since October 2012 with a confirmed diagnosis of SFN associated with either pSS (ACR/EULAR 2016 criteria, TTR-amyloidosis or idiopathic were included in this monocentric retrospective study. Diagnosis of SFN was confirmed by normal nerve conduction studies and abnormal lower limb skin biopsies (defined by a reduced intraepidermal nerve fibers (IENF) density.

Results. We included 16 patients with pSS (14 (87.5%) women, median age: 55 yrs [IQR: 49.3-66.7], 7 (43.8%) anti-SSA positive, 13 (81.3%) focus score ≥1, 17 with TTR-amyloidosis (7 (41.2%) women, median age: 47 yrs [35-56]) and 11 with Idiopathic SFN (7 (63.6%) women, median age: 47 yrs [36-56]).

Patients with pSS had a median ESSDAI of 5 [5-9], mainly due to the neurologic domain. One had monoclonal gammopathy, 5/14 (35.7%) rheumatoid factor, 2/14 (14.3%) hypergammaglobulinemia and none had cryoglobulin.

Ten TTR amyloidosis patients had MET30 mutation and 7 other mutations. Time from first neurologic symptoms to diagnosis of SFN was significantly higher for pSS (20.5 months [7.7-48.5]) and idiopathic group (35 months [11.5-65]) than for TTR group (6 months [0-15]).

Clinical presentation was length dependant in only 3 (18.7%) patients with pSS compared to 10 (58.9%) in TTR amyloidosis (p=0.03) and 3 (27.3%) in idiopathic group (p=0.66). A “patchy” presentation (defined by asymmetrical and/or proximal symptoms involving limb, trunk and/or face), was significantly more frequent in pSS than in TTR amyloidosis (743.7% vs. 15.9%; p=0.02).

This more frequent non-length dependant course was confirmed on skin biopsies with an IEF at proximal site < IEF at distal site in 8/15 (53.3%) pSS patients compared to 4 (23.5%) in TTR (p=0.14) and 1 (9.1%) in idiopathic (p=0.04) groups.

Lauria score was significantly higher in pSS than in TTR, (5 [4-8] vs. 4 [3-5], p=0.015), mainly due to sicca symptoms (n=15/16) and peripheral limb pain (n=14/16). After excluding sicca syndrome item, the score did not differ.

Conclusion. pSS patients with SFN had a low frequency of serum B cell activation biomarkers. Compared to other causes of SFN, in pSS SFN was characterized by a more frequent non-length dependant and patchy presentation and a higher Lauria score. This later was mainly driven by dryness and limb pain rather due to pSS than SFN.
Is peripheral blood lymphocyte population’s distribution different in primary Sjögren’s syndrome patients with lymphopenia?

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Background. An increasingly number of studies have given evidence for disturbances in the distribution of peripheral blood (PB) lymphocyte subsets in primary Sjögren’s syndrome (pSS) patients. pSS patients exhibit a significant decrease in proportion and absolute counts of memory B cells (affecting both switched and unswitched memory B cells) and an increase in naïve B cells. Regarding T cell populations, patients with pSS have decreased T-cell counts, mostly as a result of a decrease in CD4+ T cells (affecting memory, naïve and effector subsets of CD4+ T cells). A significant decrease in proportion and absolute counts of Th17 cells have also been reported in pSS patients. Lymphopenia is considered to be a prognostic factor and a lymphoma predictor in pSS patients. Ten patients (13.3%) had sustained lymphopenia (≤1000/mm3 PB) for pSS. We found that pSS patients with lymphopenia have a lymphopenia. The proportion of activated CD4+ T cells and CD8+ T cells, B cells, monocytes and NK cells. Our aim was to evaluate the lymphocyte population’s distribution in PB from pSS patients with lymphopenia and to compare it with that in pSS patients without lymphopenia. Methods. Seventy five patients with pSS were recruited at Vall d’Hebron University Hospital (Barcelona, Spain) for this study. pSS was diagnosed according to American-European Consensus Group Classification criteria for pSS. Ten patients (13.3%) had sustained lymphopenia (≤1000/mm3). Mean age of pSS without and with lymphopenia was 62 (±14.9) years and 54.1 (±15.8) years, respectively. There were three men in non-lymphopenic group.

Peripheral blood samples were collected in K3-EDTA anticoagulant. Main lymphocyte populations were analyzed by flow cytometry and defined according to the Human Immune Phenotyping Consortium (HIPC) (Maecker, Nat Rev Immunol 2012). Absolute counts of lymphocyte subsets were calculated using the percentages obtained by flow cytometry and the leucoocyte count obtained from an hematological analyzer. Statistical differences were analyzed using the Mann-Whitney U test. A significative difference was considered significant.

Results. No significative differences were found related to proportions of T cells, CD4+ T cells, CD8+ T cells, B cells, monocytes and NK cells. Regarding CD4+ subpopulations, we found a significative decrease in naïve CD4+ proportion (38.7% vs 26.6%, P=0.048) and a significative increase in effector memory CD4+ (25.6% vs 40.5%, P=0.012) in pSS patients with lymphopenia. The proportion of activated CD4+ T cells and CD8+ T cells were increased (3.1% vs 6.8% and 10% vs 14.9%, respectively), but this increase was only significative for CD4+ (P=0.018) and did not reach statistical significance for CD8+ (P=0.059). Consequently, absolute counts of activated CD4+ and CD8+ T cells were not significantly different between pSS patients with and without lymphopenia. No significative differences were found related to proportions of CD8+ subsets, CD4+ Treg cells, Th1, Th2, Th17, B cells sub- populations, monocytes nor NK cells.

Conclusions. We found that pSS patients with lymphopenia have a lymphocyte population’s distribution in PB similar to those pSS patients without lymphopenia, with decreased absolute count of all main lymphocyte subsets. However, pSS patients with lymphopenia have a lower proportion of naïve CD4+ and a higher proportion of effector memory CD4+ and activated CD4+ than pSS patients without lymphopenia.
Subepithelial infiltrate of the vagina in primary Sjögren’s syndrome: the cause of vaginal dryness?


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Background. Women with primary Sjögren’s syndrome (pSS) often experience vaginal dryness. Inflammation of the vaginal epithelium or endocervical mucosa might contribute to this symptom. This study evaluated whether female pSS patients have impaired vaginal health and inflammation of the vaginal epithelium or endocervical mucosa in comparison with controls.

Methods. Consecutive premenopausal women with pSS were studied using the AECG and ACR-EULAR criteria, with symptoms of vaginal dryness, and vaginal epithelium or endocervical mucosa in comparison with controls. We enrolled 41 pediatric patients who were diagnosed as having SS with unanimity by 10 pediatric rheumatologists. Twenty-five were primary SS (pSS), 11 were secondary SS (sSS) and 5 were primary to secondary SS (pSS).

Results. Vaginal health is impaired in premenopausal women with pSS. Women with pSS show significantly more infiltrating lymphocytes in the vagina, but not in the endocervix. A mild subepithelial infiltrate with aggregates in dermal papillae, consisting mostly of T lymphocytes, is present in the vagina of women with pSS.

Conclusions. Vaginal health is impaired in premenopausal women with pSS. Women with pSS show significantly more infiltrating lymphocytes in the vagina, but not in the endocervix. A mild subepithelial infiltrate with aggregates in dermal papillae, consisting mostly of T lymphocytes, is present in the vagina of women with pSS.
Table I. Scoring: Evidence for autoimmunity and exocrinopathy.

<table>
<thead>
<tr>
<th>Test Items</th>
<th>Criteria</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum IgG</td>
<td>≥97.5 percentile for age</td>
<td>1</td>
</tr>
<tr>
<td>anti-nuclear antibody</td>
<td>1:40–1:80</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>≥1:160</td>
<td>2</td>
</tr>
<tr>
<td>Rheumatoid factor</td>
<td>≥1:320</td>
<td>3</td>
</tr>
<tr>
<td>anti-SSA/Ro or SSB/La antibody</td>
<td>positive</td>
<td>3</td>
</tr>
</tbody>
</table>

Table II. Classification.

<table>
<thead>
<tr>
<th>Serological score*</th>
<th>Glandular score**</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥6</td>
<td>Defined</td>
</tr>
<tr>
<td>5</td>
<td>Probable</td>
</tr>
<tr>
<td>4</td>
<td>Probable</td>
</tr>
<tr>
<td>3</td>
<td>Possible</td>
</tr>
<tr>
<td>2</td>
<td>Possible</td>
</tr>
<tr>
<td>1</td>
<td>Possible</td>
</tr>
<tr>
<td>0</td>
<td>Need follow-up</td>
</tr>
</tbody>
</table>

*For serological score is the total score of serological parameters.

**For glandular score is the highest one: total score of salivary gland test items or lacrimal gland score.

Results. According to our criteria, patients were classified into 3 groups: definite, probable and possible. At the first visit, the number of patients in each group was 33, 2 and 6, and at the last visit, that was 38, 3 and 0, respectively. Four patients in the “possible” and 1 patient in the “probable” at the 1st visit were classified as “definite” at the last visit, and 2 patients in the “possible” at the 1st visit were classified as “probable” at the last visit. No patients were diagnosed as non-SS. In the other 4 criteria, the most sensitive was JPN, followed by A/E and ACR. However, even by using JPN criteria, 12% of pSS patients were diagnosed as non-SS at the last visit.

Conclusion. Our new criteria are useful for diagnosis and follow-up of pediatric SS, and make it possible to recognize SS-associated complications at an early stage.

P-25

Towards a better understanding of childhood Sjögren’s syndrome: evaluation of the 2016 ACR/EULAR classification criteria for use in diagnosing Sjögren’s syndrome in children

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1Stead Family Department of Pediatrics, University of Iowa, Iowa, USA.

Background. Clinical presentation of Sjögren’s syndrome in children differs from that in adults: dryness symptoms are more common in adults while parotitis is more common in children. Criteria developed for adult classification have demonstrated low sensitivity when applied to pediatric populations, and no child-specific criteria have been established. The latest adult classification criteria have not yet been evaluated for use in children. Our objective was to evaluate the applicability of these new criteria for use in children.

Methods. Retrospective chart reviews were conducted to collect individual patient level data for children diagnosed with Sjögren’s syndrome (based on clinical diagnosis at age <18 years). Data including clinical features, laboratory values, imaging studies, and test items in the 2016 ACR/EULAR criteria were collected, and de-identified data were entered into a REDCap database. This study was approved by the Institutional Review Boards or equivalent regulatory bodies at individual affiliate institutions.

Results. To date, 86 children with Sjögren’s syndrome were included from 11 institutions across 4 countries (data collection is ongoing). This constitutes the largest childhood Sjögren syndrome patient series to date. The majority of children (91%) were female with a mean age of 11.6 years at diagnosis (range 1–17.8 years). Twelve children (14%) also had another autoimmune disease (9 with SLE, 2 with uveitis, 1 with subacute cutaneous lupus). Frequency of clinical features were as follows: 51% with parotitis, 50% with dry eyes, 48% with dry mouth, 45% with arthralgias without arthritis, 24% with lymphadenopathy, 23% with arthritis, 14% with cytopenias, 14% with fever, 13% with cutaneous vasculitis, 10% with weight loss, and <10% each with recurrent vaginitis, myositis, pulmonary, renal, or neurologic manifestations. Only 3 children had testing for all 5 items included in the 2016 ACR/EULAR criteria. Most children (95%) had testing for anti-SSA antibodies, but fewer underwent minor salivary gland (MSG) biopsy (50%), Schirmer testing (51%), measurement of unstimulated whole saliva flow (UWSF, 13%), or ocular surface staining (OSS, 19%). While most children studied (96.5%) were missing at least one data point, 27 of 86 children (31%) met the 2016 ACR/EULAR classification criteria for Sjögren’s syndrome. Of these 27 children: 25 (93%) had positive anti-SSA antibodies; 16 (59%) had positive MSG biopsy; 21 (78%) had positive Schirmer test; 2 (7%) had positive UWSF; and 1 (4%) had positive OSS. Of the 59 children not meeting criteria: 39 (66%) had positive anti-SSA antibodies; 6 (10%) had positive MSG biopsy; 6 (10%) had positive Schirmer test, and 2 (3%) had positive UWSF.

Conclusions. Criteria items from the 2016 ACR/EULAR criteria are not routinely assessed in children diagnosed with Sjögren’s syndrome making formal retrospective assessment of criteria difficult. Prospective study of these criteria along with defining child-specific normal values and adding child-specific criteria items (such as recurrent parotitis) are warranted. Establishing criteria for childhood Sjögren’s syndrome is a key step toward better understanding and treating this condition.
P-26
Primary Sjögren’s syndrome in a subset of children with recurrent salivary glands enlargement – it is high time for pediatric diagnostic criteria!

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Background. The objective was to evaluate applicability of the currently used primary Sjögren’s syndrome (pSS) diagnostic criteria in pediatric population and to assess how salivary gland ultrasonography (SGUS) with elastography and salivary gland magnetic resonance with sialography (MR) might improve diagnosis in this group of patients.

Methods. Thirty-two patients, aged 2-17 years (15 females, 17 males) with recurrent salivary glands enlargement suggestive for diagnosis of pSS were enrolled in this prospective study. The assessment was based on the criteria included in 2002 AECG, 2012 ACR and 2016 ACR/EULAR classifications: ocular and oral dryness symptoms, serological tests (ANA, SAA, SSB, RF), ophthalmological examination (Schirmer test, ocular staining with fluorescein; Indirect Immunofluorescence), unstimulated whole salivary flow (UWSF) rate and labial minor salivary gland (LSG) biopsy. Moreover, parallel radiological examinations, SGUS with elastography and MR with sialography, were performed by radiology specialists. The study was approved by the Local Bioethics Committee of Medical University of Gdańsk; written informed consent of the parents of all patients and consent of patients who were older than 16 years (in accordance with polish law) were obtained.

Results. Subjective ocular and oral dryness symptoms were reported in 5/32 (16%) and 11/32 (34%) patients, respectively. Recurrent salivary gland enlargement was observed in all patients. Schirmer test was performed in 18/32 patients and in all of these children was normal. Ocular staining score (OSS) ≥3 was not observed in our patients. UWSF rate ≥1 was found in 7/30 patients (23%). Serological results revealed positive ANA (≥1:320) in 28/32 patients (87.5%), positive SSA in 2/32 (6%) and positive SSB in 1/32 (3%). RF was elevated in 15/32 patients (47%). LSG biopsy was performed in 19 patients: 13 patients had focus score (FS) ≥1 (68%); 5 patients had evidence of focal lymphocytic sialadenitis (FLS) with FS ≥2 (26%); 1 patient had FS 0.

Conclusions. Currently used diagnostic criteria for adult pSS are not applicable at the onset of the disease in children. Some of the tests used in adults are impossible to perform in children because of the young age and lack of cooperation with a child. Sicca symptoms develop with delay so they are not helpful in diagnosing pSS at the early stage of the disease. Radiological examination, especially SGUS, should be considered to be one of the criterion in the diagnosis of childhood pSS. There is an urgent need for child-specific criteria.

P-27
Characteristics of Korean primary Sjögren’s syndrome and comparison of 3 different classification criteria for primary Sjögren’s syndrome: data from Korean KISS Cohort

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Objective. The objective of this study is to introduce the clinical and laboratory characteristics of Korean primary Sjögren’s syndrome (pSS) patients enrolled in Korean Initiative of Sjögren’s Syndrome (KISS). We also sought to compare the performance of newly proposed 2016 American College of Rheumatology (ACR)/European League-Against Rheumatism (EULAR) criteria to 2002 American-European Consensus Group (AECG) and 2012 ACR classification criteria for primary pSS in a well characterized registry.

Methods. Patients with pSS from 12 university affiliated hospitals in Korea were enrolled from Oct 2013 to JAN 2017. The patients were diagnosed with pSS by either fulfilling 2002 AECG or 2012 ACR classification criteria. Data of clinical manifestations and various laboratory findings were obtained.

Results. Data at inclusion were available in 458 patients. The mean age was 51.9±11.8 years. Four hundred fifty patients (98.3%) were female. Mean disease duration was 33.9±6.7 months. Most common extraglandular manifestation was arthralgia (47.8%) followed by Raynaud’s phenomenon (15.7%) and lymphenadopathy (13.5%). Median [interquartile range] ES-SDAI and ESSPRI was 2 [0-5], 5 [4-6.7], respectively. Among 458 patients, 328 patients had sufficient data to determine the fulfillment of each criteria. All the criteria were met by 307 patients. Among 3 patients by whom 2016 ACR/EULAR criteria were not met, one 2002 AECG- 2015 ACR+ had negative anti Ro or La while positive antinuclear antibody and rheuma-
toid factor with more than 3 ocular staining score. Two 2002 AECG+ 2015 ACR- showed no ocular sign and negative anti Ro or La while had positive focus score. Ninety-six patients had results of all items in 2016 criteria, 95 met the criteria.

Table I. Characteristics of patients with primary Sjögren’s syndrome (n=41).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>oral + ocular dryness</td>
<td>21 (51%)</td>
</tr>
<tr>
<td>ocular dryness</td>
<td>28 (68%)</td>
</tr>
<tr>
<td>oral dryness</td>
<td>31 (76%)</td>
</tr>
<tr>
<td>anti-SSA (anti-Ro)</td>
<td>37 (90%)</td>
</tr>
<tr>
<td>anti-SSB (anti-La)</td>
<td>18 (44%)</td>
</tr>
<tr>
<td>RF positive &gt;2UNL</td>
<td>27 (66%)</td>
</tr>
<tr>
<td>ANA ≥1:320</td>
<td>39 (95%)</td>
</tr>
<tr>
<td>Schirmer’s test (&lt;5mm/5 min)</td>
<td>25 (61%)</td>
</tr>
<tr>
<td>stimulated SFT ≤2.5ml/5 min</td>
<td>32 (78%)</td>
</tr>
<tr>
<td>OSS ≥5</td>
<td>16 (39%)</td>
</tr>
<tr>
<td>OSS ≥3</td>
<td>16 (39%)</td>
</tr>
<tr>
<td>FS at 1 focci/4mm³</td>
<td>31 (76%)</td>
</tr>
<tr>
<td>Sialoexcess on parotid sialography</td>
<td>39 (95%)</td>
</tr>
</tbody>
</table>

33/41 (80%) fulfilled ACR (2012) criteria for pSS; 37 patients (90%) - ACR/EULAR (2016) criteria. In our small cohort of pSS patients we didn’t find any strong correlation between parameters, even among sialoexcess and FS.

Conclusion. We successfully established a nationwide pSS registry in Korea, which represents the characteristics of Korean pSS patients. The newly proposed 2016 ACR/EULAR criteria were met by most of the patients diagnosed with pSS according to previous criteria.

P-29

Transcriptomic signatures distinguish AECG-classified primary Sjögren’s from sicca control patients

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Background. There is a pressing need for biomarkers to aid selection of appropriate patients and accurately measure outcomes in clinical trials for Sjögren’s Syndrome (SS). We assessed therapeutic targets of interest, BAFF, IL-17A and IL-21 in plasma of patients classified with pSS by AECG criteria vs sicca controls (SC who met some of the criteria for pSS but not sufficient for definitive classification), vs healthy controls (HC). We also compared transcriptomes of pSS, SC and HC donors. The goals were to identify potential candidate biomarkers for more stringent selection of pSS subjects into clinical trials and potential new target identification in pSS.

Methods. Study subjects: pSS (AECG criteria), n=21, mean age 50.7 (17-71), F=21; sicca controls, n=21, mean age 49.1 (28-77), F=21; age- and sex-matched healthy controls (HC), n=41. Clinical tests were concurrent with whole blood RNA collection: Lissamine Green, Schirmer’s test, Whole Unstimulated Salivary Flow (WUSF), Labial Salivary Gland (LSG) histopathology, anti-Ro (composite), anti-La, anti-Ro60, anti-Ro52 and Rheumatoid Factor. Plasma BAFF, IL-17A and IL-21 assays were measured in validated immunoassays, pSS and SC whole blood RNA samples were run on Affymetrix HTA 2.0 arrays and gene expression compared to HCs.

Results. There was considerable overlap between plasma concentrations of BAFF, IL-21 and IL-17A in pSS and SC compared with healthy controls. Gene expression revealed fewer than expected statistically significant transcripts (unadjusted p<0.05) distinguishing pSS and SC (114 up and 194 down in pSS vs. SC). However, using a panel of pSS vs HC discriminatory genes as the basis for self-organizing maps, we observed that not all SC showed gene expression patterns similar to HC, with 6 of 21(28.6%) being more similar to pSS patients. At transcript level splicing, cumulative differences of SC and pSS patients vs HCs displayed a number of immune relevant genes (MYO1G, RAC2, CORO1A, LAPTM5, CD53 and LSP1) as top hits. Two clinical measures, LSG Focus Score and WUSF demonstrated positive and negative correlations respectively with patient differential expression scores, a measure of gene expression intensity derived from the discriminatory genes.

Conclusions. Initial plasma cytokine screening showed a substantial overlap between sicca controls and pSS compared with healthy control donors. Transcriptomic analysis, however, revealed that the pSS patients were distinct from the majority (71.4%) of sicca control donors.

P-30

Addition of salivary gland ultrasound increases the feasibility of the ACR-EULAR classification criteria in primary Sjögren’s syndrome

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Background. The ACR-EULAR criteria were recently developed to reach international consensus regarding the classification of primary Sjögren’s syndrome (pSS). Our objective was to assess whether addition of salivary gland ultrasound (SGUS) to the ACR-EULAR criteria influences the performance of these criteria in a large cohort of patients clinically suspected or diagnosed with pSS in daily clinical practice.

Methods. Included were all consecutive patients who underwent SGUS between October 2014 and July 2017, who had complete data on all ACR-EULAR items. Classification according to the criteria was determined separately in patients who underwent a labial or parotid gland biopsy. For SGUS, the average score for hypochogenic areas in the parotid and submandibular glands on one side was applied (range 0-3). The optimal cut-off value for our SGUS score was determined using ROC analysis. Clinical diagnosis by the treating physician was used as gold standard. SGUS positivity was added as an item to the original ACR-EULAR criteria. The weight of the original ACR-EULAR items was kept and SGUS positivity was given a weight of 1 point. Area under the curve (AUC), absolute agreement, sensitivity and specificity of the original and adjusted ACR-EULAR criteria sets were determined.

Results. Of the 363 patients assessed, 254 patients had a complete data set. 156 patients were diagnosed with pSS. The accuracy of SGUS to predict clinical diagnosis was good, with an AUC of 0.873 and optimal cut-off value of ≤1.5. The optimal cut-off value of the criteria to discriminate between pSS and non-pSS remained 4, irrespective of the type of biopsy used and whether SGUS was added to the criteria or not.

In patients who underwent a labial gland biopsy (n=130), the original ACR-EULAR criteria showed an AUC of 0.967. Absolute agreement with clinical diagnosis was 94.6%, sensitivity was 96.2% and specificity was 92.3%. After addition of SGUS, the adjusted criteria showed an AUC of 0.969, absolute agreement of 94.6%, sensitivity of 97.4% and specificity of 90.4% (Figure 1).

Fig. 1. ROC curves of the original and adjusted ACR-EULAR (addition of SGUS) classification criteria. Between brackets the type of salivary gland biopsy is shown that was used for classification.
In patients who underwent a parotid gland biopsy (n=208), the original ACR-EULAR criteria showed an AUC of 0.957. Absolute agreement with clinical diagnosis was 92.8% and sensitivity of 92.1% and specificity was 94.0%. After addition of SGUS, the adjusted criteria showed an AUC of 0.965, absolute agreement of 93.2%, sensitivity of 92.9% and specificity of 91.6% (Figure 1).

Conclusions. SGUS is non-invasive, cheap and easy to perform in a rheumatologist outpatient setting. Addition of SGUS to the ACR-EULAR criteria resulted in negligible changes in the performance of the criteria, irrespective of the type of biopsy performed. Thus, adding SGUS to the ACR-EULAR criteria increases its feasibility, since clinicians are offered more options that could lead to fulfillment of these criteria.

References
1. MOSELL et al.: Ann Rheum Dis 2017: Accepted for Publication.

P-31
Comparison of 2002 AECG and 2016 ACR/EULAR classification criteria and added value of salivary gland ultrasonography in a patient cohort with suspected primary Sjögren’s syndrome

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Objective. To evaluate concordance between 2002 AECG and 2016 ACR/EULAR classification criteria for primary Sjögren’s syndrome (pSS) and to assess how salivary gland ultrasonography (SGUS) might improve the classification of patients.

Methods. Patients with suspected pSS underwent a standardised evaluation, including SGUS, at inclusion into the single-centre Brittany DiaSS cohort. Agreement between the two criteria sets was assessed using Cohen’s x coefficient. Characteristics of discordant patients were detailed.

Results. We prospectively included 290 patients between 2006 and 2016, among whom 125 (43%) met ACR/EULAR criteria and 114 (39%) also met AECG criteria; thus, 11 (4%) patients fulfilled only ACR/EULAR, no patients AECG only, and 165 (57%) patients neither criteria set. Concordance was excellent (κ=0.92). Compared to patients fulfilling both criteria sets, the 11 patients fulfilling only ACR/EULAR criteria had similar age and symptom duration but lower frequencies of xerophthalmia and xeroscopy; 91% had abnormal salivary gland biopsy and 46% anti-SSA; 64% were diagnosed with pSS by the physician. SGUS was abnormal in 12% of the 165 patients fulfilling no criteria set. Including SGUS among the ACR/EULAR criteria increased sensitivity from 87.4% to 91.1% when physician diagnosis was the reference standard.

Conclusions. Agreement between AECG and ACR/EULAR criteria sets is excellent. ACR/EULAR criteria are slightly more sensitive and classified some patients without sicca symptoms as having pSS. Including SGUS into ACR/EULAR criteria may further improve their sensitivity.
Methods. The new 2017 ACR/EULAR Classification Criteria for SS allows the application of the criteria to any patient with a suspicion of SS due to systemic features with at least 1 positive ESSDAI domain. We have identified how many patients from the Big Data Sjögren Registry showed this early systemic presentation at the time of the disease diagnosis. As a control group, we selected those patients who presented with the typical sicca syndrome (subjective dry mouth and dry eyes).

Results. We have identified 240 (25.5%) patients presenting with a non-sicca systemic disease among the 9545 patients included in the Registry: 211 (88%) were women and 29 (12%) were men (female: male ratio, 7:1), with a mean age at diagnosis of 46.6 years. The frequency of fulfilment of the 2017 criteria was: 63% for positive ocular staining, 76% for abnormal Schirmer test, 79% for abnormal unstimulated whole saliva flow, 84% for positive salivary gland biopsy and 88% for Ro autoantibodies. Other immunological tests included positive ANA (88%), RF (54%), low C3 levels (25%), low C4 levels (13%), and cryoglobulins (9%). In comparison with patients presenting with the typical sicca syndrome, those presenting as systemic Sjögren’s with no sicca syndrome were younger (46.6 vs 52.9, p<0.001), more frequently males (12% vs 6%, p<0.001) and less frequently classified ethnically as White (36% vs 79%, p<0.001). Immunologically, patients with systemic Sjögren’s had a higher frequency of anti-Ro antibodies (88% vs 71%, p<0.001), positive antinuclear antibodies (88% vs 81%, p=0.014) and low C3 levels (25% vs 13%, p<0.001). Systemic activity at diagnosis was significantly higher in patients with systemic non-sicca Sjögren’s compared to those with the sicca syndrome, including higher mean ESSDAI (6.5 vs 6.0, p<0.001) and clinESSDAI (6.6 vs 6.2, p=0.003) scores. In addition, moderate systemic activity (moderate-DAS) was found in a higher frequency in patients with systemic presentation (41% vs 30%, p<0.001). With respect to the ESSDAI domains, patients with non-sicca Sjögren’s had a higher frequency of activity in the constitutional (13% vs 8%, p=0.01), renal (12% vs 4%, p<0.001), hematological (31% vs 22%, p=0.005) and biological (64% vs 49%, p<0.001) domains, but a lower frequency of activity in the glandular (12% vs 22%, p<0.001) and peripheral nervous system (3% vs 6%, p=0.05) domains.

Conclusion. Primary Sjögren’s syndrome at diagnosis is presenting as a systemic disease in the absence of the typical sicca symptoms in less than 3% of cases. These patients are characterized for being younger, less frequently women and White, with a higher frequency of immunological markers (Ro, ANA and low C3), higher activity in the hematological (cytopenias) and biological (hypergammaglobulinemia) domains, and higher activity in the constitutional (fever) and renal clinical domains in comparison with patients presenting with the classical sicca syndrome.

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Clinical and immunological disease patterns of primary Sjögren syndrome driven by gender and age at diagnosis


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senting activity at articular (OR 0.89, CI95% 0.82-0.97) pulmonary (OR 0.54, CI95% 0.42-0.70), muscular (OR 0.55, CI95% 0.33-0.94) and peri-
pheral nervous system (OR 0.59, CI95% 0.42-0.81) domains.

**Conclusion.** Gender and age at diagnosis play a key role in the sever-
ity of systemic involvement measured at the diagnosis of primary Sjögren syndrome, with men and patients diagnosed before 55 years being those presenting with the highest systemic profile. Some organs are more active with increasing age (e.g. meatal and renal involvement at younger ages) and may help identify epidemiologi-
cal subgroups with high systemic activity at diagnosis and, therefore, at high risk of suffering a complicated clinical course.

**P-34**

**How ethnicity modifies systemic activity of primary Sjögren syndrome: analysis of baseline ESSDAI scores in a multi-ethnic international cohort**

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**Objectives.** To analyse the influence of ethnicity on the clinical presentation of primary Sjögren syndrome (SjS) by measuring systemic activity (ESS-
DAI score) at the time of diagnosis.

**Methods.** The Big Data Sjögren Project is an international, multicentre reg-
istry formed in 2014 to take a “high-definition” worldwide picture of the main features of primary SjS at diagnosis by merging international SjS da-
tabases. By October 2017, the database included 9545 consecutive patients recruited from 22 countries of the five continents. Systemic involvement was defined according to the ESSDAI/clinESSDAI. Disease activity states (DAS) were categorized according to the global ESSDAI score as low-
ESSDAI<5), moderate-activity (5≤ESSDAI≤13) and high-activity (ESSDAI≥14).

**Results.** Ethnicity data were available for 8746 (95%) of 9118 patients with available information on ESSDAI: 6614 (76%) patients were classified as White, 1306 (15%) as Asian, 520 (6%) as Hispanic, 130 (1%) as Black/ African American -BA- and 176 (2%) as other ethnicities. The highest median ESSDAI score at diagnosis was found in BAA patients (6 vs 4 in W, 4 in A and 3 in H, p<0.001), as well as for median clinESSDAI score (5 vs 4 in W, 3 in A and 3 in H, p<0.001). The highest frequency of patients presenting at diagnosis with high DAS was also found in BAA (16.2% vs 15.8% in W, 11.6% in A and 8.5% in H, p<0.001). With respect to the ES-
SDAI domains, BAA patients had an enhanced risk of presenting activity at diagnosis in peripheral nervous system (OR 2, CI95% 1.27-3.15) and biological (OR 1.2, CI95% 1.03-1.4) domains in comparison with White patients. In contrast, Asian patients had a lower risk of presenting activity at diagnosis in the lymphadenopathy (OR 0.54, CI95% 0.43-0.69), glan
dular (OR 0.41, CI95% 0.35-0.49), articular (OR 0.57, CI95% 0.51-0.63), cuta
eous (OR 0.82, CI95% 0.67-0.99), muscular (OR 0.41, CI95% 0.25-0.7), periph-
eral nervous system (OR 0.54, CI95% 0.4-0.73) and central nervous system (OR 0.47, CI95% 0.27-0.81) domains, but a higher risk of having activity at renal (OR 2.58, CI95% 2.11-3.16), hemato
gic (OR 1.16, CI95% 1.05-1.29) and biological (OR 1.19, CI95% 1.13-1.26) domains in comparison with White patients. Finally, Hispanic patients had a lower risk of presenting activity at diagnosis in glan
dular (OR 0.63, CI95% 0.52-0.78), pulmonary (OR 0.49, CI95% 0.34-0.71), muscular (OR 0.35, CI95% 0.14-0.84) and hematologic (OR 0.73, CI95% 0.6-0.89) domains in comparison with White patients.

**Conclusions.** This study provides the first evidence of a strong influence of ethnicity on the systemic phenotype of primary SjS at diagnosis. BAA and White people had the highest median ESSDAI clinEES
SDAI scores and the highest frequencies of patients classified as high DAS, in contrast to Asian and Hispanic people. Organ-by-organ activity also varied signifi-
cantly across ethnicities.
Sjögren syndrome as the main maternal disease in mothers with babies affected with Ro-associated congenital heart block (Spanish Registry REBACC-GEAS-SEMI)

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Objective. To analyze both the already-diagnosed and the underlying maternal autoimmune diseases of mothers with pregnancies affected by autoimmune congenital heart block (CHB) associated with maternal anti-Ro antibodies.

Methods. The REBACC Spanish Multicenter Registry was created in March 2014. It is integrated by 12 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. On October 2017, the REBACC Registry included a total of 45 anti-Ro mothers with 50 single pregnancies with CHB. Mean maternal age at the time of first affected pregnancy with CHB was 32.97 years (range: 22-44). All mothers were anti-Ro52 (+), 17/17 anti-Ro52 (+) and 32/44 (73%) anti-La (+). The mean gestational age at diagnosis of CHB was 23 weeks (range 16-37). Information about fetal outcomes was available in 45 pregnancies: AV blocks were of type I in 2 pregnancies (4.5%), type II in 15 (33%) and type III in 27 (60%); 1 had an isolated EFE (2.5%); therapies used included dexamethasone or betamethasone (n=25), intravenous immunoglobulins (n=5), and plasma exchanges (n=3); 11 pregnancies were interrupted due to bad fetal prognosis (24%) and 34 (76%) were successfully carried to term, and pacemaker implantation was required in 18/34 babies (53%). At diagnosis of the first affected pregnancy, 31 (69%) mothers did not have any autoimmune disease and the remaining 14 (31%) had Sjögren syndrome (n=7), SLE (n=5) and undifferentiated autoimmune disease (n=2). At the last visit, of the 33 women who initially did not have any autoimmune disease and the remaining 14 (31%) developed a systemic autoimmune disease (SS in 16, SLE in 5 and SSc in 1). Also, 1 mother previously diagnosed with SLE, was subsequently diagnosed with Sjögren syndrome.

Conclusions. At the last visit, 23/45 (51%) mothers with affected babies with Ro-associated congenital heart block have a diagnosis of Sjögren syndrome (overwhelmingly primary), in contrast, 70% of mothers have no identified systemic autoimmune disease at the first affected pregnancy, as anti-Ro antibodies can be detected several years before SS is diagnosed. Autoimmune CHB is one of the first early signs of primary SS in women of childbearing age.

Re-analysis of the JOQUER trial after stratifying patients into clinical phenotypes

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Introduction. The JOQUER trial evaluated the efficacy of hydroxychloroquine (HCQ) for patients with Primary Sjögren’s Syndrome (PSS). This randomized control trial concluded that there was no overall disease benefit from the drug. However, we have recently identified four distinct clinical phenotypes in PSS each with differences in underlying pathobiological profiles - the high symptom burden (HSB), pain dominant with fatigue (PDF), dryness dominant with fatigue (DDF) and low symptom burden (LSB) phenotypes. In this study, we re-analysed the JOQUER trial after stratifying by patient phenotype.

Methods. The JOQUER trial involved 120 patients with PSS from 15 different centres in France. Participants were assessed at baseline, weeks 12, 24 and 48. Patients were randomized (1:1) to receive HCQ (400 mg once daily) or placebo from baseline until 24 weeks. Between weeks 24 and 48, all participants were prescribed HCQ. Assessment of the disease activity included ESSPRI, ESSDAI and objective measures including dryness tests (Schirmer’s and salivary flow) and systemic blood analysis. For the re-analysis of the trial, patients were stratified into four clinical phenotypes. The - sample sizes for the HSB, PDF, DDF and LSB groups were 32, 39, 19, and 14 respective.
ly. Multivariate analysis of variance (MANOVA) analysis permitted testing of changes over time from baseline, weeks 12 and 24. Contrasts were formed testing for changes in improvement in patient-reported outcome measures for HSIB, PDF, DDF, and LSB phenotypes over time.

**Results.** By just 12 weeks, there was a statistically significant improvement with HCQ in ESSPRI of 1.27 points (95% CI: 0.33, 2.22; p-value=0.0032) and improvement at 24 weeks (p-value=0.0137) compared to placebo. Patient-reported dryness scores contributed most to this ESSPRI improvement. While there were no statistically significant improvements in either pain or fatigue scores, there was a significant reduction in dryness score over the 12 weeks with improvement of 1.77 points (95% CI 0.6687, 2.8697; p-value=0.0006) and a significant improvement after 24 weeks (p-value=0.0017).

With regard to location of dryness, improvement was the most notable in nasal dryness after 24 weeks (p-value=0.029) and oral dryness at 24 weeks (p-value=0.0194).

**Conclusion.** While the mechanism of action of HCQ in PSS is not fully understood, re-analysis of the JOQUER trial suggests HCQ may benefit the HSIB subset of PSS patients. A stratified RCT powered to detect improvements in the four phenotypes is needed to confirm the efficacy of HCQ for HSIB patients.

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**Comparison of distinguishing characteristics between patients with primary Sjögren’s syndrome and non-primary Sjögren’s syndrome patients**

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**Objectives.** To evaluate potential systemic, oral and salivary distinguishing characteristics for patients with primary Sjögren’s syndrome (pSS) from non-primary Sjögren’s syndrome (non-pSS) patients.

**Methods.** Forty patients referred for diagnosis of PSS underwent an interview including exocrine and non-exocrine symptoms and manifestations, assessment of fatigue using the Multidimensional Fatigue Inventory MFI-20, an oral (dental and periodontal status) and ocular (Schirmer’s test, tear break-up time and Lissamine green staining) examination, measurements of unstimulated (UWS) and chewing-stimulated whole saliva (SWS) flow rates, a labial salivary gland biopsy and test for serum autoantibodies.

**Results.** Fourteen females and one male (aged 57±12 years) fulfilled the American-European Consensus Classification Criteria, whereas 22 females and 13 males (aged 55±14 years) did not. No significant differences were found in symptoms of oral and ocular dryness, concomitant systemic diseases, no. of prescribed medication, mean score of decayed-missed-filled-teeth, levels of plaque, gingival inflammation and probing depth. However, patients with pSS had lower UWS (0.04±0.06ml/min vs. 0.13±0.12 ml/ 
min, p=0.026) and SWS (0.47±0.50 ml/min vs. 0.84±0.60 ml/min, p=0.038) flow rates, and higher fatigue scores (p=0.046). Lymphocytic infiltration, i.e. with focus score ≥1, was found in the salivary gland biopsies from 47% of the patients with pSS and in none of those from non-pSS patients (mean focus score 2.3±4.0 vs. 0.02±0.06, p=0.001). In the remaining 53% of pSS patients, the salivary gland tissue was characterized by atrophy, fibrosis and diffuse infiltration. All patients with pSS had elevated levels of circulating anti-Ro/SSA serum autoantibodies as compared to 28% in the non-pSS group (p<0.001).

**Conclusion.** Our preliminary findings indicate that oral, ocular and sys-
temic symptoms and manifestations are poor distinguishing characteristics. A salivary gland biopsy with a focus score ≥1 had a high predictive value for the diagnosis of pSS. Presence of anti-Ro/SSA autoantibodies had a high sensitivity for pSS but a lower specificity due to several false-positives in serum samples. Our on-going study includes a larger cohort to substantiate our preliminary findings and discover more specific biomarkers for pSS.

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**The potential continuum of risk of dry eye syndrome, Sjögren’s syndrome and B-cell non-Hodgkin lymphoma**

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**Background.** Dry eye syndrome (DES) is a manifestation of Sjögren’s syndrome (SS), an autoimmune disease (AID) with a high lifetime risk of B-cell non-Hodgkin lymphoma (B-NHL). We aimed to explore whether an etiologic continuum exists from DES through SS to B-NHL by assessing en-
vironmental and infectious exposures and cytokine levels in these disorders.

**Methods.** In a clinic-based case-control study 702 participants: 91 SS, 120 B-NHL, 211 controls (age and sex-matched), and 280 B-NHL cases were re-
cruited and interviewed regarding exposures. Antibody titers to HCV, HBV, EBV, CMV, H. pylori, and C. trachomatis were tested by multiplex serol-
ergy. Serum cytokines IL4, IL6, IL10, IL12, IL17, TNFα, INFγ and IL1β were tested on SS and DES participants using multiplex ELISA.

**Results.** SS showed a female predominance (9:2). Factors inversely asso-
ciated with B-NHL, DES and SS include alcohol consumption (OR=0.47, 95%
CI=0.32-0.71; OR=0.54, CI=0.33, 0.88; OR=0.27, CI=0.15, 0.49, res-
pectively), and East European ancestry for SS (OR=0.43; CI=0.23-0.79), compared to controls. Self-reported infection requiring hospitalization was more common in B-NHL (OR=1.91; CI=1.22-2.98), DES (OR=1.22; CI= 1.93-5.35) and SS (OR=4.58; CI=2.56-8.18) than in controls. B-NHL cases were more likely to report 1st degree relatives with hematologic cancer (OR=1.91; CI=1.00-3.62), while 1st degree relatives with AID were more common among SS (OR=5.23; CI=2.58-10.58) and DES patients (OR=3.56; CI=1.84-6.89). IL10 and IL12 levels were higher in SS than in DES, while controls had intermediate levels (p<0.001). A higher proportion of SS pa-
tients had antibodies to HCV, EBV-EA-D and CMV (p=0.02, 0.02, 0.01, respectively) than B-NHL, DES or controls. CMV seropositivity was more common in SS patients than among controls (OR=3.56; CI=1.14-11.04), while that of C. trachomatis was decreased in DES (OR=0.40; CI=0.19- 
0.84) compared to controls.

**Conclusions.** While some factors (e.g. alcohol, hospitalization for infec-
tion) appear to be associated with all 3 conditions, some were specific to one or two of them. Cytokine activation does not show a continuum from controls→DES→SS. Patients with DES and SS appear distinct in terms of infectious exposures. Further work is required to understand events leading to B-NHL in autoimmune disease.

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**Depicting the spectrum of Sjögren’s syndrome patients: correlating clinical clustering characteristics with gene expression**

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**Background.** Sjögren’s Syndrome (SS) is a progressive, chronic autoim-
mune disease that is difficult to diagnose. Its trademark features include the presence of immune cells infiltrating exocrine glands, mainly the lacrimal and salivary glands, leading to symptoms of dryness. The systemic manifestations cause an array of debilitating symptoms and further confound diag-
nosis. The pathophysiology of SS is still unknown, yet there is evidence of both epithelial and immune dysfunction, leading to a heterogeneous pheno-
type. To characterize the diversity within Sjögren’s syndrome patients, we first identified patient clusters based on a number of clinical characteristics and then used whole RNA-Seq to identify the molecular pathways associ-
ated with each of these clusters.

**Methods.** Whole RNA-Seq was carried out on 44 primary (SS) patients and 13 healthy controls (HCs). Reconstructed reads were aligned to the hg19 reference genome and read counts were generated using the HT Seq python.
module. Clinical data for 8 measures – salivary flow, presence of antibodies, abnormally increased levels of C3 and C4, and (FS) focus score – were encoded and input as a numerical matrix for decomposition with principal components analysis (PCA). Coordinates of the first 3 PCs were used for k-means clustering into 4 groups. The Gene Set Enrichment Analysis (GSEA) algorithm from the Broad Institute was used to compute enrichment of pathways in the Reactome database in each patient cluster when compared to the HC cluster.

Results. The unsupervised clustering analysis defined the characteristics of 4 clusters. Cluster 1 consists mostly of healthy controls with no clinical abnormalities. Cluster 2 includes classically symptomatic patients with elevated focus scores (>4), mildly reduced salivary flow, and autoantibodies against SSA and/or SSB. Cluster 3 captures SS patients with less clinical activity among the included criteria: low focus scores, mild salivary flow, and no autoantibodies or C3/C4 elevation. Patients in Cluster 4 also have low focus scores, but they have greatly reduced salivary flows, presence of elevated C3/C4 and tend to harbor antibodies against SSA only. Pathway-level enrichment analysis revealed a downregulation of metabolic pathways and upregulation of immune and signal transduction pathways across all patient clusters. Metabolic pathways related to the respiratory electron transport chain and mitochondria protein import were downregulated specifically within Cluster 4 samples. Likewise, some immune pathways like Antigen Processing Cross Presentation were upregulated only in Cluster 2, and Potassium Channels were downregulated in Cluster 3.

Conclusions. Clustering by clinical characteristics has identified unique molecular pathways that might be important in the pathogenesis of SS. Overall, not only were previously known pathways highlighted in the analysis, but new phenotypic clusters of patients and their affected gene pathways were explored. This classification may lead to a more thorough understanding of the molecular mechanisms underlying SS as a spectrum, leading to a personalized approach based on the individual patient pathophysiology.

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Gene networks describe symptom based phenotypes in primary Sjögren’s syndrome
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Background. We have previously described a novel methodology for patient stratification in primary Sjögren’s syndrome (PSS) using five patient reported outcome measures (ISSS2014). The stratification resulted in four phenotypic groups: Low Symptom Burden (LSB), High Symptom Burden (HSB), Dryness Dominant with Fatigue (DDF) and Pain Dominant with Fatigue (PBF). There are several clinical and haematological differences (lymphocyte count and serum IgG) between the four patient phenotypes. In this study we identify further biological differences between the phenotypes using historical whole blood transcriptomics data. Transcriptional differences separate the phenotypes provide insight into the underlying biology between the four groups.

Methods. Historical whole-blood transcriptomic data from two studies from the United Kingdom Primary Sjögren’s Syndrome Registry (UKPSSR) were used to reconstruct gene networks and model phenotypes as a function of gene expression data. We used ARACNE software to reconstruct gene networks using data from 186 patients assayed in 2015 (training data). We built a partial least squares discriminant analysis (PLS-DA) model from the training data and tested this model in an independent, time-separated cohort of 119 patients assayed in 2013 (testing data).

Results. The ARACNE network reconstruction revealed three main gene networks (N1, N2, N3) (Figure 1). Network N1 is a large cluster of gene interactions, including genes known to be relevant in pSS (e.g. TROY2). Network N2 contains several genes involved in erythropoiesis and network N3 is comprised almost exclusively of IFN related genes. The PLS-DA model built with the training data performed well in predicting phenotypic group membership. The PLS-DA model predicted the phenotypic groups for the testing data with greater than 90% accuracy. Four latent variables (LV1-LV4) could be extracted from the model, which represent linear combinations of the gene expression data. LV1 and LV2 are representative of gene expression differences in DDF and LSB respectively, whereas LV3 and LV4 are representative of gene expression differences between PBF and HSB. By mapping the latent variables to the latent variables of the ARACNE network we are able to visualize differentially activated pathways for the four phenotypes. The gene expression differences in DDF phenotype are focussed in N1 (Figure 1, a), whereas the LSB phenotype shows the biggest differences in the IFN related network (N3) (Figure 1, b). The differences in gene expression between the HSB and PDF phenotypes can be observed in all three networks (Figure 1, c,d).

Conclusions. This analysis of historical whole blood transcriptomics data has granted further insight into the biological differences underpinning clinical phenotypes of pSS that our group has previously described. Further work is necessary to fully interrogate specific network interactions.

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Sjögren syndrome profile in the Brazilian population: demographic, clinical, laboratory and imaging analysis and comparison with controls
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Purpose. Sjögren syndrome (SS) is present worldwide, with unknown cause and cure. The present work describes a large series of SS patients observed in Brazil and compare the data among SS and non-SS (NSS) patients.

Methods. A spontaneous sample of individuals with sicca symptoms and controls were evaluated and were classified as SS or NSS. The demographic, clinical, laboratory and imaging data were compared among those individuals. The ODSI, PhQ-9 and neuropsychic pain questionnaires were also applied. Thirty-nine controls and 19 SS individuals we submitted to MRI on the 3.0 Tesla Magnetic Resonance Scanner. Images were analyzed for signal intensity ratio of LG and vitreous (LG/V), signal intensity ratio of ipsilateral parotid gland to vitreous (PG/V), apparent diffusion coefficient (ADC) of LG and PG (DWI sequence with b=1000 mm²/s) and Trigeminal ganglion (TG) volume (mm³).

Results. One hundred-twenty-three patients were evaluated as SS or NSS (84 and 44, respectively). The mean age is 52±15 years old, and 95% are women. The groups have a similar frequency of dry eye and dry mouth. The positivity for ANF, SSA, SSB and focus score are high in SS groups compared to non SS (p<0.05, Chi-square test). Mean values of Schirmer test, tear break-up time and salivary flow were lower and corneal staining p

Fig. 1. a, b, c, d.

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signal intensity ratios of LG/V and PG/V were significantly higher in the SS group (p=0.01, Tukey’s test). ADC of LG was higher in SS patients (p=0.003, Tukey’s test). LG volume was larger in young SS patients compared to age-matched controls (p=0.03). The volume of TG was 218.9±16.1 in the control and 158.3±11.6 mm³ in the SS group (p=0.008). The SS TG is smaller at younger ages, but the difference is attenuated after 70 years old. There was a positive correlation between cornea fluorescein staining and signal intensity ratio of LG (r=0.63 p=0.003) and PG (r=0.53 p=0.01).

Conclusions. Our data confirm the world demographic and laboratory parameters for SS. It also reveals that MRI of LG, PG, and TG identify changes in SS patients and imaging findings correlate with clinical exams. Depressive symptoms and neuropathic pain need further investigation in conjunction with the clinical signs to identify the mechanisms, potential distinctive causes and better treatment for SS and SSS sicca syndrome.

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Stability of clinical phenotype membership in primary Sjögren’s syndrome: longitudinal follow up data from 244 UK and 237 French patients

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Background. Four distinct clinical subgroups have been demonstrated in primary Sjögren’s syndrome (pSS), based on cluster analysis of pain, fatigue, dryness, anxiety and depression symptom scores. Validated in two independent cohorts, these subgroups display clinical, biological and transcriptional differences supporting the existence of pathobiological endotypes underpinning these clinical phenotypes. This is the first study examining the longitudinal stability of these clinical phenotypes and factors affecting migration between groups over time.

Methods. Retrospective data for 244 patients over a mean of 4 years in the UK Primary Sjögren’s Syndrome Registry (UKPSSR) and for 237 patients over 5 years in the French Assessment of Systemic Signs and Evolution in Sjögren’s Syndrome (ASSESS) cohort were analysed. Clinical phenotype membership was determined at two time-points using a validated Excel Macro model based on the aforementioned symptom scores. Phenotype membership at time-point 1 was compared to membership at time-point 2 to determine phenotype stability over time in each cohort. Non-parametric analysis was used to determine differences across phenotypes. Predictors of phenotype membership were analysed using nominal logistic regression modelling.

Results. The majority of patients remain the same phenotype (63% in UKPSSR, 57% in ASSESS) over this time period (Table). Clinical and biological measures (IgG, ESR, salivary flow and Schirmer’s test) remain significantly different and with a similar distribution across the four phenotypes at both time points within the UKPSSR. The primary predictor of phenotype membership in both the UKPSSR and ASSESS cohorts is the initial phenotype (p<0.001 for both). In addition, both ESR and Immunoglobulins were significantly associated with migrations from one phenotype to another (p<0.05) in the two cohorts. Other predictors included CRP (p=0.0076) and BMI (p=0.0089) in the ASSESS cohort, and Salivary Flow (p=0.0046) in the UKPSSR cohort.

Conclusions. Phenotype membership is generally stable over a 4 and 5 year period, in two independent pSS cohorts. This has implications for future studies, including clinical trials, and clinical management pathways, which may benefit from using this stratification method.
Long-term evolution to additional autoimmune diseases in patients with primary Sjögren’s syndrome

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Background. French cohort study revealed only a few percent of patients with primary Sjögren’s syndrome (pSS) developed other autoimmune diseases and those patients had active immunological profile and extra-glandular manifestations (1); however, clinical features of patients developing secondary autoimmune diseases are not fully understood. The aim of this study is to investigate clinical features of patients with pSS who develop secondary autoimmune diseases.

Methods. A total of 140 patients with pSS according to the 1999 revised Japanese criteria visited our hospital between 1989 and 2006. Clinical and laboratory data were collected from their medical charts, and 80 patients (79 women) who had visited our hospital more than 10 years were selected.

Results. Average age (SD) at the onset of pSS, at the first visit to our hospital, and at the final observation was 43.7 (13.1), 49.4 (12.0), and 66.7 (OK, USA). Department of Orthopaedics, Wilmer Orthopaedical Institute, Johns Hopkins University, Baltimore, MD, USA. 5 Department of Ophthalmology, Wilmer Ophthalmological Institute, Johns Hopkins University, Baltimore, MD, USA. 6 US Department of Veterans Affairs Medical Center, Oklahoma City, OK, USA. 14th International Symposium on Sjögren’s Syndrome  Posters

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Long-term evolution to additional autoimmune diseases in patients with primary Sjögren’s syndrome

SS and 3 incomplete SS [iSS] both by AECG and ACR-EULAR criteria participated in this pilot study in which all procedures performed in their initial evaluation were repeated.

Results. 356 (45%) questionnaires were answered (161 SS and 195 iSS). Subjectively, respondents reported equal or better status of ocular and oral symptoms but significantly worse fatigue and arthralgias (p>0.001) with no differences between SS and iSS. The 20 re-evaluated patients returned after an average of 5.4 years (range 2-9). Thirteen (65%) retained the initial disease classification, but 6 subjects (30%) went from SS to iSS and 1 (5.0%) from iSS to SS. It is noteworthy that this subject only met SS criteria by AECG and not by ACR-EULAR because his serology was anti-La (+) only with negative biopsy. Furthermore, only 2 subjects had a net increase in the number of criteria, while 18 had the same number (n=8) or fewer (n=10). There were no consistent patterns of change in the objective measures of lacrimal and salivary gland function: 5 subjects became Schirmer’s (+) and 1 reversed to (-); the opposite was the case for the ocular staining, with 5 becoming (+) and 1 becoming (-). The only unchanged results in all the recalls were the anti-Ro/anti-La status. The most intriguing results were the changes in the minor salivary gland biopsy: 4 (20%) subjects went from positive to negative biopsy resulting in a change in classification from SS to iSS in 3 cases. Moreover, the focus score was lower in the second biopsy of 10 (50%) cases, 4 (20%) had a higher score and the remaining 6 (30%) were unchanged. The morphology of the salivary gland tissue of these reversed cases showed extensive fibrosis, fatty infiltration and atrophy of the gland, precluding the lymphocytic infiltrates from meeting the definition of disease. The salivary gland biopsy results were confirmed by normal tissue. Further supporting the notion of worsened gland architecture, total focus score trended to inversely correlate with WUSF (r=-0.4; p=0.089). Complement levels and hypergammaglobulinemia did not predict stability or worsening of SS.

Conclusions. Re-evaluated patients showed little disease progression but steady presence of autoantibodies. Detrimental changes in salivary gland morphology were observed in later biopsies, even when classified as “negative” using current criteria. These results suggest that significant tissue destruction in long standing disease may lead to false negative biopsy results in spite of progressive glandular dysfunction.

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Diagnosis of keratoconjunctivitis sicca: identifying the signs of dry eye disease

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Purpose. There is no gold standard for the definitive diagnosis of keratoconjunctivitis sicca (KCS). Instead, ophthalmologists must rely on clinical signs and tests to diagnose dry eye disease. Clinical signs and tests include tear breakup time (TBUT), Ocular Surface Score (OSS), Schirmer 1, tear osmolarity, and tear osmolarity. The Sjögren’s International Collaborative Clinical Alliance (SICCA) developed new classification criteria for Sjögren’s syndrome (SS), and seeks to better characterize the SS phenotype and genotype, and establish a SS data and specimen repository to support future research.

Methods. Levels of sensitivity and specificity for each sign (variable) were assessed using latent class analysis. Our modeling was based on four predictor variables that relate to signs of KCS. Latent class analysis allowed for estimation of sensitivity and specificity using the model-based classification as a “gold standard.” We utilized R package for latent class analysis (R version 3.3.2 and RStudio 1.0.136, R, Boston, MA).

Results. A total of 3,514 participants from 9 international sites were enrolled into SICCA. Women made up the majority of participants (n=3,185 or 91%). SS as defined by ACR/EULAR criteria was diagnosed in 1,541 participants (52.9%) and 116 participants (3.3%) could not be classified. With latent class analysis, we found a best fit model with two groups, a gold standard-positive group and a gold standard-negative group. For the gold standard-positive group, having an abnormal TBUT, Schirmer 1, tear osmolarity, and OSS had a sensitivity of 100%, 19%, 43%, and 38%, respectively. In the gold standard-negative group having an abnormal TBUT, Schirmer 1, tear osmolarity, and OSS had a specificity of 40%, 100%, 72%, and 91%, respectively.

Conclusions. The Ocular Staining Score differentiated the gold standard-positive group from the gold standard-negative group better than other KCS parameters.
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In vivo confocal microscopy evaluation of corneal sub basal nerve and dendritic cell in Sjögren syndrome patients

Qin Zhang, Fangting Li, Jing He, Yuebo Jin, Yidan Zou

Background. To analyze the relationship between in vivo confocal microscopic corneal nerve density, dendritic cell density and clinical evaluation in patients with Sjögren syndrome (SS).

Methods. Seventeen patients with SS and sixteen healthy age- and sex-matched Non-Sjögren dry eye disease patients control subjects were included. A clinical evaluation of dry eye (Non-invasive tear meniscus height, non-invasive break-up time, meibomian gland) was performed. For all patients.

Results. The area of non-invasive tear meniscus height (TMH), non-invasive break-up time (NI-BUT), the loss of upper meibomian gland and corneal sub basal nerve density were significantly lower in the SS group compared with the control group (p<0.01). Dendritic cell density of central cornea increased significantly in SS group (p<0.01). The loss of upper meibomian gland was correlated to age (r=0.378, p=0.033). In the SS group, corneal sub basal nerve density was correlated to NI-BUT (r=0.543, p=0.001).

Conclusions. The dry eye, the loss of both meibomian gland and corneal sub basal nerve was more severe in SS patient than in non-Sjögren dry eye disease patients. Confocal microscopy can be an important diagnostic tool in evaluation the ocular surface change in SS patients.

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Quantification of proteoglycan 4 (PRG4) / lubricin in normal and Sjögren syndrome human tears

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Background. Sjögren’s syndrome (SS) is an autoimmune disease with hallmark clinical symptoms of dry eye and dry mouth. Proteoglycan 4 (PRG4), or lubricin is a mucin-like glycoprotein that is naturally present on the ocular surface, and in tears, where it contributes to ocular surface health. PRG4 functions as a boundary lubricant to reduce friction between ocular surfaces, and also demonstrates anti-inflammatory properties. Recently, recombinant human PRG4 was shown to be clinically effective in improving signs and symptoms of dry eye in SS patients. Confocal microscopy can be an important diagnostic tool in evaluation the ocular surface change in SS patients.

Methods. Tears were collected from 17 SS (15 F, 2 M, 56.2±16.7 years old) and 20 asymptomatic (20 M, 7 M, 13 F, 31.2±11.4 years old) participants, with approval from the Office of Research Ethics (UWaterloo). SS participants were diagnosed using the American European Consensus Criterion. Tears were collected without anaesthetic, from the inferior temporal tear meniscus of each eye, using a disposable microcapillary tube and frozen at -80°C until use. The concentration of PRG4 was determined by a sensitive, competitive amplified luminescent proximity homogenous assay using recombinant human PRG4 as the control. Total mass of PRG4 was calculated by normalizing concentration by tear volume, using 5.0 ul for normal tears and measured SS tear volume (0.1 to 2.3 ul). Data is reported as mean±SD, nonparametric statistics were employed (Mann-Whitney U & Levine tests).

Results. The concentration of PRG4 in SS tears (28.6±44.3 ug/ml) was not significantly different than that of normal tears (2.6±2.0 ug/ml, p=0.15). In the SS group, demonstrated significantly greater variation (p<0.01). The mass of PRG4 in SS tears (10.6±4.8 ng) was significantly diminished compared to normal tears (12.8±1.4 ng, p<0.05).

Conclusions. PRG4 concentration is significantly more variable in SS tears, and when normalized by volume, the PRG4 mass in SS tears is diminished compared to normal tears. These data suggest either a reduction in PRG4 production or an increase in PRG4 catabolism in SS tear relative to normal tears, which could be the cause of the variability of PRG4 concentration in SS tears. Given the role PRG4 plays in ocular surface health and its susceptibility to degradation by cathepsin S in SS tears, diminished endogenous levels of PRG4 could contribute to signs and symptoms of dry eye in SS.

P-49

Correlations between the severity of xerophthalmia and inflammation state in primary Sjögren syndrome

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Objective. To assess the relationship between ocular surface parameters and inflammation state in patients with primary Sjögren syndrome (pSS).

Methods. 62 female patients with pSS (age:49.79±12.43 years) and 16 healthy control subjects (age:55.67±13.53 years) were recruited. Clinical Characteris were documented. The immunoglobulin levels, the cytokine levels complement levels, measurements of tear break-up time (TUBT), meibomian gland (MG) evaluations, Schirmer test I, non-contact infrared meibography and lid margin morphology examination using slitlamp microscopy were performed. The MG loss, calculated as (tarsal area-MG area)/tarsal area, was evaluated in upper lids (UL). The T-test and chi-square test were used to analyze the associations between the groups. p<0.05 was considered statistically significant. Pearson and Spearman’s tests were used for correlation analysis.

Logistic regression analysis of meibomian gland damage was conducted for SS patients.

Results. (1) The ocular parameters are highly relevant and valuable for the dry eye detection. The ocular parameters of the non-invasive keratograph tear break-up time (NIBUT), Schirmer test I, tear break-up time (TUBT), the number of meibomian gland and the MG loss for the pSS group were significantly lower than those for the healthy control subjects (p<0.05). (2) The tear meniscus height (TMH) was negatively correlated with Treg and Breg level (r=-0.255, p<0.05); Schirmer test I has a negative correlation with r–globulin (r=0.29, p<0.05) and a positive correlation with transforming growth factor-beta1 (TGF-beta1) (r=0.27, p<0.05). The number of meibomian glands has a negative correlation with sCD25 and IL-17a level (respectively, r=-0.232, r=-0.201, p<0.05). The MG loss is negatively correlated with r–globulins and level Treg/Th17 level (respectively, r=-0.259, r=-0.226, p<0.05).

Conclusion. Patients with pSS has more sever xerophthalmia than controls. The severity of xerophthalmia in patients with pSS correlates with the levels of IgG, TGF-beta, IL-17a and sCD25.

P-50

IL-14 as a putative biomarker for stratification of dry eyes in primary Sjögren’s syndrome

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Background. The pathogenesis of primary Sjögren’s syndrome (pSS) is associated with abnormal B cell activation, resulting in production of excessive autoantibodies and disorder to the cytokine network. IL-14 (also known as Taxilin) is a cytokine that was shown to enhance B cell proliferation, especially of germinal center B cells. Transgenic mice overexpressing human IL-14 alpha can develop many clinical features of pSS in the same relative time frame as seen in patients. While upregulation of IL-14 gene expression has been shown in the peripheral blood leukocytes of pSS patients, till now the measurement of IL-14 serum levels has not been possible due to lack of validated assay. Our purpose was to evaluate IL-14 as a biomarker and its
correlation to B cell activating factor (BAFF), a well-established cytokine in pSS through upregulating innate immune activation and chronic autoimmune cell activation, in a cohort of patient with without-SS dry eye (NSDE), pSS, diseases and healthy controls (HC).

Methods. Total of 181 fresh serum samples were collected and stored in -80 degrees freezer. Among them, 65 were pSS patients (age 53.15±14.08 years) who meet the 2012 ACR Classification Criteria for Sjögren’s Syndrome, 20 were dry eye patients excluding SS (age 44.85±11.39 years, NSDE), 50 were Rheumatoid Arthritis patients (age 54.95±15.35 years, RA) and 46 were healthy controls (age 43.49±14.57 years, HC). Serum level of IL-14 was evaluated by quantitative Western Blots assay and BAFF level was evaluated by ELISA assay (R&D System). All clinical and laboratory data were reviewed following protocol approved by Peking University People’s Hospital IRB committee. Statistical analysis was done by software Prism 6.0 with unpaired t tests.

Results. 1) After normalized with internal control, the relative intensi-
ty ratio for serum IL-14 level in HC group was 2.13±0.81, NSDE group was 2.11±0.98 (p=0.99), pSS group was 2.92±0.93 (p=0.001) and RA group was 2.47±0.95 (p=0.15). Serum BAFF level (pg/ml) in HC group was 323.56±65.85, DE group was 355.21±87.86 (p=0.22), pSS group was 455.94±155.16 (p=0.0001) and RA group was 448.38±220.07 (p=0.0002).

2) For age <40 years, the serum level of HC group was 2.26±0.73, NSDE group was 2.21±0.93 (p=0.60), pSS group was 3.41±0.88 (p=0.003) and RA group was 3.28±0.87 (p=0.08). For age 40 to 60 years, the serum level of HC group was 2.08±0.93, NSDE group was 1.93±1.11 (p=0.69), pSS group was 2.83±0.98 (p=0.01) and RA group was 2.23±0.76 (p=0.87). For age >60 years, the serum level of HC group was 1.93±0.68, NSDE group was 2.56±0.59 (p=0.21), pSS group was 2.76±0.81 (p=0.02) and RA group was 2.49±1.04 (p=0.12).

3) In pSS patients, the serum level of IL-14 decrease as age increase (<40 years, 2.34±0.88, 40-60 years, 2.83±0.98, (p=0.048) and >60 years, 2.76±0.81, (p=0.23)). Whereas the serum level of BAFF (pg/ml) increase as age increases (<40 years, 414.22±119.94, 40-60 years, 406.22±148.29, (p=0.87), >60 years, 524.57±159.46, (p=0.008)).

Conclusions. 1) Elevation of serum IL-14 level can serve as a key cytokine biomarker for the stratification of SS vs NSDE.

2) IL-14 and BAFF may work in different fashions to maintain the normal B cell activation as seen in pSS patients.

Table 1. Demographic and phenotypic characteristics of 132 Sjögren’s syndrome patients.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) at blood draw, mean ± SD</td>
<td>53.2 ± 11.8</td>
</tr>
<tr>
<td>Female</td>
<td>120/132  (91%)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>115/132   (87.2%)</td>
</tr>
<tr>
<td>African American</td>
<td>11/132    (8.3%)</td>
</tr>
<tr>
<td>Asian</td>
<td>3/132     (2.3%)</td>
</tr>
<tr>
<td>Hispanic/Latino</td>
<td>3/132     (2.3%)</td>
</tr>
<tr>
<td>LSG Biopsy Positive</td>
<td>80/128    (63%)</td>
</tr>
<tr>
<td>Focus score, mean ± SD</td>
<td>2.43 ± 1.5</td>
</tr>
<tr>
<td>Ro60 Positive</td>
<td>82/132    (62%)</td>
</tr>
<tr>
<td>Ro52 Positive</td>
<td>80/132    (61%)</td>
</tr>
<tr>
<td>La Positive</td>
<td>44/132    (33%)</td>
</tr>
<tr>
<td>IFI16 Positive</td>
<td>40/132    (30%)</td>
</tr>
<tr>
<td>ANA ≥ 1:320</td>
<td>66/125    (53%)</td>
</tr>
<tr>
<td>Rheumatoid Factor Positive</td>
<td>44/130    (34%)</td>
</tr>
<tr>
<td>Hypergamaglobulinemia</td>
<td>35/129    (27%)</td>
</tr>
<tr>
<td>Low C4</td>
<td>14/132    (11%)</td>
</tr>
<tr>
<td>MGUS</td>
<td>12/117    (11%)</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>25/131    (19%)</td>
</tr>
<tr>
<td>Whole Unstimulated Salivary Flow, mean ± SD</td>
<td>0.87 ± 0.95</td>
</tr>
<tr>
<td>Schirmer’s Test, mean ± SD</td>
<td>5.70 ± 6.99</td>
</tr>
<tr>
<td>SICCA Ocular Staining Score, mean ± SD</td>
<td>7.56 ± 3.42</td>
</tr>
</tbody>
</table>

Numerators correspond to number of patients with indicated feature positive and denominators to total number of patients with indicated feature recorded in the cohort, followed by percent (%) positive.

LSG: Labial salivary gland; Focus score: Lymphocytic foci / 4 mm²; ANA: Anti-nuclear antibody; Hypergamaglobulinemia: IgG ≥1500 mg/dl or IgG ≥300 mg/dl; Low C4: ≤12 mg/dl; MGUS: Monoclonal gammopathy of undetermined significance; Leukopenia: WBC <4000/μl; Whole Unstimulated Salivary Flow: mL saliva / 5 min; Schirmer’s Test: mm wetting / 5 minutes.

Conclusions. AIM2 is an IFN-induced autoantigen in SS. Anti-AIM2 anti-

bodies are associated with increased labial salivary gland inflammation and serologic markers of severe disease. IFN signaling in the salivary gland may contribute to SS pathogenesis by inducing the expression of autoantigens.
Autoantibodies to ox-LDL in Sjögren’s syndrome: are they atheroprotective?

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Background. The higher incidence of atherosclerosis and cardiovascular disease (CVD) in patients with systemic autoimmune diseases cannot be attributed exclusively to traditional risk factors for CVD. Antibodies to oxidized Low Density Lipoprotein (ox-LDL) seem to have a crucial role in atherosclerosis.

Methods. Sera from 63 consecutive patients with primary Sjögren’s Syndrome (SS), 121 with Systemic Lupus Erythematosus (SLE), 79 with Rheumatoid Arthritis (RA) and 26 apparently healthy individuals were evaluated for the presence of antibodies to ox-LDL by an ELISA method. The femoral and/or carotid Intima Media Thickness (IMT) and plaque formation as well as traditional CVD risk factors and disease related features were recorded for all study participants.

Results. Anti-ox-LDL antibody levels were significantly reduced in SS and RA patients, but not in SLE patients, compared to their healthy counterparts. Subsequently, SS patients were divided into two groups according to antibody levels to ox-LDL, using as cut off the median of each group studied. SS patients with high titters of antibodies to ox-LDL displayed higher rates of autoantibodies to Ro/SSA and La/SSB antigens, purpura, low complement levels and increased SS activity index. On the other hand, the high anti-ox-LDL group was characterized by reduced rates of carotid and/or femoral plaque after adjusting for potential confounders (OR [95%CI]: 0.14 [0.03-0.72]).

Such associations were not shown in all other groups included in the study. Conclusions. These findings suggest that antibodies to ox-LDL, possibly resulting from B cell hyperactivity, may play a protective role in the development of atherosclerosis among primary SS patients.

P-54

Positivity for anti-RNP in primary Sjögren syndrome patients is associated with a more active disease and a higher risk of muscular and pulmonary involvement

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Primary Sjögren Syndrome (pSS) can be associated with anti-Sm and/or anti-ribonucleoprotein (RNP) antibodies without anti-DNA antibodies. Whether these auto-antibodies are associated with a specific phenotype is unknown.

Objectives. To describe the clinical and paraclinical characteristics of patients displaying a pSS with anti-Sm and/or anti-RNP antibodies, without anti-DNA antibodies.

Methods. Patients fulfilling ACR/EULAR 2016 criteria for pSS without other connective tissue disease diagnosis and having anti-Sm and/or anti-RNP antibodies, without anti-DNA antibodies were screened in the database from the French National Reference Center, Paris-Sud University. We compared them to all pSS patients from the Paris-Sud cohort with negative anti-Sm, anti-RNP and anti-DNA antibodies.

Results. At inclusion twenty three patients (n=18 women, 3 men) were in the anti-Sm and/or anti-RNP group (anti-Sm: n=7, anti-RNP: n=22), and 446 in the anti-Sm and anti-RNP negative group (n=426 women, 20 men). All the patients fulfilled the ACR/EULAR 2016 criteria for pSS. All patients had negative anti-DNA antibodies, and none had a diagnosis of lupus according to the SLICC criteria. In the anti-Sm and/or anti-RNP positive group, 5 patients fulfilled previously described criteria of Mild Connective Tissue Disease (Sokolow, n=1, Kasukawa, n=1, Alarcón-Segovia, n=3). Anti-Sm- and/or anti-RNP positive patients had a lower mean age at onset of pSS symptoms (40 vs 48 years, p=0.02), a higher mean ESSDAI at inclusion (13 vs 4, p<0.001), more frequent objective xerostomia or xerophthalmia (95% vs 61%, p<0.01), myositis (26% vs 2%, p=0.01), pulmonary (30% vs 6%, p=0.01), cutaneous (65% vs 36%, p=0.01) and peripheral nervous system involvement (13% vs 3%, p=0.04). Moreover, anti-Sm and/or anti-RNP positive patients had higher mean gammaglobulins (25 vs 14 g/l, p<0.01) and CPK levels (660 vs 113 U/l, p=0.02), more frequent anti-SSA antibodies (91% vs 67%, p<0.01), but less frequent lymphocytic sialadenitis with a focus score>=1 (61% vs 86%, p<0.01). Although sclerodactyly was found in 13% of patients with anti-Sm and/or anti-RNP antibodies and limited cutaneous sclerosis in 4%, no patients had a diagnosis of scleroderma.

Conclusion. pSS with anti-Sm and/or anti-RNP antibodies subjects seem to display a more active systemic disease, with a more frequent pulmonary and muscular involvement.

Table 1. Characteristics of the 23 patients with Primary Sjögren’s syndrome and anti-RNP and/or anti-Sm antibodies at inclusion, as compared to 446 pSS patients without anti-RNP and anti-Sm.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>pSS patients with anti-RNP and/or anti-Sm, n=23</th>
<th>pSS patients without anti-RNP and anti-Sm, n=446</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classification</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Objective xerostomia or xerophthalmia, n (%)</td>
<td>18/19 (94.7)</td>
<td>263/432 (60.9)</td>
<td>0.003</td>
</tr>
<tr>
<td>Positive ANA Antibodies, n (%)</td>
<td>23 (100.0)</td>
<td>328/439 (74.7)</td>
<td>0.006</td>
</tr>
<tr>
<td>Positive anti-SSA antibodies, n (%)</td>
<td>21 (91.3)</td>
<td>298/446 (66.8)</td>
<td>0.014</td>
</tr>
<tr>
<td>Positive anti-resolution, n (%)</td>
<td>5 (21.7)</td>
<td>162/443 (36.6)</td>
<td>0.183</td>
</tr>
<tr>
<td>Positive anti-SSB antibodies, n (%)</td>
<td>7 (30.4)</td>
<td>25/37 (6.8)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Positive anti-DNA antibodies, n (%)</td>
<td>1 (4.3)</td>
<td>10/307 (3.3)</td>
<td>0.049</td>
</tr>
<tr>
<td>Positive CTD, n (%)</td>
<td>15 (65.2)</td>
<td>161/442 (36.4)</td>
<td>0.006</td>
</tr>
<tr>
<td>Positive RNP, n (%)</td>
<td>15 (65.2)</td>
<td>161/442 (36.4)</td>
<td>0.006</td>
</tr>
<tr>
<td>Positive SLE, n (%)</td>
<td>1 (4.3)</td>
<td>10/307 (3.3)</td>
<td>0.049</td>
</tr>
<tr>
<td>Positive pSS, n (%)</td>
<td>1 (4.3)</td>
<td>10/307 (3.3)</td>
<td>0.049</td>
</tr>
</tbody>
</table>

Conclusion. pSS with anti-Sm and/or anti-RNP antibodies subjects seem to display a more active systemic disease, with a more frequent pulmonary and muscular involvement.

P-55

Autoantibodies to Ro/SSA, La/SSB, Sm, or Sm-RNP not detectable in the serum are often present in the saliva

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*Present affiliation: Department of Clinical Laboratory Sciences, University of Texas, El Paso, TX.
Background. The involvement of B cells in the pathogenesis of Sjögren’s syndrome (SS) is evidenced by the presence of autoantigen-specific B cells and the incidence of autoantibodies to RoSSA (Ro52 and Ro60), and La/SSB. Production of autoantibodies occurs within the salivary glands, but it is unknown if autoantibodies present in the serum originate from these cells. We hypothesize that if serum autoantibodies originate from the antibody secreting cells in the glands, they would likely be present in the saliva prior to their detectible levels in the plasma. To explore this, we tested the saliva autoantibody profiles in a group of SS patients and sicca controls and compared them to their respective IgG serum autoantibody profiles.

Methods. Following written informed consent, 27 sequential participants, all asymptomatic for dry eyes and mouth, were evaluated for American/European Consensus Group (AECG) primary SS inclusion criteria, and 1 also met the American College of Rheumatology criteria for SLE. 13 subjects did not meet the SS classification criteria and served as sicca controls. Direct enzyme-linked immunosorbent assays (ELISAs) were performed by applying saliva (1:20 dilution, in duplicate) to antigen-coated (Ro/SSA, La/SSB, Sm, and Sm-RNP; Immunovision, Inc.) plates and detected using anti-human IgA-, or IgG-alkaline phosphatase and substrate. Four control subjects negative for all SS inclusion criteria and all other measures were used to establish positive thresholds for each ELISA (mean+SD). To confirm stringency of this measure, we determined that the Q3+1.5*IQR threshold was similar. We then compared the positive specificities for the saliva ELISAs to the IgG specificities (Ro/SSA, La/SSB, Sm, and Sm-RNP) measured in the serum by Ouchterlony double immunodiffusion, INNO-LIA, and Bioplex 2200.

Results. 10/12 sicca control sera were ANA positive, but only two were seropositive for Ro. The comparison of sera and saliva results showed that 5/14 SS patients, and 6/12 sicca controls had IgA-, and 8/14 SS patients and 8/12 sicca controls had IgA-saliva specificities not detectable in the sera.

Conclusions. Saliva from some SS patients and sicca controls contain autoantibodies not present in the serum. It will be important to test these saliva positive, seronegative subjects longitudinally for future development of serum autoantibodies. If this does occur, it would be likely that certain sicca controls would then fulfill the criteria for primary SS classification, suggesting that early testing of saliva for autoantibodies may be indicated for prediction of progression to systemic disease.
Correlation studies between EIA™ Ro/SS-A antigen Well and Ro52 / Ro60 tests

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Background. Sjögren’s Syndrome is a chronic inflammatory autoimmune disease of unknown cause characterized by diminished lacrimal and salivary gland secretion resulting in keratoconjunctivitis sicca and xerostomia. In half of patients, the disease occurs as a primary pathologic entity (primary Sjögren’s syndrome). In the other half, it occurs in association with rheumatoid arthritis or other connective tissue disorders. The disease can occur at any age, but is more frequent in older women. Many patients develop Sjögren’s syndrome as a complication of another autoimmune disease, such as rheumatoid arthritis or lupus.

Detection of the Ro/SS-A antibody aids clinicians in the diagnosis of the disease. The complete Ro antigen comprises two proteins, Ro52 and Ro60. Most of the Ro positive patients show antibodies to both Ro60 and Ro52. However, there are subsets of patients who only produce antibodies to Ro52 or to Ro60.

The objective of this study was to determine the detection capability of the Ro/SSA antibodies by the EIA Ro Well compared to individual Ro 52 and Ro 60 antibody tests from another manufacturer.

Methods. Analytical runs were performed at two different laboratories. Runs for the serum panel-1 were performed at the Thermo Fisher Scientific facility in Freiburg, Germany using EIA Connective Tissue Disease assays processed on the Phadia™ Laboratory Systems by following the instructions of the manufacturer. Laboratory-2 tested the serum panel-2 using BioPlex Ro 52 and Ro 60 assays on Bio-Rad instruments by following the instructions of the manufacturer.

Results. 96 clinical defined patient samples with a known diagnosis of Sjögrens Syndrome were used in a correlation study between the EIA Ro Well, the BioPlex 2200 ANA Screen, and the individual EIA and Bio-Plex Ro52 and Ro60 parameter tests. The correlation study showed an overlap of 100% between the EIA Ro Well and the single parameter tests independent from the supplier (Thermo Fisher Scientific or Bio-Rad). Combining the test results from Ro52 and Ro60 results in a sensitivity of 84.4% which is identical with the sensitivity for the EIA Ro Well.

Conclusion. The EIA Ro Well offers the same sensitivity as the separate Bio-Rad Ro 52 and Ro60 assays ensuring detection Ro52 and/or Ro60 antibodies with the convenience and efficiency of processing a single test versus two. Compared with the single marker tests for Ro 52 and Ro 60, the risk of underdiagnosing a patient based on the results of the EIA Ro Well is very low. From viewing my poster the participants should be able to:

- Understand the use of the Ro/SS-A antibodies assay as part of the diagnosis and classification criteria of Systemic Lupus Erythematosus
- Demonstrate the clinical utility and efficiency of testing for Ro/SSA antibodies with the EIA Ro Well and mitigating the need of testing for separate antigens
- Identify how this information helps the viewer inform clinicians about disease misclassifications.

Clinical and laboratory features of primary Sjögren’s syndrome associated with anticientromere antibodies

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Background. The prevalence of ACA among patients with pSS varies from 2% to 27%. This subtype differs from the “classical” one in a number of laboratory and clinical manifestations and characterized by an increased risk of developing SSc and AMA+ biliary lesions.

Objectives, to evaluate clinical and laboratory features of ACA-positive primary pSS; to evaluate its conformity to the ACR2012 and ACR/EULAR2016 classification criteria; to evaluate prevalence of SSc according to ACR2013 criteria; to evaluate prevalence of MALT-lymphoma; to evaluate prevalence of AMA+ biliary lesions.

Table 1. Characteristics of pSS patients with positive ACA.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value (n, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sialography of parotid gland: sialodacryorrhea</td>
<td>61/64 (95%)</td>
</tr>
<tr>
<td>Xerostomia (grade I-II) stimulated saliva flow rate</td>
<td>35/56 (62.8%)</td>
</tr>
<tr>
<td>&lt;0,5ml/5min (III)</td>
<td>27/45 (60.2%)</td>
</tr>
<tr>
<td>KKS with OSS ≥5</td>
<td>36/58 (62%)</td>
</tr>
<tr>
<td>Schirmer’s test s≥5/mm5</td>
<td>27/45 (60.2%)</td>
</tr>
<tr>
<td>Focal lymphocytic sialadenitis</td>
<td>1 foci/4 mm²</td>
</tr>
<tr>
<td>anti-Ro (≥50 IU/ml)</td>
<td>14/45 (31%)</td>
</tr>
<tr>
<td>aLa (≥50 IU/ml)</td>
<td>3/6 (4.8%)</td>
</tr>
<tr>
<td>IgM RF (&gt;30 IU/ml)</td>
<td>11/25 (44.4%)</td>
</tr>
<tr>
<td>Low C4 (&lt;0.1 mg/ml)</td>
<td>2/5 (40%)</td>
</tr>
<tr>
<td>High ESR (&gt;90 mm/h Westergren)</td>
<td>3/56 (19.6%)</td>
</tr>
<tr>
<td>Hypergammaglobulinemia (&gt;20%)</td>
<td>8/34 (23.5%)</td>
</tr>
<tr>
<td>High IgM (&gt;60 g/l)</td>
<td>5/37 (13.5%)</td>
</tr>
<tr>
<td>Leucopenia (&lt;4x10⁹)</td>
<td>2/5 (40%)</td>
</tr>
<tr>
<td>Neuropathy</td>
<td>1/13 (7.7%)</td>
</tr>
<tr>
<td>AMA (&gt;1 IU/ml)</td>
<td>17/37 (46%)</td>
</tr>
<tr>
<td>AMA+ biliary lesions</td>
<td>12/6 (18.7%)</td>
</tr>
<tr>
<td>Histologic features of PBC</td>
<td>0/12</td>
</tr>
<tr>
<td>MAL-T lymphoma</td>
<td>5/4 (12.5%)</td>
</tr>
<tr>
<td>MAL-T lymphoma - RF+</td>
<td>2/45 (4.4%)</td>
</tr>
<tr>
<td>MAL-T lymphoma - aRo</td>
<td>3/9 (33%)</td>
</tr>
<tr>
<td>MAL-T lymphoma - aLa-aLa-RF</td>
<td>3/9 (33%)</td>
</tr>
<tr>
<td>Raynaud’s phenomenon</td>
<td>33/90 (36.6%)</td>
</tr>
<tr>
<td>Digital ulcers</td>
<td>1/10 (10%)</td>
</tr>
<tr>
<td>Abnormal autofluorescence</td>
<td>23/35 (65.7%)</td>
</tr>
<tr>
<td>Sclerodactyly</td>
<td>9/50 (18.3%)</td>
</tr>
<tr>
<td>Puffy fingers</td>
<td>3/50 (6%)</td>
</tr>
<tr>
<td>Interstitial lung disease</td>
<td>13/45 (28.9%)</td>
</tr>
</tbody>
</table>
**Results.** Clinical and laboratory manifestations are listed in the Table I. In our study, 58.7% ACA+ pts with pSS didn’t fulfill ACR2012 criteria, because of the lack of aRo/aLa or RF+ANA. According to the criteria 2016 also just 60-68% pts will have ≥4 scores for diagnosing pSS. Limited form of SSc due to 2013 criteria might be revealed in 20% cases with scleroderactyly, puffy fingers and digital ulcers. MALT-lymphoma occurred in 14%. AMA+ biliary lesions occurred in 18% of cases, all of which had no histological features of PBC.

**Conclusions.** ACA-positivity in pSS is associated with specific clinical and laboratory characteristics, such as combination with SSc and AMA+ biliary lesions, but only two-thirds of patients with ACA and definite pSS meet classification criteria 2012, 2016. This fact raises the question about necessity to consider ACA one of the pathogenically relevant autoantibodies for pSS.

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**P-60**

Clinicopathological characteristics of anti-centromere antibody-positive Sjögren’s syndrome in the presence or absence of systemic sclerosis

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**Objectives.** Few studies have clarified differences in sicca symptoms, ESSDAI score, and organ involvement in anti-centromere antibody (ACA)-positive Sjögren’s syndrome (SS) with and without systemic sclerosis (SSc). We compared clinicopathological characteristics between these two groups using our cohort of patients with ACA-positive SS.

**Methods.** We studied 33 patients with ACA+ primary SS and 16 with ACA+ SS with SSc in a retrospective cohort study. All SS patients met Japanese and/or ACR criteria, and those whose results exceeded the focus score by 1 underwent labial salivary gland biopsy. All SSc patients met ACR/EULAR criteria. We analyzed SS and SSc data at diagnosis and organ involvement during follow-up.

**Results.** No significant differences were seen in age at diagnosis (mean 65.2 in ACA+ primary SS vs 66.9 in ACA+ SS with SSc), sex (97.0% vs 100%), anti-SS/A/Ro antibody (42.4% vs 25.0%), anti-SS-B/La antibody (16.7% vs 0%), laboratory data including leukocytes, lymphocytes, serum IgG, and complement levels, ESSDAI score at diagnosis (1.91±2.47 vs 1.81±4.23, respectively), organ involvement, and treatment. Focus score was 3.34±2.98 in ACA+ primary SS and 3.57±2.04 in ACA+ SS with SSc (p=0.055). Germal center-like structures were 24.2% vs 18.8%; scores for Saxon's test, 0.59 g vs 0.54 g (p=0.57); Schirmer's test, 6.4 mm vs 5.4 mm (p=0.56); and Raynaud’s phenomenon, 21.2% vs 87.5% (p<0.001), respectively. No skin sclerosis developed during the follow-up period.

**Conclusion.** ACA+ SS with and without SSc showed severe sicca symptoms. Focus score was higher in the SS+ SSc group than in the ACA+ primary SS group.

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**P-61**

Anti-centromere antibody positive Sjögren’s syndrome is associated with worse sicca symptoms than primary Sjögren’s syndrome alone

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Multidisciplinary Sjögren’s Clinic. University Health Network, University of Toronto

**Background.** Sicca symptoms are the most prevalent symptoms in primary Sjögren’s Syndrome (pSS). The presence of anti-centromere antibody has been reported in 1.4 to 10.8% of patients with pSS. The purpose of our study was to determine whether the presence of anti- centromere antibodies (ACA) affects the incidence or severity of sicca symptoms in patients diagnosed with pSS.

**Methods.** Patients were pre-screened for objective evidence of dry eyes or dry mouth, abnormal serology or history of salivary gland enlargement as an adult. The evaluation included a visual analogue scale (VAS) for severity of xerophthalmia and xerostomia, Schirmer’s test (S1T), van Bijsterveld staining score and unstimulated whole saliva flow (USSF). A minor salivary gland biopsy was performed on all patients. Assessments were performed by the same pathologist on protocol for evidence of Follicular Lymphocytic Sialadenitis pathology and focus score. Serological profile included the evaluation for the presence of anti-Ro, anti-La and anti-centromere antibody (ACA). All subjects were evaluated for CREST features. pSS was classified according the American European Consensus Group (AECG) Criteria. Patients that met the pSS classification criteria were further categorized into ACA+ SS and ACA- SS. A 2- tailed student t-test with heterogeneous variance was used to compare the two groups for measures of severity of dry eyes and dry mouth.

**Results.** Within the pSS group (n=446), there were 26 patients with positive ACA serology. On a 10 cm visual analog scale (VAS), the subjective severity of ocular sicca symptoms was 7.0 (out of 10) in ACA+ SS patients and 6.4 in ACA- SS patients (p=0.197). The mean S1T in ACA+ SS patients was 3.5mm/5 mins and in ACA- SS pts was 4.1mm/5 mins. The difference was significant (p=0.038). The severity of oral sicca symptoms was 8.5 in ACA+ SS patients and 6.7 in ACA- SS patients (p=0.001). The mean value for USSF was 0.1ml/15 mins for ACA+ SS and 0.4 ml/15 mins for ACA- SS. This difference was highly significant (p<0.001). RP occurred more commonly in ACA+ SS patients (88%) than in ACA- SS patients (26%) (p<0.001). Only 35% of ACA+ SS patients were anti-Ro or anti-La positive compared with 77% of the ACA- SS patients (p<0.001).

**Table I.** Comparison between ACA+ SS and ACA- SS.

<table>
<thead>
<tr>
<th></th>
<th>ACA+ SS (n=26)</th>
<th>ACA- SS (n=429)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, mean (years)</td>
<td>55.7±10.5</td>
<td>53.2±13.4</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>0</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>26</td>
<td>381</td>
<td></td>
</tr>
<tr>
<td><strong>Clinical Differences</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prevalence of Raynaud’s phenomenon (%)</td>
<td>88</td>
<td>28</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Prevalence of elevated serum IgG ($/L$)</td>
<td>24</td>
<td>57</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Average levels of serum IgG (g/L)</td>
<td>12.4</td>
<td>19.4</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td><strong>Sero/Pathology</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prevalence of xerophthalmia (%)</td>
<td>96</td>
<td>96</td>
<td>NS</td>
</tr>
<tr>
<td>Severity of xerophthalmia (on VAS, max 10)</td>
<td>7.2±2.4</td>
<td>6.4±2.6</td>
<td>NS</td>
</tr>
<tr>
<td>Average Rose Bengal score</td>
<td>3.2±1.8</td>
<td>4.2±3.4</td>
<td>p=0.05</td>
</tr>
<tr>
<td>Duration (years)</td>
<td>5.4 (0-20)</td>
<td>7.5 (0-50)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Xerostomia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prevalence of xerostomia (%)</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Severity of xerostomia (on VAS, max 10)</td>
<td>8.5±1.4</td>
<td>6.7±2.4</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>USSF (ml/15 min)</td>
<td>0.1</td>
<td>0.4</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Duration (years)</td>
<td>5.8 (0-22)</td>
<td>6.8 (0-45)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Salivary gland biopsy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Focus score ≥1 (%)</td>
<td>92</td>
<td>84</td>
<td>p=0.011</td>
</tr>
<tr>
<td>Average focus score</td>
<td>5.5±4.3</td>
<td>4.0±3.3</td>
<td>NS</td>
</tr>
<tr>
<td>Average fibrosis score (out of 3)</td>
<td>1.0±0.82</td>
<td>1.1±0.68</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS: not significant.

**Conclusions.** ACA+ SS is associated with more severe subjective xerophthalmia and more severe subjective and objective xerostomia compared to ACA- SS. Furthermore, the majority of ACA+ SS patients meet the AECG criteria for pSS despite having negative serology for anti-Ro/SSA or anti-La/SSB antibodies.

**Reference**
Clinical and immunological features of anti-centromere antibody-positive primary Sjögren's syndrome

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1Division of Rheumatology, Department of Internal Medicine, Keio University School of Medicine, Tokyo, Japan. 2Department of Rheumatology, National Tokyo Medical Center, Tokyo, Japan.

Background. Anti-centromere antibody (ACA)-positive Sjögren’s syndrome (SS) is considered as a subtype in SS. Recent international collaborative large scale cohort study highlighted several clinical features such as Raynaud’s phenomenon, sclerodactyly and extra glandular dysfunction (Arthritis Care Res. 2016). Assessment of ACA is potentially valuable for definitive diagnosis of this subtype and medical management in a certain number of patients uncovered by current 2016 ACR/EULAR classification criteria. However, enough information of clinical and immunological features of ACA positive SS has not been accumulated and clinical significance of ACA in SS may not be fully established. The aim of this study is to clarify clinical and immunological features of ACA positive SS.

Methods. All patients with primary SS who visited to our Division of Rheumatology at Keio University Hospital in Tokyo between May 1995 and July 2017 were enrolled. Clinical information and immunological tests including immunoglobulins (g) and serum autoantibodies were collected and statistically analyzed.

Results. Six hundred and one patients were clinically classified as primary SS (female: 94%, mean age: 56±15). They were divided into 4 groups by serum ACA and anti-SS-A antibody status. Only discrete-speckled pattern in anti-nuclear antibodies (ANA) test and/or anti-centromere antibodies positive (ACA alone) were detected in 33 patients (5.5%), while only anti-SS-A antibodies with no ACA (SS-A alone) were detected in 465 patients (77.4%). Number of patients with both ACA and anti-SS-A antibody (Double positive) was 29 (4.9%), while 74 patients had neither ANA nor anti-SS-A antibody (Seronegative). Then we statistically compared these 4 groups. The proportion of dryness was no difference among 4 groups. The proportions of Raynaud’s phenomenon or sclerodactyly were higher in ACA alone and Double positive groups (p<0.01 or p<0.001). The extraglandular involvements of SS were significantly less in the ACA alone group than in the SSA alone and Double positive groups (p<0.001). The proportions of increase of serum IgG or IgA were 10% or 6% in ACA alone group, 61% or 20% in SSA alone group, 50% or 29% in Double positive group and 20% or 4% in Seronegative group (p<0.01 or p<0.001). Existence of anti-SS-A antibody, not ACA associated to high concentration of IgG or IgA, while there was no difference between 4 groups as IgM (p=0.49). Regarding the proportion of low C3, C4 or CH50, there were no differences among 4 groups. Remarkably, the proportion of leukocytopenia in ACA alone group was significantly lower than the others (p=0.011). As compared with major organ involvements, such as pulmonary, cardiac or articular involvements, no differences were found among 4 groups.

Conclusions. Our large-scale study identified distinct characteristics of ACA-positive SS patients different from anti-SS-A antibody-positive or seronegative SS patients in Japanese population.

The breakpoint of the pathogenesis of anti-centromere antibody positive Sjögren’s syndrome by analyzing MicroRNA expression from the minor salivary gland

Shinichiro Tsunoda1,2, Takahiro Yoshikawa2, Nozomu Moriya, Yuichi Yokoyama2, Masahiro Sekiguchi, Naoki Hashimoto, Kiyoshi Matsui, Hajime Sano1
1Division of Rheumatology, Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya, Japan. 2Division of Immunology & Rheumatology, Department of Internal Medicine, Sumitomo Hospital, Osaka, Japan. 3Japan Poison Information Center, Tsukuba, Japan.

Background. Anti-centromere antibody (ACA) is one of the specific autoantibodies of systemic sclerosis. Recently it is also recognized in patients with Sjögren’s syndrome (SS) who differ from classical SS patients with anti-Ro/SS-A and anti-La/SS-B antibodies in several clinical and laboratory parameters. MicroRNAs (miRNAs) are small conserved non-coding RNA molecules that post-transcriptionally regulate gene expression by targeting the 3’ untranslated region of specific messenger RNAs for degradation or translational repression. MiRNAs play an important role in innate immunity and acquired immunity. We investigated miRNAs from the minor salivary gland (MSG) to elucidate the pathogenesis of SS due to different autoantibodies between anti- centromere antibody, and anti-Ro/SSA antibody and anti-La/SSB antibody.

Method. We performed lip minor salivary gland biopsy in 12 female patients with primary SS: 7 patients have ACA and 5 patients have anti-SS-A/SS-B antibody. We extracted miRNA using RNeasy Mini Kit (QIAGEN) from MSG and did the comprehensive analysis of 2565 types of miRNA expression using miRNA oligo chip (3D-Genetrix™ from Toray Industries). Then, we compared the expression of miRNAs with the pathological classification by the extent of cell infiltration in the lip salivary glands (Greenspan classification) between ACA positive patients and anti-SS-A/SS-B antibody positive patients. Next we validated by real time PCR of these miRNAs. Finally, we used Ingenuity Pathway Analysis software to construct a molecular interaction network.

Results. We classified patients based on types of autoantibody and Greenspan’s(GS) pathological classification, then we defined them as follows: Group I: ACA positive and GS grade 1 or grade 2. Group II: ACA positive and GS grade 3 or grade 4. Group III: Anti-SS-A/SS-B antibody positive and GS grade 3 or grade 4. Group I had 4 patients and Group II had 3 patients, and Group III had 5 patients. Comparing Group II and Group III (Group III/Group II ratio) as the same degree of tissue injury, the upregulated expression of miRNA was hsa-miR-155-5p, hsa-miR-150-5p, hsa-miR-146a-5p and hsa-miR-142-3p, and the downregulated expression of miRNAs were has-miR-133b, has-miR-1-3p, has-miR-203a-3p, has-miR-144-3p and has-miR-744-3p. Comparing Group I + II and Group III (Group III/Group I + II ratio) as the differences in the autoantibodies, the upregulated expression of miRNA were hsa-miR-155-5p, hsa-miR-1-3p, hsa-miR-146a-5p, hsa-miR-142-5p and hsa-miR-142-3p, and the downregulated expression of miRNAs were hsa-miR-133a-3p, hsa-miR-1-3p, hsa-miR-133b, hsa-miR-203a-3p, and hsa-miR-144-3p. By the validation of these miRNAs, hsa-miR-744-3p and hsa-miR-144-3p in MSG from ACA positive patients were downregulated significantly than that from anti-SS-A/SS-B antibody positive patients.

From the Ingenuity Pathway Analysis, IL-6 and TNF were associated with the different pathogenesis of SS.

Conclusions. From the analysis of miRNA from MSG having different autoantibodies, the different expression of microRNA could relate the regulation of inflammatory cytokines.

P2X7 and P2Y2 receptors as therapeutic targets in Sjögren’s syndrome mouse models

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Background. Salivary gland dysfunction and other disorders related to hyposalivation affect the quality of life for millions of patients who are severely impacted by dry mouth, oral infections and poor nutrition. Loss of saliva secretion is symptomatic of Sjögren’s syndrome (SS), an autoimmune exocrinopathy associated with lymphocytic infiltration of the salivary gland, autointubation production and tissue degeneration. Clinical trials for drugs targeting P2 receptors for extracellular nucleotides have been carried out for the treatment of human diseases, including rheumatoid arthritis, cystic fibrosis and Crohn’s disease. Previously, we demonstrated that activation of the extracellular adenine 5’-triphosphate (ATP)-gated ionotropic P2X7 receptor (P2X7R) in salivary epithelium enhances salivary gland inflammation by promoting cell apoptosis, reactive oxygen species production and cytokine release. We have also shown that upregulation and activation of the G protein-coupled P2Y2 receptor (P2Y2R) for extracellular ATP and uridine 5’-triphosphate (UTP) during salivary gland inflammation contributes to proliferation and migration of immune cells through transactivation of growth factor receptors and integrins, metalloprotease-mediated growth factor release and cytokine production. Thus, we are investigating the effects of selective antagonists or deletion of the P2X7R or the P2Y2R in mouse models of SS.

Results. Selective antagonism of the P2X7R with AR-C118925 reduces inflammation and improves carbachol-induced
saliva flow in SS-like mice. Knockout of the P2Y2 in the IL-14 mouse model of SS significantly reduced immune cell infiltration in salivary glands.

Conclusions. These data suggest that targeting the P2X7 and P2Y receptors is an effective therapeutic strategy to limit inflammation associated with salivary gland disorders. This study was supported by National Institutes of Health (NIH) R01 grants DE007389 and DE023342 from the National Institute of Dental & Craniofacial Research (NIDCR).

P-65
TUDCA reduces inflammatory markers in salivary gland cells decreasing MUC1 accumulation. A potential therapeutic agent to ameliorate inflammation in Sjögren’s syndrome

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Background. Salivary glands (SG) of Sjögren’s syndrome patients produce high levels of pro-inflammatory cytokines and show evidence of endoplasmic reticulum (ER) stress, such as ER cistern dilation, mucin (MUC1) accumulation and increased levels of ER-associated protein degradation (ERAD) machinery components (1). Tauroursodeoxycholic acid (TUDCA) alleviates ER stress and decreases the expression of proteins implicated in inflammation (2). In this study, we determined the effect of TUDCA on MUC1 expression and secretion, expression of inflammatory markers and levels of ERAD components in acinar epithelial cells stimulated with pro-inflammatory cytokines.

Methods. Differentiated human salivary gland (HSG) cells were incubated with 10 ng/mL of TNF-α or IFN-γ for 6 hours, and then co-incubated with TUDCA up to 24 hours. The mRNA and protein levels of SEL1L and EDEM1 (ERAD markers) were determined by semi-quantitative reverse transcriptase (RT) qPCR and western blot analysis, respectively. The MUC1 mRNA and protein levels were evaluated in HSG cell cultures treated with cytokines and/or TUDCA, and/or stimulated with 10 mM carbamol for 45 minutes. The subcellular localization of SEL1L, EDEM1, MUC1 and RelA/p65 was determined by confocal immunofluorescence. Additionally, mRNA levels of IL-1β, IL-6, and TNF-α were determined by (RT)-qPCR.

Results. The protein and mRNA levels of SEL1L and EDEM1 were increased in HSG cells stimulated with IFN-γ or TNF-α, while the co-incubation with TUDCA up to 24 hours decreased the expression of cytokines and/or TUDCA, and/or stimulated with 10 mM carbamol for 45 minutes. The subcellular localization of SEL1L, EDEM1, MUC1 and RelA/p65 was determined by confocal immunofluorescence. Additionally, the mRNA levels of IL-1β, IL-6, and TNF-α were determined by (RT)-qPCR.

Conclusions. Our data indicate that TUDCA inhibited the expression of pro-inflammatory cytokines in HSG cells through down-regulating protein levels of the ERAD machinery components SEL1L and EDEM1. Additionally, TUDCA reversed the effects of TNF-α and IFN-γ over MUC1 mRNA and protein levels. Interestingly, TUDCA reduced NFκB nuclear translocation and pro-inflammatory markers expression in HSG cells treated by TNF-α and IFN-γ, suggesting inactivation of the NFκB pathway. Based on these findings we propose that TUDCA has an anti-inflammatory effect and alleviates the ER stress of SG from Sjögren’s syndrome patients.

Funding. Fondecyt 1160015 and Fondecyt Postdoctoral Grant 3170023.

References

P-66
Discovery of drug candidates for primary Sjögren’s syndrome that target a BAFF receptor

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1 Div. of Rheumatology, Dept. of Internal Medicine, Keio University School of Medicine, Tokyo, Japan. 2Clinical and Translational Research Center, Keio University Hospital, Tokyo, Japan. 3Research Unit/Immunology & Inflammation, Mitsubishi Tanabe Pharma Corporation.

Background and Purpose. We have reported that peripheral monocytes of patients with primary Sjögren’s syndrome (pSS) produce higher amount of IL-6 upon stimulation with BAFF in vitro as compared to healthy controls. We have also found that the expression level of a BAFF receptor (BR3) is elevated in pSS monocytes and that the level is significantly and positively correlated with serum IgG level of the patients. These data collectively suggest that the elevated expression of BR3 on monocytes is involved in the pathogenesis of pSS and that BR3 is a possible therapeutic target to treat pSS. We have successfully discovered two pyrrolopyrimidine derivatives, BIK-12 and BIK-13, as BR3 antagonists using our original high-throughput screening system. In this study, we investigated the effects of these compounds in vitro and in vivo to explore the possibility if the compounds are candidates of drugs to treat pSS.

Methods. Peripheral monocytes and B cells were co-cultured and the amount of IgG in the culture supernatant was measured. The mixed cells were stimulated with soluble BAFF (sBAFF) in the presence or absence of BIK-12 or BIK-13 to investigate if the compounds could antagonize the stimulation. The effects of the compounds on differentiation of B cells were examined by stimulating PBMC with B cell stimuli in the presence or absence of the compounds, followed by analysis of the expression levels of CD19/CD38/IgD/CD138 and activation-induced cytidine deaminase by FACS and quantitative RT-PCR, respectively. In order to show the effects of BIK-12 and BIK-13 in vivo, these compounds were administered i.p. three times a week to MRL/lpr and NZBW/F1 mice. At the end of the treatment, the mice were sacrificed by euthanasia, and immunohistochemical analysis of lacrimal and salivary glands was carried out.

Results. IgG production by B cells in a co-culture with monocytes was significantly suppressed by BIK12 and BIK13 in a dose dependent manner when the cells were stimulated with sBAFF. At higher concentrations, the compounds suppressed sBAFF-induced IL-6 production by peripheral monocytes, suggest that the compounds suppress IgG production through inhibiting BAFF-binding to BR3 on not only B cells, but also monocytes. Notably, differentiation of activated B cells into plasmablasts and/or plasma cells was inhibited by these compounds in a dose dependent manner. The analysis of in vivo effects of the compounds revealed that the compounds suppressed the increase in an anti-dsDNA antibody in both MRL/lpr and NZBW/F1 mice after 16 weeks of treatment. In addition Immunohistochemical analysis indicated that the compounds suppressed infiltration of lymphocytes to lachrymal and salivary glands of these mice.

Conclusions. Our results collectively suggest that BIK-12 and BIK-13 inhibited activation of not only monocytes but also B cells and cross talk between B cells and at least monocytes plays a role in pathogenesis of pSS and that BR3 is involved in the interaction. In addition, BR3 is a possible therapeutic target to treat pSS. Moreover, our findings strongly suggest that BIK12 and BIK13 are drug candidates for hyper-activated B cell-related autoimmune diseases, such as pSS.

P-67
Sjögren’s syndrome is associated with reduced sex hormone exposure: a cross-sectional study

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Background: Primary Sjögren’s Syndrome (SS) is an autoimmune disease with female predominance and perimenopausal onset, indicating a potential role for sex hormones in its pathogenesis. The goal of this study was to evaluate whether cumulative sex hormone exposure impacts the risk of development of SS.

Clinical and Experimental Rheumatology 2018
Methods. This is a cross-sectional study of women from the Sjögren’s Internatinal Collaborative Clinical Alliance (SICCA) registry. 1320 cases of SS satisfied the ACR/EULAR 2016 criteria. 1520 sicca controls were defined as women with sicca signs who did not meet ACR/EULAR criteria for SS, lacked anti-SS-A antibody, and had normal minor salivary gland biopsy. Composite estrogen score (CES) was calculated by point assignment for early menarche (≤10 years), high parity, hysterectomy, use of hormone therapy, and late menopause (≥53 years). Cumulative menstrual cycling (CMC) years for premenopausal subjects was calculated as the age of the subject minus date of onset of sicca symptoms minus time pregnant. CMC years for postmenopausal women was calculated as age of menopause or date of onset of sicca symptoms (whichever was first) minus age of menarche minus time pregnant. Covariates included age, referral source, race, education level, employment status, smoking status, and recruitment site. Multivariable logistic regression was used for outcomes against the predictors of interest and all results are interpreted in terms of odds ratios.

### Table 1. Odds ratios for association of sex hormone exposure and Sjögren’s syndrome.

<table>
<thead>
<tr>
<th>CES</th>
<th>OR</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>0.85</td>
<td>0.70-1.02</td>
<td>0.08</td>
</tr>
<tr>
<td>2</td>
<td>0.78</td>
<td>0.60-1.01</td>
<td>0.06</td>
</tr>
<tr>
<td>3</td>
<td>0.56</td>
<td>0.33-0.96</td>
<td>0.03</td>
</tr>
<tr>
<td>4</td>
<td>1.79</td>
<td>0.11-29.78</td>
<td>0.68</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CMC (yrs) premenopausal</th>
<th>OR</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;13-≤16</td>
<td>0.77</td>
<td>0.45-1.31</td>
<td>0.33</td>
</tr>
<tr>
<td>&gt;16-≤18</td>
<td>0.79</td>
<td>0.46-1.38</td>
<td>0.41</td>
</tr>
<tr>
<td>&gt;18</td>
<td>0.54</td>
<td>0.35-0.85</td>
<td>0.01</td>
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</table>

<table>
<thead>
<tr>
<th>CMC (yrs) postmenopausal</th>
<th>OR</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;13-≤16</td>
<td>0.6</td>
<td>0.25-1.43</td>
<td>0.25</td>
</tr>
<tr>
<td>&gt;16-≤18</td>
<td>0.6</td>
<td>0.23-1.58</td>
<td>0.23</td>
</tr>
<tr>
<td>&gt;18</td>
<td>0.61</td>
<td>0.37-0.99</td>
<td>0.047</td>
</tr>
</tbody>
</table>

OR: odds ratio; CES: composite estrogen score; CMC: cumulative menstrual cycles.

Results. SS subjects had a statistically significant reduced risk of cumulative sex hormone exposure, when compared to controls, as measured by CES of 1-3 (Table). This risk reduction increased progressively from the CES1 to CES3 strata. This association was not significant for the highest stratum of CES, but the number of subjects in this stratum was small, leading to wide confidence intervals. This finding was corroborated by the analysis of CMC. At the highest level of premenopausal CMC (>18 years), there was a 46% reduced risk of cumulative sex hormone exposure among premenopausal SS subjects relative to the control group, after adjusting for other covariates. This reduction was statistically significant (p=0.01). However, this finding was not observed at lower CMC levels. Similarly, among postmenopausal SS subjects, the risk was also significantly reduced at CMC>18 years (p=0.047).

Conclusions. Women with SS have lower estrogen exposure and cumulative menstrual cycling compared to a non-autoimmune sicca control group. As estrogen exposure and cumulative menstrual cycling increased, there was a trend toward decreased risk of SS. Further longitudinal studies of sex hormone exposure in SS are needed to confirm these findings.

### P-68

Sex hormone exposure may influence clinical characteristics of Sjögren’s syndrome: a cross-sectional study

Sara S. McCoy1, Emmanuel Sampene2, Alan N. Baer3
1Department of Medicine, University of Wisconsin School of Medicine and Public Health, Madison, WI; 2Department of Biostatistics, University of Wisconsin School of Medicine and Public Health Madison, Madison, Wisconsin; 3Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA.

Background. Primary Sjögren’s Syndrome (SS) is an autoimmune disease with female predominance and perimenopausal onset, suggesting a role for sex hormones in its pathogenesis. The goal of this study was to evaluate whether sex hormone exposure may impact disease phenotype of women with SS.

Methods. This is a cross-sectional study of women from the Sjögren’s International Collaborative Clinical Alliance (SICCA) registry. 1320 women with SS satisfied the ACR/EULAR 2016 criteria. Composite estrogen score (0-5) was calculated by points for early menarche (≤10 years), high parity, hysterectomy, use of hormone therapy, and late menopause (≥53 years). Cumulative menstrual cycling (CMC) for premenopausal subjects was calculated as the age of the subject minus date of onset of sicca symptoms minus time pregnant. CMC for postmenopausal women was calculated as age of menopause or date of onset of sicca symptoms (whichever was first) minus age of menarche minus time pregnant.

Results. Statistical analyses are reported for CES levels of 1-3, given the paucity of subjects with CES levels of 4 or higher (Table). Among SS subjects with CES levels of 3 compared to those with CES levels of 0, there was a statistically significant reduction in risk of abnormal OSS, RF, and anti-SSA antibody and increased risk of arthritis. These findings were not corroborated by an analysis of CMC.

Conclusions. Women diagnosed with SS, estrogen exposure was associated with reduced risk of abnormal OSS, RF, and anti-SSA antibody and increased risk of arthritis. CMC did not parallel these findings, potentially indicating estrogen exposure and other sex hormones involved in menstruation may differentially influence features of SS. Further longitudinal studies are required.

### Table 1. Odds ratios for association of sex hormone exposure with features of Sjögren’s syndrome.

<table>
<thead>
<tr>
<th>Feature</th>
<th>CES 1-3 vs. CES 0</th>
<th>OR</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-SSA</td>
<td>2.98 (1.55-5.76)</td>
<td>0.0016</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-DNA</td>
<td>2.15 (1.14-4.04)</td>
<td>0.0191</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RF</td>
<td>2.87 (1.56-5.32)</td>
<td>0.0009</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

OR: odds ratio; CES: composite estrogen score.

Gene expression profiles in primary Sjögren’s syndrome with and without active systemic manifestations

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Background. Different phenotypes characterize the clinical spectrum of primary Sjögren’s syndrome (SJS). Patients with a clinical expression limited to glandular features (GFs) are classically distinguished from patients with extra-glandular manifestations (EGMs). The former patients often complain higher level of fatigue and widespread pain (WP) (Segal et al. 2013). This suggests that gene expression pattern may be different in the two subgroups. The present study is aimed at investigating the differences of gene expression in SJS patients with and without EGMs.

Methods. All of the enrolled patients met the 2016 ACR-EULAR classification criteria for SS. Gene expression of peripheral blood mononuclear cells (PBMCs) from 2 patients with SJS and EGMs and 2 patients with GFs alone was preliminarily analyzed using Clariom D human Affymetrix gene chip (Affymetrix, Santa Clara, CA, USA), and compared to that found in healthy controls. Differences in gene expression were evaluated by the analysis of variance (ANOVA) and Step-Up FDR-controlling procedure, being FDR corrected p value ≤0.01 and fold change >1.5 considered as statistically significant.
Validation of the gene overexpression was performed by quantitative Real Time(qRT)-PCR in PBMCs from 9 SS patients with EGMs and 6 with GFs alone, using the ΔΔCt method for comparing relative fold expression differences.

Results. The total group of SS patients was composed by 18 females and 1 male. All of the patients had a positive lip biopsy, while anti-SSA/RO antibodies were detected in 10/11 and 6/8 of the SS patients with and without EGMs, respectively. ESSDAI value ranged from 7 to 55 in SS patients with EGMs (median 17), and from 0 to 2 in patients with GFs alone (median 1). In both types of patients, the functional analysis of the two transcriptomes showed a large number (>1000) of modulated genes that are involved in the biological processes (i.e., apoptosis, inflammatory response, immune response, type I and type II interferons, and Toll-like receptors signaling) strictly connected to the known pathological processes of SS. Genes involved in sensory perception and in nociceptive signalization (i.e., ANPEP, TNRF1, P2R1, 5HT1) were modulated only in patients with GFs alone. The significantly different expression of these selected genes in the two SS subgroups was confirmed by the qRT-PCR analysis.

Conclusions. These data indicate that in SS patients with GFs alone a dysregulation of pain signaling pathways (namely beta-adrenergic receptor signaling and Notch signaling) may play a role in the development of WP that is common in this subset of patients. The biological mechanisms underlying the activation of these genes remain to be clarified.

P-70

The role of the innate immune system in primary Sjögren’s syndrome

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1Department of Ophthalmology, 2Brain School of Public Health and Community Medicine, 3School of Medicine, 4Department of Hematology, 5Department of Internal Medicine 1Department of Health Sciences Research, Mayo Clinic, Rochester, MN, USA. 2Department of Oral Medicine & Sjögren’s syndrome Center Hadassah-Hebrew University Medical Center, Jerusalem.

Background. Cytokine-related genes are assumed to be key players in dry eye syndrome (DES) and Sjögren’s syndrome (SS) pathogenesis. However the association between specific gene variants and both DES and SS are unclear, and comparisons between these two diseases has not yet been performed. In this study we compared single nucleotide polymorphism (SNP) variation in genes encoding cytokine levels among SS and DES patients in Israel.

Methods. A total of 180 subjects were recruited, 82 with SS and 98 with DES. Using a candidate gene approach and allele-specific PCR technique for genotyping, the proportions of risk alleles in TNF-α (rs1800629), IL10 (rs1800896) and TNAIP3 (rs2230926) SNPs were compared between study groups.

Results. The allelic distribution of the study groups was found to be very similar and match to Caucasians (CEU – Northern Europeans from Utah) population distributions in these SNPs. While none of the SNPs variants were found to be statistically significant associated to SS or DES in a recessive model, in an additive model the TNFα (rs1800629)-G risk allele was found among a higher proportion of SS patients compared to DES (Homozygote-G: 70.8% vs. 64.7%; Heterozygote: 26.9% vs. 11.2%, respectively, p=0.02). After adjusting for possible confounders, none of the tested SNPs were associated with SS compared to DES.

Conclusions. The frequency of IL10 (rs1800896-A) and TNAIP3 (rs2230926-G) alleles was not found significantly differ between SS and DES patients. These findings may be due to limited power of the sample size of 180 participants. The TNF-α (rs1800629-G) SNP seems to be associated with SS in an additive model. TNF-α protein levels are known to be associated with inflammation, outcome of infection, and susceptibility to autoimmune diseases such as SS. The gene has also been associated with non-Hodgkin lymphoma, a serious complication of SS. Further comparison to healthy controls is required, as well as exploring other SNPs variants relating to the immune pathway in order to understand the genetic basis of DES and SS etiology.

P-71

Identification of dysregulated immune-related gene networks in primary Sjögren’s syndrome

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1Dept. of Oral Biology, School of Dental Medicine, State University of New York at Buffalo, Buffalo, NY, USA. 2Dept. of Biostatistics, School of Public Health and Health Professions, State University of New York at Buffalo, Buffalo, NY, USA.

Primary Sjögren’s Syndrome (pSS) is an autoimmune disease with both oral and systemic disease manifestations. Similar to pSS patients, NOD.B10Sn-H2b/J (NOD.B10) mice develop lymphocytic infiltration in exocrine tissues, lose salivary flow, and display anti-nuclear autoantibodies. While pSS is clearly mediated by immune dysfunction, the pathways and networks that mediate disease are incompletely understood. Our objective was to characterize the genetic landscape in splenic tissue from NOD.B10 female mice with clinical disease in order to identify novel immune-related pathways that may be targeted therapeutically in disease. Spleens were harvested from female NOD.B10 mice with clinical disease and age and gender-matched C57BL/10Sn controls (n=3 each). RNA was isolated and RNA-sequencing (RNA-seq) performed. Raw sequence reads were aligned and mapped to the current reference Mus musculus genome sequence using RNA-seq algorithms. Gene expression levels were calculated as fragments per kilobase of transcript per million mapped reads. We employed Cufflinks and Cuffdiff to determine gene transcript levels. Differential gene expression (DEG) analysis was carried out using DESeq2. The DEGs were analyzed by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis to determine the gene networks that were altered between pSS and control animals. We identified numerous pathways that were differently expressed between splenic tissue derived from NOD.B10 females and that from healthy controls. Many of these were related to innate and adaptive immune dysfunction, including B and T cell receptor pathways, cell adhesion molecule signaling, and networks involved in viral responses. In conclusion, our RNA-seq results identified numerous signaling pathways that are dysregulated in splenic tissue derived from a pSS mouse model. These data provide a foundation for future studies to establish the therapeutic relevance of these networks to human disease.

P-72

Genetic differentiators of Sjögren’s Syndrome subtypes in an international cohort

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1University of California, San Francisco, Russell / Engleman Rheumatology Research Center, San Francisco, CA; 2University of Washington, 3Johns Hopkins University, 4University of California, San Francisco.

Background. While much has been learned in recent years about the genetics of Sjögren’s Syndrome, little has been studied at the level of the major disease subphenotypes, which are important for understanding disease etiology and potential treatments. Our goal is to understand the genetic contributions to differences in the clinical manifestations of Sjögren’s Syndrome, particularly with respect to ocular versus oral involvement.

Methods. We studied 3,355 participants in the Sjögren’s International Collaborative Clinical Alliance (SSCCA).

1University of California, San Francisco, Russell / Engleman Rheumatology Research Center, San Francisco, CA; 2University of Washington, 3Johns Hopkins University, 4University of California, San Francisco.

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tive for the FS criteria but not the OSS criteria, compared to those who were OSS positive and FS negative.

Results. In our all-subjects and European analyses, numerous genes in the MHC region were significantly associated with both high FS (top all-subjects p=2e-25, OR=2.7; top European p=2e-22, OR=2.8) and high OSS (top all-subjects p=1e-19, OR=2.1; top European p=4e-17, OR=2.1). MHC genes were involved in the top pathways of both subphenotypes, including interferon gamma signaling and the MHC class I protein complex. As seen previously for SS, MHC variation appears to have a much lesser role in Asian patients for these subphenotypes. SNPs in IRF5 were significantly associated with high FS (top SNP kgp2820799; all-subjects OR=1.5, p=9e-10; European OR=1.7, p=2e-8) whereas SNPs in STAT4 (all-subjects top SNP kgp99676217, OR=1.4, p=1e-8; European top SNP rs7574865, OR=1.6, p=1e-9) were significantly associated with high OSS.

In a multivariate model of risk SNPs in European patients, FS-positive/OSS-negative patients were more likely to have risk alleles of GTF2I (kgp10686975, OR=1.9, p=0.021), HLA-DRA (rs9036098, OR=1.7, p=0.004), NFAT5 (rs244418, OR=1.7, p=0.003), BLK (rs2735340, OR=1.7, p=0.009), RELN (rs7341475, OR=1.6, p=0.042), and IRF5 (rs7428142, OR=1.5, p=0.026). In multivariate analysis of risk SNPs in Asian patients, FS-positive/OSS-negative patients were more likely to have risk alleles of HLA-DRA (rs6903608, OR=3.7, p=0.001) and TNP1 (rs7392785, OR=3.2, p=0.025).

Conclusions. The FS and OSS subphenotypes have a combination of shared and distinct genetic contributors, which also vary according to ancestry.

P-74

Transcription factors ETS1 and LEF1 as potential pathogenic biomarkers of Sjögren’s syndrome

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Department of Oral Medicine, Carolinas Medical Center, Charlotte, NC, USA.

Background. Sjögren’s syndrome (SS), is a chronic systemic autoimmune disease mainly characterized by severe dry eyes and dry mouth and mostly affecting women. Despite extensive research, the etiology of this disease is not fully understood. Currently, the American college of rheumatology/European league against rheumatism (ACR-EULAR) criteria are being used for the classification of primary SS. Under the ACR-EULAR classification, lymphocytic infiltration in the minor salivary gland (MSG) is defined as a focal score (FS) ≥1 can be designated as SS positive. Using a computational meta-analysis, we previously identified ETS1 and LEF1 as candidate biomarkers of SS. In this study, for the first time, dual staining immunofluorescence was used to analyze the protein expression and localization of ETS1, LEF1, and MMP9 in SS patients compared to non-SS sicca control patients.

Methods. The paraffin embedded MSVs were cut (5 μm) from 11 SS and 13 non-SS sicca patient control samples. These sections were deparaffinized and processed for antigen retrieval. Next, tissue sections were blocked and then incubated with primary antibodies anti-ETS1-Cy3 (1:10), anti-LEF1-Cy3 (1:10), and anti-MMP9-TIMP1 (1:10) for 1 hour, followed by incubation with Cy3-Cy5 (green-red) conjugated secondary antibody for dual staining. Sections were mounted and images were captured using confocal microscope. Total fluorescence intensity and H-score for individual staining were calculated.

Results. Our immunofluorescence analysis revealed that all MSV tissue sections from SS patients with FS ≥1 had most areas with moderate to high intensity of LEF1-ETS1-MMP9 colocalization, whereas MSG tissue sections from SS patients with FS <1 had mostly very low to moderate intensity areas of LEF1-ETS1-MMP9 colocalization. Since ETS1 and LEF1 are transcription factors known to increase MMP9 expression in cancer cells, it can be hypothesized that MMP9 upregulation is due to increased ETS1 and/or LEF1 expression in the MSVs of SS patients.

Conclusions. Our analysis showed that both ETS1 and LEF1 are significantly upregulated and co-localized with MMP9 expression in the MSVs of SS patients, but not in non-SS sicca control patients. These results suggest the potential importance of ETS1 and/or LEF1 upregulation as candidate biomarker(s) for the diagnosis of SS.

P-75

Functional characterization of the Sjögren’s syndrome-associated locus DDX6-CACR5

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1Arthritis and Clinical Immunology Research Program, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma, USA. 2Department of Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, USA. 3Department of Pharmacology, University of Oklahoma College of Medicine, Oklahoma City, Oklahoma, USA. 4Department of Oral and Maxillofacial Pathology, University of Oklahoma College of Dentistry, Oklahoma City, Oklahoma, USA. 5Oral Diagnosis and Radiology Department, University of Oklahoma College of Dentistry, Oklahoma City, Oklahoma, USA. 6Clinical Immunology Unit, Department of Internal Medicine, Stavanger University Hospital, Stavanger, Norway. 7Department of Medicine, Karolinska Institute and Karolinska University Hospital, Stockholm, Sweden. 8Sjögren’s Syndrome Clinic, National Institute of Dental and Craniofacial Research, Bethesda, MD, USA. 9Department of Clinical Immunology and Rheumatology, Hannover Medical School, Hannover, Germany. 10Department of Rheumatology, Haukeland University Hospital, Bergen, Norway. 11Broedelmann Research Laboratory, Department of Clinical Science, University of Sydney, Sydney, Australia. 18Department of Rheumatology, The Queen Elizabeth Hospital and Discipline of Medicine, University of Adelaide, South Australia. 10Department of Medicine, University of Oklahoma Health Sciences Center, Oklahoma City.
Background. Sjögren’s syndrome (SS) is a chronic, heterogeneous disease with hallmark features of auto-inflammation and autoantibody production. We previously identified association between the DDx6-CXCR5 locus and SS surpassing genome-wide significance. The goal of this study is to determine the mechanism by which this association contributes to disease.

Methods. Fine mapping and imputation approaches were used to enrich existing genetic datasets using 1916 SS cases and 3684 controls to include ~2x the testable variants as previous, 971, in the DDx6-CXCR5 interval. Statistical approaches and bioinformatics data were used to prioritize candidate variants. Electromobility shift assays (EMSA) and pull-downs (PDs) followed by mass spectrometry were used to determine allele-specific differences in binding using lysates from HSB-2 (T), Jurkat (T), Reh (B), Ramos (B), Daudi (B), THP-1 (monocyte), and HEK 293T (epithelial) cells.

Results. In the DDx6-CXCR5 region, imputation showed a pattern of associations consistent with the length of the DDx6 coding sequence to the proximal promoter of CXCR5. Bioinformatic analysis of the top associated variants (rs7125066 and rs7119038) did not yield evidence of regional functionality. However, 46 other candidates that span the region of association were identified. Chromatin methylation pattern data from the Roadmap database showed several variants in this region were within transcription start sites or enhancer elements depending on the cell type and state. Using RegulomeDB, Haploreg, and other databases, 4 variants showed strong evidence they affect binding and/or expression of one or more target genes in the region. Thus, rs9438572, rs12365699, rs75494551, and rs10892294 were selected for further study. Using EMSSAs, the risk allele resulted in a statistically significant decrease in binding when compared to the non-risk allele for rs9438572 (p<0.01) using cell lysates from HSB-2, Reh, THP-1, Ramos, Jurkat, and Daubi but not HEK 293T cells. However, the differences were more pronounced for rs10892294 and rs75494551, which decreased binding with the risk allele only using Ramos cells. Moreover, the risk allele decreased binding at rs10892294 and rs75494551 increased binding at rs12365699. The rs75494551 risk allele also decreased binding within the THP-1 cell lysate. Preliminary mass spectrometry analysis showed several immune related transcription factors likely binding this region, including: IRF8, GTF1, FXR5, IKZF3, PA5X, and IKZF1. Cell type-specific expression of the genes in the region shows the expression of DDx6 spans many immune cell subsets while the expression of CXCR5 is more restricted.

Candidate variants in the DDx6-CXCR5 locus likely alter both protein coding genes in a cell/context specific manner. Ongoing studies will assess the role of these sequences as promoters and/or enhancers using luciferase assays and CRISPR/Cas9 deletions of the elements of interest in various cell types.

P-77

The bacterial community of the periparotid epithelium in patients with Sjögren’s syndrome


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Background. Sjögren’s Syndrome (SS) is a common autoimmune exocrinopathy that affects primarily the salivary and lacrimal glands, causing hyposalivation and chronic dryness of the mouth and eyes. Because saliva secretions are critical for oral health, SS patients report xerostomia, dryness of mouth, and risk of dental caries and oral candidiasis, which is partly attributable to the fundamental role of saliva in the metabolism of oral microorganisms. 16S rRNA has previously been used to study the oral microbiome of SS patients using DNA extracted from whole saliva, but, at best, only mild disease-specific alterations were reported. Here, we examine phylogenetic diversity in bacterial communities present on the oral mucosa adjacent to the parotid gland of SS patients and healthy controls (HCs).

Methods. DNA was extracted from buccal swabs of the parotid periglandular region of SS patients (n=12) and controls (n=13). 16S rRNA sequencing was performed using primers directed to the hypervariable V1V2 region on the Illumina MiSeq platform. Sequenced reads were processed according to standard procedures for mothur analyses. Reads were filtered for length and quality, then clustered into Operational Taxonomic Units (OTU: species defined by rRNA gene sequence) according to the reeated SEED database from SILVA version 123, provided with mothur. The phyloseq package in R was used to perform statistical analyses.

Results. On average, 134 species were observed in each sample. While no significant difference was found between patients and controls (p=0.19), SS patients tended to have fewer species. Further, no statistically significant differences were found by the alpha diversity measures Chao1, Shannon, Simpson, Fisher, and ACE indices. However, principal coordinates analysis (PCoA) of the Jensen-Shannon (JS) distance – a measure of beta diversity – showed differences between patients and healthy controls. Statistical testing on the Jaccard and JS distances between SS and HC samples proved the differences to be highly significant (p=0.001). Linear discriminant effect analysis (LDA) demonstrated SS patients to have a greater community structure.
presence of OTUs belonging to taxa in the Escherichia/Shigella group and in Enterobacteriales than did the HC, while OTUs within phylum Firmicutes and family Erysipelotrichaceae were more important in HC.

**Conclusions.** Statistically significant differences were found in the oral flora of the glandular epithelium between SS patients and HC. Differences in beta diversity (the relative distribution of organisms), but not in alpha diversity (number and types of organisms), between health and disease suggests that the communities differ significantly. The taxa enriched in SS patients are representative of non-resident bacteria, such as enterococci and coliform bacteria, whereas typical oral taxa (some being minor components of the oral flora) were more abundant in healthy subjects. Given the proximity of the sample collection site to the parotid gland, a major target organ in SS, a potential exists that the SS-associated organisms have a role in the severity or progression of the disease through their intimate relationship with the glandular region.

**P-78**

**Dysbiosis of the Oral Microbiome in Patients with Primary Sjögren’s Syndrome**


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**Background.** Early differentiation of primary Sjögren’s syndrome (pSS) patients from patients with oral dryness due to other causes is a diagnostic challenge. If the oral microbiome is specific for pSS, then the oral microbiome might serve as an early biomarker to distinguish pSS from non-SS dry mouth patients (non-SS). Therefore, the aim of our study was to assess the oral microbiome of pSS and non-SS patients in relation to healthy controls and disease controls.

**Methods.** The oral microbiome of 72 pSS patients, 90 non-SS sicca patients, 33 systemic lupus erythematosus (SLE) patients and 14 healthy controls (HC) was assessed with 16S rDNA sequencing of oral washing samples. All pSS patients fulfilled the 2016 ACR/EULAR classification criteria. Patients referred for a diagnostic workup for SS not fulfilling the classification criteria were grouped as non-SS patients. All SLE patients fulfilled the 2012 SLICC criteria. Sequencing data and summary diversity analyses were processed in QIIME 1.9.1. Samples contained at least 4000 reads/sample. Bacterial richness, diversity, overall composition and relative abundance of bacterial taxa were compared between the four groups.

**Results.** Oral washings from pSS patients showed a lower bacterial richness (number of different bacterial taxa/sample) than those of non-SS patients, SLE patients and HC (p=0.001, p=0.03, p=0.01 respectively, Wilcoxon test, Figure 1). Also bacterial diversity (Shannon index) was lower in pSS patients compared to non-SS patients. Only genus Lactobacillus was higher in pSS patients (statistics: MaAsLin in R, false discovery corrected p-value<0.1). Thus, the oral microbiome of pSS patients is characterized by a lower bacterial richness, lower diversity and an increased relative abundance of Lactobacillus. Therefore the oral microbiome of pSS patients can be classified as dysbiosis.

**Conclusions.** The oral microbiome of pSS patients is characterized by dysbiosis and differs from the oral microbiome of non-SS patients, SLE patients and healthy controls.

**P-79**

**Oxidative damage in Sjögren’s syndrome**

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Sjögren’s syndrome (SS) is a lingering inflammatory, autoimmune condition with reduced lacrimal and salivary gland secretion resulting in keratoconjunctivitis sicca and xerostomia. Autoantibodies targeting the 60 kDa Ro/60 kDa/SS-A and La/SS-B/SS-B antibodies, as well as other autoantibodies, are found in SS, with the last being the most specific for SS. Autoantibodies to 60 kDa Ro/60 kDa/SS-A and SS-B/60 kDa Ro/60 kDa are found in SS, with the last being the most specific for SS. Autoantibodies to 60 kDa Ro/60 kDa/SS-A and SS-B/60 kDa Ro/60 kDa are found in SS, with the last being the most specific for SS.

**Materials.** We studied 69 primary SS subjects, 25 age and sex matched subjects that did not meet criteria (incomplete SS), and 18 normal controls. Indices of oxidative damage, namely conjugate diene formation, and HNE or MDA-protein adducts were investigated in the sera of SS, incomplete SS and normal controls. Diluted sera from SS subjects or normal controls were coated on ELISA plates as antigen source. HNE or MDA adducts in serum proteins bound to the plates was determined with rabbit anti-HNE or anti-MDA antibodies purchased commercially. To identify specific proteins modified by HNE, sera from SS or normal controls were electrophoresed, transferred by electroblotting and subjected to immunoblotting with rabbit anti-HNE antibody. For determination of conjugate diene formation, conjugate diene formation and modification of serum proteins by the lipid peroxidation by-product 4-hydroxy-2-nonalen (HNE) or malondialdehyde (MDA) in SS, and age-and sex matched controls.

**Results.** Oxidative damage was found in the sera of primary SS subjects compared to normal controls by ELISA and immunoblotting. Serum proteins from SS subjects were found to contain HNE adducts. There was significantly more HNE-modified proteins in SS sera (n=10) compared to controls (n=10) by ELISA (0.07±0.017 versus 0.04±0.007; p=0.00015; average OD±SD). However, there was no sig-
significant difference in MDA-modified proteins between SS and controls by ELISA. When SS sera (n=34) were analyzed by immunoblotting, we found HNE adducts in several serum proteins, and significantly in a protein migrating at 18 kD. Normal control sera did not show significant HNE-modification (n=8). Our preliminary results for conjugate diene formation show that there is no significant difference between conjugate diene levels in the Sjögrens’s syndrome patients (n=25) and incomplete SS subjects (n=25).

We are pursuing HNE modification in the sera of incomplete SS subjects and also identifying the protein bands in SS subjects with HNE adducts by matrix assisted time of flight mass spectrometry.

Conclusion. Oxidative damage occurs in SS, as evidenced by the significantly elevated HNE protein adducts—but not MDA-protein adducts—in the sera of SS subjects compared to normal controls.

P-80
Transcriptomic profiling of pDCs from patients with pSS indicates an activated phenotype, enhanced anti-viral state, and increased susceptibility to apoptosis

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Background. Type-I interferons (IFN) are thought to play an important role in pSS pathogenesis. Plasmacytoid dendritic cells (pDCs) produce large amounts of type-I IFN upon activation via Toll-like receptors. pDCs infiltrate the salivary glands of patients with pSS and their numbers correlate with local IFN-production. Furthermore, pDCs are decreased in number in the peripheral blood of patients with pSS. To understand the molecular mechanisms behind systemic dysregulation of pDCs, we performed RNA sequencing on pDCs isolated from peripheral blood of patients with pSS, incomplete SS (iSS) and healthy controls.

Methods. We established two independent cohorts (each n=31), of patients and controls. pSS patients (n=25) were classified according to the 2002 AECG criteria. iSS patients (n=20) were included in both cohorts. These individuals presented with dryness complaints without a known cause, did not have any generalized autoimmune disease including pSS, and did not fulfill the classification criteria for pSS. Healthy donors (n=17) were included as control group. Peripheral blood pDCs were isolated using magnetic bead-associated cell sorting. ±20 million paired-end sequencing reads per sample were obtained using Illumina HiSeq 2500 platform.

Results. 8556 genes were differentially expressed (p-value <0.05) between all three groups in the discovery cohort. Of these, 3144 genes were also differential in the replication cohort. We generated gene modules from both discovery and replication cohorts, and found 5 gene clusters comprising 1259 genes that were consistently dysregulated in both cohorts. Interestingly, this dysregulation was regulated by a few key transcription (co)facets. Pathway analysis showed that the 5 modules contain genes associated with cellular activation, including a group of IFN-induced genes, as well as genes involved in regulation of apoptosis and intracellular transport. Generally, pDCs from patients with iSS displayed an intermediate phenotype but a few iSS patients exhibited similar degree of dysregulation in their pDCs transcriptome compared to the pSS patients.

Conclusions. Using blood pDC transcriptomics, we identified 5 gene clusters that show that pDCs from pSS patients exhibit an activated phenotype. These gene clusters are robustly replicated in two independent cohorts. Taken together, our data indicate that pDCs from the blood of patients with pSS, and to a lesser extent those with iSS, have an activated phenotype and are more susceptible to induction of migration and apoptosis.

P-81
Immune activation following seasonal influenza vaccination in patients with primary Sjögren’s syndrome

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Background. Immune triggering by environmental factors in genetically predisposed individuals has been suggested as an underlying pathogenic mechanism in development of autoimmune diseases. Although highly warranted, studies of primary infections in humans pose great logistic and temporal challenges. However, monitoring responses following vaccination enables systematic studies of immune activation to microbial agents in vivo under standardized and safe conditions. In the present study, we therefore longitudinally observed untreated and hydroxychloroquine (HCQ) treated patients with primary Sjögren’s syndrome (pSS) and healthy controls (HC) vaccinated against seasonal influenza to identify differences in immune activation and resulting cellular and serological responses.

Methods. The study included pSS patients without treatment (n=17), pSS patients receiving HCQ treatment (n=8), and HC (n=16). All participants were female and all pSS patients were positive for Ro/SSA autoantibodies. All individuals received vaccination with a non-adjuvanted trivalent seasonal influenza vaccine as part of the standard vaccination program. Blood samples were collected before vaccination, 24 hours after, on day 28, and on day 90. Clinical parameters were registered using a questionnaire based on ESSPRI. RNA expression analysis was performed in CD14+ monocytes using nanoString. Antibody titers were analysed by ELISA.

Results. Differences in the immediate response to immune activation was observed in terms of a significant decrease in the total leukocyte count 24 hours after vaccination in untreated patients with pSS (p<0.02), which was not observed in the HCQ treated or HC group. Investigation of mRNA expression in CD14+ monocytes before and 24 hours after vaccination as expected demonstrated an IFN-signature in patients before vaccination, and revealed a differential transcriptional response to vaccination in untreated patients compared to HC. In particular, higher activation of genes in the NFkB signaling pathway was observed in untreated patients following vaccination, with the top hit being the NF-kappa-B essential modulator (NEMO) (p<0.001). Further, untreated patients with pSS, but not HCQ treated patients, responded with significantly higher vaccine specific IgG titers than HC (p<0.03), and there was a positive, significant correlation of the early, higher expression of genes in the NFkB signaling pathway and the higher serological response to the vaccine one month after vaccination (Spearman R=0.44, p<0.02). Notably, levels of anti-Ro52 autoantibodies increased in untreated patients, but not in HCQ-treated patients after vaccination. No significant changes in self-reported clinical parameters were registered.

Conclusion. We observed augmented innate and adaptive immune responses to vaccination in untreated patients with pSS, suggesting an underlying hyper-responsiveness to immune challenges. The higher serological response was associated with a higher early activation of the NFkB pathway in monocytes, indicating the importance of NFkB signaling in enhanced immune responses of autoimmune individuals.

P-82
Optimized footprinting profile for profiling diseased cell state changes


Background. Sjögren’s syndrome is a complex multifactorial disease with an associated loss of salivary tissue function. Salivary gland tissues of individuals with Sjögren’s syndrome have altered transcription compared to tissues of healthy volunteers. To profile the regulatory state changes concomitant with Sjögren’s syndrome, we have optimized a pipeline for analyzing footprinting information against known protein binding information from ChIP-seq.

Methods. To optimize the pipeline, two replicates each of DNase1- and ATAC-seq data for the lymphocytic cell line GM12878 were tested in pipeline lines varying across eight parameters for a total workload of 106 computational years. Pipelines with different combinations of parameters were tested for the correlation between intermediate files of the two replicates,
replicates in intermediate files, so pipelines optimized for reproducibility are reported alongside pipelines optimized for recapitulation of biological information.

Results. Reproducibility-optimized pipelines showed a nearly twofold increase in correlation between alignments, called regions of open chromatin, and footprint placement between replicates. ChIP-seq-optimized pipelines showed a roughly 16% increase in AUC against ChIP-seq relative to default parameters when using the footprinting algorithm HINT-BC and recovered non-random prediction when using the footprinting algorithm Wellington. Conclusions. These pipelines can be used similarly to ensemble machine learning, where multiple models vote on predictions. The results from well-validated pipelines of different stringencies and strengths adds information about footprints that are gained or lost between diseased and healthy individuals, and this paves the way for understanding how changes in gene expression can be used to identify new therapeutic targets.
IDO2 was largely observed in classical dendritic cells and B-cells. These data show that IDO1 and IDO2 are expressed by distinct cells and might play different roles during SS pathogenesis. Gene expression profiling in inflamed murine SGs at different stages of the inflammatory process demonstrated IDO2 upregulation during the early and peak phases of inflammation. In contrast, IDO1 showed significant increase towards the resolution of inflammation. Differential inhibition of IDO1 versus IDO2 with MethyL-DL-Tryptophan (1-MT) given at different stages of inflammation clearly showed that IDO2 had a pro-inflammatory role wherein it was important for induction of Th17 cells and aggravation of the inflammatory process. In contrast, 1-MT treatment at later time-points (coinciding with increased IDO1 expression) resulted in persistence/retention of lymphocytic aggregates. This anti-inflammatory IDO1 function was mediated by impairing the survival/proliferation of lymphocytes in situ.

Conclusions. These data demonstrate engagement of IDO1 and IDO2 in different cell populations and moreover very distinct functions in SS pathogenesis.

P-85

RANK/RANK-ligand interaction regulates pathogenic T cell recruitment in Sjögren’s syndrome


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Background. The RANK (ligand)-RANK-OPG triad, members of the TNF(R) superfamily, is implicated in lymphoid organ development and bone homeostasis. It has recently been demonstrated that RANK-activated osteoclasts release CCL20 and attract T cells to the central nervous system in a model of Multiple Sclerosis and that transgenic RANK expression in the skin promotes aberrant epithelial cell proliferation and is sufficient to induce ectopic formation of tertiary lymphoid structures (TLS). Ductal epithelial cells (SGEs) have been implicated in Sjögren’s Syndrome (SS) pathogenesis where they mediate immune recruitment by expression of pro-inflammatory chemokines and support the formation of pre-malignant myoepithelial lesions.

Objective/Methods. A combination of human and mouse studies were used to address the role of RANK-RANKL interaction in primary (p) SS. Salivary glands (SGs) and saliva samples from patients recruited in the OASIS cohort (University of Birmingham) were studied to evaluate this pathway in human disease. Consecutive stimulated saliva samples (n=69) were analysed using Proseek Multiplex INF, covering 92 unique inflammation-related protein biomarkers. Taking advantage of a viral induced model of pSS we studied the effect of this pathway with a RANKL blocking antibody in human disease. Consecutive stimulated saliva samples (n=69) were analysed using Proseek Multiplex INF, covering 92 unique inflammation-related protein biomarkers. Taking advantage of a viral induced model of pSS we studied the effect of this pathway with a RANKL blocking antibody in human disease.

Results. Fourteen proteins in saliva were significantly separated between early and peak phases of inflammation. In contrast, IDO1 showed significant increase towards the resolution of inflammation. Differential inhibition of IDO1 versus IDO2 with MethyL-DL-Tryptophan (1-MT) given at different stages of inflammation clearly showed that IDO2 had a pro-inflammatory role wherein it was important for induction of Th17 cells and aggravation of the inflammatory process. In contrast, 1-MT treatment at later time-points (coinciding with increased IDO1 expression) resulted in persistence/retention of lymphocytic aggregates. This anti-inflammatory IDO1 function was mediated by impairing the survival/proliferation of lymphocytes in situ.

Conclusions. These data demonstrate engagement of IDO1 and IDO2 in different cell populations and moreover very distinct functions in SS pathogenesis.

P-86

T follicular-helper cells (TFH) enrichment and follicular T regulatory cells (TFR) exclusion from ectopic germinal centers in salivary glands of Sjögren’s Syndrome patients.


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Background. Lymphocytic aggregates in the salivary glands (SG) of Sjögren’s syndrome (SS) can organize in ectopic lymphoid structures (ELS) forming functional germinal centers (GCs), which are linked to the development of MALT lymphoma (MALT-L). T follicular-helper cells (Tfh) and follicular T regulatory cells (Tfr) are specialized CD4+ T helper cells that positively and negatively regulate, respectively, the magnitude of the GC response and possibly the development of autoimmunity.

Objectives. To characterize the infiltration of Tfh and Tfr in the SG of patients with SS in the presence/absence of ectopic GCs and MALT-L.

Methods. SG biopsies with matching histology and RNA from 37 SS and 38 non-specific chronic sialadenitis (NSCS) patients were stratified as ELS-/ELS+ based on CD3/CD20/CD138 immunostaining (IHC). Histological samples and mRNA from 12 parotid MALT-L were also studied. Gene expression was performed with Taqman rTPCR. Multicolor immunofluorescence/confocal microscopy for CD3, CD4, CD45RO, ICOS, PD1, BCL6 and FoxP3 was used to identify Tfh and Tfr cells. Follicular T helper cells (CD4+CD45RO+PD1+ICOS+Bcl6+) and Tfr cells (CD4+CD45RO+PD1+ ICOS+FoxP3+) are significantly increased in the ELS+ SG tissues from SS patients vs ELS- and NSCS. Tfh cells densely infiltrate the B cell rich areas and preferentially localize within ectopic GCs in the SS tissues. Furthermore, Tfh infiltration correlated with SG IL-21 mRNA expression, which in turn was strongly correlated with CD3+, CD20+ and CD138+ IHC scores and with CXCL13, Ltb, BAFF, AID and Pax5 gene expression. Finally, MALT-L tissues displayed 10-fold higher IL-21 mRNA and twice as much PD1+ICOS+Bc16+ Tfh-cells/field compared to ELS+ SS samples. The Tfh:Tfr ratio in ELS+ SG was approximately 2:1. Interestingly, while in tonsils Tfr were routinely detected within GC, in ELS+ SG Tfr were predominantly excluded from the B cell follicles and accumulated in the T cell rich areas at the periphery of the B–cell aggregates.

Conclusions. Within the SG of SS patients Tfh cells closely segregate with lesional IL-21 expression, localize within ELS and are strongly enriched during MALT-L development. Conversely, although Tfr cells are also present in ELS- and SS patients, they are excluded from ectopic GCs. This suggests that Tfr in SS SG fail to exert their physiological immunoregulatory function in controlling the magnitude of the GC response and B cell autoreactivity, as observed in secondary lymphoid organs.

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P-87

A preliminary exploration of the role of follicular T helper cell subsets in primary Sjögren’s syndrome

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Objective. Primary Sjögren’s syndrome(pSS) is an autoimmune disease characterized by the production of autoantibodies due to abnormal humoral immunity. Follicular T helper cells (Tfh) play an important role in the pathology of autoimmune diseases by directing the differentiation of B cells into memory B and plasma cells in secondary lymphoid follicles. To examine abnormalities of Tfh cells in SS patients, the percentage of Tfh cells were compared to particular clinical manifestations in patients with pSS.

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Methods. Peripheral blood was collected from 57 SS patients and 16 healthy controls. Tfh cell subsets were analyzed via flow cytometry. The clinical data and laboratory parameters were collected. Serum levels of cytokines were determined by ELISA.

Results. The proportion of Tfh cells in peripheral blood of patients with SS was significantly higher than that of healthy controls (24.08%±3.57% vs. 8.7%±2.0%, p<0.05). The frequency of Tfh cells was correlated with sCD25, Cxc, eGFR, platelet count, γ-globulin (r=0.254±0.337; r=0.338; r=0.365; rPlt=0.266; γ-globulin =0.280; p<0.05). This was significantly positively correlated with Th17 and IgA (rIgA=0.334; p<0.05). Th1 and IgG (rIgG=0.325; p<0.05).

Conclusion. Tfh cells are elevated along with increases in sCD25, IgA, IgG and decreases in platelet counts and GFR in patients with SS. Tfh cells may play an essential role in the development of SS and sustaining antibody production, which ultimately results in the activation of disease and organ damage, esp. kidney.

P-88

A potential role of NR4A2 overexpression in CD4⁺ T cells in the pathogenesis of Sjögren’s syndrome

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Background. In Sjögren’s syndrome (SS), emerging roles of Th17 cells in the pathogenesis of SS were suggested in some studies. Among Th17-related molecules, nuclear receptor subfamily 4, group A, and member 2 (NR4A2), was reported to promote Th17 differentiation via IL-21 production. In an animal model of experimental autoimmune encephalomyelitis, the purpose of this study is to clarify a potential role of NR4A2, which was up-regulated in labial salivary glands (LSGs) of patients with SS, in the pathogenesis of SS.

Methods.
1) Gene expression was analyzed by DNA microarray in LSGs of SS patients with SS was significantly higher than that of healthy controls (24.08%±3.57% vs. 8.7%±2.0%, p<0.05). The frequency of Tfh cells was correlated with sCD25, Cxc, eGFR, platelet count, γ-globulin (r=0.254±0.337; r=0.338; rPlt=0.266; γ-globulin =0.280; p<0.05). There was a significantly positive correlation between Th17 and IgA (rIgA=0.334; p<0.05). Th1 and IgG (rIgG=0.325; p<0.05).

Conclusion. Tfh cells are elevated along with increases in sCD25, IgA, IgG and decreases in platelet counts and GFR in patients with SS. Tfh cells may play an essential role in the development of SS and sustaining antibody production, which ultimately results in the activation of disease and organ damage, esp. kidney.

P-89

Sjögren’s syndrome minor salivary gland CD4⁺ T cells associate with oral disease features and have a T follicular helper-like transcriptional profile

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Background. The predominant salivary gland (SG) T cell types contributing to disease in Sjögren’s syndrome (SS) are unclear. This study assessed the frequency and number of SG CD3⁺ T cell subtypes for association with SS disease features and compared the SG CD4⁺ T cell transcriptome of primary SS subjects to that of sicca controls not meeting criteria for SS.

Methods. CD3⁺ T cells from SG biopsy tissue of subjects with primary SS and non-SS sicca were evaluated for proportion (n=45 SS, n=46 non-SS) and number/mg biopsy tissue (n=34 SS, n=56 non-SS) of T cell subsets defined by CD3, CD4, CD8 and CD45RA. Memory CD4⁺/CD8⁺ T cell ratios were evaluated for correlation with clinical and oral disease parameters. Sorted memory CD4⁺ T cells from a subset of focus score positive SS cases (n=17) and focus score negative non-SS controls (n=15) were evaluated for global gene expression by microarray.

Results. Proportions of CD4⁺/CD45RA T cells (mean±SEM SS: 31.6%±2.0, non-SS: 20.0%±1.1, p<0.001) but not that of other CD3⁺ T cell subsets were increased in SS cases compared to non-SS sicca controls. Ratios of SG CD4⁺/CD8⁺ memory T cells positively correlated with SG focus score (r=0.49, p<0.0001), morphologic area of SG fibrosis (r=-0.35, p=0.006), and vanBjsterveld corneal damage score (r=-0.37, p<0.0001), with relationships remaining after age correction. Differentially expressed (DE) genes in SS cases versus non-SS sicca controls were enriched for T follicular helper (Tfh), interferon, T cell homeostasis, resistance to apoptosis, atypical lymphoid trafficking and elevated inflammatory response pathways but not Th17 profile. Predicted upstream drivers of the DE genes included CXCL13, CADy0/CADy0 ligand and Bcl3, while predicted decreased effects included Foxp3, Fox, Stat6 and mTOR.

Conclusions. Proportion and number of SG memory CD4⁺ T cells selectively associate with key SS disease features, and SG memory CD4⁺ T cells are enriched for a Tfh-like cell profile.

P-90

Single cell TCR analysis of Sjögren’s syndrome salivary gland CD8⁺ T cells reveals extensive clonal expansions and the presence of viral-specific cells

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Background. In Sjögren’s syndrome (SS) an autoimmune disease characterized by lymphocytic infiltration of the salivary and lacrimal glands, severe dry eyes and mouth, fatigue and musculoskeletal pain. How the T cell-dominated salivary gland (SG) inflammation is linked to the clinical and cellular features of SS is poorly understood. We reported that SG CD4⁺ T cell clonal expansion is antigen-driven and correlated with reduced sali-
vary flow and increased SG fibrosis. To determine whether the extent of SG CD8+ T cell clonal expansion is related to pathologic features of disease, we evaluated the SG CD8+ TCR repertoire of the same subjects. Approach. Multiplex single cell RT-PCR was used to retrieve paired TCR α and β sequences from SG and peripheral blood (PB) memory CD8+ T cells from 11 subjects meeting the 2016 ACR/EULAR classification criteria for primary Sjögren’s syndrome. The extent of SG and PB CD8+ T cell clonal expansion was compared between SG and PB and evaluated for relationships with pathologic and clinical disease features using Mann-Whitney and Spearman rank correlation tests.

Results. Our cohort repertoire consisted of over 2,800 TCR sequences isolated from a median of 81 (range 15-119) SG and 100 (range 40-146) PB cells evaluated per subject. Clonal expansions of SG and PB CD8+ T cells were detected in all subjects. Expansions were extensive in both tissue compartments, ranging from 8.3-66.3% in SG (mean 34.9) and 5-48.5% in PB (mean 23.1). Although the percentage of clonally expanded CD8+ T cells did not differ significantly between SG and PB, there were significantly higher levels of clonally expanded SG CD8+ T cells compared to SG CD4+ T cells from the same individuals. Further, many subjects exhibited large expansions, with 60% of individuals having at least one clonal expansion of 4 or more cells. In contrast to our prior observations in CD4+ T cells, the degree of SG CD8+ T cell clonal expansion did not significantly correlate with measures of oral disease. However, CD8+ T cell expansions were more extensive in patients with ESSDAIs ≥2. Identical clonal expansions were detected in SG and PB from 5/11 patients (45%), indicating significant trafficking of CD8+ T cells in both tissues. Further, several clonotypes of expanded cells in SG and PB were identified as EBV viral-specific, indicating the presence of viral-specific CD8+ T cells in the SG of SS patients. Conclusion. Clonally expanded CD8+ T cells were abundant in both the SG and PB of SS patients, but did not correlate with measures of oral disease. Known viral-specific T cells were found in SG and PB from SS patients, adding evidence for viral-initiated or -driven CD8+ T cell proliferation in SS.

P.91
Transcriptomics of CCR9-expressing pathogenic T helper cells from primary Sjögren’s syndrome patients reveal dysregulated pathways involved in effector T cell function

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Background. CCR9+ T cells produce large amounts of IFN-γ and IL-10 (Papadakis H, 2003), lack CXCR5 expression but have features similar to T follicular helper cells and the recently described pathogenic PD1+CXCR5+ cells, including expression of ICOS, PD-1, IL-21 and Bc6, but no CXCR5 expression (MCGuire Immunity 2011, Rao Nature 2017). CCR9+ T helper (Th) cells and their ligand CCL25 are elevated in salivary glands of primary Sjögren’s syndrome (pSS) patients (Blokland A&R 2017). Since CCR9+ T cells strongly induce antibody production and robustly respond to IL-7 (Blokland A&R 2017), which is indicated to play an essential role in pSS pathogenesis (Van Rooij A&R 2013) and in formation of ectopic lymphoid structures (Seo J Virol 2014), these cells may play an important role in pSS immunopathology. The goal of this study was to identify the molecular dysregulation of CCR9 Th cells in pSS patients.

Methods. CCR9+ and CCR9-CXCR5+ T cells from peripheral blood of 7 healthy individuals and 7 pSS patients and RNA sequencing of these sorted cell subsets was performed. Computational analysis was used to investigate significantly differentially expressed genes (DEG) and to identify gene expression networks. Pathway enrichment analysis was performed in order to assess differentially regulated pathways. Target genes identified using these analyses are being validated on protein level by flow cytometry. Knockdown experiments will assess the functional role of identified targets.

Results. The sorted Th subsets could robustly be distinguished based on their transcriptomes. In the CCR9+ Th cell subset 2777 DEG (1249 up and 1528 down) were identified between healthy controls and pSS patients, and 1450 and 1077 in the CXCR5+ and CCR9-CXCR5- subsets, respectively. Using network analysis 15 modules were constructed, consisting of genes showing coherent expression patterns. Four modules of interest were selected based on pathway enrichment analysis, revealing pathways involved in, e.g., cytokine and chemokine production, proliferation and migration. DEG of interest within these networks were selected, including upregulated expression of integrin αE, integrin αL and downregulation of regulatory T cell associated genes FoxP3 and IL2RA. Expression of these markers is being validated using flow cytometry. In addition, knockdown of predicted key transcription factors is studied to reveal their role in the pathogenic potential of CCR9+ T cells.

Conclusion. Transcriptomic analysis of CCR9+ Th cells from pSS patients reveal a multiplicity dysregulated pathways previously shown to be involved in increased effector T cell function. Upregulation of genes associated with pathogenicity and downregulation of regulatory T cell associated genes were found in pSS patients. Targeting predicted key molecules might reveal (novel) therapeutic targets to halt pathogenic processes induced by CCR9+ T cells.

P.92
Single cell based phosphorylation profiling identifies alterations in Toll-like receptor 7 and 9 signaling in patients with primary Sjögren’s syndrome

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Background. Primary Sjögren’s syndrome (pSS) is associated with polymorphisms and mRNA expression profiles that are indicative of an exaggerated innate and type I IFN immune response. Excessive activation potential of signaling pathways may play a role in this profile, but the intracellular signaling profile of the disease is not well characterized.

Methods. To gain insights into potential dysfunctional intracellular signaling profiles of pSS patients, we conducted an exploratory analysis of MAPK/ERK and JAK/STAT signaling networks in peripheral blood mononuclear cells (PBMCs) from primary Sjögren’s syndrome (pSS) patients and healthy controls (HC), using single cell based immunophenotyping and cellular signaling analysis (ISCAN).

Results. In ISCAN, single cells were labeled with a panel of antibodies recognizing different signaling proteins and analyzed for intracellular signaling using cell-permeabilization and immunostaining. Single cell based phosphorylation profiling revealed a high degree of variability between individual cells. Pathway analysis revealed significant alterations in the downstream signaling pathways of two Toll-like receptors (TLR), TLR7 and TLR9. TLR7 signaling, which is involved in the recognition of single-stranded RNA, was found to be upregulated in pSS patients, while TLR9 signaling, involved in the recognition of unmethylated CpG-DNA, was downregulated in pSS patients. These findings were validated by qPCR and western blot analysis.

Conclusion. Single cell based phosphorylation profiling identified alterations in TLR7 and TLR9 signaling in primary Sjögren’s syndrome patients, providing insights into potential dysregulation of intracellular signaling pathways in this disease. Further studies are needed to validate these findings in larger cohorts and to understand the functional implications of these alterations.
onuclear cells (PBMC) from 25 female pSS patients and 25 female age-matched healthy donors using phospho-specific flow cytometry. We analysed unstimulated samples, and samples during a 4-hour time period following activation of Toll-like receptor (TLR) 7 and 9. Expression levels of MxA, IFI44, OAS1, GBP1, and GBP2 in PBMC were analysed by real-time PCR. We will confirm the flow cytometry results using mass cytometry.

Results. Principal component analysis (PCA) showed that basal phosphorylation profiles could be used to differentiate pSS patients and healthy donors. Including clinical parameters such as extraglandular manifestations (EGM), PCA revealed stronger responses through NF-κB and STAT3 in pSS-negative patients than in patients with EGM and healthy donors. In addition, 70% of the patients had a positive IFN score. These patients differed from the IFN score-negative patients regarding their phosphorylation profiles. Mass cytometry data will be shown to confirm this.

Conclusion. We here report increased signaling potential in PBMC from pSS patients after TLR7 and -9 stimulation, mainly through STAT3.

P-93
DNA microarray analysis of salivary gland in Sjögren’s syndrome indicates a role for innate immune responses in its pathogenesis via Toll like receptor 8

Mizuki Sakamotô, Masafumi Moriyama1,2, Keiko Oyama1, Akiko Tana- kō1, Takashi Maehara1, Sachiko Furukawa1, Miho Ohta1, Noriko Ishiguro1, Haeque A. S. M. Rafa1l, Akira Chinju1, Keita Mochizuki1, Ryuusuke Munemura3, Jun-Nosuke Hayashida3, and Seiji Nakamura4.

Background. Sjögren’s syndrome (SS) is an autoimmune disease characterized by lymphocytic infiltration into the salivary and lacrimal glands with concomitant autoantibody production and destruction of the glandular tissue. Recent studies suggest that Toll-like receptors (TLRs) play a important role in innate immune responses and are associated with pathogenesis of autoimmune diseases including systemic lupus erythematosus and psoriasis. However, the involvement of TLRs in the pathogenesis of SS is still not clear. In this study, we thus addressed to identify the disease-associated genes, especially TLR-related molecules.

Methods. Gene expression was analyzed by using DNA microarray in a salivary gland (LSG) from patients with typical SS (n=3) and healthy controls (n=3). DNA microarray analysis was performed in three groups to screen for TLR family (TLR1-TLR10). Up-regulated TLRs were validated by real-time PCR and immunohistochemical staining in SS (n=19), and healthy controls (n=10).

Results. Gene expression patterns in the 2 groups were quite different from each other by the pvclust method and principal components analysis. In SS, 2361 up-regulated genes and 1388 down-regulated genes were identified (adjust p-value<0.01, and ratio ≥2 or ratio ≤0.5). Functional analysis indicated that the up-regulated genes in SS encoded proteins involved in T/B cell activation and chemotaxis. Regarding to TLR family, the gene expressions of TLR1, TLR7, TLR8, TLR9, MyD88, IRF1, IRF7, and IRF8 in SS were up-regulated compared with that of healthy control. PCR validated that the expression of TLR8 in SS was significantly higher than that of healthy controls (p<0.01). TLR8 is considered to play an important role in the innate immune response by recognizing viral RNA, self- RNA, and several classes of small molecule agonists. Furthermore, immunofluorescence double staining and flow cytometry analysis confirmed that the expression of TLR8 almost merged with that of the monococyte/macrophage marker CD68.

Conclusions. TLR8 was identified as a disease-associated molecule in SS by DNA microarray. Moreover, TLR8+ monocytes/macrophages might be involved in the pathogenesis of SS.

P-94
Activated M2 macrophage via its Toll-like receptor 7 contributes to the pathogenesis of IgG4-related disease

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Background. IgG4-related dacrocyoadenitis and sialoadenitis (IgG4-DS) is a unique inflammatory disorder characterized by the elevation of serum IgG4 and infiltration of IgG4-positive plasma cells in lacrimal and salivary glands (SGs). Regarding the immunological aspects of this disease, it is well known that IgG4 is induced by T helper type 2 (Th2) cytokines such as IL-4 and IL-13. We previously reported that these Th2 cytokines contributed to IgG4 production in IgG4-DS. In addition, recent studies indicated that the activation of innate immunity also plays a key role in the IgG4 production upon stimulation with toll-like receptor (TLR) ligands. In this study, we thus examined the expression of innate immune molecules, especially TLRs in SGs from patients with IgG4-DS.

Methods. Gene expression was analyzed by DNA microarray in submammary salivary glands (SMGs) from patients with IgG4-DS (n=6), chronic sialoadenitis (CS) (n=3), and controls (n=3). TLR family (TLR1-TLR10) was validated by real-time PCR and immunohistochemical staining in SGs from patient with IgG4-DS (n=15), Sjögren’s syndrome (SS) (n=15), CS (n=9), and controls (n=9). Up-regulated TLRs TLR7 is considered to express on macrophages and dendritic cells (DCs) in secondary lymphoid organs (SLOs), we examined the distribution of macrophages (CD68, CD163), DCs (CD11c, CD123), and TLR7 in the SMGs from patients with IgG4-RD and normal SLOs such as lymph nodes and tonsils. Finally, the phenotype of human TLR7 (huTLR7) transgenic (Tg) C57BL/6 mice before/after stimulation with TLR agonist (R848) was assessed compared with that of wild-type C57BL/6 mice.

Results. In patients with IgG4-RD, TLR4, TLR7, TLR8, and TLR9 were overexpressed. PCR validated the up-regulation of TLR7 in IgG4-RD compared with the other groups. Immunohistochemical analysis confirmed strong infiltration of TLR7-positive cells in the SGs of patients with IgG4- RD. Double immunofluorescence staining showed that TLR7-positive cells mainly co-localized with CD123-positive cells in SLOs. In contrast, in IgG4-RD tissues, TLR7-positive cells mainly co-localized with CD163-positive cells.

The mRNA expression of TLR7 in IgG4-RD was positively correlated with that of IL-33, which is a Th2 activating cytokine. In huTLR7 Tg mice, the focus and fibrosis score in SGs, pancreas, and lungs were significantly higher than those in wild-type mice. Moreover, the concentration of serum IgG and IgG1 in huTLR7 Tg mice was significantly higher than that in wild-type mice and distinctly increased upon stimulation with TLR7 agonist.

Conclusions. TLR7-expressing M2 macrophages might promote the activation of Th2 immune responses via the local inflammation with IL-33 secretion in IgG4-DS. At more thorough understanding of the role of TLR7/Cd163+ M2 macrophages in IgG4-RD could lead to the establishment of a mouse model of IgG4-RD and to the eventual development of novel pharmacological strategies to interrupt TLR7 or TLR7-downstream signals as a further means of inhibiting disease initiation or progression.

P-95
Withdrawn

Clinical and Experimental Rheumatology 2018
Background. The pathophysiology of Sjögren’s Syndrome is mainly described as a focal lymphocyte infiltration in exocrine glands. One of the most accepted mechanisms to the development of the disease is the involvement of epithelial cells as a target called “autoimmune epithelitis”. The epithelium activation leads to the secretion of pro-inflammatory cytokines which can attract lymphocytes and induce inflammatory responses. These cytokines are mediated by Th1 helper (Th) lymphocytes, being the Th17 subtype abundantly found in minor salivary glands with a severe destruction. These histological changes are often associated with the decrease in salivary flow and xerostomia, however, there’s still the necessity of understanding the mechanisms that lead to the salivary gland secretion impairment and the development of a specific biomarker to differentiate SS from other autoimmune diseases.

Methods. Twenty-seven (27) patients diagnosed with SS were selected using the American-European consensus criteria. All patients underwent a minor salivary gland biopsy and healthy controls. The expression levels of CCL21 and CXCL13 within the lymphocytic infiltrate characteristic of the condition have been reported to contribute to ectopic lymphoendothelial tissue. The minor salivary glands of patients with SS varied in pathological features and were positively stained for anti-SS-A and -SS-B titers. A higher focus score and ESSDAI value at the time of biopsy were also associated with these chemokines. In patients with extraglandular manifestations of SS, the prevalence of lymphadenopathy increased with increasing CCL21 levels.

Results. The minor salivary glands of SS patients stained positively for CCL21 and CXCL13 in 46.2% (49/106) and 70.7% (75/106) of all cases, respectively. Higher-level expression of CCL21 and CXCL13 was associated with increased esR, IgG and rheumatoid factor levels, as well as anti-SS-A and -SS-B titers. A higher focus score and ESSDAI value at the time of biopsy were also associated with these chemokines. In patients with extraglandular manifestations of SS, the prevalence of lymphadenopathy increased with increasing CCL21 levels.

Conclusion. The expression levels of CCL21 and CXCL13 within the lymphoendothelial tissue of SS patients are associated with several laboratory features of the disease as well as lymphadenopathy and the extent of clinical disease activity. CCL21 and CXCL13 levels can therefore serve as useful markers to predict the disease activity and prognosis of patients with SS.

**P-98**

**Distinct and persistent cytokine profiles associated with pathological features in primary Sjögren’s syndrome**

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Background. The cytokine network in Sjögren’s syndrome is highly dysregulated, with local and systemic overexpression of pro-inflammatory cytokines.

Methods. Seventy SS patients were recruited in a follow-up study, 43 of whom were classified as GC−, 15 as GC+ and 12 with negative focus score at the time of study inclusion. Serum samples obtained at time of study inclusion and at 8-year follow-up were analysed for cytokines using a Luminex assay (Human Cytokine 25plex Panel, Invitrogen, #LHC0009M).

Results. Of 25 measured cytokines, overall levels of IL-5, IL-8, IL-10, IL-12, IL-17a, GM-CSF and MIG were significantly increased at follow-up, whereas IL-4, MCP-1 and TNF-α levels were decreased (Wilcoxon p-values ranging between 0.3 and <0.0001, Fig. 1). To assess cytokine level variations in GC−, GC+ and focus score (FS)− patients, we conducted two-way repeated measure ANOVA analyses and identified IL-1RA, IL-13, IL-17, MIP-1α and TNF-α as significantly associated with GC-status at both time-points (Fig. 2). We also found that GM-CSF, IL-13, IL-8 and MCP-1 levels significantly changed with time across all groups. Further, using mixed linear model analyses we identified between-group differences in Eotaxin (p=0.009), IL-12 (p=0.04) and MIG (p=0.01) level changes with time (Fig. 3). We confirmed GC-status associated with IL-1RA (p=0.02), IL-17a (p=0.007) and MIP-1α (p=0.04) levels, as well as time effect on GM-CSF (p<0.0001), IL-8 (p=0.006), IL-13 (p<0.0001) and MCP-1 (p=0.0001) levels. No differences were observed between patients with normal and hyposalivation.

Conclusions. Our study confirms that serum cytokine levels are abnormal in SS, and only vaguely fluctuate with time. GC+ patients had overall higher

**P-96**

**Th17 cytokines as a potential salivary biomarker for morphological changes in Sjögren’s Syndrome**

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Background. Chemokines, which control inflammatory cell migration, have been shown to play important roles in the inflammatory processes associated with Sjögren’s Syndrome (SS). CCL21 induces the homing of T cells, while CXCL13 determines the positioning of B cells in specific follicles and facilitates B-cell maturation. CCL21 and CXCL13 within the lymphocytic infiltrate characteristic of the condition have been reported to contribute to ectopic lymphoendothelial tissue. In the current study, we investigated whether CCL21 and CXCL13 expression levels in the minor salivary gland are associated with the laboratory and clinical manifestations of SS.

Methods. Sociodemographic data on 106 SS patients were obtained and the glandular and extraglandular manifestations of the disease were documented. In addition, minor salivary gland biopsies were performed and the patients’ laboratory findings were analyzed. European League Against Rheumatism SS disease activity index (ESSDAI) values of SS disease activity at the time of biopsy and the SS disease damage index (SSDDI) values were also recorded. An immunohistochemical approach was used to semiquantitatively measure the CCL21 and CXCL13 expression in the minor salivary glands.

Results. The minor salivary glands of SS patients stained positively for CCL21 and CXCL13 in 46.2% (49/106) and 70.7% (75/106) of all cases, respectively. Higher-level expression of CCL21 and CXCL13 was associated with increased esR, IgG and rheumatoid factor levels, as well as anti-SS-A and -SS-B titers. A higher focus score and ESSDAI value at the time of biopsy were also associated with these chemokines. In patients with extraglandular manifestations of SS, the prevalence of lymphadenopathy increased with increasing CCL21 levels.

Conclusion. The expression levels of CCL21 and CXCL13 within the lymphoendothelial tissue of SS patients are associated with several laboratory features of the disease as well as lymphadenopathy and the extent of clinical disease activity. CCL21 and CXCL13 levels can therefore serve as useful markers to predict the disease activity and prognosis of patients with SS.
serum cytokine levels. With the exception of pro-inflammatory, autoimmunity associated cytokines IL-17a and the IL-23 subunit IL-12p40, chemokines rather than inflammatory cytokines were the discriminating factors between GC-status subgroups both at individual time-points and over time.

Fig. 1. Serum cytokine and chemokine levels were over all relatively constant across all three subgroups between the two time points, though levels of IL-4, MCP-1, and TNF-α dropped slightly, whereas IL-5, IL-8, IL-10, IL-17a, GM-CSF, and MIG increased.

Fig. 2. Differentially expressed serum cytokine levels in patients with and without ectopic GC-like structures in minor salivary glands (GC+ and GC-) and patients with negative focus score (FS) at time of inclusion (S2A) and follow-up (S2B).

Fig. 3. Changes in serum levels of Eotaxin (CCL 11), Monokine induced by gamma interferon (MIG/CXCL9), and Interleukin 12 (IL-12) at time of inclusion (S2A) and time of follow-up (S2B) was significantly different between pSS patient with and without ectopic GC-like structures in minor salivary glands (GC+ and GC-) and patients with negative focus score (FS).

Table.

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<th>Patients</th>
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<tr>
<td>Lymphoma</td>
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P-99
Role of CXCL13 and CXCL12 in Sjögren’s syndrome: association with histological, clinical and laboratory features.

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Background. Ectopic production of the lymphoid chemokines CXCL13 and CXCL12 has been described in tertiary lymphoid structures (TLS) that harbour in the salivary glands of patients with Sjögren’s Syndrome (pSS).Whilst CXCL13 expression was found to correlate with clinical features, its potential role as a biomarker to monitor the organization and severity of the salivary gland infiltrate has been hampered by the lack of sensitive tools to describe TLS extent and features.

Methods. We studied histological features of the minor salivary glands (MSG) and sera of respectively fifty and seventy (Table) unselected consecutive patients with pSS (AECG criteria) enrolled in the Sapienza University of Rome, Italy. Concentration of CXCL13 and CXCL12 were evaluated by ELISA in patient sera and eleven healthy age-matched controls (HC). Paraffin embedded MSG were studied by haematoxylin/eosin and anti-CD3, anti-CD20, anti-CD21 staining. Images analysis was used to calculate focus score (FS), mean foci area, percentage of infiltration (%i), segregated foci (%SF), %GCs and lymphoepitelial lesions (%LEL). GCs from MSG and tonsils were microdissected and quantitative PCR was used to test CXCL12 and CXCL13 transcripts.

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P-100
Germ-free environment worsens lacrimokeratoconjunctivitis in a mouse model of Sjögren’s syndrome

De Paiva, CS 1, Zaheer, M 1; Yu, Z 2; Bian, F; Swennes, AG 3, Britton, RA 3, Pflugfelder, SC 1, 2, 3
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Background. To investigate the role of the gut microbiota in the appearance and development of dacyrocytitis in the IL-2 receptor alpha knock-out (CD25KO) model of Sjögren Syndrome.

Methods. Eight-week old germ-free (GF) KO mice were compared to KO mice raised in specified pathogen free (SPF) conditions. Corneal barrier function was assessed by dye staining. Eyes and adnexae were excised and prepared for histology. Conjunctival goblet cell density was counted in PAS stained sections. Total cell infiltrates in lacrimal gland (LG) were visualized in histologic sections. T helper (Th) phenotype in LG and cervical lymph nodes (CLN) of recipients was investigated by flow cytometry. IL-12, TNF-α and IL-1β mRNA transcripts by real-time PCR.

Results. GF KO mice have significantly lower number of goblet cells and significantly greater corneal barrier disruption and LG infiltration than SPF KO mice. There was a greater percentage (% of CD4+ IFN-γ in GF KO LG and CLN compared to SPF KO group (7.65±3.26 vs. 5.23±3.13 and 4.90±1.07 vs. 3.49±1.07, p<0.05 for both, respectively). Adoptive transfer of GF KO CD4+T cells recapitulated the most severe phenotype in RAG-1 KO recipients compared to SPF CD4+T donors 5 weeks post-transfer. GF KO animals gavaged with normal mouse fecal slurry had a significantly higher number of goblet cells and significantly lower corneal barrier disruption than GF KO mice while displaying decreased LG infiltration score. This was accompanied by a 50% or greater decrease in IFN-γ, MHC II, IL-12, TNF-α and IL-1β mRNA levels in the LG of recolonized animals.

Conclusions. Lack of commensal bacteria accelerates the onset and severity of dacyrocytitis and generates autoreactive CD4+T cells with greater pathogenicity. These results indicate the commensal bacteria or products secreted by them have immunoregulatory properties that protect exocrine glands in the CD25KO SS model.

Support. This work was supported by the NIH/NEY EY026893 (CSDP), Alkek Center for Metagenomics and Microbiome Research (CSDP), Biology of Inflammation Center (SCP and CDP), NIH Training Grant T32-AI053831 (FB), RPB Stein Innovation Award (RAB), RPB Research to Prevent Blindness (SCP), The Oshman Foundation (SCP), William Stamps Farish Fund (SCP), The Hamill Foundation (SCP), and by the Cytometry and Cell Sorting Core at Baylor College of Medicine, which is funded by the NIH NIAID P30AI036211, NCI P50CA125123, and NCRR S10RR024574.

P-101
Analysis of suppressive ability and its mechanisms of rice seeds expressing altered peptide ligands against M3 muscarinic acetylcholine receptor (M3R) induced sialadenitis

Hanez Kudo 1, Hiroto Tsuibo 2, Hiromitsu Asashima 1, Hiroyuki Takahashi 1, Yuko Ono 1, Suori Abe 1, Yuya Kondo 1, Yuya Wakasa 1, Fumio Takaiwa 2, Isao Matsumoto 3 and Takayuki Sumida 1

1Department of Internal Medicine, Faculty of Medicine, University of Tsukuba. 2Plant Molecular Farming Unit, Division of Biotechnology, Institute of Agrobiological Sciences, National Agriculture and Food Research Organization.
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Bone morphogenetic protein 6 receptor inhibition restores salivary gland function in a mouse model of primary Sjögren’s syndrome

Hongen Yin1, Lovika Kalra 1, Arif Karim1, Zhennan Lai 1, Maria C. Guima- ro1, Lauren Aber1, Blake Warner1, Bill Swaim 1, Sandra Afione, Alexandra Vagia1, Cuong Q Nguyen2, Paul Ye1, Donald B. Bloch3 and John A. Chiorini1. 1Molecular Physiology and Therapeutics Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD, USA. 2Department of Pathology and Infectious Diseases, University of Florida, Gainesville, FL, USA. 3Cardiovascular Division, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA. 4Center for Immunology and Inflammatory Diseases and the Division of Rheumatology, Allergy, and Immunology of the Department of Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA, Boston, MA.

Background. Bone morphogenetic protein 6 (BMP6) plays a critical mech- anistic role in decreasing salivary gland dysfunction in primary Sjögren’s syndrome (pSS) patients. BMP6 is reported to be overexpressed in the salivary glands of over 50% of pSS patients and has been linked to a decrease in salivary gland function in patients. BMP6 signals through type 1 receptors, which results in phosphorylation of SMAD1/5/8 transcription factors that ultimately alter gene expression within the nucleus. Two inhibitors, LDN-212854 and LDN-193189, have been developed to selectively target the ALK2 and ALK3 BMP type 1 receptors. This study examined the ability of these inhibitors to block BMP6 signaling, and their effect on expression of key proteins involved in salivary gland function and inflammation.

Methods. BMP6 expression was detected in minor salivary glands (MSG) from female pSS patients by immunofluorescent staining. In vitro water permeability was tested by regulated volume decrease (RVD) assay in BMP6 treated HS cells with or without LDNs treatment, followed by observation of change of phosphorylation of pSMAD1 expression as detected by Western blotting. BMP6 overexpression mice were generated by retrodural cannulation of AAV5-BMP6 in submandibular salivary glands (SMG) of 6-8Wks old mice. In vivo activity of the ALK inhibitors was tested in C57BL/6.NOD-Aec1Aec2 mice and BMP6 overexpressing mice, which have established SS like disease, by daily i.p. injection for 24 days or 3 days respectively. Subsequent pilocarpine stimulated saliva flow were assessed during and at the end of the study. Local and systemic immune response was investigated by flow cytometry. The levels of pSMAD1, ID3 and aquaporin-5 (AQP-5) expression in SMG from LDN treated C57BL/6, NOD-Aec1Aec2 mice were measured by immunofluorescent staining.

Results. Elevated BMP6 was found in N=43/49 (54.4%) of pSS patients examined in this study. In humans, ALK2 and ALK3 receptors were found to be involved in ducal and acinar cells. In vitro treatment of HS cells with ALK2/3 inhibitors resulted in decreased BMP6 signaling and SMAD 1/5/8 phos- phorylation and led to a recovery of fluid movement. Furthermore, daily treatment of BMP6 overexpressing mice or C57BL/6.NOD-Aec1Aec2 mice with either inhibitor restored salivary flow rate. Associated with this increase in salivary flow was an increased expression of AQP5, a protein critical for membrane water permeability in salivary glands, and decreased pSMAD1 and ID3, which are downstream signaling pathways for ALK2/3. LDN treatment also decreased infiltrating IgN-gamma producing CD4+ T cells in submandibular glands from C57BL/6.NOD-Aec1Aec2 mice.

Conclusions. BMP6 expression is increased in a majority of pSS patients. Treatment with BMP6 inhibitors can reverse the loss of function within the salivary gland as well as decrease inflammation. These findings suggest that inhibition of BMP6 is a promising approach to the treatment of primary Sjögren’s syndrome.

References.

P-103

Targeting B-cell activating factor (BAFF) impairs ectopic lympho- phoneogenesis in murine models of Sjögren’s syndrome

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Background. Tertiary lymphoid structures (TLS) characterised by germina centre formation and B cell proliferation represent the histological hallmark of primary Sjögren’s syndrome (pSS). However, the events preceding the formation of such ectopic structures and factors driving their persistence are unknown. Overexpression of BAFF, also known as B cell lymphocyte stimulator (BLyS), in pSS patients has been linked with the presence of autoreactive B cells and autoantibody production (1). Furthermore, in pSS salivary glands BAFF is associated with the expansion of specific B cell subsets, and with B cell repopulation post rituximab treatment (2). In this work we aimed to dissect the dynamics of B cell subsets within tertiary lymphoid structures following BAFF-targeted treatment in both inducible and chronic animal models that mimic the histological features of pSS.

Methods. Submandibular salivary glands of C57BL/6 mice were intra-duct- tally treated with luciferase-encoding replication-deficient adenovirus to induce TLS formation as previously described (3). Prior to salivary gland cannulation, mice were treated with two doses (i.p.) of either anti-BLyS mAb or isotype control. Salivary glands were dissected at day 15 post- canulation and TLS formation in both groups was assessed. NOD.B10. H2b mice were similarly treated with anti-BLyS mAb at 26 weeks old and salivary gland infiltrates assessed 21 days later.

Results. Histological analysis of salivary glands from anti-BLyS treated C57BL6 animals unveiled severely compromised TLS formation. Post anti-BLyS treatment, salivary glands were infiltrated by T cell clusters but only few, and scattered, B cells were present, contrasting with fully developed and organised TLS in the salivary glands of mice treated with iso- type control. Significantly lower numbers of B cells, particularly from the B2 subset, as well as plasmablasts, infiltrated salivary glands of anti-BLyS treated mice. However, treatment with anti-BLyS did not affect numbers of infiltrating T cells (both CD4 and CD8), proliferative T cells, or plasma cells in normal salivary glands. In a chronic setting, salivary glands from NOD.B10.H2b mice were also infiltrated with only few, low numbers of B cell subsets following anti-BLyS treatment.

Conclusions. Our data highlights BAFF as a key player in ectopic lympho- phoneogenesis during inflammation as well as a subset-specific role for BAFF in B cell maturation. Furthermore, these results support future studies of BAFF-targeted therapeutics in pSS.
Acknowledgement.
This research is funded by Idorsia Pharmaceuticals Ltd.

Background/Objective. Tertiary lymphoid structures (TLS) often develop in target tissues of autoimmune diseases (AID) such as systemic lupus erythematosus (SLE), multiple sclerosis (MS), rheumatoid arthritis, and Sjögren’s syndrome (SS). These structures consist of aggregates of B and T cells with varying degree of organization and are proposed to promote the generation of autoreactive effector cells and autoantibody production. Modulation of the S1P1 receptor inhibits egress of pathogenic lymphocytes from lymphoid organs and reduces their availability in circulation. This has proven to be an effective target for the treatment of AID including MS and is currently being considered for early phase clinical trials in SLE and SS. Here we investigated the functional targeting of the S1P1 receptor in a murine model of SS.

Method. Cenerimod, an orally active, selective S1P1 receptor modulator, was used either preventively (early in inflammation) or therapeutically (established inflammation) in an inducible model of SS to evaluate the efficacy of cenerimod in vivo.

Results. Cenerimod induced disaggregation of the lymphocytic structures and resolution of salivary gland (SG) inflammation with a concomitant decrease in focus score, lymphoid structure size and T/B-cell follicular organization. Mice treated with cenerimod displayed significantly decreased T (naïve, central memory and effector) and B (including CD138+ plasma cells) lymphocyte infiltration in cannulated SG, relative to vehicle treated mice. Interestingly, the lymphocytes from cenerimod treated mice exhibited significantly reduced proliferation as well as reduction in pro-inflammatory cytokine RNAs such as IL-17, IL-21, and IL-6. Furthermore, the gene expression profile associated with TLS formation (LTx, LIT, TNF-α, CXCL13, CCL19) was less pronounced in cenerimod treated samples. The cervical lymph nodes draining the salivary glands showed a slight reduction in T lymphocytes, but no significant defects were observed in structure, organization and production of lymphoid chemokine/cytokines, suggesting that homeostatic regulation of tertiary and physiological lymphoid organs differentially relies on lymphocyte/stromal cell cross-talk during inflammation.

Conclusion. Together, these data demonstrate that the S1P1 receptor modulator cenerimod regulates TLS in mice and might therefore be a potential treatment option for SS.

Acknowledgement. This research is funded by Idorsia Pharmaceuticals Ltd.

P-104
Effect of cenerimod, a sphingosine-1-phosphate receptor 1 (S1P1) modulator, on the formation of tertiary lymphoid structures in a mouse model of Sjögren’s syndrome
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Methods.
Sodium butyrate (1g/kg) or vehicle (saline) was intraperitoneally injected three times per week from 11 weeks after birth. Salivary flow rate (SFR) was addressed on every 2 weeks between 11 and 23 weeks. Histological analysis was performed on 23 weeks.

Results. The SFR of NOD mice in both groups decreased over time. However, SFRs of sodium butyrate-treated mice were greater than those treated with vehicle. Histopathologic evaluation of salivary gland and lacrimal gland showed markedly reduced lymphocytic infiltration in the mice treated with sodium butyrate.

Conclusion. Butyrate has suppressive effect on SFR decrease and lymphocytic infiltration in salivary gland and lacrimal gland of NOD mice. Butyrate may be a novel therapeutic approach in SS.

P-105
Butyrate suppresses salivary flow rate decrease and lymphocytic infiltration in salivary and lacrimal glands of non obese diabetic mice
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Background. Gut microbiota has been introduced as an important environment factor in the pathogenesis of autoimmune inflammatory diseases, such as rheumatoid arthritis and inflammatory bowel disease. Gut microbiota participates in the development of the immune system and produces various metabolites. Metabolites secreted by gut microbiota maintain homeostasis through various mechanisms, of which butyrate is known to have an anti-inflammatory effect. The purpose of our study was to investigate the effect of butyrate on clinical and histopathologic aspects of Sjögren’s syndrome (SS) in non-obese diabetic (NOD) mice, an animal model of SS.

Methods. Sodium butyrate (1g/kg) or vehicle (saline) was intraperitoneally injected three times per week from 11 weeks after birth. Salivary flow rate (SFR) was addressed on every 2 weeks between 11 and 23 weeks. Histological analysis was performed on 23 weeks.

Results. The SFR of NOD mice in both groups decreased over time. However, SFRs of sodium butyrate-treated mice were greater than those treated with vehicle. Histopathologic evaluation of salivary gland and lacrimal gland showed markedly reduced lymphocytic infiltration in the mice treated with sodium butyrate.

Conclusion. Butyrate has suppressive effect on SFR decrease and lymphocytic infiltration in salivary gland and lacrimal gland of NOD mice. Butyrate may be a novel therapeutic approach in SS.

P-106
Low-dose IL-2 administration expands regulatory T cells and protects against experimental Sjögren’s syndrome
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Objective. Treg dysfunction or deficiency results in unopposed immune activation and contributes to the development of autoimmune diseases such as Sjögren’s syndrome. Interleukin-2 (IL-2) is critical for the expansion, suppressive function, and maintenance of Tregs. This study is to evaluate the effect of low dose IL-2 treatment in SS.

Methods. 8-week old female NOD mice were administered daily subcutaneous injections of humanized recombinant Interleukin-2 (30,000 IU) every day or PBS as a control. Immunized mice were analyzed at week 16. Solecnocytes were incubated with fluorophore-conjugated monoclonal antibodies and Treg (Foxp3+CD25+CD4+) cells was analyzed by flow cytometry. The animals were analyzed for the presence of anti-SSA, anti-SSB, RF, ANA by immunofluorescence or ELISA. The salivary flow rate was measured. Salivary glands were examined by H&E staining.

Results. The number of Foxp3+CD4+CD25+ regulatory T cells was higher in the IL-2 groups compared to the PBS control groups (p<0.05). SS related antibodies titers and lymphocytic infiltration in the salivary glands were decreased in the IL-2-treated group. Salivary flow rate increased in the IL-2 treatment group.

Conclusion. Our data demonstrate that low dose IL-2 effectively inhibited the progression of experimental Sjögren’s syndrome autoimmunity in NOD mice and expanded Tregs in vivo. Low dose IL-2 may be an appropriate treatment for SS patients.

P-107
Contributions of CXCL12 and its receptor to the T cell autoimmune response in a Sjögren’s syndrome murine model
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Dysregulated chemokine signaling contributes to autoimmune diseases by facilitating aberrant T cell infiltration into target tissues, but the specific cytokines, receptors, and T cell populations still remain largely unidentified. Contributions of the potent chemokine CXCL12 and its receptor CXCR4 to the T cell autoimmune response were evaluated in allograft rejection (a/hy/aly mice, a Sjögren’s syndrome (SS) model) bearing a point mutation of the nuclear factor (NF)-κB-inducing kinase gene. Salivary gland (SG), a major target of SS pathology in a/hy/aly mice exhibited higher concentrations of effector memory T (TEM) cells than a/hy/+ mouse SG. TEM cells from a/hy/aly mice demonstrated higher in vitro migratory activity toward CXCL12 than a/hy/+ TEM cells. Moreover, CXCL12 expression was specifically upregulated in SS target organs of a/hy/aly mice, and a/hy/aly TEM cells with higher CXCR4 surface expression. TEM cells from ReBl+/− mice but not NF-κB1+/− mice also demonstrated greater migratory activity toward CXCL12, implicating a non-classical NF-κB2/ReBl pathway in regulation of TEM cell migration. TEM cells from a/hy/aly mice also overexpressed...
transforming growth factor (TGFβ) receptors I and II, and TGFβ enhanced both CXCR4 expression and migratory activity to a greater degree in alky/alve TEM cells than alky+ TEM cells. The CXCR4 antagonist AMD3100 suppressed autoimmune lesions in alky/alve mice by reducing TEM cell infiltration. Collectively, these results suggest that NF-kB/RelB regulates T cell migration to autoimmune targets through TGFβ/TGFβR-dependent regulation of CXCL12-CXCR4 signaling, and highlights these signaling pathways as potential therapeutic targets for autoimmune diseases.

P-108
Reduced corneal innervation in the CD25KO model of Sjögren syndrome
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Purpose. Decreased corneal innervation is a frequent finding in patients with Sjögren Syndrome (SS). The purpose of this study was to investigate the density of intraepithelial corneal nerves (ICNs) and corneal sensitivity using the well-characterized IL-2 receptor alpha chain (CD25KO) model of SS.
Methods. Corneal barrier function was examined by uptake of a fluorescein dye and graded by two masked observers in CD25KO and wild-type (WT) mice. Whole-mount corneas were used to quantify ICN density and thickness using bII tubulin staining. Mechanical corneal sensitivity was measured using a modified Cochet-Bonnet esthesiometer. Quantitative PCR was performed to quantify expression of beclin 1, LC3, Lamp-1, Lamp-2, CXCL1, BDNF, TNF1, DCC, Unc5b1, Efn4a, Efn5a, Rgma, and p21 in corneal epithelial mRNA.
Results. CD25KO mice had significant greater corneal barrier disruption than WT mice. This was accompanied by a significant reduction in axon density and mechanical corneal sensitivity. Real-time PCR results indicated that CD25KO mice have increased expression of genes regulating phagocytosis and autophagy (beclin-1, LC3, Lamp-1, Lamp-2, CXCL1 and BDNF) while no change was observed in genes related to axonal targeting and extension (TNF1, DCC, Unc5b1, Efn4a, Efn5a, Rgma, and p21).
Conclusions. At 8 weeks of age, mice lacking CD25 show decreased corneal innervation accompanied by reduced corneal sensitivity and increased expression of genes regulating phagocytosis and autophagy.

P-109
Pathogenic role of interleukin 27 in the nonobese diabetic mouse model of Sjögren syndrome
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Background. Interleukin 27 (IL27) is a heterodimeric cytokine with immunostimulatory and immunomodulatory properties depending on the context. Patients with Sjögren syndrome have elevated levels of IL27 in serum, yet whether IL27 contributes to the pathogenesis of Sjögren syndrome is not known. Similar to Sjögren syndrome in humans, nonobese diabetic (NOD) mice develop spontaneous autoimmune dacryoadenitis and sialoadenitis. The role of IL27 in lacrimal and salivary gland autoimmunity in NOD mice has not been reported. Our objective was to evaluate the role of IL27 signaling in the development of dacryoadenitis and sialoadenitis in the NOD mouse model of Sjögren syndrome.

Methods. NOD mice with deletion mutations disrupting expression of heterodimeric p28 component of IL27 (IL27) or the alpha chain of the IL27 receptor (IL27ra) were developed through Zn-finger nuclease or CRISPR/Cas9 mediated gene editing, respectively. Development of dacryoadenitis and sialoadenitis were determined by histological analyses, and T cell phenotypes were characterized by flow cytometry. In vivo regulatory T cell (Treg) depletion with anti-CD25 monoclonal antibody (PC61) and adoptive transfers were performed to determine the effects of disrupted IL27 signaling on development of dacryoadenitis. Studies were approved by the Institutional Animal Care and Use Committee of the University of Iowa.

Results. NOD mice lacking IL27 or IL27Ra failed to develop spontaneous autoimmune dacryoadenitis or sialadenitis. Phenotypically, T cells from IL27-deficient or IL27Ra-deficient NOD mice showed no evidence of defective T cell activation based on expression of T cell activation markers analyzed by flow cytometry and intracellular cytokine (IFNγ) by Treg cells in IL27-deficient NOD mice failed to drive dacryoadenitis in these mice. In our adoptive transfer model, wild-type T cells transferred dacryoadenitis to NOD-SCID recipient mice, but IL27Ra-deficient T cells failed to transfer dacryoadenitis. When co-transferred with wild-type cells, IL27Ra-deficient CD4 effector T cells demonstrated a significant competitive disadvantage in their ability to infiltrate lacrimal glands.

Conclusions. IL27 is required for development of dacryoadenitis and sialadenitis in NOD mice. T cell-intrinsic IL27 signaling is required to transfer disease. Defective infiltration of lacrimal glands by IL27Ra-deficient effector CD4 T cells suggests IL27 signaling may drive upregulation of homing receptors required for lacrimal gland inflammation.

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P-110
Activation of the STING pathway is involved in the induction of Sjögren’s syndrome-like disease in mice
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Background/Purpose. Patients with Sjögren’s syndrome (SS) often present with a heightened type I IFN response. Recognition of DNA within the cytosol by a multitude of cytosolic DNA sensors and downstream activation of the stimulator of interferon gene (STING) protein is a key pathway for the induction type I IFN. The major objective of this study was to investigate the role of STING activation in the etiopathogenesis of SS.

Methods. Female C57BL/6 mice were injected with a STING agonist dimethylxantheno-4-acetic acid (DMXAA) and control mice were treated similarly with the vehicle. Salivary glands were monitored for gene expression by real time PCR and for inflammatory cell infiltration by immunohistochemistry and flow cytometry. Salivary gland function was evaluated by measuring pilocarpine-induced salivation. Sera were analyzed for cytokines and autoantibodies. Cultured primary salivary gland cells were used to study the expression and activation of IFN-γ.

Results. DMXAA treatment rapidly upregulated the expression of Ifnβ and pro-inflammatory cytokines, both systemically and locally in the salivary glands. The murine submandibular glands, STING expression was detected mainly in interstitial cells. In vitro activation of STING in cultured primary salivary gland cells, rapidly phosphorylated TBK1, IRF3 and induced the expression of Ifnβ and TNF-α. At 4 weeks of treatment, in comparison with the vehicle group, DMXAA treated mice developed significantly higher incidence of sialoadenitis (1/7 versus 2/7, p=0.009). At early stages of the disease, significantly increased numbers of NK1.1+ NK cells (CD49b+CD49a-), tissue type I innate lymphoid cells (ILC1) (CD49a+CD49b-), and epithelial salivary gland ILC1 (CD49a+CD49b+) were observed in the salivary glands. The mean saliva amount in DMXAA treated group (56±14 mg) was significantly lower (p=0.001) than the uninfected (80±20 mg) and vehicle treated groups (78±25). The incidence of high titer ANA (>400) was significantly higher (p=0.02) in the DMXAA group (8/16) than in the vehicle treated group (0/8).

Conclusion. This study demonstrates that activation of STING protein induces certain features of SS in mice. Our study also suggests that follow-up activation of innate immunity, type I innate lymphoid cells might be involved in the initial stages of salivary gland disease in SS. We would like to propose that apart from viral infections, conditions that cause cellular perturbations and accumulation of host DNA within the cytosol should be considered as possible endogenous triggers for SS.
P-111

Metabolic changes in the evolution of Sjögren’s syndrome in a mouse model.

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Background. Previous studies have identified metabolic abnormalities in patients with Sjögren’s syndrome (SS) and fatigue (Clin. Immunol. 162 (2017). Deletion of the P2Y2 nucleotide receptor (P2Y2R) alleviates symptoms and signs of SS in IL-14 alpha transgenic mice (IL14aTG). Stimulation of P2Y2R requires cellular release from the cytoplasm of ATP that is derived from mitochondria. The current studies were designed to evaluate changes in metabolism that occur in IL14aTG mice during the evolution of SS.

Methods. Microarray studies were performed on the spleens and submandibular glands of IL14aTG mice and control mice at 6 months of age. Abnormally expressed genes were confirmed by qPCR studies during both early disease (6 months) and late disease (12 months). Serum metabolites were evaluated by gas chromatography at 6 and 12 months of age.

Results. At 6 months of age, IL14aTG mice expressed two mitochondrial respiratory chain enzymes in their spleens at high levels compared to normal controls, NADH dehydrogenase and ubiquinol cytochrome C reductase. Their salivary glands express Pr-3K at high levels, which can induce mTOR1 and p70S6K. Expression of tyrosine receptor 1 (Ryr1) was decreased. Serum studies revealed enhanced activity of the citric acid cycle, mitochondrial respiratory chain and aerobic glycolysis, consistent with the activation of mTOR1. In contrast, serum studies at 1 year of age, when IL14aTG mice are at an advanced stage of disease, revealed less activity of the mitochondrial respiratory chain and increased amino acid metabolism. Elevated C5 carnitine, and hydroxyglutarate were consistent with acquired glutaryl-CoA dehydrogenase deficiency.

Conclusions. The nature of metabolic abnormalities differs in the early and late stages of SS in IL14aTG mice. At 6 months of age, high energy requirements are met with increased mitochondrial respiration and aerobic glycolysis. At 1 year of age, energy requirements are altered and are met more with increased amino acid metabolism and fatty acid oxidation. A potential defect in fatty acid metabolism is noted at 1 year of age. Because of the potential central importance of mTOR1 in early metabolic events, current studies are evaluating the effects of the mTOR1 inhibitor, rapamycin, on early events in SS.

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P-112

RORγt antagonist suppressed spontaneous sialadenitis in RORγt transgenic mice via inhibition of IL-17 production with increase of regulatory T cells

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Objective. Our previous studies demonstrated that T cells specific RORγt-transgenic-mice under human CD2 promoter (RORγt-Tg mice) developed spontaneous sialadenitis like Sjögren’s syndrome (SS) in which reduced regulatory T cells (Tregs) and RORγt-overexpressed CD4+T cells contributed to the pathogenesis. The purpose of this study is to clarify suppressive ability and its mechanisms of RORγt antagonist (A213) for sialadenitis in RORγt-Tg mice.

Methods. 6-week aged RORγt-Tg mice orally received 300 mg/kg of A213 (10 μL/g body weight) or vehicle (PBS, 10 μL/g body weight) every three days for 2 weeks. We analyzed 1) saliva volume, 2) histopathology of salivary glands (Hematoxylin and Eosin-staining and focus score), 3) populations of T cells subsets (naïve, central memory, and effector memory T cells) in splenocytes, 4) percentages of Tregs(Foxp3+/CD4+CD25+) in populations of T cells subsets (naïve, central memory, and effector memory T cells), 5) percentages of Tregs(Foxp3+/CD4+CD25+) in populations of B cells (LSGs) of primary SS (pSS) patients and non-pSS controls. The technology uses an array of 84,672 nanowells to capture an individual cell per well. Single-cell suspensions of LSG cells were prepared from pSS and non-

Results. 1) The ratio of saliva volume at 2 weeks to that at baseline was significantly increased in A213-treated group (1.4±0.1) compared with PBS-treated group (0.9±0.1) (p<0.05).
2) Infiltration of mononuclear cells in salivary glands in Hematoxylin and Eosin-staining were dramatically improved in A213-treated group compared with PBS-treated group. The focus score of sialadenitis at 2 weeks was significantly lower in A213-treated group (0.2±0.2) than in PBS-treated group (2.3±0.6) (p<0.05) (Fig. 1).
3) The population of effector memory T cells (CD44+CD62L+) was 96.9%, central memory T cells (CD44+CD62L+) was 1.99%, and naïve T cells (CD44−CD62L−) was 0% in RORγt-Tg mice before administration of A213. After administration of A213, the population of effector memory T cells tended to be decreased (73.2%) and central memory and naïve T cells tended to be increased (26.0% and 0.51%, respectively), whereas PBS did not alter the population of these T cell subsets.
4) The percentage of Treg (Foxp3+CD4+CD25+) in RORγt-Tg mice tended to be increased from 47.4% at baseline to 75.5% at 2 weeks after administration of A213, while PBS did not alter the percentage of Treg (40.9%).
5) In splenocytes at 2 weeks after treatment, the mRNA expression of IL-17A was significantly decreased in A213-treated group compared with PBS-treated group (p<0.05), while that of IFNγ was comparable between groups.

Conclusion. A213 could suppress the sialadenitis in RORγt-Tg mice via inhibition of IL-17 production with increase of Tregs.

Fig. 1. Comparison of histological focus score of inflammatory lesions in salivary glands between A213- and PBS-treated RORγt-Tg mice.

P-113

B cell receptor repertoire of signature Sjögren’s syndrome anti-gen specific autoantibodies in labial salivary glands

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Objectives. Sjögren’s syndrome (SS) is characterized by massive lymphocytic infiltrations in the salivary and lacrimal glands. Additionally, anti-SSA/Ro and anti-SSB/La autoantibodies are the signature diagnostic biomarkers for the disease; Ro antigens have been implicated in the pathogenesis of SS. As an essential etiological feature of the autoimmune process, it remains unclear what B cell repertoires are expressed by these antigen-specific and autoreactive B cells. Here we examine the infiltrating B cells of SS patients ex vivo using single-cell microengraving analysis and high-throughput sequencing to determine the B cell receptor (BCR) repertoires of the individual antigen-specific B cells.

Methods. Single-cell microengraving analysis is a soft lithographic technology that is used here to isolate single cells and identify specific subsets of B cells that secrete reactive antibodies against Ro-52, Ro-60, and La antigens, as well as identify the antibody isotype, in the labial salivary glands (LSGs) of primary SS (pSS) patients and non-pSS controls. The technology uses an array of 84,672 nanowells to capture an individual cell per well. Single-cell suspensions of LSG cells were prepared from pSS and non-
pSS samples labeled with CD19, CD27, and CD138, then dispersed into nanowells. Individual B cells in the nanowells were identified using automated epifluorescent microscopy to identify the presence of plasma cells, plasmablasts, and memory B cells in each well. Individual B cells were microengraved for secreting IgG isotypic anti-SSA/Ro52, anti-SSA/Ro60, and anti-SSB/La. Individual secreting B cells were isolated and subjected to high-throughput sequencing for BCR repertoire analysis.

**Results.** The data indicate that there was a disproportionately high frequency of plasmablasts present in the LSGs of pSS patients compared to non-pSS subjects. The frequency of plasmablasts is significantly higher than plasma cells and memory B cells in pSS. Interestingly, both pSS patients and non-pSS controls produced IgG isotypic anti-SSA/Ro52, anti-SSA/Ro60, and anti-SSB/La with higher levels of these autoantibodies found in pSS patients. Lastly, high-throughput sequencing of individual BCRs of these antigen-specific B cells revealed a more restricted repertoire in the pSS patients when compared to non-pSS controls.

**Conclusion.** The results indicate an antigen-driven B cell response in the LSGs of pSS patients. Further work will be needed to understand the underlying mechanisms that govern the clonal expansion during the autoimmune process of SS.

**P-114**

**Somatically introduced N-glycosylation of immunoglobulin – a potential non-specific mechanism for B cell activation in Sjögren’s syndrome**

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**Background.** Sjögren’s syndrome (SS) is a systemic autoimmune disease characterized by lymphocytic infiltration of the salivary and lacrimal glands resulting in pathological dry mouth and dry eyes and in increased risk for lymphoma. The presence of ectopic germinal centers, clonally related B cells and autotolymphopoiesis production from antibody-secreting cells (ASCs) isolated from the salivary glands indicate that at least some of the ASCs arise from an antigen-driven immune response. Analyses of immunoglobulin sequences can be instrumental for determining somatic mutational patterns shaped by selective pressures, where positive selection in the Complementary Determining Regions (CDRs) and negative selection in the framework regions (FWRs) would indicate antigen-driven antibody production. An alternative would be a non-specific mode of B cell activation. Immunoglobulin variable region N-linked glycosylation acquired by somatic hypermutation (AcN-glycs) has been strongly correlated to follicular lymphoma. Bacterial lectins can bind and activate B cells and autoantibody production from antibody-secreting cells (ASCs). Immunoglobulins with AcN-glycs have an increased frequency of plasmablasts present in the LSGs of SS patients and sicca controls in our cohort undergone positive selection in the CDRs and negative selection in the FWRs, indicating antigen-driven immunoresponses. Immunoglobulins with AcN-glycs have significantly less positive selection than those without AcN-glycs (p=0.05 and p=0.04, respectively).

**Conclusions.** Overall, immunoglobulins from ASCs infiltrating the minor salivary glands of SS patients and sicca controls in our cohort undergo positive selection in the CDRs and negative selection in the FWRs, indicating a non-specific mechanism for activation in these ASCs that could potentially give rise to autoantibodies or lymphoproliferative neoplasms.

**P-115**

**RNA Sequencing detection of gene dysregulation in epithelial sorted cells from salivary gland tissue reveals interesting pathways involved in Sjögren’s syndrome pathophysiology**

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**Background.** Primary Sjögren’s syndrome (pSS) is a chronic autoimmune disorder characterized by lymphocytic infiltrates and destruction of the salivary glands. Several lines of evidence support the hypothesis that salivary gland epithelial cells (SGECs) are not only the target of autoimmunity in pSS patients but may also play a role for its initiation and maintenance.

**Objective.** To establish high-resolution molecular maps of SGEC from pSS patients compared to controls using RNASeq analysis.

**Methods.** SGEC, B, T CD4 and CD8 lymphocytes were sorted from salivary gland biopsies from 9 pSS patients and 4 controls, using a FACS ARIA cell sorter. Total RNASeq profiling was performed using MiSeq (Illumina). For SGEC subset, 4 samples were excluded due to a contamination by B lymphocytes, thus analysis was performed on 5 pSS and 4 controls using R software, to identify transcriptional differences between pSS and control SGEC. Functional Enrichment analysis was performed using Ingenuity Pathway Analysis software.

**Fig. 1.** Volcano Plot representation of differential expression analysis of genes in the pSS SGEC versus controls. Each dot corresponds to a gene, and dots are represented in a 2D chart, where the x-axis shows fold-changes expressions and the y-axis shows the p-values –(represented as -log10).
Table I. Pathways identified a statistically over-represented with Ingenuity Pathways Analysis in epithelial cells from pSS patients compared to controls.

<table>
<thead>
<tr>
<th>Pathways</th>
<th>log p-value</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Immuno deficiency</td>
<td>4.08</td>
<td>PTPRC, BTH, IGHH1, CD8A, TAPI,</td>
</tr>
<tr>
<td>Interferon Signaling</td>
<td>3.52</td>
<td>IFIT3, OA51, IFI6, STAT1, TAPI</td>
</tr>
<tr>
<td>8 Cell Development</td>
<td>2.89</td>
<td>PTPRC, HLA-DRA, CD66, IL7</td>
</tr>
<tr>
<td>Role of JAK2 in Hormone-like</td>
<td>2.73</td>
<td>STAT5A, IRS1, SH2B3, STAT1</td>
</tr>
<tr>
<td>Cytokine Signaling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-7 Signaling Pathway</td>
<td>2.51</td>
<td>STAT5A, SLC2A1, IRS1, IGHH1, STAT1, IL7</td>
</tr>
</tbody>
</table>

Results. In SGEC, 495 genes were differentially expressed between pSS and controls. 280 genes were up-regulated, and 215 genes were down-regulated (Figure 1). Enrichment analysis (Table I) highlighted IL7 signaling pathways (including IL7, STAT5A, STAT1 genes) and interferon signaling (including OAS1, IFIT3, IFI6, TAP1 genes). Other genes potentially involved in immune responses and interactions between SGEC and lymphocytes were significantly up-regulated, including bone marrow stromal cell antigen 2, HLA-DRA, BAFF-R and IL23 A (Table II). CD86, a costimulatory molecule, was found to be significantly down-regulated. These results need to be confirmed by RT qPCR. However, congruent results have already been obtained in our laboratory, showing that IL7 serum level is increased in pSS patients compared to controls and that SGEC produce IL7 after interferon stimulation. The analysis of the non-coding RNA part and the other sorted cells subtypes is ongoing.

Conclusions. Immune interactions between SGEC and B or T lymphocytes could represent a key in the understanding of the initiation and/or maintenance of autoimmunity in pSS. Our study highlights the key role of epithelial cells in activation of immune cells. In vitro experiments are needed to confirm these results and elucidate the molecular mechanisms.

P-116

IL-7 in primary Sjögren syndrome (pSS) is secreted by salivary gland epithelial cells after IFN stimulation and is associated with B-cell activation

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Background. pSS is characterized by a strong IFN signature, ectopic germinal centers formation and a chronic blood lymphopenia. IL-7 plays a central part in T cells homeostasis and in lymphoid structures organization. We aimed to assess the role of IL-7 in pSS pathogenesis.

Methods. IL-7 serum level was assessed in 372 pSS patients and 73 paired controls. Primary cultures of salivary gland epithelial cells (SGEC) from patients and controls were stimulated by Poly I: C30 ng/ml, IFN-α 600UI/ml, IFN-γ 5ng/ml and IFN-λ (IL-28) 25ng/ml for 72 hours. IL-7 secretion after a 72-hour-stimulation of SGEC.

Results. In SGEC, 495 genes were differentially expressed between pSS and controls. 280 genes were up-regulated, and 215 genes were down-regulated (Figure 1). Enrichment analysis (Table I) highlighted IL7 signaling pathways (including IL7, STAT5A, STAT1 genes) and interferon signaling (including OAS1, IFIT3, IFI6, TAP1 genes). Other genes potentially involved in immune responses and interactions between SGEC and lymphocytes were significantly up-regulated, including bone marrow stromal cell antigen 2, HLA-DRA, BAFF-R and IL23 A (Table II). CD86, a costimulatory molecule, was found to be significantly down-regulated. These results need to be confirmed by RT qPCR. However, congruent results have already been obtained in our laboratory, showing that IL7 serum level is increased in pSS patients compared to controls and that SGEC produce IL7 after interferon stimulation. The analysis of the non-coding RNA part and the other sorted cells subtypes is ongoing.

Conclusions. Immune interactions between SGEC and B or T lymphocytes could represent a key in the understanding of the initiation and/or maintenance of autoimmunity in pSS. Our study highlights the key role of epithelial cells in activation of immune cells. In vitro experiments are needed to confirm these results and elucidate the molecular mechanisms.
than A20WT/WT mice after correction for pilocarpine dose (Figure 1A). The level of mucin 10 in saliva of A20FL/FL mice decreased compared to A20WT/WT mice at 30 weeks of age, whereas mucin 19 appeared to increase. CD45+ and CD3+ periductal lymphocytic infiltrations started to emerge in A20FL/FL mice from 20 weeks of age, and were significantly higher than those in the control mice at 30 weeks of age. Characterization of B cell content of A20FL/FL mice is in progress. Lymphocytes also infiltrated the striated ducts of A20FL/FL mice by 50 weeks of age, (Figure 1B). Characterization of the dominant cell type in these structures is underway.

Conclusions. We present a mouse model for early epithelial cell involvement in pSS pathology development, whereby immune activation via the NF-κB pathway of epithelial cells is enough to generate the main characteristics of pSS, namely saliva reduction, altered composition of saliva, periductal lymphocytic infiltration and lymphoepithelial lesions. In our study, we emphasize the critical role of epithelial cells in the early stage of this autoimmune disease and open the door for further studies into the initiation of salivary gland pathology development in pSS.

**Background.** The aim of this study was to analyze whether Sjögren’s syndrome is triggered by patient’s intrinsic pathomechanisms or in cooperation with coincident hidden viral infection.

**Methods.** In 126 patients with sicca conditions immunohistochemical analysis of immune response to suggested silent persistence of mumps virus in the minor salivary glands biopsies and cytometric analysis of blood cells was done.

**Results.** Marks of mumps virus together with protein IFI16, interferons gamma and beta, dendritic cells, interleukin-3 receptor, receptor for natural killer cells and autophagy products were detected in the minor salivary gland biopsies from the patients with Sjögren’s syndrome, rheumatoid arthritis, but also in the non autoimmune sicca patients. Cytometric analysis of the blood cells from these patients revealed dropping amount of circulating natural killers and dendritic cells as supposed result of their central exhaustion due to massive intraglandular entrapping and degradation.

**Conclusions.** Occurrence of abundant intraglandular immunohistochemical marks of mumps virus protein known by strong tropism to some epithelial cells accompanied by depletion of circulating immune cells make firm background for thought of presumable mumps or/and other viruses participation in specific epithelial damage in predisposed patients sicca syndrome.

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**P-119**

**Local glandular interferon activation in primary Sjögren's syndrome is associated with systemic interferon activation**

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**Background.** Interferon (IFN) is considered a pathogenic factor in subsets of primary Sjögren's syndrome (pSS) patients. Upregulation of both IFN type I and IFN type II are described locally in the glands and systemically in the blood. However, there are limited data on how local and systemic IFN activation are correlated. It is critical to define the correlation between peripheral blood and minor salivary gland IFN activation patterns in the same individual as this can prevent repeated collection of salivary gland biopsies. In this pilot study we analyzed systemic IFN activation in the peripheral blood samples of pSS patients and correlated this with the local glandular IFN activation which was previously characterized in simultaneously collected salivary gland biopsies of patients.

**Methods.** Frozen PBMC samples were obtained from 11 pSS patients and 2 healthy controls without pSS from the Sjögren’s International Collaborative Clinical Alliance (SICCA) registry. Local IFN type I and type II activation was previously determined in frozen labial salivary gland lysates by Western blotting by us (Hall et al. 2015). IFIT3 protein expression was used to indicate IFN type I activation (alpha) and GBP1 was used to indicate IFN type II activation (gamma). Peripheral blood monocytes were isolated from PBMC samples followed by RQ-PCR as described previously (Briek et al. 2013). Systemic modular IFN expression was analyzed (Chiche et al. 2014, Bodewes et al. in press). M1.2 indicated systemic activation of IFN type I only and M5.12 indicated systemic activation of both IFN type I and IFN type II.

**Results.** Patients with local upregulation of IFN showed systemically higher M1.2 (IFN type I activation) and M5.12 scores compared to patients with local gamma only. M1.2 and M5.12 scores of patients without local IFN activation were comparable to scores of healthy controls.

**Conclusions.** This small sample study indicates that pSS patients with local glandular IFN activation also have systemically higher IFN scores. Systemic IFN activation might therefore be a useful biomarker to monitor local inflammation.

**Acknowledgements.** The SICCA registry and participating patients.

**Funding.** The study was supported by a grant of the Dutch Arthritis Foundation (RF14-3-404) and the Jerome L. Greene foundation.
TBK1 inhibition downregulates expression of interferon type I and the upregulated expression of RIG-like receptors and DNA-sensing receptors in interferon positive primary Sjögren’s syndrome patients

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Background. Upregulation of type I interferons (IFN-I) is a hallmark of systemic autoimmune diseases like primary Sjögren’s syndrome (pSS). Expression of IFN-I is induced by three different receptor families: Toll-like receptors (TLRs), RIG-like receptors (RLRs) and DNA-sensing receptors (DSRs). Previously we have shown increased mRNA levels of TLRs and RLRs in plasmacytoid dendritic cells (pDC) and CD14+ monocytes of IFN-I receptors (TLRs), RIG-like receptors (RLRs) and DNA-sensing receptors (DSRs) in patients with primary Sjögren’s syndrome. Expressed in pDC of IFNpos pSS patients, 1. TANK-binding kinase (TBK1), an important signaling hub downstream of RLRs and DSRs leads to production of IFN-I and subsequent induction of interferon-stimulated genes (ISGs). The objective of this study was to study RLRs and DSRs in pSS and explore the potential of a TBK1 inhibitor to downregulate IFN-I activation.

Methods. Expression of RLRs and DSRs was assessed by RT-PCR and flowcytometry in CD14+ monocytes, BDCA4+CD123+ pDC and CD19+ B cells from IFNpos pSS patients. pDCs from IFNpos pSS patients were analyzed by flowcytometry for phosphorylated TBK1 (pTBK1). PBMCs of pSS patients were cultured with a TBK1 inhibitor, BX795, followed by analysis of IFN-I production and expression of ISGs.

Results. In addition to upregulated mRNA levels of RLRs IFI18 (encoding for MDA5) and DDX58 (encoding for RIG-I), which we previously observed in pDC and monocytes of IFNpos pSS patients, gene expression of IFI18 and DDX58 was also upregulated in B cells. Upregulation of mRNA levels of the DSRs IFI16 and ZBP1 was observed in monocytes and B cells from IFNpos patients. In pDC protein expression of MDA5, ZBP-1, IFI16 was increased in IFNpos pSS, while there were no differences in RIG-I. In monocytes protein expression of MDA5 was increased and a trend was visible for RIG-I and IFI16. B cells showed increased protein expression of MDA5 and a trend was observed for RIG-I, ZBP-1 and IFI16. These data indicate upregulation of RLRs and DSRs, particularly in pDC of IFNpos pSS patients. To further look into the signaling of RLRs and DSRs, phosphorylation of TBK1 was studied in pDC, the main IFN-I producers, of pSS patients. Increased expression of pTBK1 was observed in pDCs from IFNpos pSS. Similar upregulation of pTBK1 was observed in IFNpos systemic lupus erythematosus (SLE) and systemic sclerosis (SSc) patients. Upon treatment with BX795, PBMCs from IFNpos pSS (and SLE and SSc) downregulated the production of IFN-I and mRNA expression of the ISGs MxA, IFI44L, IFI16 and IFIT3.

Conclusions. RLRs and DSRs are upregulated in IFNpos pSS. Signaling of these receptors could be blocked using a TBK1 inhibitor, which reduced IFN-I protein production and expression of ISGs in PBMCs of IFNpos pSS patients. As patented pharmacological inhibitors, amongst others a small molecule inhibitor, are available TBK1 inhibition is indicated as a potential future treatment target for IFNpos pSS.

Funding. The study was supported by a grant of the Dutch Arthritis Foundation (RF14.3-404).

Reference


Sjögren’s-like syndrome in APECED patients is associated with down-regulated IFN pathways and impaired salivary fluid secretion; Calcium signaling attenuation in patients and NOD Aire +/- mice

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Background. Recognized as a rare autoimmune disorder; Autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy (APECED) is caused by mutations in the AIRE (autoimmune regulator) gene. Mutations in AIRE cause disruption of negative selection in the thymus, creating impaired autoreactive T cells leading to endocrine and non-endocrine autoimmune dysregulation. Diagnosis of APECED requires the presence of two of the three traditional hallmarks which include chronic muco-cutaneous candidiasis, hypoparathyroidism, and Addison’s disease. 42.9% of our American APECED cohort developed Sjögren’s-like syndrome (JCI Insight. 2016;1(13): e88782) based on established American-European consensus criteria; manifesting chronic sicca symptoms (eyes and mouth) and positive focus score on minor salivary gland biopsies. In contrast to Sjögren’s syndrome; Sjögren’s-like syndrome in APECED patients lack ENA autoantibodies, suggesting the pathogenesis of the syndrome in APECED is distinct from primary Sjögren’s syndrome. The objectives were to identify key targets altered in the pathophysiology of Sjögren’s-like APECED patients. We performed RNA-Seq of patient salivary glands and we completed functional imaging studies on salivary glands from patients and the APECED mouse model NOD Aire-/-.

Methods. Total RNA was isolated from 4 APECED patients with Sjögren’s-like syndrome and 6 Healthy Controls. Sequenced data were preprocessed on the Ion Torrent server with Torrent Suite. Genomic features were counted using the HTSeq-count tool, and raw counts were analyzed for differential expression using the DESeq2 package in R. Ingenuity Pathway Analysis (IPA) was used to analyze pathway enrichment. Functional data was acquired from patient salivary gland biopsies and submandibular glands from NOD Aire-/- mice, and minced to obtain cell clusters. Loaded clusters with the cytosolic calcium indicator (Fluo-2AM) were used for live-cell imaging in order to quantify cytosolic calcium fluxes induced by parasympathetic stimulation.

Results. IPA analysis displayed enrichment of immune-related pathways including downregulation of IFN pathways and genes associated with saliva secretion in the patients. The most significant down-regulated gene was KCNMA1; a calcium-calcium-activated channel critical for maintaining fluid secretion. In support of this finding, the functional data displayed attenuation of calcium signaling at the level of calcium release in two of three patients and in the NOD Aire-/- mice. Aquaporin 3 (AQP3), a highly AIRE-dependent gene in medullary thymin epithelial cells, was also downregulated; AQP3 localizes on the basolateral side of acinar cells is known to participate in transepidermal osmotic water flow.

Conclusion. Our study has identified specific target genes that may be implicated in the pathogenesis of Sjögren’s-like syndrome in APECED patients. Ongoing experiments are aimed at validating these target genes, at examining the transcriptome profile of Aire-/- salivary glands, and in the presence of the presence of autoantibodies against KCNMA1 or AQP3 in this population.

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A Novel Graph-Approach Applied to Modular-Analysis Identifies Shared Gradual Whole Blood IFN Signatures in Primary Sjögren’s Syndrome and Systemic Lupus Erythematosus, and Reveals New IFN-related Modules in Disease Progression

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Background. There is significant clinical and molecular heterogeneity among patients suffering from systemic autoimmune diseases, such as primary Sjögren’s Syndrome (pSS). Deciphering this heterogeneity could allow the molecular stratification of patients in terms of prognosis and therapeutic targets. Our previous work using a Modular Repertoire Analysis (MRA) has demonstrated that the IFN signature observed in systemic lupus erythematosus (SLE) patients is not restricted to a mere type I IFN signature, but involves the gradual activation of 3 distinct IFN modules driven by various IFN types including IFNg. Although type I and type II IFN signatures have been described in patients with pSS, a detailed MRA in pSS is still lacking. Here we aimed to refine MRA and discover new transcriptional signatures in pSS, by applying a novel Graph-Theoretic-Approach (GTA) to reveal the progression of module activation patterns.

Methods. Blood transcriptomic microarray datasets, including that of pSS patients (n=133, UKPSSR) fulfilling American European Consensus Group (AECG)-criteria and SLE patients (n=157 samples; LUPUCE cohort) fulfilling ACR-criteria, were analyzed using MRA followed by GTA. MRA was performed using a blood modular framework comprising 260 modules. A novel GTA, based on the Extended Suppex Bays Causal Network (ESBCN), was used to generate an ordered, branching progression model of modular activation. Disease-specific causal graphs in selected datasets were built in order to generate hypotheses regarding disease progression for a particular disease. Significance to clinical characteristics was evaluated using Fisher’s exact test and ANOVA, for categorical and continuous characteristics, respectively.

Results. The GTA-to-MRA analysis confirmed the previously described pattern of gradual activation of IFN modules in SLE patients: first M1.2 (81.5%), then M3.4 (67.5%) and finally M5.12 (22.3%). Interestingly, this gradual modular IFN signature was similarly observed in pSS patients who exhibited activation of I (64%), 2 (37%) or all 3 (8%) IFN modules. Additionally, GTA-to-MRA identified a dual mode of disease progression in SLE after the activation of the IFN modules M1.2 and M3.4: either completion of the IFN signature, to include the more IFN-related module M5.12 or with completion of a newly identified 4th IFN-related module M8.59, or the activation of a neutrophil module M5.15 associated with renal involvement. In pSS, a dual mode of progression identified comparable completion of IFN signature to include M5.12, ending with IFN module M8.59, or activation of a 5th IFN-related module M8.95. Solely 7% of pSS patients portrayed a neutrophil signature.

Conclusion. With the application of GTA to blood MRA, we are the first to show a detailed modular IFN signature in pSS. Here we show the sharing of gradual activation of IFN modules between pSS and SLE and the identification of new IFN-related modules through the observation of progression patterns, with both shared and distinct downstream progression patterns in pSS and SLE. Defining distinct molecular subgroups in pSS will aid in development of more tailored therapeutic regimens.

P-124
Aberrent cell signaling in peripheral blood mononuclear cells upon interferon alpha stimulation in patients with primary Sjögren’s syndrome associates with type I interferon signature

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Background. Primary Sjögren’s syndrome (pSS) is a complex heterogeneous systemic autoimmune disease. Biomarkers for patient stratification are scarce. Several single nucleotide polymorphisms within type I interferon (IFN) signaling pathways are associated with pSS. To define novel biomarkers for pSS patient stratification, we analysed the temporal profile of MAPK/ERK and JAK/STAT signaling networks in peripheral blood mononuclear cells (PBMC) upon stimulation with IFNα by flow cytometry.

Methods. PBMC from pSS patients and healthy matched donors were stimulated for 15, 30, 60, 120, 180, and 240 min with IFNα at 100 ng/ml. Nine different phosphorylation epitopes were analysed: STAT1(pY701), ERK1/2(pY204), NF-κB(p65)p52(p529), STAT1(pS727), STAT1(pY701), p38 MAPK(pT180/pY182), STAT3(pS727), STAT3(pY705) and STAT5(pY694).

To define leukocyte populations, scatter properties and cell surface markers CD3, CD20 and CD56 were utilized.

Results. Cells from pSS patients displayed significant differences in basal and IFNα induced phosphorylation levels of numerous signalling proteins compared to cells from healthy donors. Principal component analysis (PCA) using IFNα induced phosphorylation levels after 15 minutes showed clustering of pSS patients and pSS patient subgroups. PCA visualization showed a positive shift for pSS samples away from healthy donor samples with positive movement influenced by changes in phosphorylation of STAT1 Y701 in T, NK and B cells in PC1, and PC2 positive movement influenced by STAT3 Y701 in NK and B cells, and negative movement by STAT3 S727 in T cells. Medicated and autoantibody negative patients grouped closer to healthy donors than non-medicated or autoantibody positive patients.

Conclusion. pSS patients show increased responses to IFNα through STAT1. Increased responses to IFNα may in part drive an up-regulation of interferon induced genes.

P-125
Imbalance in subsets of circulating innate lymphoid cells is associated with disease activity and type I interferon signature in primary Sjögren’s syndrome

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Background. Recent studies indicate an important role for innate lymphoid cells (ILCs) in the pathophysiology of rheumatic diseases. In rheumatoid arthritis and spondyloarthritis elevated numbers of subsets of ILCs have been found at the site of inflammation producing cytokines including IFN-γ and IL-22 and in addition, group 3 ILC have been suggested to be involved in formation of ectopic lymphoid structures in rheumatic diseases (Shikhar et al Rev Rheumatol 2017, Wenink A&R 2017). ILC3-like cells producing IL-22 have been found in the salivary glands of pSS patients (Ciccia ARD 2012). However, circulating ILC have not yet been studied in primary Sjögren’s syndrome (pSS) and systemic lupus erythematosus (SLE). SLE and pSS are characterized by presence of a type I interferon (IFN) signature in a large proportion of the patients. Animal studies in HIV and asthma implicates type I IFN, produced by plasmacytoid dendritic cells (pDCs), to regulate the survival of group 2 and group 3 ILCs (ILC2 and ILC3) via increased Fas engagement. ILC2 and ILC3 may be involved in the pathogenesis of pSS.

Methods. Frequencies and phenotypes of ILC subsets and pDCs were asayed by flow cytometry in peripheral blood of patients with pSS (n=20), SLE (n=20) and healthy controls (n=17). Patients were stratified by the...
Y RNA and other circulating RNAs in Sjögren’s syndrome

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Results. CLR1C numbers were increased in peripheral blood of patients with SLE as compared to healthy controls and in pSS patients ILC1 numbers correlated with disease activity (ESSDAI score), serum IgG levels and anti-SSB autoantibodies (all p<0.05). Numbers of ILC1, ILC2 or ILC3 did not significantly differ between patients with SLE and pSS. However, patients with a high expression of the type 1 IFN signature had significantly decreased numbers of ILC2 and ILC3 (p=0.04 and p=0.02, respectively). The decrease of ILC2 and ILC3 was related to increased expression of Fas (CD95) on these cells in patients with a high type 1 IFN signature (both p<0.01).

Conclusion. Both in SLE and pSS, the presence of a type 1 IFN signature is related to reduced numbers of circulating ILC2 and ILC3 in association with increased Fas expression on these cells possibly rendering them more susceptible to Fas/FasL-dependent apoptosis at peripheral sites.

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Y RNA and other circulating RNAs in Sjögren’s syndrome

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Results. CLR1C numbers were increased in peripheral blood of patients with SLE as compared to healthy controls and in pSS patients ILC1 numbers correlated with disease activity (ESSDAI score), serum IgG levels and anti-SSB autoantibodies (all p<0.05). Numbers of ILC1, ILC2 or ILC3 did not significantly differ between patients with SLE and pSS. However, patients with a high expression of the type 1 IFN signature had significantly decreased numbers of ILC2 and ILC3 (p=0.04 and p=0.02, respectively). The decrease of ILC2 and ILC3 was related to increased expression of Fas (CD95) on these cells in patients with a high type 1 IFN signature (both p<0.01).

Conclusion. Both in SLE and pSS, the presence of a type 1 IFN signature is related to reduced numbers of circulating ILC2 and ILC3 in association with increased Fas expression on these cells possibly rendering them more susceptible to Fas/FasL-dependent apoptosis at peripheral sites.

P-127

Decreased expression of microRNA 130a indicates dysregulation of classical dendritic cells in patients with primary Sjögren’s syndrome

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Background. Primary Sjögren’s syndrome (pSS) is a systemic autoimmune disease characterized by lymphocytic infiltration of the exocrine glands and dryness of mouth and eyes. Classical dendritic cells (cDCs) are very potent antigen presenting cells known to induce strong T-cell proliferation and cytokine production. Considering the critical role of microRNAs (miRNAs) in regulation of gene expression, we investigated miRNA expression in isolated CD1c-expressing cDCs of patients with pSS.

Methods. Two independent cohorts consisting of pSS patients and healthy controls were established: a discovery cohort (15 pSS, 6 HC) was used to screen the expression of a large panel of 758 miRNAs, while a validation cohort (8 pSS, 11 HC) was used to test the reproducibility of the results. CD1c-expressing cDCs were isolated from peripheral blood using MACS and miRNA profiling of 758 targets was performed using the OpenArray platform in the donors included in the discovery cohort. A selection of 16 miRNAs found to be differentially expressed in the pSS group compared to the control group (p<0.05, with a difference between the groups of >log2) was measured in the independent validation cohort using a custom-made array. Isolated cDCs from HC were stimulated with a panel of Toll-like receptor (TLR) ligands and the expression of miR-130a and miR-708 was measured by qPCR.

Results. Nine variants of Valine tRNA were upregulated by an average of 6.6 fold (logFC=2.7) in SS vs DES, several other RNAs were down regulated (Table 1). Y RNA was depleted in SS secondary to RA vs DES while it was slightly upregulated in the other SS groups. Only the R4 variant change was statistically significant, but there is a general trend (Figure 1).

Conclusions. Circulating miRNA has the potential to uncover SS biomarkers. Further large scale multinational clinical trials are needed to validate this observation. The depletion of Y RNA in serum of SS patients is particularly alarming since this highly conserved sequence is part of the Ros60 RNP and this depletion could be explained by autoimmune mediated degradation. The relevance of Y RNA in SS was recently hypothesized by Kabeerdoss et al.: “Y RNA derived small RNAs in Sjögren’s syndrome: Candidate biomarkers?”. Int J Rheum Dis. 2017 Nov 19.
isotope labeling by amino acids in cell culture (pSilAC) method (quantitative mass spectrometry-based technique) in a HEK-293T cell-line.

Results. mRNA was downregulated in pSS patients versus HC in the discovery cohort. Of the 16 selected targets for replication, decreased miR-130a and miR-708 were validated. cDC activation through TLR3 and TLR7/8 downregulates the expression of both mRNA-130a and mRNA-708. Transfection with miR-130a resulted in downregulation of proteins involved in NF-κB pathway. As such, these miRNAs seem to be involved in cDC activation and reflect enhanced activation of cDCs from peripheral blood of pSS patients.

Conclusions. mRNA-130a and miR-708 are significantly downregulated in cDCs of patients with pSS. We show that the expression of these miRNAs is decreased upon cDC activation and that transfection with miR-130a downregulates the expression of proteins involved in the NF-κB pathway. As such, these miRNAs seem to be involved in cDC activation and reflect enhanced activation of cDCs from peripheral blood of pSS patients.

P-128
Identification of dysregulated Interferon-inducible non-coding RNAs in Sjögren’s syndrome
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Background. Sjögren’s syndrome (SS) is a chronic, heterogeneous disease with hallmark features of auto-immunization and autoantibody production. Upregulation of type I and II interferon-stimulated genes (ISGs), known as the “Interferon (IFN) Signature” is correlated with anti-Ro titers and has been observed both in the salivary glands and peripheral blood of SS patients. Within the 2p25.2 genomic interval, the long non-coding RNA (IncRNA) negative regulator of the interferon response (NRIR) has been identified as inducible by type I IFN and is responsible for the downregulation of the ISGs CMPK2 and RSAD2 (Kambara et al., Nucleic Acids Res 2014). We sought to identify additional unannotated ISGs IncRNAs that are differentially expressed (DE) in SS patients utilizing correlated expression of RSAD2.

Methods. In this study, we evaluated and compared the transcriptome of anti-Ro(+) patients (n=27) and healthy controls (n=27) using RNA-seq with DE defined as q < 0.05 and a fold change (FC) ≥2 or ≤0.5. qRT-PCR to measure the protein coding genes MX1, OAS1, and GBP5 along with hallmark features of auto-inflammation and autoantibody production. Since RSAD2 plays a role in the type I IFN pathway, pair-wise correlation coefficients between all the DE transcripts and RSAD2 expression were calculated. A total of 24 miRNAs was downregulated in pSS patients utilizing correlated expression of RSAD2 and miRNA-708 were validated. cDC activation through TLR3 and TLR7/8 downregulates the expression of both mRNA-130a and mRNA-708. Transfection with miR-130a downregulates the expression of proteins involved in the NF-κB pathway. As such, these miRNAs seem to be involved in cDC activation and reflect enhanced activation of cDCs from peripheral blood of pSS patients.

Conclusions. mRNA-130a and miR-708 are significantly downregulated in cDCs of patients with pSS. We show that the expression of these miRNAs is decreased upon cDC activation and that transfection with miR-130a downregulates the expression of proteins involved in the NF-κB pathway. As such, these miRNAs seem to be involved in cDC activation and reflect enhanced activation of cDCs from peripheral blood of pSS patients.

P-130
Involvement of gap junctional communication in salivary gland dysfunction related to primary Sjögren’s syndrome
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Background. Gap junctions and distinct connexin (Cx) isoforms play a crucial regulatory role in various aspects of glandular function. The acinar cells of salivary glands are coupled by Cx26 and Cx32 channels and it has been suggested that these gap junction channels may control salivary secretion via the inhibition of capacitative Ca2+ entry. To date few data are available regarding the role of connexins in salivary dysfunction related to primary Sjögren’s syndrome (pSS).

Aim of this study, to characterize the expression of Cx26 in minor salivary gland biopsies (MSGBs) of patients with pSS in order to investigate their potential role in salivary gland inflammation and hypofunction.

Methods. We analyzed the expression of Cx26 in MSGBs from pSS patients and from non-immune mediated sicca subjects, (no-SS). MSGB were obtained as part of the routine diagnostic procedures when pSS was suspected. In all cases, part of the MSG specimen was fixed in neutral-buffered formalin for the assessment of the focus score (FS), and the remaining part was quickly frozen and stored at -80°C for immunohistochemical experiments. Unstimulated salivary flow was measured as well in all the subjects enrolled. Cx26 expression was evaluated by indirect immunofluorescence on acetone/ methanol fixed cryosections. Double immunofluorescence, performed using rabbit anti-human Cx26 and mouse anti-human ASMA primary antibodies was observed under confocal laser scanning microscopy by two blinded, independent researchers. We defined according to a semiquantitative scale 4
levels of Cx26 expression in both acinar and myoepithelial cells (1=severely decreased, 2=moderately decreased, 3=slightly decreased, 4=not decreased). Results. We analyzed the expression of Cx26 in 29 MSGBs:18 from pSS patients and 11 no-SS subjects. Immunolabeling for Cx26 was observed at level of luminal, lateral and basal borders of secretory and myoepithelial cells. The expression of Cx26 in both acinar (p=0.002) and myoepithelial cells (p=0.03) from pSS patients was significantly decreased when compared to no-SS subjects. In pSS MSG with a FS≥3, the acinar (p=0.01) and myoepithelial expression (p=0.05) of Cx26 was significantly lower than in pSS samples with a FS<3. Finally, a significant association (p=0.01) was observed between Cx26 expression in MSGB and salivary flow impairment in pSS.

Conclusions. This pilot study has shown that gap junctional communication may be involved in pSS-salivary gland dysfunction. Further studies are necessary to clarify the pathogenetic role of Cx proteins in salivary gland hypofunction and whether these proteins may represent a novel target for future therapies.

P-131
Role of Nuclear factor of activated T-cells 5 in hyperosmolar stress-induced osmoadaptive and inflammatory responses in human salivary glands cells

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Background. Cells can be subjected to hyperosmotic stress (HOS) under physiological and pathological conditions. Indeed, during the steps leading to saliva secretion, acinar cells are exposed to a transepithelial osmotic gradient, resulting from NaCl secretion and accumulation in the acini lumen, which drives an important transcellular water flux through apically-localized aquaporin-5 (AQ5) and basolaterally localized AQ1-AQ4 with the ductal lumen, the composition of the primary secreted isotonic fluid is modified, leading to the secretion of a final hypotonic saliva. In salivary glands of patients suffering from Sjögren’s syndrome, characterized by gland destruction and in some cases altered aquaporin-5 subcellular localization, acinar cells are hypothesized to be subjected to chronic HOS. In response to HOS, cells rapidly initiate an osmoadaptive program that includes the activation of the transcription factor Tonicity Enhancer Binding Protein (TonEBP), also called Nuclear factor of activated T-cells 5 (NFAT5), and subsequent transactivation of osmoregulatory genes (such as aldose reductase (AKR1B1) and sodium- and chloride-dependent taurine transporter (TAUT)). Under pathological conditions, HO5 may significantly contribute to disease progression by triggering proinflammatory cytokines release. The aim of the present study was to investigate the role of NFAT5 in osmoadaptive and inflammatory responses triggered by HOS in HSG cells.

Methods. Human salivary gland HSG cells were stably transduced by electroporation with either a plasmid coding for a scrambled RNA sequence (shCTN) or a plasmid coding for NFAT5 gene silencing short hairpin RNAs (shNFAT5). shCTN- and shNFAT5-stably transfected HSG cells were subjected to iso-osmolar condition (iso-osmolar medium; ISO) or HOS (iso-osmolar medium supplemented with various concentrations of NaCl or sucrose). NFAT5 transactivation activity was measured in shCTN- and shNFAT5-stably transfected HSG cells that were subsequently co-transfected with the reporter plasmid pTonE-SEAP (two NFAT5 binding sites (TonE) upstream of a modified secreted alkaline phosphatase (SEAP) gene) and finally exposed to ISO or HOS. mRNA levels were quantified by real time quantitative PCR (RT-qPCR). Statistical analysis was performed to compare group means using t-test for unique sample and repeated measure ANOVA with post-hoc Bonferroni t-tests.

Results. As compared to ISO, HOS significantly increased NFAT5 transactivation activity; NFAT5, AKR1B1 and TAUT mRNA levels; as well as CCL2, IL6, IL8, TGFβ mRNA levels in HSG cells. As compared to shCTN, shNFAT5 significantly decreased NFAT5 transactivation activity; NFAT5, AKR1B1, CCL2, IL6, IL8, TGFβ (but not TAUT) mRNA levels in HSG cells. Conclusions. Our data suggest that NFAT5 is involved in HOS-triggered osmoadaptive and inflammatory responses in HSG cells. In patients suffering from Sjögren’s syndrome, HOS may therefore significantly contribute to disease progression by triggering proinflammatory cytokines release.

P-133
The levels, subcellular distribution and posttranslational processing of salivary mucins are affected by pro-inflammatory cytokines in Sjögren’s syndrome patients

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Background. Sjögren’s syndrome (SS) is a chronic autoimmune disease characterized by inflammation of the exocrine glands and symptoms of oral and ocular dryness. The persistent sensation of oral dryness in SS-patients may be attributable to changes in the quality of mucins, the major component of the viscoelastic layer covering all mucosal surfaces in the body. Mucins are large glycoproteins synthesized in the rough endoplasmic reticulum (RER) and post-translationally processed in the RER and Golgi apparatus where they are N- and O-glycosylated, respectively. O-glycosyla- tion consists of the attachment of sialylated and sulfated oligosaccharides conferring mucins the property of retain water. It has been described that pro-inflammatory cytokines induce the expression of certain mucins and modulate post-translational processing of these and other glycoproteins in several cell types. Salivary glands (SG) from SS-patients show high levels of pro-inflammatory cytokines, accumulation of MUC1 and decreased sialation of MUC5B. We hypothesized that pro-inflammatory cytokines alter the secretory pathway of SG from SS-patients, inducing changes in levels, localization and post-translational processing of salivary mucins.

Methods. We evaluated the expression and subcellular localization of MUC1, the expression and activity of Golgi glycosyltransferases and Gal3-O-sulfotransferases (Gal3ST), and the effect of pro-inflammatory cytokines on levels and localization of mucins. Relative mRNA and protein levels of mucins and Golgi enzymes were determined by real-time RT-PCR and Western blotting in SG from SS-patients and controls and in HSG-3D acini. Localization analyses were performed by immunofluorescence. Enzymatic activities were quantified in SG using in vitro assays with radioactively la- beled donor substrates and specific acceptor substrates.
Results. MUC1 was overexpressed and accumulated in the endoplasmic reticulum where unexpectedly it was also observed the Golgi tethering protein Giantin. A significant decrease of Gal3ST activity without expression changes was observed in SG from SS-patients. Gal3ST activity directly correlated with MUCSB sulfation and inversely correlated with inflammation. Stimulation of HSG-3D acini with TNF-α or IFN-γ induced MUC1 expression and accumulation.

Conclusions. Our results suggest that pro-inflammatory cytokines induced the expression of MUC1, which accumulated in the RER of SG from SS-patients, likely by the stress condition of this compartment. As Gal3ST activity was decreased without expression changes, we speculate that altered localization of Giantin could affect the correct targeting of Gal3ST thereby explaining the hyposulfation of mucins observed in SG from SS-patients. The decrease of glandular inflammation could help to restore the secretory pathway of salivary mucins, improving the hydration of the oral mucosa in SS-patients.

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P-134

Acinar atrophy and adipose tissue infiltration in salivary gland biopsy are associated with IFN-γ pathway inflammatory bio-markers

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Background. Acinar atrophy and adipose infiltration are late findings in the salivary glands histology of primary Sjögren’s syndrome (pSS). We hypothesized that they could be an evidence of severe disease and associated with disease activity. We assessed inflammatory biomarkers of the IFN-γ pathway and its association with acinar atrophy and/or adipose infiltration in the minor salivary gland biopsy in patients with pSS.

Methods. Cross-sectional study including patients with pSS (AEGC 2002 or ACR/EULAR 2017) submitted to minor salivary gland biopsy and histological analysis. Kyurenine, and neopterin were measured in plasma by liquid chromatography–tandem mass spectrometry. Non-parametric statistics was applied with p-value of 0.05.

Results. 99 patients with pSS performed labial salivary gland biopsy (LSGB), 95.9% were women, 51.49±12.3 years, Anti-Ro positive in 68% (n=70/103) and Eular Sjögren’s Syndrome Disease Activity Index (ESSDAI) 3.6±5.12. LSGB histopathology showed 73.7% (n=73/99) focal lymphocytic sialadenitis with focus score ≥1, non-specific chronic salivary gland atrophy 1.2% (n=15/125), hypothyropic chronic sialadenitis in 10.1% (n=10/99) and within normal limits (no lymphocytes) in 1% (n=1/99). No case of sclerosing chronic salivary glanditis, granulomatous inflammation or marginal zone (MALT) lymphoma. The majority of patients had some grade of acinar atrophy (70.4%, n=57/81), ductal dilatation (86.4%, n=70/81), adipose infiltration (51.2%, n=39/78). Acinar atrophy was present in older individuals (53.46±10.09 vs. 42.83±13.3 years, p=0.001), associated with a higher frequency of menopause (57.9% vs. 33.3%, p=0.043), dryness (ESS-SPRI >3) (78.2% vs. 50%, p=0.018), more active disease (ESSDAI ≥5) (33.3% vs. 8.3%, p=0.017) and higher levels of neopterin (25.5±4.125 vs. 20.67±12.58 nmol/L, p=0.040). There was no association with disease duration, anti-Ro, glandular dysfunction (unstimulated whole saliva flow) and smoking. Adipose infiltration was also present in older individuals (53.49±12.33 vs. 47.51±11.29, p=0.016), associated with lacrimal dysfunction (Schirmer ≤5mm) (69.2% vs. 41%, p=0.012) and higher quinolinic acid (503.35±193.30 vs. 427.35±285.76 nmol/L, p=0.029), kyurenine (1.99±0.6, 54 vs. 1.61±0.46 µmol/L, p=0.006), kyurenine/tryptophan ratio (KTR) (0.030±0.09 vs. 0.025±0.01, p=0.031) and antrichonic acid (0.6±4.96 vs. 16.4±5.24 nmol/L, p=0.003).

Conclusions. Acinar atrophy and adipose infiltration are associated with disease activity symptoms, and activation of the INFγ pathway. Kyurenine pathway metabolites are associated with adipose infiltration and neopterin is associated with acinar atrophy.

Background. Sjögren’s syndrome (SS) is a complex autoimmune disease associated with lymphocytic infiltration and secretory dysfunction of salivary and lacrimal glands that are frequently accompanied by pro-fibrotic changes in the underlying stromal compartment. Although the current model postulates that primary SS is a secondary effect of lymphocytic infiltrates, increasing evidence suggests that structural defects in the salivary gland epithelia precede and contribute to pathological immune and fibrotic responses in this disease. To gain insights into the molecular changes underlining SS epithelia that may impact immune and fibrotic responses, we carried out a global gene expression analyses of labial salivary gland epithelia from SS and non-SS patients using laser capture microdissection (LCM) followed by computational genomic analyses.

Methods. Labial salivary glands were obtained from biopsies from 16 patients with sicca symptoms. Based on the American-European Consensus Criteria, these patients were sub-divided into 8 SS (focus score (FS) 1-2) and 8 non-SS sicca controls (FS<1). RNA was isolated from laser capture microdissected epithelia and analyzed by RNAseq followed by computational interrogation of gene expression signatures using genome-wide differential expression testing (DEseq2) for multiple group comparisons.

Results. Analyses of RNAseq-based gene expression profiles derived from SS and non-SS samples revealed three distinct subclusters. These included a SS subcluster (n=3), a non-SS subcluster (n=3) and a subcluster that consists of SS and non-SS samples (n=10). Differential expression analysis between the different subclusters revealed signaling events that may be associated with differential stages of SS epithelial pathology. Notable signals included the enrichment of INFgamma and JAK/STAT-regulated genes, and the induction of genes encoding secreted factors implicated in immune responses.

Conclusion. Our study has identified potential molecular subtypes of SS pathology, defined by gene expression signatures of salivary epithelia that are associated with mixed clinical and histopathological characteristics. Our observations also suggest that gene expression alterations arising in the salivary epithelia contribute to the etiology of SS. We postulate that these molecular sub-groups reflect varying stages of disease predisposition and may offer novel insight into the signals contributing to the progression of SS.
by immunofluorescence. Correlation analysis was performed by Pearson’s test. The effect of IFN-γ on UPR pathways was evaluated in 3D-acini.

**Results.**
Significant decrease of IRE1α, XBP-1u, XBP-1s, total XBP-1, and GRP78 mRNAs (IRE1α pathway) was observed in LSG of SS-patients, which was correlated with increased methylation levels of their respective promoters. Consistently, the protein levels were decreased. In 3D acini IFN-γ decreased the mRNA and protein levels of XBP-1s, IRE1α, and GRP78 and increased the methylation of their promoters. PERK pathway was activated and ATF4 protein was overexpressed in LSG from SS-patients. The ATF4 mRNA levels were decreased, which was correlated with increased promoter DNA methylation. SS-patients showed a significant increase of Xc-system (antioxidant response) expression. ATF4 protein levels correlated with scintigraphy data and serological markers while Xc-system protein levels correlated with ATF4 protein levels, serological markers, and mouth and eye dryness. In 3D acini IFN-γ decreased the ATF4 mRNA and increased its promoter’s methylation.

A significant increase of ATF6α mRNA (ATF6α pathway) was observed in LSG of SS-patients, correlating with decreased DNA methylation of its promoter. The protein levels of ATF6α and ERAD (ER-associated degradation) machinery components were increased. A balanced expression of cIAP2 and cleaved-caspase-3 was observed in LSG of SS-patients. IFN-γ decreased ATF6 DNA promoter methylation increased ATF6α mRNA levels, ATF6α protein levels, ATF6α nuclear translocation and ERAD machinery components, without increasing apoptosis.

**Conclusions.** The attenuation of IRE1α/XBP-1 signaling pathway could explain the glandular dysfunction in SS-patients, because this pathway is involved in biogenesis of the secretory machinery. Considering that ATF4 and ATF6 regulate the expression of genes involved in adaptive responses to cellular stress and ERAD, respectively, their increased expression and correlation with clinical data could be partially explained by partial resistance of patients to alleviate the stress arising in LSGs of SS-patients, thereby promoting cell survival. Glandular stress signals, including IFN-γ, could modulate the expression of the UPR pathways, likely by promoter DNA methylation.

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**P-138**

**RANK-L is expressed by salivary gland stromal and epithelial cells in primary Sjögren’s syndrome and could represent a key player in ectopic lymphoid structures neogenesis**


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**Introduction.** Tertiary Lymphoid Organs (TLOs) can be observed in target tissues of various autoimmune diseases such as salivary glands in primary Sjögren’s syndrome. TLOs are mimicking secondary lymphoid organs (SLOs) structure and strikingly share common features with lymph nodes. SLOs organogenesis is coordinated by a complex stromal network and RANK-L (Receptor Activator of NF-κB Ligand) has been recently involved as a pivotal cytokine in precoce steps of their formation. Nethertheless, the contribution of stromal cells in TLOs neogenesis remains unclear. Thus, we hypothesized that RANK-L could be expressed by salivary gland stroma and could therefore play a critical role in TLOs establishment.

**Materials and Methods.** Stromal cells and RANK-L expression were analysed in salivary glands’ TLOs by immunofluorescence on frozen sections in the NZB/NZW F1 mouse model and in minor salivary gland biopsies of patients fulfilling 2016 ACR-EULAR Sjögren’s syndrome criteria and by flow-cytometry after enzymatic digestion of NZB/NZW F1 salivary glands. RANK-L expression has also been assessed by Real Time quantitative Polymerase Chain Reaction (RT-qPCR) and immunofluorescence on primary cultures of salivary gland epithelial cells (SGECs) with or without IL-1α stimulation.

**Results.** Most of SLOs stromal cells populations: FRCs (Fibroblastic Reticular Cells), FDCs (Follicular Dendritic Cells), LECS (Lymphatic Endothelial Cells), BECs (Blood Endothelial Cells) and HEVs (High Endothelial Veins) were identified in salivary TLOs of both NZB/NZW F1 mice and patients. FRCs were dominant in salivary TLOs and their number correlated with the degree of lymphocytic infiltration (r=0.7; p=0.007). The main difference between TLOs and SLOs was the lack, in salivary gland, of MRCs (Marginal Reticular Cells) which are a major source of RANK-L in lymph nodes. Despite the absence of MRCs in TLOs, we have observed an expression of RANK-L by a few T-cells within the infiltrates and strikingly by blood vessels and epithelial cells. Moreover, RANK-L expression by SGECs in primary cultures was increased after INF-α or IL-1β stimulation.

**Conclusion.** To our knowledge, this is the first report of a RANK-L expression in Sjögren’s syndrome. These results suggest that RANK-L could be an important actor of ectopic lymphoid structures neogenesis and its inhibition might represent, in the future, an alternative immuno-modulatory strategy in primary Sjögren’s syndrome.
P-139
Salivary gland damage in Sjögren’s syndrome: are endothelial and vascular alterations associated with the secretion of IL-17?
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Background. Sjögren’s syndrome (SS) is a systemic, inflammatory autoimmune disease that primarily affects the exocrine glands. The pathophysiology of the disease is mainly described as an autoimmune epithelitis, that, mediated by cytokines, growth factors and metalloproteinases leads to the destruction of the glandular parenchyma, causing a sclerotic process, which ends up impairing the salivary secretion. Recent studies have shown that systemic vascular changes are also part of the SS; these alterations involve endothelial dysfunction and vascularitis and are described in multiple organs, however the description of these changes in salivary glands are scarce. Saliva production is complex; it involves water and ionic changes throughout salivary gland ducts and the vascular flow is an important factor for saliva formation. Interleukin-17 (IL-17), a pro-inflammatory cytokine, which has been associated with a pro-thrombotic effect in multiple inflammatory and autoimmune diseases, is also frequently associated with SS, for this reason, this study aimed to correlate the concentrations of IL-17 family in the saliva and their alterations found in minor salivary gland specimens.

Methods. The morphological characteristics of biopsied specimens from 27 patients previously diagnosed with SS, were analyzed for the presence of vascular alterations such as vasculitis, thrombosis, congestion and hemorrhage. These patients also underwent a saliva collection for a multiplex analysis of the concentrations of IL-17A, IL-17E and IL-17F. Statistical analysis were performed to observe the concentration differences among the morphological alterations.

Results. Morphological analysis showed vessels congested in every specimen analyzed, and the presence of thrombi in 11 (40%) of the cases. Vasculitis was also found in 16 (59%) of the specimens. Binary logistic regression showed an increase of 1.012 in IL-17E when associated with vasculitis (p=0.034; 95%CI 1.001-1.022) with statistically significant higher concentration in patients with signs of vasculitis (p=0.0058). However, there was no statistically significant differences or associations between the IL-17A and IL-17F with the vascular changes. There was a higher concentration of IL-17E when compared to IL-17A (p=0.0001).

Conclusions. The IL-17A and its isoform IL-17F are often associated with larger vessels inflammation such as takayasu arteritis (1), IL-17E was recently renamed to IL-25, for its antagonist function to IL-17. The functions of this cytokine are still being described, however, it is known that IL-17E is capable of mediating Th2 responses, and was found present in a higher concentration in patients with Churg-Strauss syndrome, associated with vascular changes (2), however, its role in mediating the autoimmun process in SS and the association with vascular alterations in the disease must be further investigated.

References

P-140
Functional analysis of saliva secretion in a mouse model of X-linked hypohidrotic ectodermal dysplasia
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Background. X-linked hypohidrotic ectodermal dysplasia (X-LHED) is the most common form of more than 150 types of ectodermal dysplasias. Over 200 mutations in Ectodysplasin A (EDA), a gene inherited on the X-chromosome, have been associated with abnormal development of the hair, teeth, and sweat glands. Like Sjögren’s syndrome, X-LHED patients often report salivary gland dysfunction and nearly 30% of them perceive xerostomia. Thus, the purpose of this study is to further our understanding of the fluid secretion process and to investigate the mechanism of hyposalivation in X-LHED.

Methods. Tabby (Ta/Y) male mice, an X-LHED disease model that has a single nucleotide deletion mutation in the Eda gene, were used. Their male unaffected littermates (X/Y) served as controls. The amount of secreted saliva from submandibular and sublingual glands (SMGs and SLGs) in response to 0.3μM carbamol and 1.0μM isoproterenol was determined using the ex vivo salivary gland perfusion technique, while in vivo experiments measured the amount of secreted saliva from parotid glands (PGs), SMGs and SLGs in response to 10 mg pilocarpine/kg body weight. The concentration of ions (Na+, K+ and Cl-) in the secreted saliva were determined. The cross-sectional areas of acini and ducts in the three major salivary glands were calculated from HE-stained images using ImageJ (NIH) software. Epithelial sodium channel (ENaC) subunits, α-, β- and δ- mRNA expression was measured by qPCR.

Results. The body and gland weights of both SMG and SLG in Ta/Y mice were significantly less than in X/Y mice, whereas PG weight was comparable between X/Y and Ta/Y mice. The amount of secreted SMG saliva in both ex vivo and in vivo experiments was significantly reduced in Ta/Y mice, however, when normalized to gland weight, it was significantly increased in Ta/Y mice. In contrast, the amount of secreted saliva from SLGs and PGs was comparable between X/Y and Ta/Y mice, both for total saliva volume and when normalized to gland weight. The concentration of Na+ in the saliva of Ta/Y mice were significantly higher than in X/Y mice, and this observation was most remarkable in SMGs. The cross-sectional area of SMG ducts in Ta/Y mice was less than in X/Y mice. Furthermore, mRNA expression of all ENaC subunits in Ta/Y mice was less than in X/Y mice. In contrast, the Na+ and Cl- concentrations in the secreted saliva were increased, while both the cross-sectional area of ducts and transcript levels for ENaC subunits were reduced in Ta/Y mice. Together, these results are consistent with a compromised NaCl reabsorption mechanism in X-LHED Ta/Y mice.

P-141
Increased expression of lysosomal associated membrane protein (LAMP) 3 in the salivary glands of Sjögren’s syndrome patients can stimulate stalled autophagy and apoptosis
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Background. Environmental insults (e.g., viral or bacterial infection) may induce the accumulation of misfolded proteins and activation of the unfolded protein response (UPR) pathway. Triggering of the UPR can result in endoplasmic reticulum (ER) stress, autophagy or cell death. Recent studies reported that salivary glands (SG) in primary SS (pSS) patients are under ER stress. To understand gland dysfunction in pSS we performed microarray analysis that revealed overexpressed UPR genes in minor salivary glands (MSGs) including lysosome associated membrane protein 3 (LAMP3). LAMP3, a UPR-responsive gene and inductive member the LAMP family (e.g., LAMP1/2), is expressed in several cancers, neurodegenerative disorders, and inflammatory conditions; its role in pSS has not been defined. Given the link of the UPR in pSS, we assessed LAMP3 overexpression in cellular function.

Methods. Changes in epithelial gene expression were measured by microarray analysis of complimentary RNA (cRNA) isolated from MSGs of female patients with pSS. Results were compared with those obtained from the MSGs of sex-matched healthy volunteers or patients with an unrelated autoimmune disease (e.g., IgG4 disease). Changes in autophagy, ER stress, and apoptosis were studied in the context of two 5G-derived cell lines, HSG and A253 cells, by treatment with either chloroquine (CQ), cycloheximide (CHX), e-aib inhibitor (inmatimib), Cathepsin D (CTSD) inhibitor (pestatin A), or pan-caspase inhibitor (Z-VAD-FMK).

References
Results. Microarray analysis of differentially express genes indicated that LAMP3 expression was significantly increased in the MSGs of pSS patients when compared with healthy individuals or IgG4 disease patients. Expression of LAMP3 impeded cell growth relative to control cells and led to the accumulation of LC3-LAMP3 co-localized vesicles. When the effect of LAMP3 on autophagy was examined, LAMP3 decreased reporter protein degradation and increased the LC3II/LC3I ratio independent of treatment with CQ. Although no change in ER stress was measured via AIP expression, LAMP3 expression reduced p-crk:crkL, increased degradation of LAMP1, and increased cytoplasmic CTSD. This data supports that LAMP3 expression alters autophagic flux leading to stalled autophagy. In addition, the LAMP3 induced an increase in cytoplasmic CTSD, resulting in activation of the BID-caspase 3 pathway and an increase in apoptosis.

Conclusions. Markers of autophagy and apoptosis have long been associated with pSS but a fundamental mechanistic understanding of the gene expression changes associated with this change in state has not been identified. Our study of LAMP3 expression in SG cells signifies a connection between these two observations and suggests increased LAMP3 expression can trigger stalled autophagy via degradation of c-abl and induced degrada
tion of LAMP1. Furthermore, the change in lysosomal integrity results in the release of CTSD into the cytoplasm, initiating cellular apoptosis via the BID-Caspase 3 pathway independent of ER stress markers. Given significantly increased expression in pSS, inhibition of LAMP3 expression may represent a novel therapeutic strategy in the treatment of pSS.

P-143
Orofacial myofunctional status and temporomandibular disorder symptoms in patients with Sjögren’s syndrome

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Purpose. Sjögren’s syndrome (SS) induces difficulty in chewing, swallowing, which is attributed to saliva deficit. However, the orofacial myofunctional and the frequency of temporomandibular disorder (TMD) and its relationship with the patients’ perception of impairment is unknown in SS. In the present study, we investigated whether SS negatively affects the stomatognathic system and functions and whether the patients’ perception of their functional limitations and TMD symptoms is related to orofacial myofunctional performance.

Methods. 19 women with SS based on the American-European Criteria and 20 healthy volunteers were compared by the orofacial myofunctional evaluation with scores protocol (OMES), Iowa Oral Performance Instrument (IOPP) model 2.2, electromyography (EMG) of masticatory muscles and the Eating Assessment Tool (EAT-10). TMD were compared by pain to palpation of muscles and temporomandibular joint (TMJ), noise and range of jaw movements, using a digital caliper, and Jaw Functional Limitation Scale (JFLS-20).

Results. The orofacial myofunctional condition was worse in SS group. SS patients presented lower scores of all categories of OMES protocol (p<0.0001), tongue strength (p<0.001) and muscular activities of the temporals and masseter measured by EMG (p<0.01 and 0.05, respectively). SS group manifested higher scores of muscles and TMJ pain, eating disorder (EAT-10) and JFLS-20 (p<0.0001).

Conclusions. The results showed that patients with Sjögren’s Syndrome present impaired muscle and orofacial functions, TMD signs/symptoms, eating and jaw limitations. Those disorders may prejudice to the disease control and must be addressed in the clinical evaluation to prevent nutritional and metabolic comorbidities in SS patients.

P-142
Do I sound dry?: voice analysis and affecting factors in patients with primary Sjögren’s syndrome

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Objective. Saliva plays an important role in voice acuity, speech, and articulation. Therefore, we hypothesized that the reduced salivary rate might affect voice of patients with primary Sjögren’s syndrome (pSS). The purpose of this study was to investigate whether patients with pSS have voice impairment compared to controls and to determine the affecting factors.

Methods. Subjects who complained of voice related symptoms underwent acoustic analysis, aerodynamic study, voice handicap index (VHI) questionnaire from September 2016 to January 2017. In cases of pSS patients, various disease-related parameters such as disease duration, EULAR Sjögren’s syndrome disease activity index (ESSDAI), EULAR Sjögren’s syndrome patients reported index (ESSPRI) were obtained by retrospective chart re-

Results. Fifty-four pSS patients and 52 controls were recruited. The subjects were all female, and mean age was 53.9±9.73. VHI score was significantly higher in patients group (17.11±17.286 vs 9.35±10.518, p=0.006). However, the results of acoustic analysis and aerodynamic study were not different between the two groups, except that the proportion of subjects with abnormal mean flow rate (MFR) value in aerodynamic study was higher in pSS patients (68% vs. 50%, p=0.052), although it did not reach statistically significant difference. Disease-related parameters were available in 47 pSS patients. High VHI score was associated with low quality of life measured by EQ-5D (spearman’s rho=-0.39, p=0.006). Patients with abnormal MFR value showed higher physician global assessment (23.03±14.79 vs 10.63±13.995, p=0.008), xerostomia inventory (XI) score (40.32±4.93 vs 34.06±8.12, p=0.006), and higher ESR (30.06±20.797 vs. 17.75±12.835, p=0.016). However, ESSPRI, ESSDAI, salivary flow rate, or schirmer’s test was not associated with voice related parameters.

Conclusion. Patients with pSS show higher VHI score, which was associated with low quality of life. MFR value tends to be abnormal in pSS patients and correlated with physician global assessment and XI score.

P-144
Relationship between caries and salivary flow rates in Sjögren’s syndrome

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Background. Accelerated caries in Sjögren’s (SS) subjects (Scts) is frequently attributed to reduced salivary flow (SF). Current guidelines for caries prophylaxis mention “stimulation of saliva flow” as a therapeutic option. Methods. Two hundred and twenty-five Scts with sicca symptoms underwent testing (excluding parotid sialography) between 2012–15 as per the 2002 AECG criteria and were classified as SS or non-SS (NSS). Evaluation included measurement of whole mouth unstimulated and parotid stimulated SF and dental examination or record review. Scts with incisal or cervical/ root caries were identified. For ordinal regression models, 3 categories of carious involvement were defined (0, 1-2 or >3 caries). Differences in the number of incisal, cervical/ root, & total caries in SS vs. NSS were compared. Statistical analysis was performed to evaluate differences in continuous & categorical variables and to identify clinical risk factors associated with caries. Because of a pre-hypothesized relationship between SF and total caries, measurements of unstimulated and stimulated salivary flow were included in multivariable models.

Results. Ninety-nine Scts were classified as SS and 126 as NSS. SS Scts had a significantly higher mean and median number of incisal, cervical/ root and total caries vs. NSS (Figure 1). Clinical evaluation is summarized in Table 1. No significant differences in unstimulated whole or parotid stimulated SF (Table II), patient demographics, symptom prevalence, use of anticholinergic drugs, secretagogues, tooth loss, or dental visits in the last year were observed between the two groups.
A focus score >1/4 mm² was the only risk factor associated with total caries in SS. Within the SS cohort, there was no significant correlation between the total number of carious surfaces and low unstimulated whole or parotid stimulated SF.

**Conclusions.** Sjögren’s Scts have a greater risk of caries compared to individuals with salivary hypofunction from other causes. While most SS Scts exhibit reduced SF, the rate itself may not be the sole determinant of caries risk. The overall risk may also be influenced by host factors and/or quantitative & qualitative differences in sialochemistry related to the underlying inflammatory process. Further research is needed to optimize strategies for caries prophylaxis in SS.

**Background.** A prominent feature of Sjögren’s syndrome (SS) is chronic autoimmune inflammation of the salivary glands, manifested by oral dryness, major salivary gland swelling and a predisposition to dental caries and oral microbial infections. The aim of this study is to learn more about the dryness aspect of today’s patients with SS.

**Methods.** Patients were recruited from the Department of Rheumatology, Haukeland University Hospital, University of Bergen, Norway (August-November 2017). As part of clinical follow-up encompassing major salivary gland ultrasonography (SGUS) and unstimulated (UWS) and stimulated sialometry (SWS) patients (n=15) were invited to a free dental check-up with radiographic examination (panoramic and two bite-wing images).

**Results.** By November 2017, thirteen patients had attended the dental appointment. Pathological SGUS (SGUS+) was determined in 7/13 patients; 5/7 and 3/6 had pathological levels of unstimulated (UWS+) and stimulated whole saliva (SWS+), respectively. UWS and SWS levels correlated (n=12, r=0.581, p=0.048). Xerostomia was reported by 12/13 patients; 9/12 and 9/11 with UWS+ and SWS+, respectively. In 4/9 and 3/5 patients with xerostomia, SGUS+ co-occurred with UWS+ and SWS+. In patients with xerostomia, hyposalivation was linked to problems eating dry food in 9/10 patients (p=0.007), two patients with altered taste and 4/5 patients having to get up to drink at night. No patients were current smokers, but previous smoking was reported by 2/8 patients with xerostomia. Patients with hyposalivation and xerostomia presented with dry lips in 7/9 cases, 5/7 manifested angular cheilitis, and 4/5 patients had increased facial mimics. All patients used fluoride toothpaste; 2/12 patients with xerostomia and hyposalivation used additional oral fluoride rinse, 5/6 used fluoride tablets, 7/9 used dental floss. Tooth picks were used by 8/9 patients, and one patient used interdental brushes. For relief of oral dryness, 3/5 patients with xerostomia and hyposalivation had found a product such as gel or spray that they were content using. Using Challacombe’s scale for the clinical assessment of oral dryness, 6/13 had no dryness, 5/13 had mild dryness, and 2/13 had moderate dryness. Challacombe’s score correlated with stimulated whole saliva levels (n=12, r=0.636, p=0.026) and with SGUS score (n=13, r=0.626, p=0.022) and (n=13, r=0.719, p=0.006), parotid and submandibular glands, respectively. Gingivitis was observed in 9/12 patients with xerostomia, and dental erosion in 10/12 patients. The number of decayed, missing, and filled teeth (DMFT) was observed in 9/12 patients with xerostomia, SGUS+ co-occurred with UWS+ and SWS+. In patients with xerostomia, hyposalivation was linked to problems eating dry food in 9/10 patients (p=0.007), two patients with altered taste and 4/5 patients having to get up to drink at night. No patients were current smokers, but previous smoking was reported by 2/8 patients with xerostomia. Patients with hyposalivation and xerostomia presented with dry lips in 7/9 cases, 5/7 manifested angular cheilitis, and 4/5 patients had increased facial mimics. All patients used fluoride toothpaste; 2/12 patients with xerostomia and hyposalivation used additional oral fluoride rinse, 5/6 used fluoride tablets, 7/9 used dental floss. Tooth picks were used by 8/9 patients, and one patient used interdental brushes. For relief of oral dryness, 3/5 patients with xerostomia and hyposalivation had found a product such as gel or spray that they were content using. Using Challacombe’s scale for the clinical assessment of oral dryness, 6/13 had no dryness, 5/13 had mild dryness, and 2/13 had moderate dryness. Challacombe’s score correlated with stimulated whole saliva levels (n=12, r=0.636, p=0.026) and with SGUS score (n=13, r=0.626, p=0.022) and (n=13, r=0.719, p=0.006), parotid and submandibular glands, respectively. Gingivitis was observed in 9/12 patients with xerostomia, and dental erosion in 10/12 patients. The number of decayed, missing, and filled teeth (DMFT) correlated with Challacombe’s dryness score (n=13, r=0.615, p=0.025). Surprisingly, quality of life was somewhat higher in patients with hyposalivation compared to the patients with normal salivary secretion (p=0.050).

**Conclusions.** Oral dryness is common in patients with SS and linked to pathological SGUS changes, hyposalivation and caries experience. Patients with SS may benefit from additional information on caries prophylactic measures such as fluorides and regular oral health supervision.

**Table I.** Basic characteristics among Sjögren’s and Non-Sjögren’s.

<table>
<thead>
<tr>
<th></th>
<th>Sjögren’s</th>
<th>Other sicca</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>99</td>
<td>126</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>54.1 (13.8)</td>
<td>51.5 (13.1)</td>
<td>0.14</td>
</tr>
<tr>
<td>Female</td>
<td>87.8%</td>
<td>89.8%</td>
<td>0.64</td>
</tr>
<tr>
<td>Currently smoking</td>
<td>5.0%</td>
<td>5.4%</td>
<td>0.90</td>
</tr>
<tr>
<td>Duration dry mouth sx</td>
<td>40 (17.93)</td>
<td>36 (12.91)</td>
<td>0.55</td>
</tr>
<tr>
<td>Duration of dry eye sx</td>
<td>48 (22.15)</td>
<td>48 (14.12)</td>
<td>0.55</td>
</tr>
<tr>
<td>ANA or RF+</td>
<td>64%</td>
<td>23%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SSA+</td>
<td>63%</td>
<td>9.1%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Schirmer’s s &lt;5mm/5 min</td>
<td>44%</td>
<td>37%</td>
<td>0.27</td>
</tr>
<tr>
<td>Focus score ≥1/4mm²</td>
<td>1 (1.1)</td>
<td>0 (0.0)</td>
<td>=0.001</td>
</tr>
<tr>
<td>Anticholinergic drugs</td>
<td>53%</td>
<td>56%</td>
<td>0.65</td>
</tr>
<tr>
<td>Cholinomimetic drugs</td>
<td>24%</td>
<td>17%</td>
<td>0.19</td>
</tr>
<tr>
<td>Antimalarial drugs</td>
<td>34%</td>
<td>18.6%</td>
<td>0.008</td>
</tr>
<tr>
<td>Number of incisal caries</td>
<td>0 (0.2)</td>
<td>0 (0.0)</td>
<td>0.004</td>
</tr>
<tr>
<td>Number of cervical/root caries</td>
<td>0 (0.2)</td>
<td>0 (0.1)</td>
<td>0.05</td>
</tr>
<tr>
<td>Total caries (%)</td>
<td>56%</td>
<td>44%</td>
<td>0.08</td>
</tr>
<tr>
<td>Dentures</td>
<td>11%</td>
<td>9.3%</td>
<td>0.67</td>
</tr>
<tr>
<td>Missing teeth</td>
<td>58%</td>
<td>30%</td>
<td>0.21</td>
</tr>
<tr>
<td>Oral Exam in last year</td>
<td>82%</td>
<td>85%</td>
<td>0.58</td>
</tr>
<tr>
<td>Dentist Visit in last year</td>
<td>80%</td>
<td>85%</td>
<td>0.29</td>
</tr>
<tr>
<td>Unstimulated whole mouth SFR*</td>
<td>0.62 (0.26, 1.16)</td>
<td>0.75 (0.28, 1.40)</td>
<td>0.33</td>
</tr>
<tr>
<td>% Abnormal USFR</td>
<td>12%</td>
<td>9%</td>
<td>0.51</td>
</tr>
<tr>
<td>Parotid stimulated SF*</td>
<td>0.001 (0.0002)</td>
<td>0.001 (0.003)</td>
<td>0.96</td>
</tr>
<tr>
<td>% Abnormal stimulated SF</td>
<td>68%</td>
<td>75%</td>
<td>0.24</td>
</tr>
</tbody>
</table>

* (ml/min).

**Table II.** Factors associated with greater total caries among all subjects and those diagnosed with Sjögren’s Syndrome.

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>Sjögren’s patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B (95% CI)</td>
<td>p</td>
</tr>
<tr>
<td>Age</td>
<td>1.02 (1.00, 1.04)</td>
<td>0.02</td>
</tr>
<tr>
<td>Focus score &gt;1/4 mm²</td>
<td>2.16 (1.28, 3.64)</td>
<td>0.004</td>
</tr>
<tr>
<td>Anticholinergic drugs</td>
<td>1.62 (0.96, 2.73)</td>
<td>0.07</td>
</tr>
<tr>
<td>Low parotid stimulated</td>
<td>0.99 (0.54, 1.75)</td>
<td>0.92</td>
</tr>
<tr>
<td>Salivary Flow</td>
<td>0.59 (0.44, 0.77)</td>
<td>0.03</td>
</tr>
<tr>
<td>Low whole mouth unstimulated</td>
<td>0.94 (0.55, 1.58)</td>
<td>0.80</td>
</tr>
<tr>
<td>Salivary Flow</td>
<td>1.03 (0.45, 2.33)</td>
<td>0.95</td>
</tr>
</tbody>
</table>

*also tested and not-significant: sex, duration of xerostomia, duration of dry eyes, abnormal ocular staining, RF, ANA, SSA, abnormal Schirmer’s test, and current smoking status.
such as Sjögren’s syndrome (SS) are the main causes of this condition and the presence of this symptom in patients with lupus erythematosus (LE) is usually referred as secondary Sjögren’s syndrome (2). Although these diseases share many clinical and laboratory aspects, some authors consider that both disorders as different conditions (3, 4). Based on this query, the aim of the present study was to analyze and compare morphological findings of minor salivary glands in patients with xerostomia diagnosed with primary SS or LE.

Background/Purpose. The geographic variation in healthcare service utilization and access in cohorts of patients with primary Sjögren’s syndrome (pSS) to collect information on access to and intensity of treatment over time. 14th International Symposium on Sjögren’s Syndrome Posters

Methods. We describe the development and preliminary validation of questionnaires to assess health-care utilization and access in cohorts of patients with primary Sjögren’s syndrome in the diagnosis and during the disease course

Results. The pilot version of the two questionnaires were administered to 50 pSS in the clinical centre of Pisa (Italy) and counted 21 and 32 closed-ended questions, respectively. A narrative-based medicine section was also included in the questionnaires to collect stories about patients’ care pathways and their health status before the diagnosis. Three questionnaires out of 47 were returned incomplete. Mean (SD) age was 60 (12.5) years and 96% of the sample was female. The majority of the respondents had a primary or secondary school (59%). Construct validity was supported by the questionnaire’s ability to discriminate between groups with different levels of activity of the disease and socio-demographic characteristics. Disease activity was significantly associated with frequency of rheumatologic visits and diagnostic tests (p<0.001). Conclusions. Preliminary results confirm that the questionnaire is a valid instrument to assess patterns of care for pSS in terms of access and utilization and in relation to clinical and socio-demographic characteristics of patients. Further analysis are ongoing in other clinical centers to verify the generalizability and additional psychometric properties of the instrument.
Development of high systemic activity in primary Sjögren syndrome: analysis of 1487 Spanish patients (GEAS-SS Registry)


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Background. To characterize high systemic involvement in primary Sjögren syndrome (SS) in a large cohort of Spanish patients using the EULAR-SS disease activity index (ESSDAI).

Methods. The GEAS-SS Study Group was formed in 2005 with the aim of collecting a large series of Spanish patients with primary SS. Systemic involvement was characterized using ESSDAI definitions for the 12 domains, and patients scoring as high in the domains that specifically contain high activity (lymphadenopathy, articular, cutaneous, pulmonary, renal, peripheral nervous system, central nervous system and muscular) were identified and analysed.

Results. Of the 1487 patients included in the Registry, 186 (12.5%) presented with 197 systemic features classified as high according to the corresponding organ-by-organ ESSDAI domains. There were 159 women and 27 men, with a mean age of 59.06 yrs (range 15-89 yrs) at the time of the diagnosis of high activity. High systemic activity was scored in the lymphadenopathy (n=53), peripheral nervous system (n=50), central nervous system (n=43) and muscular (n=39) domains. Men were overrepresented in the muscular (33%), lymphadenopathy (19%) and cutaneous (19%) domains. The domains diagnosed at younger ages included muscular (mean age of 45 yrs), CNS (49.7 yrs), articular (54.3 yrs) and hematological (55.4 yrs), while cutaneous (63.2 yrs) and pulmonary (67.1 yrs) were diagnosed at older ages. The main clinical syndromes responsible of high systemic activity were lymphoma (n=53), atracne neumopathy (n=14), pulmonary fibrosis (n=12), diffuse leukocytoclastic vasculitis (n=10), polynuropathy (n=10), myelitis (n=8), meningitis (n=7), multiple mononeuropathies (n=7), membranous/membranoproliferative glomerulonephritis (n=6) and severe thrombocytopenia (n=5). Notably, 13 (81%) out of the 16 patients presenting with high activity in the articular domain (≥6 joints involved) were finally diagnosed with rheumatoid arthritis during the follow-up.

Conclusions. Primary Sjögren syndrome at diagnosis is presenting with high systemic activity in 12.5% of cases; half the cases were related to either the development of lymphoma or severe neurological features. Nearly all the patients presenting with a severe polyarthritis were finally diagnosed with associated rheumatoid arthritis.

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Methods. We have quantified at diagnosis systemic involvement (defined according to the 10 domains of the ESSDAI score) in patients included in the Big Data Sjögren Registry. We quantified how many patients had activity in more than one domain, how many patients had involvement of an isolated domain and we measured the degree of overlap between the main pairs and triplets of associated groups of active domains. Using a mathematical model, we represented the overlap between groups by means of Venn diagrams.

Results. 9118 patients were included in the Registry with ESSDAI values available at the time at diagnosis: 5758 (63%) had activity in at least one ESSDAI domain. Among them, 3167 (55%) had only one active domain, 1532 (27%) two, 628 (11%) three and 431 (7%) four or more active domains. Three domains showed activity at diagnosis in at least 10% of patients: articular (37.9%), glandular (21.4%) and pulmonary (10.4%). The frequency of patients having concomitant activity in other organs varied widely in each domain: among patients with articular involvement, 55% of cases had another domain active, a figure that was 68% for patients with glandular involvement; 88% of patients with muscular involvement had concomitant activity in other organs, 78% of those with peripheral neuropathy and 74% of those with renal involvement. Numerically, the most frequent associations among two different domains were between articular and glandular (n=944), pulmonary (n=87), constitutional (n=409) or cutaneous (n=410) domains, while the most frequent associations among 3 organs were between articular/glandular plus pulmonary (n=224), constitutional (n=216) or lymphadenopathy (n=173) domains. Mathematical model identified the highest degree of overlap among the different domains for the following pairs: % of patients with muscular activity who had concomitant glandular activity (Δ22.9%), those with lymphadenopathy who had concomitant glandular activity (Δ21.6%) or glandular (Δ21.1%) activities.

Conclusions. Primary Sjögren syndrome is presenting at diagnosis as a systemic disease in two thirds of cases; in more than half of cases, there was only one active domain, although 7% of patients presented with a multisystemic disease affecting 4 or more different organs. An enhanced degree of overlap in comparison with the expected values found for the total cohort varied widely in each domain: among patients with articular involvement, 55% of cases had another domain active, a figure that was 68% for patients with glandular involvement; 88% of patients with muscular involvement had concomitant activity in other organs, 78% of those with peripheral neuropathy and 74% of those with renal involvement. Numerically, the most frequent associations among two different domains were between articular and glandular (n=944), pulmonary (n=87), constitutional (n=409) or cutaneous (n=410) domains, while the most frequent associations among 3 organs were between articular/glandular plus pulmonary (n=224), constitutional (n=216) or lymphadenopathy (n=173) domains. Mathematical model identified the highest degree of overlap among the different domains for the following pairs: % of patients with muscular activity who had concomitant glandular activity (Δ22.9%), those with lymphadenopathy who had concomitant glandular activity (Δ21.6%) or glandular (Δ21.1%) activities.

Fig. 1a

Fig. 1b

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Predictors of fatigue in 608 patients from the United Kingdom Primary Sjögren’s Syndrome Registry

Katie L Hackett1,2, Kristen Davies1, Rebecca Bragg1, Sheryl Mitchell1, Samira T Miyamoto2, Dennis Lendrem1, Wan-Fai Ng1,2 on behalf of the UK primary Sjögren’s Syndrome Registry.

Background. Fatigue is a dominant symptom for 70% of primary Sjögren’s syndrome (PSS) patients. Many PSS patients are likely to have comorbidities associated with fatigue and/or take drowsiness inducing medication. This study reports on fatigue symptoms of a large cohort of 608 patients diagnosed with PSS using the American European Consensus Group criteria. We identify predictors of fatigue in patients with assignable causes (AC) and in those with no assignable cause for their fatigue.

Methods. We calculated physical and mental fatigue scores from the Profile of Fatigue and Discomfort tool. We collected measures of mood (Hospital Anxiety and Depression Scale (HADs)), daytime sleepiness, dryness (1-10 scale and Schirmer), pain (1-10 symptoms (ESSPRI), disease activity (ESSDAI), BMI and comorbidity and polypharmacy scores (CPS).

We allocated patients with AC of fatigue (comorbidities associated with fatigue), those taking drowsiness inducing medications and/or a HADS anxiety or depression score ≥8) to the AC group. We named the remaining cohort the CORE group. We made comparisons between the two groups and conducted a multiple regression analysis to identify potential predictors of physical and mental fatigue. We constructed a dummy variable permitting us to test directly for differences between the AC and CORE groups.

Results. 21% of patients were taking medication associated with drowsiness and 33% of patients had comorbidities associated with fatigue. 465 patients had AC for their fatigue. We saw no differences between groups for age, ESSDAI or BMI. The AC group had significantly greater symptom burden (physical fatigue, mental fatigue, pain, depression, and daytime sleepiness all p<0.001) and dryness (p=0.0012)). The main predictors of physical fatigue were pain, depression and dryness (all p<0.0001). Whilst the relationships between pain and depression were slightly different (p=0.0023 and p=0.0269 respectively), the relationships with the main predictors were broadly similar in both groups. Depression, daytime sleepiness and pain were the main predictors of mental fatigue (all p<0.0001). There was a significant difference in the relationship with depression between the groups (p=0.0311) but again, the relationships with the main predictors of mental fatigue were similar for both groups.

Conclusion. Large numbers of PSS patients have AC for their fatigue, including drowsiness inducing medication and comorbidities. While pain and depression are both predictors of physical and mental fatigue, dryness is an important predictor of physical but not mental fatigue. Reviewing medications, treating comorbidities (including depression) and addressing pain may all have a positive impact on PSS fatigue.

P-152

Depressive symptoms, fatigue, and dry eye in patients with Sjögren’s syndrome

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Background. Fatigue is a dominant symptom for 70% of primary Sjögren’s syndrome patients. Many PSS patients are likely to have comorbidities associated with fatigue and/or take drowsiness inducing medication. This study reports on fatigue symptoms of a large cohort of 608 patients diagnosed with PSS using the American European Consensus Group criteria. We identify predictors of fatigue in patients with assignable causes (AC) and in those with no assignable cause for their fatigue.

Methods. We calculated physical and mental fatigue scores from the Profile of Fatigue and Discomfort tool. We collected measures of mood (Hospital Anxiety and Depression Scale (HADs)), daytime sleepiness, dryness (1-10 scale and Schirmer), pain (1-10 symptoms (ESSPRI), disease activity (ESSDAI), BMI and comorbidity and polypharmacy scores (CPS).

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Conclusion. Large numbers of PSS patients have AC for their fatigue, including drowsiness inducing medication and comorbidities. While pain and depression are both predictors of physical and mental fatigue, dryness is an important predictor of physical but not mental fatigue. Reviewing medications, treating comorbidities (including depression) and addressing pain may all have a positive impact on PSS fatigue.
Primary Sjögren Syndrome (pSS): a randomized trial
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Introduction. Primary Sjögren Syndrome (pSS) is an autoimmune inflammatory systemic disease that affects exocrine glands and less frequently internal organs. Inflammation can affect any system, including the musculoskeletal system, resulting in reduction in physical functions with consequent decrease in muscle strength, aerobic capacity, joint mobility, and static balance. In addition, psychosocial impairment with potential worsening of quality of life and functional capacity (FC) is described.

Objective. Analyze the effectiveness of resistance exercise on daily motor behavior and FC in women with pSS.

Methods. Fifty nine patients were randomized; of which 51 completed the study (26 assigned to the exercise group - EXG and 25 to the control group - CG). The EXG participated in a 16 week supervised exercise program, including 2 sessions per week and 3 sets of 10 maximal repetitions per exercise. Both before and at the end of the intervention protocol four variables were evaluated: the daily motor activity index (DMAI) was analyzed by an actigraph, which records body movements; the FC by the Fullerton Functional Fitness Test that consists of a sequence of 6 tests that mimic the neuromotor and cardiorespiratory needs involved in the activities of daily life; the disease activity (ESSDAI questionnaire); and quality of life (SF-36 questionnaire).

Results. After the intervention period in the EXG group, all FC parameters showed improvement over basal and final times, except the upper limb (UL) flexibility test (p=0.896): UL strength (p<0.001), lower limb (LL) strength (p<0.001), LL flexibility (p<0.001), aerobic capacity (p<0.001), agility (p<0.001). A similar situation occurred with the SF-36 where all domains improved, except for the emotional aspect (p=0.710): functional capacity (p<0.001), limitation by physical aspects (p=0.005), pain (p<0.001), general condition (p=0.006), vitality (p<0.001), social aspects (p<0.001), and mental health (p<0.001). There was no change in the DMI (p=0.2) or ESSDAI (p=0.284). In the CTRL group, the only variable that showed a difference when compared to baseline was aerobic capacity of the FC test, presenting a tendency to improve (p=0.05).

Conclusion. Thirteen of the 17 variables evaluated showed improvement after the resistance exercise program. The intervention protocol did not change the daily motor behavior (DIMA), flexibility parameter of UL of the FC test, or the domain emotional aspects of the quality of life questionnaire. Resistance exercise did not worsen disease activity (ESSDAI). In this sense, we can conclude that the program that included resistance exercise was effective in improving functional capacity and quality of life in women with pSS.

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Correlation between sleeping quality with fatigue, quality of life and disease activity (ESSDAI) in patients with primary Sjögren’s syndrome
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Introduction. Fatigue is a prominent and disabling feature in the majority of primary Sjögren’s Syndrome (pSS) patients. Although the presence of sleep disorders in pSS patients has been previously confirmed in other studies, its relationship with fatigue and disease activity needs to be further assessed.

Objectives. To assess the sleep quality in pSS patients using the Pittsburgh sleep quality index (PSQI) and actigraphy (PSQI), and its relationship with fatigue, quality of life, and disease activity (ESSDAI).

Methods. 50 pSS patients and 50 controls with sleep disorders according to the PSQI were included. The pSS patients used an actigraph for 15 days and filled in the following questionnaires and tools: PSQI, Profile of fatigue and disability (PROFAD), Visual Analog Scale (VAS pain and VAS fatigue), EULAR Sjögren’s Syndrome Patients Reported Index (ESSPRI), Restless Legs Syndrome International Rating Scale (EIGSPI), Mini-Mental State Examination (MMSE), and EULAR Sjögren’s Syndrome disease activity index (ESSDAI).

Results. The pSS patients were on average aged 56.4 years, had suffered from the disease for 12 years, and 100% presented fatigue. The ESSDAI showed that 40 patients (80%) demonstrated low disease activity and 10 (20%) moderate activity. Controls were on average aged 56.5 years, and all presented sleep disorders, (PSQI average total score 10.5). The actigraphy in pSS patients demonstrated means of 26.2 minutes for sleep latency, 48.2 minutes for total sleep time, 89.7% for sleep efficiency, and 398.5 minutes (approximately 6.5 hours) of sleep. There was a positive and significant correlation between PSQI and the following variables: VAS pain (p=0.020), VAS fatigue (p=0.006), PROFAD total score (p=0.005), and fatigue and mental ESSPRI (p=0.012, p=0.009). There was a positive correlation between the number of nightly awakenings assessed using actigraphy and disease activity by ESSDAI (p=0.012). A comparison between groups demonstrated a significant difference regarding the VAS fatigue outcome, (p=0.011), where the pSS group presented higher scores than the control group.

Conclusion. This study showed a positive and significant correlation between sleep disorders with pain, fatigue, and disease activity. It may also be concluded that other factors independent of sleep have influence on fatigue in pSS patients, since when compared with the controls with sleep disorders, the pSS patients maintained higher scores for fatigue.

P-154
Effectiveness of Resistance Exercise in women with primary Sjögren’s syndrome (pSS): a randomized trial
Paulo A Minali, Luciana Dardin, Carolina FMGP Mota, Virginia FM Trevisani

Posters 14th International Symposium on Sjögren’s Syndrome

Clinical and Experimental Rheumatology 2018
P-155

Pilocarpine spray for the treatment of xerostomia: a randomized, double-blind, placebo-controlled trial

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Background. Xerostomia secondary to hyposalivation is a significant burden for patients with Sjögren’s Syndrome (SS), since it may affect taste, speech, swallowing, and cause oral discomfort and burning. Management of xerostomia remains an unresolved topic for SS patients. Oral pilocarpine has showed to be very effective for controlling keratoconjunctivitis in several clinical trials, but with variable side effects. Topical formulation could be an approach for getting local effect with minimal side effects. The aim of this study was to evaluate the effectiveness of pilocarpine spray as a treatment for keratoconjunctivitis in patients with SS.

Methods. This was a randomized, double-blind, crossover placebo-controlled clinical trial (NCT02982577) of patients with SS complaining of xerostomia. Patients were randomly assigned to either placebo or pilocarpine (5%) spray and instructed to use 3 times a day for 4 weeks (equivalent concentration to 5mg of oral pilocarpine). Patients used pilocarpine spray or placebo for one month, with a one-month washout period between treatments. Outcomes measures were salivary flow (Unstimulated Whole Saliva Flow - UWSF), keratoconjunctivitis (Xerostomia Inventory - XI) and quality of life (Oral Health Impact Profile - OHIP-14), which were performed at baseline, one hour (only UWSF) and at one month of treatment.

Results. Twenty-four patients were enrolled and randomized to receive pilocarpine (n=12) or placebo spray (n=12). Pilocarpine spray significantly increased UWSF after one hour of using the spray (Wilcoxon test; p<0.01), but no changes were observed at one month. Xerostomia symptoms (XI) improved at one month of treatment (Friedman test; p<0.05), and OHIP-14 scores declined 1 month after using pilocarpine spray related to functional limitation dimension (Friedman test; p<0.05). Sprays were well tolerated and no clinical side effects were observed.

Conclusions. Topical application of pilocarpine spray is effective and safe for managing xerostomia in patients with SS, and should be considered as a therapeutic option.

Table I. Dynamics of parameters on treatment.

<table>
<thead>
<tr>
<th></th>
<th>Before treatment</th>
<th>1 week</th>
<th>1 mo</th>
<th>3 mo</th>
<th>6 mo</th>
<th>12 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vision (Mc±SD)</td>
<td>0.2±0.9</td>
<td>0.5±1.0</td>
<td>0.8±1.0</td>
<td>0.8±1.0</td>
<td>0.8±1.0</td>
<td>0.8±1.0</td>
</tr>
<tr>
<td>Width of the optic fissure (Ms±SD), mm</td>
<td>5.0±0.6</td>
<td>9.5±0.5*</td>
<td>9.9±1.0*</td>
<td>9.9±0.9*</td>
<td>9.1±0.8*</td>
<td>9.2±0.7*</td>
</tr>
<tr>
<td>Schirmer test (Ms±SD), mm</td>
<td>3.4±1.1</td>
<td>3.7±1.2</td>
<td>4.8±1.4</td>
<td>6.1±0.6*</td>
<td>7.1±1.0*</td>
<td>7.9±1.0*</td>
</tr>
<tr>
<td>Osmometry of tears, mOsm/L</td>
<td>316.3±381</td>
<td>275.31±0.5</td>
<td>275.30±0.5</td>
<td>275.30±0.5</td>
<td>275.30±0.5</td>
<td>275.30±0.5</td>
</tr>
<tr>
<td>pH of tears (Ms±SD)</td>
<td>6.5±0.2</td>
<td>6.5±0.2</td>
<td>6.5±0.2</td>
<td>6.5±0.2</td>
<td>6.5±0.4</td>
<td>6.5±0.4</td>
</tr>
</tbody>
</table>

Tests with the dry (Ms±SD), ball 3.1±0.5 1.5±0.5* 0.5±0.2* 0.2±0.2* 0.4±0.1* 0.4±0.1*

OSDI (Ms±SD), ball 86.8±10.3 38.7±17.2* 38.7±17.2* 17.2±5.5* 15.8±4.7* 13.8±4.6%

* (p<0.05).

P-156

A new treatment method of severe keratoconjunctivitis sicca with designed soft contact lens filled with cyclosporin A

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Background. The aim is to improve the treatment methods of severe forms of keratoconjunctivitis sicca (KCS) with the help of the newly designed soft contact lens (SCL).

Material and methods. We created an original SCL made of silicone hydrogel material with individual optical power and multiple grooves filled with 0.05% Cyclosporin A (CyA).

This SCL saturated with CyA was examined in 31 patients (4 male and 27 female (43 eyes)) aged from 32 to 67 years with severe KCS, who were constantly wearing them for 7-14 days. Additionally, they instilled artificial tears with hyaluronic acid without preservatives up to 6 times a day. Standard ophthalmic examination, Schirmer test, tear breakup time, tests with the vital dye, which was assessed by the Oxford scale, osmometry of tears (TearLab System, USA), pH-metry tears on test basis with a litmus test of high sensitivity, seeding content conjunctival cavity of the microflora, the measurement of the width of the optic fissure were carried out, lesion index of the ocular surface (OSDI) was determined. Results of treatment were monitored after 1 week, 3, 6 months from beginning of SCL wearing.

Results. Wearing SCL saturated with CyA, facilitated complete epithelialization of the cornea and helped to reduce inflammation from 1 week up to 1 month (Table I). This significantly reduced time of treatment of severe forms of KCS compared with standard therapy. After clinical improvement to 1 month (Table I). This significantly reduced time of treatment of severe forms of KCS compared with standard therapy.

Conclusion. This new treatment method of severe forms of KCS was found on application of originally designed therapeutic SCL which provided output of CyA 0.05% on ocular surface, while instilling artificial tears without preservatives. The results have shown high efficiency of this method in terms of reducing corneal epithelialization and relieving inflammation on the ocular surface.

P-157

Dry eye therapeutics: approvals finally, pipeline bursting, novel delivery, new mechanisms

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Dry eye disease (DED) therapy has entered a new golden era of innovation, funding, marketing and awareness. Clinical choices will empower prescribers, benefit patients, challenge payers and foster awareness.

Approvals. Topical Lifitegrast 0.1% preservative free solution (Xiidra, Shire) welcomes the first addition to topical DED therapeutics in 12 years. Novel molecular engineering created an active totally unique to ocular surface application. A successful launch prompted growth in numerous ocular surface markets, including Restasis.

Neurostimulation (True Tear, Allergan) introduces a totally new mechanism of action thought to be complimentary to existing allopatic surgical and pharmaceutical therapeutics. The handheld device emulates natural tripartite tear production through afferent Trigeminal nerve stimulation, controlled by the patient.

Pipeline. There are over 40 entities in Phase I through Phase III evaluation for DED therapy. These agents include semi-fluorinated alkane or nanoparticle vehicle delivery of cyclosporine, an enhanced mucus penetrating nanoparticle vehicle with loteprednol etabonate for treatment of episodic dry eye, epithelial sodium channel blocking agent, neuropeptide agonist, topical pure hyaluronic acid, and an aldehydes sequestration agent.

Topical biologics include recombinant human serum albumin (rHSA), a
naturally occurring secretagogue (lacratin), and a large surface-active endogenous complex mucinous glycoprotein (lubricin).

Delivery. New methods for drug delivery include a wide variety of topical vehicles: emulsions, mucus penetrating particles, semiuminorized alkanes, nanoparticles, and gels. Iontophoresis can deliver charged particles to the cornea, sclera and anterior segment. Neurostimulation is delivered through a single use biodegradable polymer for nasal mucosal interface. Thermal pulsation therapy provides deep Meibomian gland warming, massage and extirpation. Intense pulsed light (IPL) and electrolytic probing (Maskin) liberate posterior lamellar lid secretions.

Novel mechanisms. Include LPA1-ICAM blockade, anterior ethmoidal nerve electrostimulation, ENaC blockade, and topical monocycline induced physiochemical changes in Meibomian secretion content.

Landscape. Payers will see more challenges as numerous new entities enter the marketplace after the intensely costly approval process. Despite costs, early intervention, lifestyle and advocacy efficiency, decreased absenteeism, and reduced morbidity from ulceration, keratitis, pain, refractive error will undoubtedly benefit society through reduced clinic visits, fewer wasted empirical prescriptions, and lower medication costs overall.

P-158 Superior inhibition of disease-relevant inflammatory mediators and cell biology by combined leflunomide and hydroxychloroquine supports rationale for combination therapy in pSS patients

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Background. 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. LEF and HCQ robustly induce cytokine production 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. Hence, the additive effects of LEF and HCQ were assessed in vitro on CD4 T- and B-cell activation and production of interferons α and γ, Tfh-related cytokine CXCL13, as well as IgG and IgM. Inhibition was compared to IFNα receptor (IFNAR) blockade.

Methods. PBMCs of healthy individuals (n=9) and of pSS patients (n=8) were cultured with antigen (SEB) and TLR9L, in presence or absence of IFNα, as well as LEF and HCQ and their combination in clinical relevant concentrations. Anti-IFNα antibody was tested at the maximal inhibitory concentration. Production of T- and B-cells and release of IFN-α, IFN-γ, CXCL13, IgG and IgM were measured.

Results. SEB/TLR9 robustly induced T and B-cell activation, IFNγ, IFNα, CXCL13, IgG and IgM production (all p<0.001). LEF dose dependently inhibited B and T-cell proliferation, IFN, CXCL13 and Ig production. HCQ dose dependently inhibited B-cell proliferation, IFN-α, CXCL13, and immunoglobulin production. T-cell proliferation and IFN-γ production were inhibited by HCQ only at higher concentrations. At several suboptimal concentrations LEF and HCQ additively inhibited T-cell proliferation both in healthy individuals and in pSS patients. Significant additive effects were seen for all outcome measures except IFN-α. Since IFNα was already robustly inhibited by HCQ alone (eg. for pSS 90% at 3.3 μM, p<0.001), only trends towards additive effects were observed. Interestingly, anti-IFNAR treatment strongly inhibited IFNα production and CXCL13 production (both p<0.001), but did not inhibit T and B cell proliferation or IFNγ production.

Conclusion. More potent than IFNAR blockade LEF and HCQ robustly inhibited proliferation of T and B-cells, cytokine production and immunoglobulin production with clear additive efficacy in both healthy individuals as in pSS patients. These data support the potential surplus value of combination therapy with LEF and HCQ for patients with pSS.
Results. Among the 19 patients included, 11 received pilocarpine treatment for the whole 3 months period, 6 of the 8 remaining patients stopped the pilocarpine due to side effects. Among the 11 patients with a follow-up evaluation at 3 months, 5 had primary Sjögren’s syndrome according to the American-European’s classification criteria. The differences of RI before and after lemon stimulation was on average of -0.04 at baseline and -0.04 at M3. The sum of ultrasound’s grades average of the four glands was 3.47 at M0 and 4.18 at M3. The non-stimulated salivary flow was on average of 1.96 mL/min at M0 and 5.23 mL/min at M3, whereas the average of stimulated salivary flow was 2.84 mL/min at M0 and 8.51 mL/min at M3. None of these observations were statistically significant: RI before and after lemon stimulation (p=0.953), the sum of the four glands’ grades (p=0.858), the non-stimulated (p=0.26) and stimulated salivary flow (p=0.139).

Concerning the 3 patients with Sjögren’s syndrome, the differences in RI before and after lemon stimulation was lower probably due to a lower initial RI.

Conclusion. Preliminary results showed no significant differences between these patients and controls.

References
1. CORNEJC et al.: « Contribution of Salivary Gland Uronanography to the Diagnosis of Sjogren’s Syndrome ».
2. Jousse-Joulin et all: « Ultrasound Assessment of Salivary Glands in Patients with Primary Sjogren’s Syndrome Treated with Rituximab ».

P-161 Sjögren’s Syndrome Foundation Clinical Practice Guidelines for pulmonary involvement in Sjögren’s

Scofield RH1, Lee AS2, Baraf HS3, Gupta N4, Lynch J5, Meehan R6, Moua T7, St. Clair EW8, Dunleavy K9, Carteron N10, Carsons SE11, Hammitt KM12 on behalf of the Sjögren’s Syndrome Foundation.

Background. Management guidelines for Sjögren’s patients remain a major unmet need, particularly for potentially severe extraglandular (systemic) manifestations. To meet this patient and clinician need, the Sjögren’s Syndrome Foundation (SSF) launched a major initiative to develop Clinical Practice Guidelines for Sjögren’s in 2012. Goals include: improving quality and consistency of care, creating guidance documents for US clinicians, obtaining broad acceptance of guidelines by key professional and government organizations, educating payers and identifying gaps in evidence to spur much needed research. Following 4 recent publications by the SSF on initial Clinical Practice Guidelines under the rheumatology/systemic, oral, and ocular topics, Phase 2 is now well underway. This phase aims to comprehensively collate scientific evidence and expert opinion on the management of pulmonary manifestations of Sjögren’s.

Methods. Guideline members developed a rigorous and transparent process based on American College of Rheumatology quality of care standards and protocols developed by other professional organizations such as the American Society of Clinical Oncology. A unique aspect of these guidelines is the inclusion of multiple specialists. The Pulmonary Topic Review Group (TRG) includes an equal number of rheumatologists and pulmonologists as well as an oncologist with expertise in Sjögren’s. The process involved drafting and ranking clinical questions, defining the literature search and study criteria, and outcome measures to be addressed a priori to execution of the search. Members then identified eligible abstracts, and a minimum of 2 TRG members extracted data into tables, which included study characteristics and evidence and quality assessment. Recommendations will be drafted and finalized with input from at least 30 oral healthcare professionals who were not involved in the guidelines development.

Results. Guidelines for oral mucosal management and use of secretagogues in Sjögren’s

Al-Hashimi1, Papas A2, Alevizos I3, Brennan M4, Navazesh M5, Pinto A6, Stewart C7, Sweier D8, Tanzer J9, Vivino F10, Shiboski C11, Zero DT12, Carsons SE13, Hammitt KM14 on behalf of the Sjögren’s Syndrome Foundation.

Background. Oral complications in Sjögren’s can lead to painful oral infection/lesions/irritation, rampant caries and loss of teeth, in addition to difficulty eating and talking, which compromise the quality of life of patients with Sjögren’s. There is a clear unmet need for managing the lack of saliva and its consequences in Sjögren’s. The Sjögren’s Syndrome Foundation (SSF) launched a major initiative to address this need in 2012, and clinical practice guidelines for caries prevention were published in 2016 in the Journal of the American Dental Association (JADA) and included in the Rheumatic Diseases Clinics of North America. Phase 2 oral guidelines are now well underway and include coverage of mucosal management and treatment and use of secretagogues in Sjögren’s. Guidelines on caries restoration and management and parotid gland swelling also will be developed.

Methods. Guidelines members developed a rigorous and transparent process based on standards set by the American Dental Association, American College of Rheumatology and American Society of Clinical Oncology. The methodology utilizes a Delphi-type consensus process and includes drafting clinical questions and defining literature search parameters and study criteria a priori to execution of the search. Eligible abstracts then are identified, and a minimum of 2 guidelines members extract the data on study characteristics, evidence and quality assessment. Recommendations will be drafted and finalized with input from at least 30 oral healthcare professionals who were not involved in the guidelines development.

Results. The Mucosal Management and Treatment Topic Review Group (TRG) drafted clinical questions for its outline covering oral symptoms of mucosal pain and/or inflammation in Sjögren’s, including prevalence and identification of oral cancer. Prevention and management of burning mouth; oral candidiasis; lichenoid reactions or hypersensitivity-induced mucositis; and oral trauma associated with oral dryness were discussed. The Secretagogues TRG’s clinical questions explore whether studies have demonstrated that secretagogues improve salivary flow and composition; reduce the occurrence of candida, other oral infections and burning mouth; and translate to better outcomes for the incidence of caries, periodontal disease, taste disturbance; hoarseness, chronic cough, and subjective and objective dryness and discomfort.

Conclusions. Initial clinically relevant questions for Clinical Practice Guidelines for oral mucosal management and use of secretagogues for dry
P-163
Sjögren’s Syndrome Foundation Clinical Practice Guidelines for neurological involvement in Sjögren’s

Mandel S1, Vivino FB2, Fox R1, Deboo A1, Binhuma J1, Bloch D3, Brauning R1, Brown ES4, De Sousa E1, Gelfand G1, Gromesh G1, Lange DJ1, Lawrence-Ford T1, Lewis J1, Liao Y1, Maiz E1, Maiz S1, Nosei G1, Pavlikis P1, Sarka G1, Sicotte N1, Varadhachary A2, Wallace DJ1, Wilson JW2, Winter WC3, Scofield RH4, Carteron N1, Carsons SE5, Hammitt KM6, on behalf of the Sjögren’s Syndrome Foundation.

1 Hofstra Northwell, 2University of Pennsylvania, Penn Presbyterian Medical Center, 3Scripps Memorial Hospital, 4Lewis Katz School of Medicine at Temple University, 5The Johns Hopkins University School of Medicine, 6Partners Healthcare; Massachusetts General Hospital; Harvard Medical School. 7Washington University in St. Louis, 8University of Texas Southwestern Medical Center, 9University of California San Francisco, 10University of Kansas, 11Washington Hospital Center, 12Hospital for Special Surgery; Cornell, 13North Georgia Rheumatology Group; Philadelphia College of Medicine; Emory University, 14Johns Hopkins University School of Medicine, 15Partners Healthcare; Massachusetts General Hospital; Harvard Medical School; 16University of California San Francisco, 17University of Washington, 18University of Texas Southwestern Medical Center, 19University of California at Los Angeles, 20University of California at Irvine, 21University of Texas Southwestern Medical Center, 22University of California at Los Angeles; 23Charlottesville Neurology and Sleep Medicine; 24American Society of Clinical Oncology, 25New York University School of Medicine.

Background. Sjögren’s Syndrome is a systemic autoimmune disease that affects the entire body. The purpose of this major national patient survey was to gain an understanding from adults who have been diagnosed with Sjögren’s about the impact of the disease on their quality of life, including the physical, emotional and financial burdens.

Methods. The Sjögren’s Syndrome Foundation (SSF) conducted the Living with Sjögren’s survey between May 11 and July 11, 2016. Participants were recruited by Harris Poll from a pool of 9,252 active SSF patient members. The survey was conducted among adults aged 18 years or older who reported having been clinically diagnosed with Sjögren’s by a physician or other medical professional. The survey asked closed-ended questions about patient experiences with Sjögren’s and the impact it has on their quality of life.

Results. There were 3,072 survey responses (33% response rate), 2,963 of which were included in the analyses. Survey respondents were 96% female and 4% male; 32% were aged 60 years or less. On average, respondents saw 4.6 different healthcare professionals annually and used 8.8 medications and treatments to help manage their Sjögren’s symptoms. Nearly all respondents (96%) indicated they wished that additional treatments for Sjögren’s were available. The most common symptoms experienced by survey respondents on a weekly or more frequent basis were dry mouth (92%), dry eyes (92%), fatigue (80%), dry or itchy skin (76%), and morning stiffness (69%). Respondents reported having been diagnosed by a health care provider with an average of five other health conditions, the most frequent of which were GERD (45%), Raynaud’s (38%), neuropathy (38%), and sinusitis (33%). Most respondents (71%) reported Sjögren’s interferes with their daily activities, and 79% and 54% indicated the disease has led them to make at least one day-to-day change at home or work, respectively. Sjögren’s was identified as adding a significant emotional burden to the lives of 74% of respondents (80% of patients >= 60 years of age vs 71% of >60 years). A negative impact on relationships with friends and family (63%), sex life (59%), spouse and partner relations (55%) was reported for children (19%) who were also reported. 66 percent of respondents indicated Sjögren’s adds a significant financial burden to their life. Those aged 60 years or less were more likely to view Sjögren’s as a significant burden in their lives compared to those older than 60 (72% vs. 63%).

Conclusions. The findings from this national survey highlight the complex impacts of Sjögren’s and its impact on patients and their families. Sjögren’s is burdensome to patients and affects their well-being and quality of life in a variety of ways, including physical, emotional, and financial challenges. Patients reported the need for new treatment options to treat the various manifestations of Sjögren’s. The findings from this survey will help to inform and support future SSF efforts to increase public and professional awareness of Sjögren’s and encourage research into new treatments and a cure.

P-164
A summary of key findings from the Sjögren’s Syndrome Foundation’s National Patient Survey

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Is elastography a new tool to differentiate Sjögren syndrome to sicca syndrome: results of the ELSA (elastography of salivary glands) study


Background/Purpose. Ultrasoundography (US) has been developed in salivary glands (SG) and particularly in primary Sjögren syndrome (pSS) for 10 years. However, the training curve is long and the reliability is not completely achieved (1). A new tool procedure has been developed to study the elasticity of the tissue parenchyma using elastography and could be implemented in the evaluation of SGUS pSS patients.

The objective was to evaluate SGUS using grey scale (GS) and the elasticity salivary glands parenchyma using elastography to differentiate pSS from sicca syndrome (SS) patients in a longitudinal consultation of sicca syndrome in Doppler Unit of Brest (France).

Methods. 63 patients complaining of sicca syndrome were enrolled in the ELSA study. At inclusion, all patients underwent a standardized workup including a clinical evaluation, laboratory tests, SG histology, SGUS and ELSA study. At inclusion, all patients underwent a standardized workup including a clinical evaluation, laboratory tests, SG histology, SGUS and ELSA study. At inclusion, all patients underwent a standardized workup including a clinical evaluation, laboratory tests, SG histology, SGUS and ELSA study. At inclusion, all patients underwent a standardized workup including a clinical evaluation, laboratory tests, SG histology, SGUS and ELSA study.

Results. 15 patients fulfilled the AECG criteria. The clinical characteristics of the patients in terms of ocular dryness and shirmer test were not significant between pSS and Sicca patients. There was a significant difference (p=0.04) concerning oral dryness (p<0.04), immunological data: Antinuclear antibodies and anti SSA and anti minor salivary glands biopsy (p=0.007). We found significant differences between the 2 groups in GS for the USLPG (p=0.021) and USLSMG (p<0.05). Evaluation of sublingual glands using US in grey scale and elastometry of the 6 SG showed no significant differences between the two groups.

Conclusion. Grey scale SGUS seems to be more sensitive to differentiate pSS to Sicca patients compared to elastometry. US of sublingual glands showed no involvement in the structural damage in pSS patients and suggest not examining these glands in the USpSS evaluation. Elastometry has been described to be a new tool and might be added as a new imaging technique to ultrasonography in pSS patients (3). However, ELSA study results showed no differences between pSS and Sicca patients. We need to follow the evolution of the Sicca population with US in GS and elastometry to detect potential echostructural parenchymal damage which might not yet be present at inclusion.

References
Results. Fifty patients diagnosed with SjS were included (45 female, age: 56 years). In 2011, the mean ESSPRI score was 8.3 (SD=4.6) and the mean ESSDAI score was 5.6 (SD=7.5). Initially, the sono graphic evaluation of the parotid gland (PG) resulted in a mean score of 1.6 (SD=0.6) and in the submandibular gland (SMG) of 1.7 (SD=1.0), the mean ARFI value of PG was 2.99m/s (SD=0.93) and the mean ARFI value of the SMG was 2.15m/s (SD=0.57).

Clinical examination and sonoelastographic evaluation was repeated after five years in 2016, revealing a mean ESSPRI score of 6.1 (SD=3.7, p=0.002) and a mean ESSDAI score of 4.6 (SD=7.0, p<0.001). The mean sono graphic score of the PG was 1.4 (SD=0.7, p=0.001) and of the SMG was 1.9 (SD=1.0, p=0.034). There was a decline in the sono graphic score of the PG of 0.27 (SD=0.5) and increase of the SMG of 0.1 (SD 1.1) on average. After five years a significant decline of ARFI values could be observed in the PG (2.33m/s, SD=0.70, p<0.001) while no significant changes of the ARFI of the SMG could be observed. Results of RTTE and VTTI did not change signifi cantly. The mean time interval between onset of first symptoms and first sono graphic examination in 2011 was 57.3 months (SD=60.8).

Conclusion. The five-year sonoelastographic follow up of salivary gland alterations in patients with SjS revealed a decline in the severity of sono graphic alterations of the parotid gland in BMUS and ARFI imaging, indic ating a certain capability for modulation of salivary gland affection in SjS.

Table I. Inter-observer correlation (Spearman correlation).

<table>
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<tr>
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<th>Video</th>
<th>Still-image</th>
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<tr>
<td>Right parotid</td>
<td>.807-.831-.838</td>
<td>.691-.742-.757</td>
</tr>
<tr>
<td>Right parotid</td>
<td>.764-.769-.780</td>
<td>.658-.765-.912</td>
</tr>
<tr>
<td>Left parotid</td>
<td>.760-.843-.864</td>
<td>.702-.727-.866</td>
</tr>
<tr>
<td>Left parotid</td>
<td>.724-.735-.795</td>
<td>.739-.824-.831</td>
</tr>
<tr>
<td>Right submandib.</td>
<td>.718-.780-.791</td>
<td>.736-.828-.934</td>
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<tr>
<td>Right submandib.</td>
<td>.670-.743-.831</td>
<td>.741-.787-.849</td>
</tr>
<tr>
<td>Left submandib.</td>
<td>.718-.743-.870</td>
<td>.575-.748-.811</td>
</tr>
<tr>
<td>Left submandib.</td>
<td>.603-.721-.739</td>
<td>.370-.642-.800</td>
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Conclusions. Both intra- and inter-observer variations were good. In general, bed-side scoring correlated better with longitudinal video as compared to still-images. If possible, we suggest scoring to be performed bed-side, with a video as documentation and possibility to monitor disease progression.

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Diagnostic and predictive evaluation using salivary gland ultrasonography in primary Sjögren's syndrome

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Background. We aimed to assess the diagnostic accuracy of salivary gland ultrasonography (SGUS) as a single test for the detection of primary Sjögren’s syndrome (pSS) and examine the prognostic factors for severe structural damage of the salivary glands based on SGUS score.

Methods. Patients with pSS (n=94) and idiopathic sicca syndrome (n=44) were evaluated using the SGUS 0-48 scoring system, which comprises five parameters: parenchymal echogenicity, homogeneity, hypoechoic areas, hyperechoic reflections, and clearance of posterior borders. The salivary gland volume and intraglandular power Doppler signal (PDS) were also assessed. A multivariate linear regression analysis was performed to determine the factors associated with SGUS score.

Fig. 1. Representative images showing salivary gland ultrasonography in primary Sjögren’s syndrome. (A-D) Parotid ultrasonographic grade of homogeneity. (A) Normal homogenous parotid gland (Grade 0). (B) Mild inhomogeneous parotid gland (Grade 1). (C) Evident inhomogeneous parotid gland (Grade 2). (D) grossly inhomogeneous parotid gland (Grade 3). (E-H) Submandibular ultrasonographic grade of homogeneity. (E) Grade 0. (F) Grade 1. (G) Grade 2. (H) Grade 3.
Sjögren’s Syndrome Clinic were reviewed. All subjects received a minor salivary gland biopsy for diagnostic purposes. The objective of this project was to determine the clinical significance of US findings consistent with changes seen in SS and to establish an independent predictor for structural damage of the salivary glands.

**Introduction.** In recent years, there has been an increase in the use and perceived clinical utility of salivary gland ultrasonography (SG-US) in the evaluation of patients for Sjögren’s syndrome (SS). SG-US core items and scoring methodology was established by the European League of Rheumatism (EULAR) in 2017. The panel identified 10 SG-US characteristics that demonstrated reliability when considering a diagnosis of SS. The minor salivary gland biopsy is a well-established component for the objective evaluation of SS and is heavily weighted by the most recent iteration of the American College of Rheumatology/EULAR classification criteria. Many clinical settings do not routinely have capability to provide or perform salivary gland biopsies but may have access to US. Thus, it is important to determine the clinical significance of US findings for diagnostic purposes. The objective of this project was to determine the correlation of SG-US findings with surgical pathology scores.

**Methods.** Clinical data from fifty (n=50) consecutive previously undiagnosed subjects evaluated for salivary gland dysfunction at the NIDCR/Sjögren’s Syndrome Clinic were reviewed. All subjects received a minor salivary gland biopsy and SG-US. Clinical findings were assessed for correlation between SG-US findings consistent with changes seen in SS and patients whose minor salivary gland biopsies demonstrated a focus score (FS) of at least 1 lymphocytic focus per 4mm² (1a focus/4mm²) of salivary gland tissue. The data were divided into 3 subject groups: 1) those with normal SG-US findings and no foci, 2) those with a focus of at least 1 and normal or indeterminate SG-US findings, and 3) those with at least 1 focus and SG-US findings consistent with SS. Salivary function across these groups using unstimulated and stimulated (e.g., parotid and submandibular/sublingual) unstimulated and stimulated were compared using Kruskal-Wallis at a p-value of 0.05 as significant.

**Results.** Twenty-two of the 50 (44%) of the subjects satisfied diagnostic criteria for a diagnosis of SS following routine work up, including both SG-US and minor salivary gland biopsy. Approximately 27% of the 22 subjects (6 of 22) had positive US and FS consistent with SS, while 50% (11 of 22) had FS consistent with SS, but normal or indeterminate US scores. Analyses of stimulated and unstimulated salivary gland function between FS and SG-US are being assessed.

**Conclusions.** Minor salivary gland biopsy remains an important tool in the diagnosis of SS. Here, we present a pilot analysis of our ongoing efforts on the implementation of SG-US in the diagnostic armamentarium of SS. Based on our findings, US imaging alone is not a reliable diagnostic predictor for SS, but can provide clinical information on salivary gland function. Although these results support the use of SG-US scoring as an adjunctive measure when considering a diagnosis of SS, it is possible that additional findings may be apparent when using a larger sample size, and correlations with other clinical characteristics might be established.

**P-171 Ultrasound imaging of salivary glands correlates with labial gland biopsy scores in Sjögren’s syndrome**

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**Objectives.** Ultrasound imaging (US) is a non-invasive, reproducible method for assessing the major salivary glands and results can be quantified. The purpose of this study was to determine the relationship in Sjögren’s Syndrome (SS) between US scoring of major glands and focal lymphocytic scores (FS) of minor salivary glands

**Methods.** Biopsies of labial-glands and Ultrasounds of major-glands were performed on 199 patients (mean age 55 years, 18 male, 181 female) attending the Sjögren’s Clinic at Guy’s Hospital. Patients were categorised into 3 groups: those diagnosed with SS according to the American-European classification criteria, those with no specific sialadenitis (sialadenitis-group), and those with normal biopsy results (control-group). FS of labial-glands were assessed by two calibrated histopathologists. Ultrasounds were carried out using a single Philips-AI22® Ultrasound, and a disease severity-score determined.

**Results.** There was a highly significant positive correlation between FS (mean=1.8, SD=2.4) and US scores (mean=2.7, SD=2.9) (n=199 r=0.708 p<0.0001) in the whole series. For the SS group (mean FS score=3.6, SD=2.5; mean US score=5.1, SD=2.4) there was a significant positive correlation between FS and US scores (r=0.458 n=87 p<0.0001). For the Sialadenitis-group (mean FS score=0.4, SD=0.6; mean US score=1.1, SD=1.7) there was a significant correlation between FS and US scores (r=0.656 n=66 p<0.0001). For the control-group (mean FS=0.2, SD=0.5; mean US score=0.5, SD=1.3).

**Conclusion.** A highly significant positive correlation was found between ultrasound scores of major salivary glands and focus scores of minor salivary (labial) glands.

**Relevance.** This is the first large-scale study to analyse the relationship between US scores of major salivary glands, and Focus Scores of minor salivary glands. Ultrasound analysis should prove to be an extremely important investigation in both cross-sectional and longitudinal studies of Sjögren’s syndrome.

**P-172 Ultrasongraphy of salivary glands in primary Sjögren’s syndrome and association with symptoms of dryness, disease activity and biopsy of minor salivary glands**

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**Objective.** To analyse ultrasonography (US) changes of salivary glands (SG) in patients with primary Sjögren’s syndrome (pSS) and association with symptoms of dryness, disease activity and biopsy of minor salivary glands (MSG).
Ultrasound of the salivary gland mostly showed grade 1 or grade 2. Our early arrangement of ultrasound of salivary gland with further biopsy. Index) were 14.6. ESSDAI (EULAR Sjogren syndrome disease index) were 4.4 0 123. Ultrasound score Grade 1 or Grade 2.

Conclusions: This study underlines the ultrasound of salivary gland maybe a diagnostic tool of Sjogren’s syndrome, but not relation with clinical severity of Primary Sjogren syndrome with ESSDAI and ESSPRI.

Methods. This study included 205 pSS patients (mean age 53.9±11.5, disease duration 5.6 years) and 87 healthy controls (mean age 52.3±14.7). All pSS patients fulfilled the AEGC diagnostic criteria. The disease activity was evaluated by EULAR SS disease activity index (ESSDAI), Sjogren’s Syndrome Disease Damage Index (SSDDDI) and EULAR Sjogren’s syndrome patient reported index (ESSPRI). Parotid and submandibular glands on both sides were assessed by US for size, parenchymal echogenicity and inhomogeneity, posterior glandular border and presence of intraglandular lymph nodes. Inhomogeneity of the SG were graded according to the De Vita scoring system. The global SGUS score (0-6) was the sum of the scores of each pair of SG. Statistical analysis was performed by SPSS v19.

Results. The mean ages were 50.5 years respectively. Sicca symptoms mean duration were 197/205 (96.9%) pSS patients and in 16 (18%) controls (p<0.0001).

Table I. 28 Primary Sjögren syndrome patient.

| Male | 3.5% (1/28) |
| Age (yrs) | 50.5 yrs |
| Seca duration (yrs) | 1.3 yrs |
| Anti-SSA | 291.5 (AU/mL) |
| Anti-SSB | 178.4 (AU/mL) |
| RF-IGM | 25.6 (IU/mL) |
| RF-IGM | <6 |
| IgG4 | 173.6 (mg/dL) |
| IgG4 | 700-1000 |
| C3 | 65.6 (mg/dL) |
| C4 | 3-201 |
| ESSPRI | 14.6 |
| ESSDAI | 4.4 |

Background. Yellow fever is a viral haemorrhagic fever transmitted by mosquitoes in patients with primary Sjogren’s syndrome (PSS) in the short and long term. Methods. This is a phase IV controlled prospective study, including 50 patients with PSS and 29 healthy controls. All of them were vaccinated for the first time by the 17DD YF vaccine (Biomanguinhos, Brasil) after individual clinical evaluation. Patients who presented CD4 <200 cells/mm3, neoplasia, HIV, primary immunodeficiency, using cyclophosphamide, prednisone >20mg/d, azathioprine >2mg/kg/d, chlorambucil, mycophenolate or biological therapy were excluded. Patients were evaluated at baseline, after 3, 4, 5, 6, 7, 14 and 30 days of the vaccination for viremia and humoral response. Plaque reduction neutralization test (PRNT) was measured at baseline and after 28 days. Disease activity was evaluated at baseline and after 6 months. Serum and cell samples were frozen at -70°C. The analyses of viremia and PRNT will be processed by Instituto Fiocruz-BH and Fundação Biomanguinhos-RJ.

Results. The mean age was 53 (±15.5) years, ESSDAI 1.2 (±2.4), ESSPRI 3.6 (±2.9), PCR 3.3 (±4.2), C3 174.2 (±266.2), C4 26.3 (±10.7) and IgG 1555.8 (±769.5). Most patients showed disease under control before and after 6 months (p>0.556). Forty four percent of the patients were using hydroxychloroquine, 18% methotrexate, 4% leflunomide, 2% sulfasalazine and 2% corticosteroid. Nine patients had adverse events from the 21 who were using only one medication and all 4 patients who were using 2 medications had an adverse event, and the patient who was using 3 medications did not have any adverse event (p=0.066). There was no difference between patients and controls in the occurrence of adverse events (46% vs 27.6%, p=0.106). The local main symptoms were: pain (16%), nodule (2%), oedema (4%) and heat (2%). The main systemic adverse events were: malaise (24%), myalgia (20%), headache (12%), arthralgia (12%), low back pain (10%), weakness in limbs (10%), productive cough (10%), dry cough (8%), pruritus (8%), abdominal pain (6%), nausea (6%), vomit (6%), fever (4%), dyspnoea (4%) and diarrhoea (2%).

Conclusion. The yellow fever vaccine is safe in patients with PSS with low disease activity and under low immunosuppression. It is necessary to await the studies of vaccine kinetics for further conclusions.
Activation of RANKL system in Sjögren’s syndrome related lymphoma

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Introduction/Objective. Sjögren’s syndrome (SS) is an autoimmune exocrine disease characterized by chronic dysfunction and destruction of the salivary and lacrimal glands, resulting in mucosal dryness. Approximately 5-10% of patients are identified as a high-risk group for the development of lymphoproliferative disease, most commonly B-non Hodgkin’s lymphoma. Previous studies support the activation of the receptor activator of nuclear factor kappa-B ligand (RANKL) pathway, as an important contributor in both systemic autoimmune diseases and haematological malignancies. The purpose of this study was to investigate the role of RANKL-pathway in SS-related lymphoma.

Methods. RANKL and osteoprotegerin (OPG) titers were determined in sera derived from 66 SS patients and 12 sicca controls (SC) by ELISA. In addition, miRNA expression levels of RANKL and OPG were determined in cDNA isolated from minor salivary gland tissues of 34 SS patients, 19 patients with SS complicated with lymphoma and 11 SC using RT-PCR. In order to identify potential associations of the components of the RANKL/RANK/OPG system with disease related characteristics, demographics, clinical and serological features of SS patients were recorded after thorough chart review. For statistical analysis SPSS 24.0 software has been implemented.

Results. SS patients displayed increased RANKL and OPG serum titers, as well as salivary gland tissue RANKL mRNA levels compared to SC. At salivary gland tissue level, OPG mRNA levels were decreased in SS patients associated with lymphoma compared to both SS and SC groups. Patients with higher serum OPG levels displayed significantly higher erythrocyte sedimentation rate (ESR) values, and a trend toward increased rheumatoid factor titers and Tarpley scores in minor salivary gland biopsies.

Conclusion. Decreased OPG and increased RANKL mRNA expression levels in salivary gland tissues derived from SS patients complicated by lymphoma compared to controls might imply an important role of deregulated RANKL pathway in SS related lymphomagenesis.

Interleukin-22 and associated genes are targets of microRNAs dysregulated in primary Sjögren’s syndrome associated lymphoma

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We aimed to explore whether Three-prime Repair Exonuclease 1 (TREX1) genetic variants could influence the risk of primary Sjögren’s syndrome (SS) and SS-related lymphoma. Three TREX1 variants (rs11797, rs3135941 and rs3135945) were evaluated in 229 SS, 89 SS-lymphoma (70 SS-MALT and 19 SS non-MALT) and 240 healthy controls (HC) by PCR-based assays. In available 52 peripheral blood and 26 minor salivary gland (MSG) SS samples, mRNA expression of type I interferon (IFN) related genes and TREX1 was determined by real-time PCR. Significantly decreased prevalence of rs11797 A minor allele was detected in SS patients complicated by non-MALT lymphoma compared to HC (OR [95% CI]: 0.4 [0.2-0.9], p-value: 0.02). SS patients carrying the rs11797 AA genotype had increased prevalence of anti-Ro/SSA autoantibodies and type I IFN related gene mRNA expression in MSG tissues. These data support genetically related development of type I IFN production as an additional mechanism for SS-related lymphomagenesis.
Thymic stromal lymphopoietin expression and function in lymphoproliferation and lymphoma in primary Sjögren’s syndrome

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Background. Thymic stromal lymphopoietin (TSLP) is an epithelial lymphoepoietic cytokine mainly expressed at interfaces between the body and the environment, showing immunoregulatory properties and acting as a B cell growth factor. Two isoforms of TSLP have been described, showing opposite biological actions. The short form (sTSLP) is constitutively expressed at body barriers, where it regulates the immune tolerance. Conversely, the expression of the long TSLP is inducible and upregulated in several inflammatory diseases. Recent studies highlighted the contribution of TSLP to the immunopathology of human autoimmune and systemic inflammatory disorders and of both solid and hematological malignancies.

Objective. To investigate TSLP expression and function in primary Sjögren’s syndrome (pSS) patients, stratified according to the lymphoproliferative status, from fully benign (fbSS) to myoepithelial salaladinitis (MESA) and to B-cell non-Hodgkin lymphoma (NHL).

Methods. On our preliminary TSLP serological data in 79 pSS patients and 100 controls (21 non-autoimmune sicca-syndrome, nSS; 79 sex and age-matched healthy blood donors, HBDs), TSLP expression was also studied in salivary glands (SG) biopsies from 38 pSS patients (13 fbSS; 13 MESA; 12 NHL) and from 13 nSS by RT-PCR, immunohistochemistry and immunofluorescence. TSLP or in vitro functional effects were also evaluated on peripheral B lymphocytes collected from 5 patients in each pSS subgroup, and from 5 HBDs.

Results. Serum studies showed significantly higher TSLP levels in pSS compared to controls, significantly increasing from fbSS to MESA and to NHL. In SG biopsies, TSLP-positive B lymphocytes increased with the progression of lymphoproliferation, maximally in NHL, consistently with the detection of inducible HTSLP mRNA only in MESA and NHL. Constitutive sTSLP mRNA levels also increased in pSS compared to controls, but with no statistical difference among pSS subgroups. TSLP promoted a significant B-cell activation and immunoglobulin production by peripheral B lymphocytes in pSS-related MESA and NHL.

Conclusions. A pathogenetic role of TSLP is herein suggested in pSS for the first time. TSLP, which promotes B-cell expansion, progressively increases from benign to malignant B-cell lymphoproliferation in pSS. With the progression of lymphoproliferation, the B-cells, rather than the salivary epithelium, appear as the major TSLP source, in its long isoform. Further studies and the analysis of TSLP as a biomarker in pSS are worthwhile.

References
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Single cell RNA sequencing of B cell subsets from parotid glands of patients with Sjögren’s syndrome identifies the expression profile of epitheliocell-associated FcRfL4^+ B cells

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Background. A subset of B cells expressing the inhibitory Fc receptor-like protein 4 (FcRfL4) is found in salivary gland lesions of patients with primary Sjögren’s syndrome (pSS). FcRfL4^+ B cells are associated with ductal epithelial cells forming lymphoepithelial lesions (IELs), in particular within parotid glands. Furthermore, FcRfL4 is expressed by macrophage-associated lymphoid tissue (MALT) lymphoma B cells. We aimed to investigate, by single cell and bulk RNA sequencing, how the transcriptome of FcRfL4^+ B cells differs from FcRfL4-negative naïve and memory B cells in salivary gland tissue of pSS patients. We hypothesize that FcRfL4^+ B cells contribute to IEL formation and are prone to lymphomagenesis.

Methods. Parotid gland biopsies of 5 pSS patients without MALT lymphoma and 1 pSS patient with MALT lymphoma were obtained. Single cell suspensions were prepared by mechanical disruption and enzymatic digestion. The cells were incubated with anti-CD19, anti-CD27 and anti-FcRfL4 antibodies and sorted into single cell and bulk (5 cells per well) based on the following definitions: CD19^+CD27^−FcRfL4^− (‘naïve’), CD19^+CD27^+FcRfL4^− (memory) and CD19^+FcRfL4^+ (FcRfL4^+). Library preparation was done using an in-house SMARTer RiboSEQ protocol and sequencing was done on an Illumina HiSeq 2500. Expression data were analyzed using Seurat or DESeq packages in R.

Results. Samples from 4 pSS patients and the MALT lymphoma patient passed quality control and were included. A total of 206 single cells and 450 cells in bulk were included in the analysis. Genes identified by differential expression were compared to gene pathway analysis. Both in single cell and bulk analysis, multiple genes coding for integrins, such as ITGAX (CD11c) were significantly upregulated in FcRfL4^+ B cells. Gene Ontology pathways that showed the highest upregulation in FcRfL4^+ B cells (both single cell and bulk) were receptor binding, GTPase and protein kinase pathways. Analysis of bulk samples further revealed that expression levels of CXCR3, NFKB1, AK2 and LYN, among others, were significantly upregulated in FcRfL4^+ B cells, compared with either naïve or memory B cells. LCK was, for example, increased in the FcRfL4^+ compartment. Interestingly, preliminary single cell analysis of single cell negative ‘naïve’ B cells of the MALT-lymphoma patient showed upregulation of MX1, OAS1, ISG15, BTK and TIF1, among others.

Conclusions. FcRfL4^+ B cells in salivary glands of pSS patients show upregulation of genes involved in homing and cell adhesion, consistent with their tissue location close to the epithelium. FcRfL4^+ B cells also show increased upregulation of genes that promote inflammation. These cells exhibit all characteristics of chronically stimulated CD11c^+ memory B cells, and we speculate that these cells contribute significantly to the epithelial damage seen in the glandular tissue of pSS patients.

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Usefulness of 18F-FDG positron emission tomography (PET) for lymphoma diagnosis in patients with primary Sjögren’s syndrome

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Background. Primary Sjögren’s syndrome (pSS) is the autoimmune disease having the highest risk of lymphoma. The differential diagnosis between benign and malignant lymphoproliferation is sometimes difficult. Among imaging procedures, 18F-FDG PET could be useful for that purpose. The objectives were to compare 18F-FDG PET results between patients with and without lymphoma to identify PET pattern associated with lymphomas in pSS.

Methods. Retrospective study conducted in 2 centers including pSS patients (ACR/EULAR 2016 criteria) who undergo 18F-FDG PET. PET abnormalities were compared between patients with and without lymphoma. Two independent readers analyzed PET blind to lymphoma diagnosis. ESSDAI-PET score previously described by Cohen et al. was calculated.

Results. 45 patients were included; 15 had lymphoma: MALF (n=12), nodal marginal zone with plasmacytic differentiation (n=2), diffuse large B-cell (n=1). Patients with lymphoma had more frequently parotid gland swelling (67% vs 20 %, p=0.003) and higher ESSDAI score (24 [13.5-29] vs 9 [5-20], p=0.003), even after exclusion of lymphoma item (19 [11-27] vs 9 [5-20], p=0.03). Compared to non-lymphoma patients, mean size (45.5 [38-56] mm vs. 40 [37-41] mm; p=0.048) and maximum standardized uptake value

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Long-term results of R-CHOP therapy in patients with diffuse large B cell lymphoma (DLBCL) associated with Sjögren’s syndrome (SS)
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Background. Development of DLBCL in SS is a major outcome, since it is the second most common type of lymphoma in SS, with the higher risk of adverse events during follow-up and significantly poorer outcome for patient survival compared to the other common SS-associated lymphomas. First line treatment is R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone), which was empirically based on the therapeutic experience of the DLBCLs of the general population with similar 5-year survival rates. In this study, we evaluated the 10-year survival of SS-DLBCLs treated with R-CHOP.

Methods. Twelve cases with DLBCL from total 92 patients with SS-associated lymphomas that are included in our institution database were retrospectively studied. Eleven patients received R-CHOP and one CHOP. Demographic, clinical, laboratory and histological features of SS disease and lymphoma were recorded and analyzed by appropriate statistical approaches.

Results. Patients with SS-associated DLBCL were monitored for a long-term follow-up since their SS- and DLBCL-diaognosis. Median follow-up from the diagnosis of SS was 38 years and a total of 211.15 person-years at risk, was reached and median follow-up from the diagnosis of DLBCL was 5.88 years and a total of 77.70 person-years at risk, was reached. During this follow-up, 6 patients died, but only 3 deaths were lymphoma-related. Seven patients achieved a complete remission (CR; CR rate: 58.3%) and 6 of them remained in CR and are still alive with a median duration of CR response reaching to 11.08 years. In all patients that presented an event during follow up, this was observed during the first five years. From the diagnosis of SS, the 5-year and the 10-year OS% was 58.3% and 48.61%, respectively, while the median OS was 5.88 (range: 0.01-13.92) years. The 5-year and 10-year EFS% was 33.3% and 22.22%, respectively and the median EFS was 3.26 (range: 0.01-13.92) years. The 5-year and 10-year OS% was 58.33% and 48.61%, respectively, while the median OS was 5.88 (range: 0.01-13.92) years.

Conclusions. The survival rates of SS-associated DLBCLs are similar to those described (10-year OS%: 43.5%) by the first study of Groupe d’Etude des Lymphomes de l’Adulte (GELA) investigating the addition of rituximab to CHOP and support the use of R-CHOP as treatment of choice.

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Cigarette smoking is a risk factor for developing primary Sjögren’s syndrome with Ro/SSA and La/SSB autoantibodies
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Background. Cigarette smoking is a well-established risk factor for several systemic autoimmune disorders, including rheumatic diseases. However, only a limited number of studies have investigated the effect of smoking on the risk of developing primary Sjögren’s syndrome (pSS), reporting contradictory results. This may relate to factors such as the smoking being conducted before development of current classification criteria, including few patients or using mixed controls for comparison. The aim of this study was therefore to investigate the impact of smoking on the development of pSS in a large, clinically well characterized cohort of patients with pSS.

Methods. A case-control study using prevalent cases of pSS classified according to the American-European Consensus Criteria (606 cases and 5,925 population controls) was performed. Smoking habits prior to diagnosis were obtained from questionnaire data and cases and controls were classified into ever-smokers or never-smokers. The impact of ever-smoking on pSS was assessed by calculating odds ratios (OR) with 95% confidence intervals (CI) employing logistic regression. Estimates were adjusted for age, sex, time period and area of residence.

Results. Ever-smokers had an increased risk of developing pSS (OR 1.3, 95% CI 1.1–1.5). The risk of ever-smokers to develop pSS was somewhat higher for men (OR 2.0, 95% CI 1.0–4.1, compared to OR 1.2, 95% CI 1.0–1.5 for women). Stratifying the analysis according to Ro/SSA and La/SSB autoantibody positivity revealed that the increased risk of pSS associated with smoking was limited to individuals with autoantibodies; both in pSS positive for Ro/SSA and La/SSB autoantibodies (OR 1.5, 95% CI 1.2–2.0) as well as in Ro/SSA and/or La/SSB positive pSS (1.4, 95% CI 1.1–1.7), but not in pSS negative for these autoantibodies (OR 1.1, 95% CI 0.8–1.6).

Conclusions. We observed a significantly increased risk for ever-smokers of both sexes to develop pSS in the largest study to date. The increased risk was only evident for development of Ro/SSA and/or La/SSB positive pSS, and not for pSS negative for these autoantibodies. The data indicate that underlying genetic factors predisposing for autoantibody positivity may be of importance for the increased risk.

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Tobacco smoking effects on clinical, serological and histological manifestations of Sjögren’s syndrome, a cross-sectional study of the OASIS cohort
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Background. Previous studies showed that tobacco smoking in patients with primary Sjögren’s syndrome (pSS) is negatively associated with a histopathological focus score and anti-Ro/anti-La serological positivity (1). We aimed to evaluate the association of tobacco smoking history with clinical, serological and histological features of patients with Sjögren’s syndrome. Methods. Cross-sectional study of patients at the time of their inclusion in the OASIS cohort between 2014 and March 2017. This UK prospective research cohort includes patients with suspected pSS or known pSS. Patients included in the analysis a the pSS gene expression analysis in the ACR-EULAR (2016) classification criteria for pSS. We excluded patients with secondary Sjögren’s syndrome. Characteristics of pSS patients with and
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Influence of smoking and obesity on the risk of developing primary Sjögren’s syndrome: a population-based cohort study

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Objective. Cigarette smoking and obesity have been identified as risk factors for developing several autoimmune diseases, and may be protective for others. We aimed to explore the role of these risk factors in primary Sjögren’s syndrome (pSS).

Methods. A cohort of Olmsted County, Minnesota residents diagnosed with pSS between January 1, 2000 and December 31, 2015 was identified based on individual medical record review. Each of the cases was matched to 3 age- and sex-matched comparators without pSS randomly selected from Olmsted County residents, indexed to the date of pSS diagnosis. Smoking status was divided into three categories of current smoker, ex-smoker, and never smoker. The body weight and height closest to date of diagnosis/ index date (±1 year) were used. Obesity was defined as a body mass index (BMI)≥30 kg/m².

Results. 106 incident cases of pSS and 318 controls were identified. The odds ratio (OR) of pSS comparing current smokers with never smokers adjusted for age and sex was 0.34 (95% confidence interval (CI): 0.14, 0.85; p<0.05), while the age- and sex-adjusted OR for former smokers compared to never smokers were 1.27 (95% CI 0.80, 2.03). The OR of pSS comparing obese subjects with non-obese subjects was 0.79 (95% CI, 0.45, 1.30), while the OR of pSS for BMI analyzed as a continuous variable was 0.97 (95% CI, 0.94, 1.01).

Conclusions. In this population-based study, current smokers have a lower risk of developing pSS while BMI does not affect this risk.
History and physical examination with stimulation and unstimulated salivary flow (WUSF) and a lip biopsy, ocular examination with a slit lamp by an ophthalmologist and a dentist. History and physical examination with stimulation were performed. Patients with pSS positive for SSA and/or SSQ, the risk was even more striking for deep venous thrombosis (HR 2.7, 95% CI 1.8-4.0) and pulmonary embolism (HR 2.6, 95% CI 1.5-4.3).

Conclusions. We observed a significantly increased risk for both arterial and venous cardiovascular events occurring after pSS diagnosis, compared to matched population controls. Patients with pSS positive for SSA and/or SSQ, the risk was even more striking for deep venous thrombosis (HR 2.7, 95% CI 1.8-4.0) and pulmonary embolism (HR 2.6, 95% CI 1.5-4.3).

Results. The mean age at pSS diagnosis was 54.4 years. Patients with pSS had a significantly increased risk of myocardial infarction (5.6%) compared to controls (3.2%), (HR 1.8, 95% CI 1.4-2.4), occurring at a mean of 9.8 years after pSS diagnosis. The risk was even higher in pSS patients positive for SSA and/or SSQ autoantibodies (n=679 pSS, versus n=6790 controls) (HR 2.0, 95% CI 1.4-2.9). A non-significant trend of increased risk was observed in pSS patients without these autoantibodies (n=282 pSS, versus n=2820 controls) (HR 1.5, 95% CI 0.9-2.5). Stratifying the SSA and/or SSQ positive pSS for gender, the increased risk was significant in females (HR 2.0, 95% CI 1.4-3.0) with only a trend in males (HR 1.9, 95% CI 0.8-4.5). pSS could not be reliably associated with an increased risk of cerebral infarction, occurring in 4.2% of pSS patients, compared with 3.2% among controls (HR 1.3, 95% CI 0.9-1.8). However, pSS patients positive for SSA and/or SSQ autoantibodies had a small increased risk of cerebral infarction (HR 1.4, 95% CI 1.0 – 2.1) while the risk in antibody negative pSS was in parity with the general population (HR 1.1, 95% CI 0.6 – 2.1). In affected patients, the cerebral infarction occurred after a mean of 12 years post diagnosis.

Venous thromboembolism was also more frequent in pSS compared with controls and deep venous thrombosis was observed in 4.4% of patients at a mean of 7 years after pSS diagnosis, versus 1.9% in controls (HR 2.3, 95% CI 1.7-3.3). Pulmonary embolism presented in 2.6% of patients at a mean of 10 years after pSS diagnosis, versus 1.1% in controls (HR 2.3, 95% CI 1.5-3.6). In pSS positive for SSA and/or SSQ, the risk was even more striking for deep venous thrombosis (HR 2.7, 95% CI 1.8-4.0) and pulmonary embolism (HR 2.6, 95% CI 1.5-4.3).

Ethnicity of Sjögren’s syndrome in the USA

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Background. We undertook this study to describe the racial and ethnic diversity of primary Sjögren’s syndrome (pSS) compared to SLE, which is known to be more common and severe in Black Americans than White Americans.

Methods. Individuals with sicca were evaluated by a rheumatologist, ophthalmologist and a dentist. History and physical examination with stimulation and unstimulated salivary flow (WUSF) and a lip biopsy, ocular examination with a slit lamp by an ophthalmologist and a dentist. History and physical examination with stimulation were performed. Subjects were classified using ACEG and ACR criteria for pSS. We compared the non-Hispanic Black pSS to the non-Hispanic White pSS subjects with one to four age and sex match in terms of clinical and serological manifestations. We also compared pSS subjects with non-Sjögren’s Sicca (nSS) and those in a SLE cohort followed in the same facility. P values were corrected for multiple comparisons. Due to low representation of other ethnic groups in our study population we only considered non-Hispanic Blacks and non-Hispanic Whites in the study.

Results. We classified 327 subjects in the clinic as pSS of which 201 were considered for the study. Among these 187 (92.1%) were self-identified as White, while only 14 (6.9%) were self-identified as Black. There were 7 (3.05%) Blacks and 223 (96.95%) Whites in nSS group. Among the SLE subjects, there were 106 (29.5%) Black and 253 (61.5%) as White. Thus, we found that black Americans were 5 times more likely to have SLE compared to pSS (g=3.6, 0.0001, OR=5.45), while there was no such difference when compared to pSS subjects with nSS (control group) (g=2.76, p=0.06, OR=0.41). We also compared the ethnic make-up of the pSS to the population and found no difference, while among SLE patients Black Americans were several times more common than in the general population. Concerning the classification criteria, we found that Black subjects had higher incidence of corneal erosion as evident by Lissamine green test (p=2.7x10^-4 by Fisher’s exact test). We also evaluated pSS subjects for systemic manifestations. Black subjects were found to have higher incidence of parotid gland enlargement (p=0.01 by Fisher’s exact test) and hypergammaglobulinemia IgG type (p=0.02 by Fisher’s exact test).

Conclusions. In contrast to SLE, Black Americans were enriched among subjects with pSS. This is likely due to a biological difference in the risk of the two diseases between these ethnic groups. However, socioeconomic differences leading to health care disparity may be responsible for some of the difference in representation among subjects with these diseases. We found minor differences in the manifestations of pSS between Black and White Americans, suggesting pSS is not more severe in Blacks. Similar to risk of disease, this is in contrast to the situation in SLE where Black Americans have more severe disease.