Ultrasonography of major salivary glands in primary and juvenile Sjögren's syndrome

Daniel S. Hammenfors

Thesis for the degree of Philosophiae Doctor (PhD)
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1. SCIENTIFIC MILEU

The present work was performed at the Department of Rheumatology at Haukeland University Hospital, Bergen in 2011-2018. The project was planned and executed in close collaboration with the Broegelmann Research Laboratory (BRL), especially Professor Roland Jonsson (Head of BRL) and Professor Silke Appel. I have also been a part of the Bergen group of Epidemiology and Biomarkers in Rheumatic Diseases (BEaBIRD).

During the course of my PhD training, I had a close collaboration with Professor Valéria Valim, Head of the Rheumatology Division at Federal University of Espírito Santo Hospital, without whom paper II would not have been feasible.

Throughout my work, I have been a part of the research group "Immunology and Rheumatology" and enrolled in the Bergen Research School in Inflammation. My supervisors have been Professor Malin V. Jonsson and Professor Johan G. Brun.

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4. ABBREVIATIONS

ANA	Antinuclear Antibodies
ACR	American College of Rheumatology
AECG	American-European Consensus Group
AIDS	Acquired Immunodeficiency Syndrome
BAFF	B-cell Activating Factor
CMV	Cytomegalovirus
CRP	C-Reactive Protein
EBV	Epstein-Barr Virus
EGM	Extraglandular manifestations
EULAR	European League Against Rheumatism
GC	Germinal Center
HepC	Hepatitis C
HCQ	Hydroxychloroquine
HIV	Human Immunodeficiency Virus
jSS	Juvenile Sjögren's Syndrome
kD	kiloDalton
MALT	Mucosa-associated Lymphoid Tissue
mHz	mega Hertz
MRI	Magnetic Resonance Imaging
MSG	Minor salivary gland
NHL	Non-Hodgkin's Lymphoma
pSS	Primary Sjögren's Syndrome
SGUS	Salivary Gland Ultrasonography
RA	Rheumatoid Arthritis
US	Ultrasonography
Ro/SSA	Robert/Sjögren's-syndrome-related Antigen A
La/SSB	Lane/Sjögren's-syndrome-related Antigen B
SLE	Systemic Lupus Erythematosus

5. ABSTRACT

Background

Primary Sjögrens's syndrome (pSS) is a systemic autoimmune disease, mainly affecting the salivary and lacrimal glands. Primary SS can also affect the younger population and when it does, the denomination is Juvenile SS (jSS). The salivary gland component in SS is commonly evaluated by histopathological detection of focal mononuclear cell inflammation in a minor labial salivary gland. Other methods are needed when a lip biopsy is inconclusive or cannot be performed. Interest in non-invasive major salivary gland ultrasonography (SGUS) is increasing for both clinical practice and research.

Aim

To investigate the use of SGUS in the diagnostic evaluation of pSS and jSS.

Methods

In *Paper I,* we included 97 well-characterized pSS patients. *Paper II* included patients diagnosed with jSS at the age of 18 years or younger, and younger than 25 years at inclusion. The cohort in *Paper III* consisted of 32 consecutive SS patients.

In *Paper I-III,* we collected clinical data and performed a clinical examination including Schirmer's I-test and sialometry. In *Paper I* and *Paper III,* we also included new serology/laboratory tests. For all three papers, SGUS of the parotid and submandibular glands was performed and evaluated using a simplified scoring system (0-3). In *Paper I,* the SGUS examinations were digitally stored as images and evaluated and scored at a later time-point. In *Paper II,* the SGUS examinations were evaluated and scored bedside. In *Paper III*, the SGUS examinations were evaluated and scored both bedside and post-examination. Post-examination evaluation and scoring was performed on both images and videos, at two different time-points.

Results

In *Paper I,* the female:male ratio was 91:6 (15.2:1), and the mean age 61.3 years. All patients fulfilled the AECG classification criteria for pSS. We found pathological SGUS findings (SGUS+) in 51/97 patients (52.6% sensitivity). SGUS+ was associated with hyposalivation, reduced tear secretion, and elevated titers of anti-Ro/SSA and anti-La/SSB.

In *Paper II,* the female:male ratio was 58:9 (6.4:1). Mean age at first symptom was 10.2 years and mean age at diagnosis was 12.1 years. Ocular and oral symptoms were noted in 42/67 and 53/66 patients, respectively. The AECG or ACR/EULAR classification criteria for pSS were fulfilled by 42/67 patients. SGUS+ was observed in 41/67 patients (61.2% sensitivity). The AECG and/or ACR/EULAR criteria was fulfilled by 26/41 SGUS+ patients. SGUS+ was associated with hyposalivation and elevated titres of anti-Ro/SSA, anti-La/SSB and rheumatoid factor. Salivary gland enlargements/parotitis was noted in 37/58 patients and non-significantly associated with SGUS+.

In *Paper III*, the female:male ratio was 7:1, and the mean age 56 years. The ACR/EULAR classification criteria for pSS was fulfilled by 24/42 patients. SGUS+ was observed in 14/32 patients (43.8% sensitivity). Results from the first round of video and image evaluations were used to calculate interobserver agreement and was compared to the bedside evaluations. The second round of evaluations were compared to the first round to calculate intraobserver agreements. The inter- and intraobserver agreement was overall good to excellent. Image and video evaluation compared to bedside evaluation revealed a slightly better agreement for longitudinal video of the parotid gland.

Conclusion

Pathological SGUS findings correlate with several clinical and laboratory findings for both pSS and jSS, adding important information to the diagnostic evaluation. Using a simplified scoring system, both inter- and intrarater correlations were excellent for experienced examiners, with longitudinal video of the parotid gland proving most reliable for post-examination evaluation.

6. LIST OF PUBLICATIONS

The thesis is based on the following papers:

I

Daniel S. Hammenfors, Johan G. Brun, Roland Jonsson, Malin V. Jonsson. Diagnostic utility of major salivary gland ultrasonography in primary Sjögren's syndrome. *Clinical and Experimental Rheumatology 2015; 33(1):56-62*.

II

Daniel S. Hammenfors, Valéria Valim, Blanca E. R. G. Bica, Sandra G. Pasoto, Vibke Lilleby, Juan Carlos Nieto-González, Clovis A. Silva, Esther Mossel, Rosa M. R. Pereira, Aline Coelho, Hendrika Bootsma, Akaluck Thatayatikom, Johan G. Brun, Malin V. Jonsson. Juvenile Sjögren's syndrome: clinical characteristics with focus on salivary gland ultrasonography. *Arthritis Care and Research 2019, e-pub ahead of print*.

III

Daniel S. Hammenfors, Haris Causevic, Jörg Assmus, Johan G. Brun, Roland Jonsson, Malin V. Jonsson. Assessment of major salivary gland ultrasonography in Sjögren's syndrome - A comparison between bedside and post-examination evaluations. *In manuscript*.

7. Table of contents

8. INTRODUCTION

8.1 Primary Sjögren's syndrome

Sjögren's syndrome (SS) is a chronic, autoimmune condition, mainly affecting the salivary and lacrimal glands. The hallmark complaints are of dry eyes and mouth (keratoconjunctivitis sicca and xerostomia), but patients also frequently present symptoms of fatigue, and muscle and joint pain (1, 2). Extraglandular involvement such as dry skin, interstitial lung disease, autoimmune hepatitis, primary biliary cirrhosis and interstitial nephritis, occur in 70-80% of patients with pSS (3-5). Furthermore, approximately 7.5% of the patients with primary SS (pSS) also develop Non-Hodgkin's lymphoma, 48-75% being MALT-lymphomas (6-9).

Focal mononuclear cell inflammation in the minor labial salivary gland biopsy, (Figure 1) is an important finding in pSS (10). It is one of the main components of the classification criteria addressed later in the thesis. Germinal center (GC)-like morphology (GC+) with local and systemic autoantibody production, (11) can be observed in 20-25% of lip biopsies attained from pSS patients. Some studies indicate that presence of GC-like morphology is associated with increased risk of lymphoma (12, 13) whereas one study could not support these findings (14). GC+ patients present a distinct salivary proteome (15) closely linked to the biologic state of the target organ (16).

Important immunological markers in pSS include antinuclear antibodies (ANAs) such as anti-Ro/SSA and anti-La/SSB, with anti-Ro/SSA as the most specific autoantibody for pSS (17). Anti-Ro/SSA is also associated with several other autoimmune diseases and may be present in the serum of patients with rheumatoid arthritis, primary biliary cholangitis, systemic lupus erythematosus, systemic sclerosis, idiopathic inflammatory myopathies, and mixed connective tissue disease. Pregnant women

with anti-Ro/SSA have an increased risk for having a child with neonatal lupus and/or congenital heart block (18, 19). Maternal serum anti-SSA may recognize one or two of Ro/SSA cellular proteins with a molecular weight of approximately 52 and 60 kiloDalton (kD). These antigens are called Ro52 and Ro60. Anti-Ro52 seems to have an important role in the development of congenital heart block (20) whereas it has been suggested that Ro60 plays an important role in preventing autoimmune diseases (21).

Göransson and colleagues proposed a 0.05% point estimate of pSS in a Norwegian study from the western part of Norway (22). In a 2015 meta-analysis, the pooled incidence rate for pSS was 6.92 per 100.000 person-years, and the overall prevalence rate was 60.82 per 100.000 inhabitants (23) making pSS the second most prevalent autoimmune rheumatic disease. Primary SS predominately affects middle-aged women with a 9:1 female:male ratio (23). The different estimates of incidence and prevalence of SS may partially be a result of different classification criteria for patient inclusion over time (Table 1).

At present, there is no causal treatment for Sjögren's syndrome. Clinicians often concentrate on symptomatic relief, for instance by using artificial tears and saliva, and prophylactic measures focusing on good oral hygiene, fluorides, and stimulation of saliva (24).

Management of Sjögren's syndrome is also influenced by the presence of extraglandular manifestations, which may prompt more specific treatment regimes. The most commonly used systemic treatment is hydroxychloroquine (HCQ), but a randomized controlled trial by Gottenberg et al (25) in 2014 could not document improvement of patient reported symptoms of dryness, pain and fatigue after 24 weeks of treatment with HCQ. For severe pSS related conditions and organ manifestations other empiric treatments such as intravenous methylprednisolone,

cyclophosphamide, azathioprine, cyclosporine, mycophenolate mofetil, or chlorambucil may be used (25).

There has also been two large randomized controlled trials investigating the efficacy of rituximab in pSS (26, 27). The follow-up period was for 24 and 48 weeks. Neither study met their primary endpoint, but significant reductions in fatigue, dryness VAS and an increase in unstimulated salivary flow were observed. The effect of rituximab on salivary gland ultrasonography score has also been investigated (28, 29), as will be described in more detail in a later section of the thesis.

8.2 Juvenile Sjögren's syndrome

Juvenile Sjögren's syndrome (jSS) is a rare, poorly defined and possibly underdiagnosed condition (30, 31). Reports of jSS were noted as early as the 1960s (32). Mean age at time of diagnosis is around 10 years (33). The clinical presentation is slightly different from pSS, with salivary gland swelling/parotitis seeming more common and sicca symptoms less prominent in jSS compared to pSS (33-35). As for pSS several organ systems may be affected in jSS, resulting in neurological, dermatological, musculoskeletal, vascular, gastrointestinal, respiratory, renal, and haematological manifestations (36, 37). Similar to pSS, extraglandular manifestations occur in approximately 50.0-84.6% of children with jSS (30, 38).

8.3 Classification criteria for Sjögren's syndrome

Since the triad of dry eyes, oral dryness and pain in several joints was first described by Henrik Sjögren in 1933, several classification criteria for pSS have been suggested

(Table 1), and applied in research papers. Until recently, the most commonly used criteria was the American-European Consensus Group (AECG) criteria (10) (Table 2). The main components in the AECG criteria are the salivary gland component assessed by focal mononuclear cell infiltrates in the minor labial salivary glands (Figure 1) and detection of autoantibodies in serum (anti-Ro/SSA and/or anti-La/SSB). Measures of reduced levels of unstimulated salivary secretion (sialometry), the detection of morphological and functional abnormalities of the glands by scintigraphy or sialography, and the measurement of reduced tear secretion or corneal damage are also considered. In addition, subjective symptoms of oral and ocular dryness are taken into account (10), representing important symptoms that may actually bring about a clinical suspicion of SS.

In 2012, the American College of Rheumatology (ACR) presented an alternate version of the criteria based on the Sjögren's International Collaborative Clinical Alliance (SICCA) cohort. Where the AECG classification criteria (10) consist of both subjective symptoms and objective findings, the ACR 2012 criteria (39) favor the autoimmune and eye components, in practice excluding the hallmark sicca symptoms.

To give researchers a single set of consensus measures to classify pSS, a novel set of classification criteria for SS were recently presented (17), aiming to replace both the provisional 2012 ACR criteria (39) and the revised 2002 AECG classification criteria (10). These criteria, termed the ACR/EULAR classification criteria, are based on five objective tests/items, resulting in a total score ≥ 4, derived from the sum of the weight assigned to each positive test/item (Table 2).

A Japanese study (40) compared the 1999 revised Japanese Ministry of Health criteria for diagnosis of SS (JPN), 2002 American-European Consensus Group classification criteria for SS (AECG) and 2012 ACR classification criteria for SS (ACR), to the 2016 American College of Rheumatology (ACR)-European League

Against Rheumatism (EULAR) classification criteria for primary Sjögren's syndrome (SS) in Japanese patients. It showed that the ACR/EULAR criteria had significantly higher sensitivity, but lower specificity for the diagnosis of pSS, compared to the other currently available three sets of criteria. These results were different compared to Shibowski et al (17), which showed a higher specificity. It was speculated whether this difference was caused by the eight pSS and 11 non-pSS patients in the Japanese study (40), which only fulfilled the ACR/EULAR classification criteria (17).

Table 1. An overview of previous and current classification criteria for SS.

Classification criteria	Published	Reference
Sjögren's Disease Research Committee review on research reports: Annual report of the Ministry of Health and Welfare. Tokyo, Japanese Ministry of Health	1977	(41)
Sjögren's syndrome. Proposed criteria for classification	1986	(42)
Criteria for Sjögren's syndrome in Japan	1986	(43)
The Copenhagen criteria for Sjögren's syndrome	1986	(44)
European Community Study Group Criteria (ECSG)	1993	(45)
Revised Japanese Diagnostic Criteria for Sjögren syndrome	2000	(46)
American European Consensus Group Criteria (AECG)	2002	(10)
ACR criteria	2012	(39)
2016 ACR/EULAR classification criteria for primary Sjögren's syndrome	2016	(17)

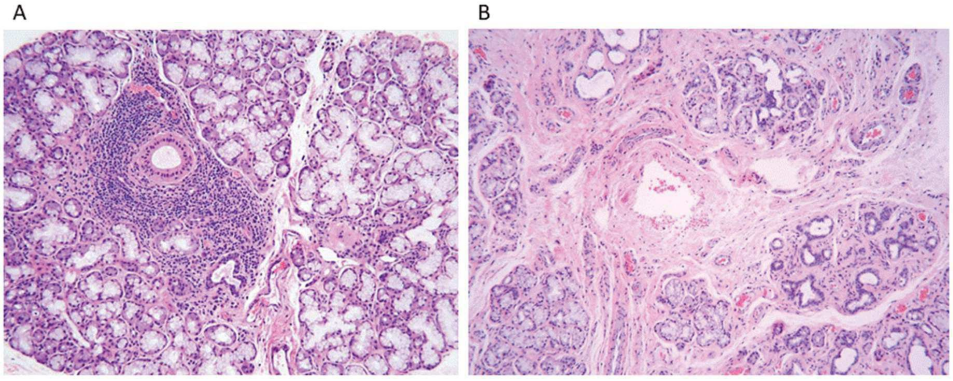

Figure 1. Minor labial salivary gland tissue section (H&E) from a patient with pSS (A). Dense mononuclear focal infiltrates surround the larger ducts and there is adjacent normal-appearing acini. Minor salivary gland from a patient with sicca symptoms (B). Negative biopsy without focal infiltrates. Atrophy of acinar glands and prominent interstitial fibrosis. Scattered lymphocytes and plasma cells mainly located in the atrophic areas. *Images by Kathrine Skarstein.*

Table 2. AECG and ACR/EULAR classification criteria for pSS. Modified from (47).

2002 AECG Criteria (10)	2016 ACR/EULAR Criteria (17)	Points
Items		
I. Ocular dryness symptoms	1. Focus score ≥1 foci/4 mm^2 in labial MSG biopsy	3
II. Oral dryness symptoms	2. Anti-Ro/SSA antibodies	3
III. Ocular signs: Schirmer's I-test ≤ 5 mm/5 min or van Bijsterveld score ≥ 4	3. Ocular Staining Score ≥5 (or van Bijsterveld score ≥ 4) in at least one eye	1
IV. Focus score ≥ 1 foci/4 mm^2 in labial MSG biopsy	4. Schirmer's test ≤ 5 mm/5 min	1
V. Salivary gland involvement: unstimulated whole salivary flow ≤ 0.1mL/min or characteristic sialography, sialoscintigraphy findings	5. Unstimulated whole salivary flow ≤ 0.1mL/min	1
VI. Antibodies to Ro/SSA or La/SSB antibodies or both		
Rules for classification		
Absence of exclusion criteria*	Applies to individuals with signs/symptoms that may be suggestive of pSS, such as ocular or oral dryness, or ESSDAI ≥1	
Presence of any 4 of 6 items with at least item 4 or 6, or presence of any 3 of 4 objective items (III, IV, V or VI)	Absence of exclusion criteria#	
	Cut-off ≥4 points	

* Head/neck radiation, HepC, AIDS, pre-existing lymphoma, sarcoidosis, graft-versus-host disease, use of anticholinergic drugs
# Head/neck radiation, active HepC, AIDS, sarcoidosis, amyloidosis, graft-versus-host disease, IgG4-related disease

8.4 Classification criteria for juvenile Sjögren's syndrome

Established diagnostic or classification criteria for jSS are not available in current literature (48-50). Diagnostic criteria for use on children and adolescents have been suggested (Table 3), but have not yet been validated in a large study population (34). Neither the AECG criteria (10) nor the ACR/EULAR criteria (51) were validated in a juvenile population. In addition, the AECG criteria may not be applicable due to diverse clinical symptoms and manifestations in children compared to adults (31, 50). For instance recurrent parotitis is often the presenting symptom in jSS and is included in the suggested criteria for jSS (Table 3).

A study by Yokogawa et al (52) in 2016 included 26 jSS patients diagnosed by a pediatric rheumatologist. The patients were evaluated with the AECG, ACR and Japanese criteria for pSS. They were also evaluated with the suggested jSS criteria (34). Interestingly, none of the patients had performed all the necessary test required to calculate the sensitivity of the criteria. Only nine (35%) patients met the AECG criteria, seven (27%) met the ACR criteria and seven (27%) met the Japanese criteria. Clearly, there is a need for distinctive jSS criteria. None of the pSS criteria performed better than the suggested jSS criteria (34). In the paper by Yokogawa et al (52), the jSS criteria performed better than noted in other studies (48, 50).

Table 3. Proposed jSS criteria (34). Table modified from (52) and (34).

	Criteria item
1	Recurrent parotitis or parotid enlargement
2	Recurrent conjunctivitis (non-allergic and non-infectious), or keratoconjunctivitis sicca
3	Recurrent vaginitis
4	Systemic: fever of unknown origin, arthralgias, hypokalemic paralysis, or abdominal pain
5	Ocular dryness (ocular staining or Schirmer's I-test)
6	Abnormal sialography
7	Elevated serum amylase
8	Leukopenia or elevated ESR
9	Hyperimmunoglobulinaemia (polyclonal)
10	Renal tubular acidosis
11	At least one of: anti-Ro/SSA, anti-La/SSB, high titer ANA (speckled pattern), RF
12	Lymphocytic infiltration of minor labial salivary glands or other organs

Diagnosis requires at least 4 of 12 items and exclusion of other autoimmune diseases.

8.5 Salivary gland ultrasonography

Ultrasonography (US) is an imaging method based on sound waves emitted and received by Piezo-electric crystals. The parotid and submandibular major salivary glands are superficial and well situated for high frequency ultrasonography examination (53, 54). High frequency transducers provide high-resolution images, with a more detailed image compared to low frequency transducers. Implementation of SGUS into clinical practice as a non-invasive imaging technique may aid the physician in the diagnostic process, reduce the time-delay associated with other diagnostic imaging techniques, confirm a clinical suspicion of disease and in some cases even replace the minor labial salivary gland biopsy. Utilization of ultrasonography may also prove as a valid method for assessing disease severity and progression. The method may prove of special importance in seronegative (ANA-negative) patients (55).

Characteristic SGUS findings in pSS consist of inhomogeneity and hypoechogenic areas, hyperechogenic reflections and diffuse gland borders (image c and d in Figure 2). In short, such findings are associated with increased signs and symptoms indicative of systemic complications, higher disease activity measured by ESSDAI (56), salivary gland swelling, reduced levels of unstimulated whole salivary flow (57), higher MSG focus score (57), and markers of lymphoma development such as skin vasculitis, GC-like structures in minor labial salivary gland biopsy and CD4+ T-lymphocytopenia (58).

Utilizing SGUS is reported to improve classification criteria for SS (47, 59, 60), but one has yet to reach a consensus on standard scoring method for clinical practice. In comparison to imaging methods traditionally used for the major salivary glands, findings from non-invasive SGUS (Figure 2) compare well with both contrast

sialography and scintigraphy (61), and MRI (62). Salivary gland SGUS is also convenient for repeated examinations in a clinical setting (63).

In 2008, Shimzu et al. (64) published a study investigating whether Doppler added to SGUS would improve the diagnostic value. Significantly higher vascularity was detected in pSS patients compared to controls. Increased vascularity also correlated with hisopathological findings in labial MSGs. Adding Doppler, increased the sensitivity from 44% to 63%, whereas the specificity decreased from 97% to 90%.

Other US techniques can also be used to evaluate the salivary gland component of pSS. Real-time sonoelastography (RTS) investigates the elasticity of soft tissues, based on the principle that tissue compression produces strain (displacement) within the tissue that is less pronounced in harder compared to softer tissues (65). The tissue elasticity can also be measured using Acoustic Radiation Force Impulse (ARFI) ultrasonography (66). When using this technique, the local tissue displacement is acquired by a brief acoustic radiation, causing emission and propagation of shear waves. The shear wave velocity (SWV) is measured in meters per second and increases with tissue stiffness. Studies show that pSS patients have increased stiffness in their major salivary glands compared to healthy controls (66, 67).

As mentioned in an earlier section of this thesis, the effect of rituximab was investigated by SGUS (28, 29). The study by Fisher et al (28) in 2018 showed improvement in glandular border/definition after 16 and 48 weeks of treatment, but could not confirm improvements in homogeneity or hypoechogenic areas as shown by Jousse-Joulin et al (29) in 2015. In 2007, Jousse-Joulin et al (68) demonstrated altered blood flow in the salivary glands upon lemon stimulation after 12 weeks of treatment with rituximab, using Doppler SGUS in pSS patients. The size of the submandibular and parotid glands was also reduced. In major salivary gland biopsies, a reduction in lymphocytic infiltrations and presence of GC-like structures was

observed following treatment with rituximab (69-71), but unfortunately, these studies did not include SGUS examination.

8.6 Applied scoring systems for salivary gland ultrasonography

Throughout the years, several scoring systems for SGUS have been described in the literature. One of the first scoring systems was published by Hocevar et al in 2005, presenting the diagnostic value of a novel scoring system consisting of five parameters (72). The study included 68 patients with confirmed pSS according to the AECG classification criteria and 150 patients with suspected pSS. The parameters assessed were echogenicity, inhomogeneity, and number of hypoechogenic areas, presence of hyperechogenic reflections, and clearness of the salivary gland borders. The grades of the five parameters were summarized for all four major salivary glands, with a final US score ranging from 0 to 48. Patients with confirmed pSS had a significantly higher US score, and with the cut-off at ≥17, the specificity and sensitivity was 98.7% and 58.8%, respectively. Simplified scoring systems based on Hocevar's study have since been suggested (58, 73-75).

To our knowledge, two meta-analyses have been published on SGUS scoring systems in the last five years (73, 75). The most recent meta-analysis (73) noted 1301 articles describing different scoring systems using the AECG criteria. Out of these, 24 met the criteria for quality-assessment and 14 for meta-analysis. Eight of the 24 studies that met the quality-assessment criteria used a scoring system of 0-4 and six studies used scoring 0-3. Two studies used 0-12, six studies 0-16, five studies 0-48. The 0-4 scoring system seemed to have the least variations in specificity and diagnostic OR (Table 4). Studies using the 0-4 systems had a pre-specified cut-off, whilst the cut-off varied in studies using 0-16 and 0-48 systems, resulting in relatively large heterogeneity of sensitivity and specificity for the two latter systems. The 0-4 system

also displayed a shorter operation time and more manageable performance, which is of large importance in a clinical setting.

Using a simplified scoring system, Baldini et al. (57) found that a cut-off of 2 provided the best ratio of specificity (98%) and sensitivity (66%), in accordance with both current and early studies (58, 76). Baldini et al. also found a positive predictive value of 97%, and a negative predictive value of 77% for the diagnosis of pSS (57).

Studies have since indicated that hypoechogenic areas and homogeneity in the parotid gland seem to have the highest interobserver reliability (74, 77). Mossel et al (78) even found that scoring the parotid and submandibular glands on only one side, was sufficient to predict classification of patients in accord to the ACR/EULAR classification criteria. In the same paper, Mossel demonstrated that scoring only hypoechogenic areas showed close to similar results as scoring all components in the previous scoring systems (72); parenchymal echogenicity, homogeneity, hypoechogenic areas, hyperechogenic reflections and salivary gland posterior border. These findings further support a simplified scoring system for clinical use (78).

	Scoring System		
	0–4	**0–16**	**0–48**
Sensitivity			
Pooled Sensitivity (95% CI)	0.75 (0.71–0.79)	0.84 (0.81–0.87)	0.75 (0.70–0.80)
Chi-square (Degree of Freedom)	74.65 (6)	13.74 (5)	32.83 (3)
P Value	0.0000	0.0174	0.0000
Inconsistency (I^2)	92.0%	63.6%	90.9%
Specificity			
Pooled Specificity (95% CI)	0.93 (0.90–0.95)	0.88 (0.85–0.91)	0.95 (0.91–0.97)
Chi-square (Degree of Freedom)	21.04 (6)	14.47 (5)	18.69 (3)
P Value	0.0018	0.0129	0.0003
Inconsistency (I^2)	71.5%	65.4%	83.9%
Diagnostic Odds Ratio			
Pooled Diagnostic Odds Ratio (95% CI)	71.26 (42.29–120.09)	46.3 (19.95–107.44)	66.07 (33.73–129.42)
Cochran-Q (Degree of Freedom)	4.25 (6)	19.07 (5)	2.11 (3)
P Value	0.6430	0.0019	0.5507
Inconsistency (I^2)	0.0%	73.8%	0.0%
Tau-squared	0.0000	0.7812	0.0000

Table 4. The meta-analysis results of three scoring systems (AECG as the diagnostic criteria). Cl = confidence interval. Table from (73).

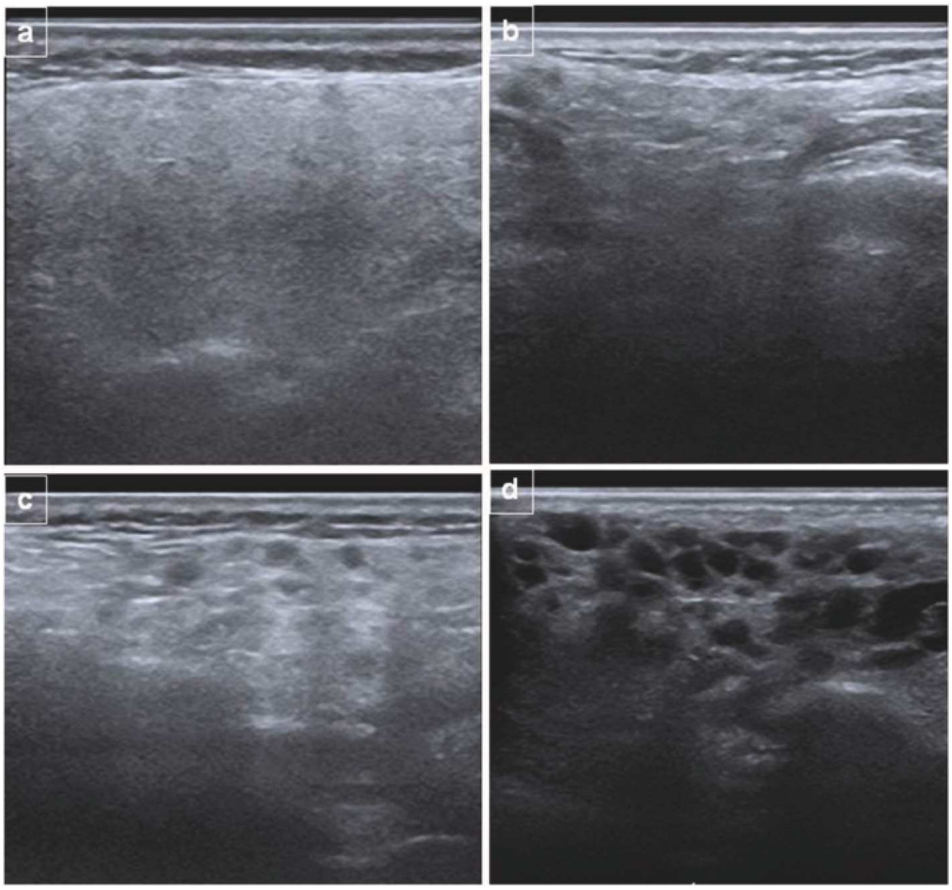

Figure 2. Parenchymal inhomogeneity in the parotid glands demonstrated by ultrasonography. Normal parotid gland, grade 0 (a). Parotid gland with mild non-specific inhomogeneity, grade 1 (b). Parotid gland with evident inhomogeneity, grade 2 (c). Parotid gland with gross inhomogeneity, grade 3 (d). Figure and text from (57).

8.7 Literature search

The literature search was completed March 21st, 2019.

9. AIMS

The overall aim of this thesis was to investigate SGUS in pSS and jSS. Moreover, we wanted to study the use of SGUS in diagnostic evaluations and the correlation of pathological SGUS changes to clinical and laboratory findings.

The specific aims were

I. To investigate SGUS findings in an adult pSS cohort and to determine correlation with symptoms, and clinical and laboratory findings.

II. To characterize symptoms and clinical findings of jSS, and to explore the clinical application of SGUS in patients with jSS.

III. To determine the gland, projection and format most applicable for reproducible image analysis, by comparing inter- and intra-rater correlations of SGUS bedside and as digitally stored still-images and videos.

10. MATERIALS AND METHODS

10.1 Patient populations

10.1.1 Paper I

Patients with pSS were recruited from the Department of Rheumatology, Haukeland University Hospital (n=97) and were either previously diagnosed with pSS (n=82) and part of a well characterized pSS cohort, or newly diagnosed (n=15) and included in the cohort. All patients met the criteria set by the American-European Consensus Group (AECG) in 2002 (79). Daniel S. Hammenfors (DH) examined all patients alone or together with Malin V. Jonsson (MVJ).

10.1.2 Paper II

The study design was a cross-sectional multicentre study. Patients were recruited from Haukeland University Hospital in Bergen, Norway, Oslo University Hospital, Norway, Hospital General Universitario Gregorio Marañón in Madrid, Spain, Hospital Universitário Cassiano Antônio Moraes of Federal University of Espírito Santo in Vitória, Brazil, Hospital Universitário Clementino Fraga Filho, Federal University of Rio de Janeiro in Brazil and Hospital das Clinicas, Faculdade de Medicina da Universidade de Sao Paulo (HCFMUSP) in Brazil, University Medical Center Groningen, the Netherlands, and Department of Pediatrics, University of Florida, Gainesville, Florida.

Patients had previously received the diagnosis jSS, by a specialist in rheumatology or paediatric medicine, at the age of 18 years or younger. At the time of inclusion, all patients were younger than 25 years. The local specialist in rheumatology or paediatric medicine performed identification of patients at each clinic.

Patients included in Norway (n=11), Spain (n=5) and Brazil (n=40) were examined and included by the primary investigators (DH and MVJ), in cooperation with the local specialist. Patients recruited from the Netherlands (n=8) and USA (n=3) were examined in collaboration with local experts and included retrospectively.

10.1.3 Paper III

We currently have an ongoing enrolment of patients in the SS cohort denoted S2C, including patients diagnosed with SS by a rheumatologist but not necessarily fulfilling the 2002 AECG (10) or the 2016 ACR/EULAR classification criteria (17). The patients are recruited from the outpatient clinic at the Department of Rheumatology, Haukeland University Hospital, Bergen. For this project, data and examinations were from the first included patients in S2C (n=32). All patients were examined and included by Haris Causevic (HC).

10.2 Salivay gland ultrasonography examination and evaluation

10.2.1 Paper I

We examined the parotid and submandibular glands by US using a GE LogiqE9 with a linear high-frequency transducer (6-15 MHz). The parotid glands were evaluated in a longitudinal and cross-sectional plane and the submandibular glands in a longitudinal plane (Figure 3). One out of two clinical investigators (DH and MVJ) performed the US examination. Representative SGUS images were stored digitally as .jpg images and evaluated later on, in a darkened room and on a standard high-quality computer screen. All images were evaluated separately by two investigators (DH and

MVJ) who were both blinded for clinical findings such as subjective sicca symptoms, sialometry, serum autoantibodies and minor salivary gland inflammatory focus score.

Glandular homogeneity and presence of hypoechogenic areas were evaluated and graded (0-3) using a simplified scoring system, as previously described by Hocevar et al 2005 (72) and applied in other studies (58). Grades 0-1 were considered to correspond to normal/non-specific changes and grades 2-3 to correspond to pathological changes. The number and size of hypoechogenic and/or anechoic areas as well as the degree of inhomogeneity presented in both submandibular and parotid glands were evaluated and graded by both investigators (DH and MVJ). A final overall score was then determined for all glands combined.

In cases where hypo-/anechoic areas were not detected, the score was set to 0. If SGUS showed a few minor focal hypo-/anechoic areas that were considered within normal, the score was set to 1. When at least one of the glands was more severely affected with multiple focal hypo-/anechoic areas but some homogenous and normal appearing salivary gland tissue remained, the score was set to 2 and changes considered pathological. In cases of severe generalized affection of at least two of the glands with minimal normal appearing glandular tissue remaining, as well as at least a grade 2 affection of the remaining gland(s), the score was set to 3.

10.2.2 Paper II

The SGUS examination of the right and left parotid and submandibular glands was performed using linear high-frequency transducers (6-15 MHz). The available local hardware was used. Glandular homogeneity and presence of hypoechogenic areas were evaluated and graded using a simplified scoring system (0-3) as described for *Paper I* (Figure 2). The SGUS examinations were conducted and scored bedside by

local experts in the USA and the Netherlands, and by DH/MVJ in Norway, Spain and Brazil.

10.2.3 Paper III

Upon inclusion, one clinical investigator (HC) performed SGUS on all patients using a GE Logic E9 (GE, USA) with a linear high-frequency transducer (6-15 MHz). Both right and left parotid and submandibular glands were examined. All four glands were examined in the longitudinal and transversal planes (Figure 3). The projections were evaluated bedside by the clinical investigator (HC), and each gland was given a composite score (0-3) based on overall impression. Representative videos and static images for both the longitudinal and transversal planes of all four glands were concurrently stored for documentation and later re-evaluation, in total 16 images (eight videos and eight static images) per patient. Using the local institution media storage database (Digital Media Archive), videos and images were extracted in dis-identified format and randomized using a random number generator for evaluation in equal consecutive order by three investigators (DH, HC, MVJ), securing a blinded evaluation of images and videos. The images and videos were scored using the simplified scoring system as described for *Papers I&II*.

In a minority of cases where two videos and/or images were available from the same gland and projection, and pathological findings for some reason differed, the highest/more severe score was noted. All videos and images were evaluated and scored by all three investigators at two separate times (round 1 and round 2, November 2017 and January 2018). The scores registered in round 1 were used for interclass correlation assessment (bedside score compared to image and to video scores), and interobserver reliability. The scores registered in round 2 were used for calculating intraobserver reliability.

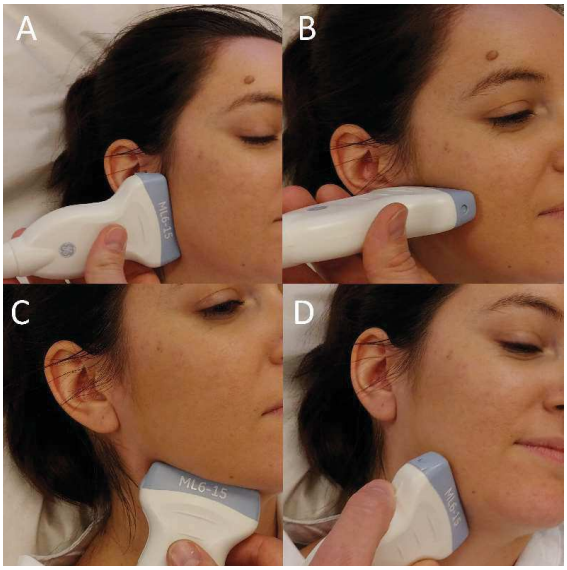

Figure 3. Probe placement when examining the parotid gland in the longitudinal (A) and transversal (B) planes, and when examining the submandibular gland in the longitudinal (C) and transversal (D) planes. *Images by Daniel Hammenfors*.

10.3 Sialometry

Salivary gland functional capacity was evaluated by unstimulated and stimulated sialometry of whole saliva, in ml/15 minutes and ml/5 minutes, respectively. The clinical investigations were performed in a consecutive setting, starting with the unstimulated whole saliva, (UWS) with the patients fasting at least 90 min before examination (Figure 4). Stimulated whole saliva (SWS) was obtained by letting patients chew on paraffin for five minutes (80). Both unstimulated and stimulated

saliva was collected in pre-weighed containers and the volume of secreted saliva determined by weighing, with 1 gram of saliva corresponding to 1 ml. Levels ≤ 1.5 ml/15 minutes of unstimulated saliva and ≤ 3.5 ml/5 minutes of stimulated saliva were considered pathologically reduced.

Figure 4. Unstimulated and stimulated sialometry. Necessary equipment constitute tract, measuring tube or pre-weighed containers, and a timer. Figure from (81). For representative and reproducible results, unstimulated saliva is measured for 15 minutes, and stimulated saliva for 5 minutes (81, 82).

10.4 Schirmer's I-test

Tear secretion was evaluated by the Schirmer's-I test. Wetting of the strip was recorded in mm, with levels of 5 mm or less wetting of the paper strip considered as pathologically reduced tear secretion (Figure 5).

Figure 5. Schirmer's I-test. The illustration shows an individual with normal tear secretion. *Image by Daniel Hammenfors*.

10.5 Minor labial salivary gland biopsy and evaluation

Minor labial salivary gland tissue biopsies were not performed as a part of the study, and were only obtained if deemed necessary for medical and diagnostic reasons as evaluated by the treating rheumatologist/physician. In most cases, the biopsy had been performed prior to inclusion.

The local oral pathologists routinely performed determination of focus score. Sections were not re-evaluated for the current research project. Minor salivary gland focus score denominates the number of focal mononuclear cell infiltrates with ≥ 50 cells per 4 mm^2 of otherwise normal appearing minor salivary gland tissue (83) (Figure 1). In *Paper I*, data regarding lymphoid neogenesis/germinal center (GC)-like structures was available for 72/82 patients and was added to data analyses as a patient characteristic.

10.6 Patient Reported Outcome Measures (PROMS) and Disease Severity Scores

EULAR Sjögren's Syndrome Patient Reported Index (ESSPRI) (84) was determined in all patients upon inclusion. Severity of sicca symptoms of the mouth and eyes were reported by patients on a visual analogue scale (VAS) ranging from 0-10 and the level of fatigue on a VAS ranging from 0-100, with a higher number representing more severe symptoms.

EULAR Sjögren's syndrome disease activity index (ESSDAI) (85) was registered upon inclusion for most patients. The haematological and biological domains were determined from the most recent blood samples available, if possible obtained the same day as inclusion.

10.7 Statistics

The statistical analyses for all papers were performed using IBM SPSS Statistics software (Armonk, NY, IBM Corp), version 19.0 (*Paper I-II*) and version 24 (*Paper III*).

The Student's t-test with Welch's correction was used to study differences between groups (*Paper I-III*). For categorical data, Chi-square analysis was employed (*Paper I-III*). Spearman (monotonic) and/or Pearson (linear) correlation analysis were used to evaluate the relationship between two variables (*Paper I-III*). The inter- and intra-observer reliability was assessed by intraclass correlation coefficient (ICC), two-way mixed, absolute agreement (*Paper III*). ICC less than 0.40 were considered poor, 0.40-0.59 as fair, 0.60-0.74 as good, and greater than or equal to 0.75 as excellent (86).

In general, *P* values less than 0.05 were considered statistically significant. In *Paper II*, Benjamin-Hochberg adjustment was applied to reduce type 1 errors due to multiple comparisons.

Primarily, DH performed statistical analyses in cooperation with MVJ, Johan G. Brun and HC. For *Paper II,* Jannicke Igland at Department of Global Public Health and Primary Care, University of Bergen provided statistical advice. In *Paper III*, statistician and co-author Jörg Assmus was the main statistical advisory.

10.8 Ethical considerations

The studies (*Paper I-III*) were approved by the Regional Medical and Health Research Ethics Committees (145/96-44.96, 242.06 and 2009/686) and reported to the Data Inspectorate (NSD number 9646) in Norway. For patients included abroad (*Paper II*), the inclusion was according to the Regional Medical and Health Research Ethical regulations and necessary applications were approved by the regional committees in the participating centers/countries. All patients and/or legal guardians had oral and written information of the study and provided a signed consent form.

11. SUMMARY OF RESULTS

11.1 Diagnostic utility of SGUS in pSS (Paper I)

In *Paper I* the female:male ratio was 91:6 (15.2:1), the mean age was 61.3 years. Pathological SGUS findings (SGUS+) were determined in 51/97 patients. Oral dryness symptoms (VAS) correlated with SGUS score and unstimulated and stimulated saliva levels.

Positive Schirmer's I-test in one or both eyes was observed in 42/50 (84%) patients with pathological US findings, compared to 24/45 (53%) patients with normal/non-specific US findings, and SGUS scores correlated with tear secretion in both eyes.

Focus score was available in 81/97 (84%) patients, and correlated with SGUS score.

Serum autoantibody status was associated with SGUS+ in 44/50 (88%) patients. Specifically, serum titers of anti-Ro/SSA were elevated in 41/50 (82%) SGUS+ patients compared to 23/46 (50%) SGUS- patients. Anti-La/SSB titers were elevated in 29/50 (58%) SGUS+ patients compared to 8/46 (17%) SGUS- patients.

11.2 Clinical characteristics and SGUS in jSS (Paper II)

In *Paper II* the female:male ratio was 58:9 (6.4:1). Mean age at first symptom was 10.2 years, and 12.1 years at diagnosis.Ocular and oral symptoms were noted in 42/67 (63%) and 53/66 (80%) patients, respectively, with salivary gland enlargements/parotitis in 37/58 (64%) patients. AECG or ACR/EULAR classification criteria for pSS was fulfilled by 42/67 (63%) patients. Pathological SGUS findings (SGUS+) were observed in 41/67 (61%) patients; 26/41 (63%) SGUS+ fulfilled pSS criteria. Mean level of saliva was 5.6 ml/15 minutes in SGUS- patients compared to

3.3 ml/15 minutes in the SGUS+ patients. Among the SGUS+ patients, 36/41 (88%) patients were anti-Ro/SSA+ or anti-La/SSB+ compared to 14/26 (54%) SGUS- patients. Among the SGUS+ patients 24/39 (62%) patients were RF+, whereas only 5/25 (20%) SGUS- patients were RF+. Mean ESSDAI was non-significantly higher in the SGUS+ compared to the SGUS- patients.

The majority (54/59, 92%) of the patients with jSS were currently (n=40) or had previously (n=14) been treated with HCQ. Most SGUS- patients (22/25, 88%) had current treatment with HCQ whilst approximately half of the SGUS+ (18/34, 53%) had no current HCQ treatment. Various extraglandular manifestations (EGM) were registered for 56/62 (90%) patients and associated with systemic treatment.

11.3 Real-time and post-examination SGUS (Paper III)

In *Paper III* the female:male ratio was 28:4 (7:1), the mean age was 56 years. The cohort consisted of 32 consecutive patients with a clinical pSS diagnosis. ACR/EULAR classification criteria for pSS was fulfilled by 24/32 (75%) patients.

Bedside SGUS examination revealed pathological findings (score 2-3) in at least two glands in 14/32 (44 %) patients. The bedside evaluation was defined as the gold standard for this project, and used for comparison to the evaluation of the digitally stored images and videos.

Comparing interobserver agreement, i.e. evaluation of videos and images to bedside evaluation, the ICC between investigators was overall excellent with scores ranging from 0.81 to 0.93 in round 1, and from 0.84 to 0.94 in round 2.

The intraobserver reliability scores had a wider range, from 0.46 to 0.96, depending on the gland (parotid/submandibular), projection (longitudinal/transversal) and image

format (video/image). In general, the scores were good, with a slightly higher intraobserver reliability ICC mean (for all investigators) for images (0.81) compared to video (0.74), and longitudinal (0.80) compared to transversal (0.76) projection. The ICC for the parotid and submandibular glands were similar (0.78 and 0.78).

When comparing round 1 to bedside evaluation, we found that the ICC ranged from 0.131 to 0.882. The average ICC scores for the three investigators were 0.577, 0.641 and 0.674. The best agreement between bedside assessment and image/video re-evaluation was observed for longitudinal video of the right parotid gland, with average score 0.772. The poorest agreement between bedside scoring and image/video re-evaluation was found for transversal video of the left submandibular gland with average score 0.209. The ICC average for longitudinal video was 0.683, transversal video 0.510, longitudinal image 0.667 and transversal image 0.662 (Figure 6).

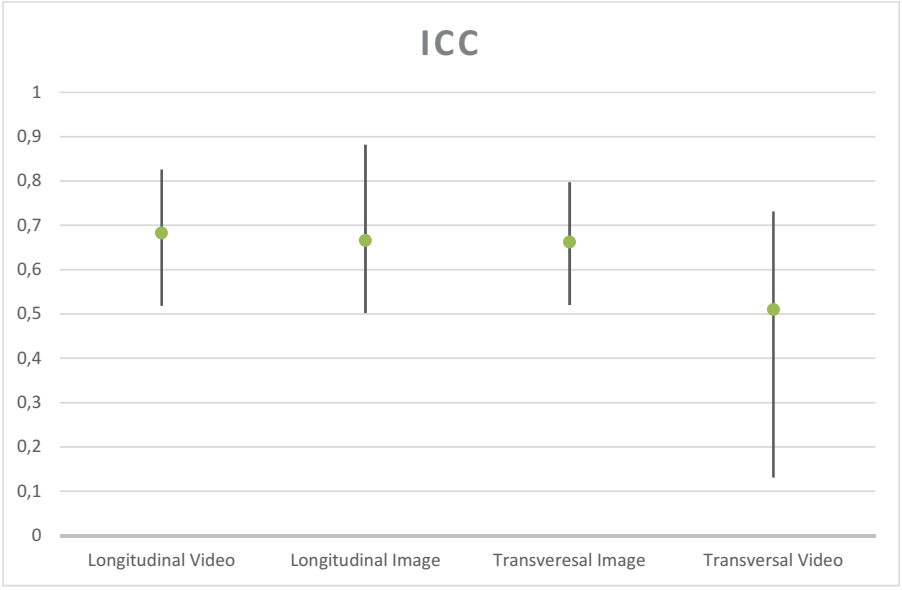

Figure 6. Maximum, minimum and average ICC scores for image/video in both longitudinal and transversal planes.

12. DISCUSSION

12.1 Methodological considerations

Since first described in 1988 (87) the application of SGUS for pSS has increased in both research studies and clinical work (53-55, 61, 63, 74). The imaging modality is non-invasive, can be considered low-cost compared to other currently available imaging techniques, but is highly user-dependent, which contributes to the diagnostic evaluation (53, 55). Regarding pSS, one major challenge is the multitude of scoring systems suggested in the literature (73, 75), all with separate advantages. Important aspects from our point of view were short operation time and easy application in the clinic. For the papers in this thesis, one of the less complex scoring systems mainly focusing on homogeneity and echogenicity was applied. Other studies have since confirmed the reliability of this approach (74, 77, 78).

Our evaluation methodology evolved during the course of the project. In *Paper I*, we only used post-examination evaluation of still-images, whilst in *Paper II* and *Paper III* we performed a bedside evaluation. At the start of patient SGUS examinations for *Paper I*, both main investigators (DH and MVJ) were still learning the technique. Although in reality we did evaluate the SGUS examination bedside, we considered the scoring process for *Paper I* to be more reliable as post-examination evaluation. Also, *Paper I* was the first step to congregate images for documentation, later evaluation and comparison. In *Paper III* we combined both bedside evaluation and post-examination evaluation of images, with the addition of videos.

In retrospect, a more ideal design in *Paper I* could have contained a record of the scoring done bedside, in addition to the scoring of digitally stored images. When performing the SGUS for *Paper II,* technical challenges limited the possibility for storing digital images and/or videos of all the examinations. We were able to store

52/67 (78%) for later re-evaluation. In a pilot study presented as a poster at the ACR Annual meeting in 2017 (88), we found that we classified images as containing pathologic changes (grade 2-3) more often than we did bedside (Figure 7).

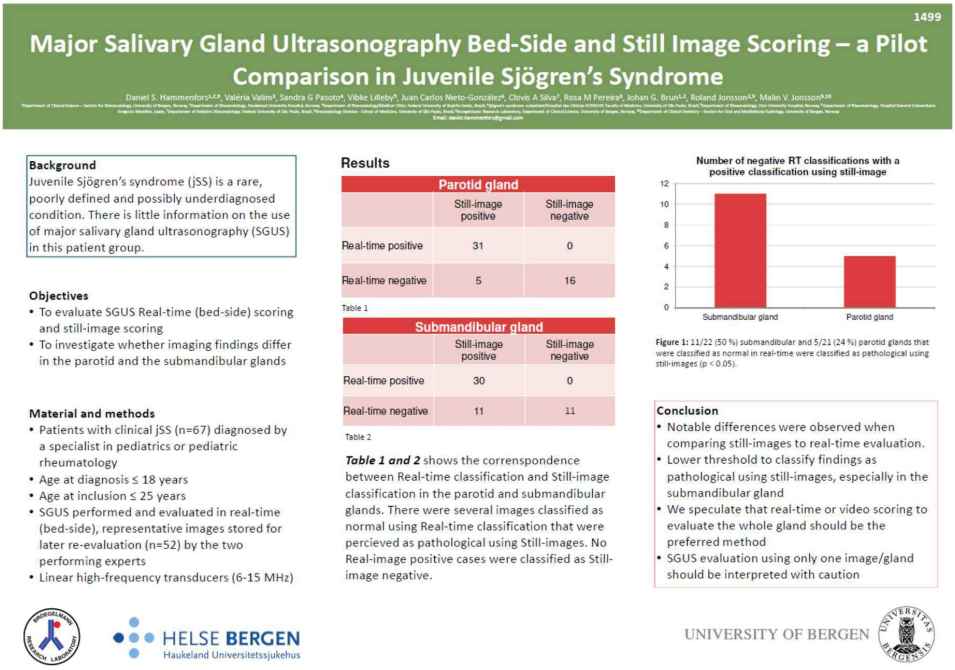

Figure 7. A pilot study presented as a poster at ACR 2017 demonstrating a higher degree of pathological classification of SGUS findings in still-images compared to bedside (88).

Different US machines were also used at the various centers (*Paper II*). Also, at two centers (The Netherlands and the USA), local specialists and not the main investigators performed the SGUS examination and evaluation. Although we use

similar, high-grade US machines, and in discussion agree on SGUS scores, these two factors might have influenced the results. In general, the hardware used was of good quality and produced high-quality images. Variation in image contrast and resolution were noted, which might influence the ability to spot early, minuscule changes. With regard to different examiners, interrater correlations have been reported good to excellent between experts when using a simplified scoring system focusing on homogeneity and hypoechogenic areas (89).

In *Paper III*, we performed both a bedside evaluation and evaluation and scoring of images/videos. The main limitation to this methodology was that only one of the three investigators performed the bedside examination, evaluation and image and video acquisition. Ideally, one could argue that all three should have conducted the bedside evaluation twice per patient to define the gold standard, as well as scoring the images and videos. The evaluation of images and videos was performed in a random order, blinded for patient identity and characteristics. We evaluated the images and videos on two separate occasions, two months apart (November 2017 and January 2018). The first evaluation was used to compare our evaluation of images and videos to the gold standard and each other (interobserver). The second evaluation was used to compare re-evaluation (intraobserver).

12.2 Study population

In *Paper I,* the cohort studied consisted of well-characterized pSS patients that all fulfilled the AECG classification criteria (10). The patients were recruited from the Department of Rheumatology at Haukeland University Hospital, Bergen, Norway. Being recruited from a university hospital clinic, it is possible that the patients with low disease activity and not needing follow-up by a specialist might be underrepresented, and that the cohort thus represents patients with a more severe

disease profile. The cohort characteristics were however similar to other studies (90, 91).

In *Paper II,* the cohort consisted of younger patients recruited from three continents, five countries and eight hospitals. The included patients had previously received the jSS diagnosis by a pediatrician or specialist in rheumatology. The patients were younger than 18 years at the time of jSS diagnosis and not older than 25 years when included in the study. The age-limits were set to include both children and adolescent with pSS and to exclude patients with long standing disease. Exclusion criteria were currently known viral diseases (CMV, EBV, Hepatitis C, HIV), current eating disorders (bulimia and anorexia nervosa) or substance abuse (alcohol). Patients considered to have a disease profile more concurring with other connective tissue disorders such as SLE, without an associated jSS, were excluded from the cohort.

When planning *Paper II*, lack of established diagnostic and classification criteria for jSS presented a major challenge. The disease manifestations of jSS are in some cases difficult to distinguish from other connective tissue diseases or IgG4 related conditions (92). Inclusion of patients from several continents also introduced possible genetic influences and environmental variations. Juvenile SS is however, a rare condition and too a rigorous patient selection, without established standardization or criteria to rely on, would probably have yielded too few patients compared to what was needed for a representative and sizeable cohort. As stated in several papers (34, 50, 52), verified classification and diagnostic criteria are vital to improve patient selection in future studies.

In *Paper II,* studying children and adolescents, we took on a non-invasive approach. Suggested jSS criteria (Table 3)(34) include five items (Items 7-11) based on laboratory tests. We did not have ethical approval to perform invasive procedures such as blood tests/serology or biopsies and therefore had to rely on results from the

patients' medical history. Consequently, we did not base our inclusion of jSS patients was on these criteria.

For the benefit of this thesis, however, the implementation of previously suggested criteria for jSS was assessed retrospectively for the cohort in *Paper II* (Table 5). In the immediate data available, seven items at the most could be evaluated (Table 3 and 5, items 1, 2, 4, 5, 6, 11, 12).

For *item 2,* we applied AECG item I (Ocular dryness symptoms), which is based on daily, persistent, troublesome dry eyes for more than 3 months and/or recurrent sensation of sand or gravel in the eyes and/or tear substitutes more than 3 times a day. This criteria was fulfilled by 42/67 patients.

In addition, for *Item 2* we supplemented with xerostomia, AECG item II (Oral dryness symptoms), which is based on daily feeling of dry mouth for more than 3 months, recurrent or persistent swollen salivary glands or the need to frequently drink liquids to aid in swallowing dry food. This item was noted in 48/66 patients. Seventeen of these patients did not fulfill *item 2* based on AECG item I alone. Altogether, 59 patients fulfilled the supplemented *item 2*.

Item 5, which is ocular dryness, was based on AECG item III (Ocular signs) and ACR/EULAR item 3; Schirmer's I-test and Ocular staining score (OSS), or Schirmer's I-test alone; only three patients had not performed any of these tests.

For *item 6*, sialography, we also added ACR/EULAR item 5 and AECG item V (hyposalivation by unstimulated sialometry; UWS). Only three patients had performed sialography, whereof one was positive. UWS was positive in 20/60 patients.

For *item 11,* AECG item VI (Antibodies to Ro/SSA and/or La/SSB) and ACR/EULAR item 2 (antibodies to Ro/SSA) was applied; all patients had measured

anti-Ro/SSA, anti-La/SSB, RF and ANA, except two patients who lacked RF titers. We had however no information on exact ANA-titers, only positive/negative. For 13/63 patients, a positive ANA was the only test to fulfill *item 11*.

Out of the seven possible items we were able to obtain data for, any one patient had data available for three to seven items. When applied, 30/67 patients had at least four positive items (Table 5), which is considered suggestive of jSS (34). This frequency is lower than 39/67 attained with the ACR/EULAR (17) and 35/67 with the AECG classification criteria (10). For the jSS criteria (34), the presence of other autoimmune diseases is an exclusion criterion. In our cohort, eleven patients had a prior diagnosis of MCTD, SLE or thyroid disease, with a concomitant SS-like disease profile.

The addition of xerostomia to *item 2* increased the number of patients fulfilling the jSS criteria with additionally five. If applying SGUS+ as an item to the jSS criteria, eleven additional patients fulfilled at least four items. Adding SGUS alone thus provided a diagnosis of jSS in 41/67 patients, which is slightly higher compared to the number of patients fulfilling the AECG and/or ACR/EULAR criteria. Xerostomia and SGUS together makes the number of patients fulfilling the criteria 45/67.

Most of the patients had not performed a sialography (*item 6*), and substituting sialography with ACR/EULAR item 5 (hyposalivation by unstimulated sialometry; UWS), did indeed add four more patients that fulfilled the jSS criteria (34). Of note, reference values for UWS (Table 5) are based on levels for adults (81, 82). For children and adolescents, published UWS levels seem to vary depending on age, gender, and body composition, as well as health and climate (93, 94).

Taking all alterations to the criteria (xerostomia, hyposalivation and SGUS+) into consideration, in total 50/67 patients would fulfill the jSS criteria, which is more than both the AECG and the ACR/EULAR classification criteria. Among the 50 patients, thirteen did not fulfill the AECG or the ACR/EULAR classification criteria.

Five patients however, fulfilled either the AECG or the ACR/EULAR classification criteria, but not the jSS criteria. Twelve patients fulfilled neither criteria. Of these twelve patients, seven were SGUS+. They had however not performed sialography or sialoscintigraphy. Only one had a low UWS, three had sicca symptoms and none had a positive Schirmer's I-test. Two had performed OSS, which were negative. All were anti-Ro/SSA+.

Including sialoscintigraphy to *item 6*, as well as UWS and sialography, would add eight more patients fulfilling four items of the jSS criteria (34). Involving ionizing radiation, sialoscintigraphy is not a preferred imaging technique to perform in children without carefully considering its justification. Including sialoscintigraphy in jSS diagnostic/classification criteria would thus be inappropriate.

The applied modification to the jSS criteria, with several added items could indeed render the cut-off of four items invalid. Nonetheless, it does imply that several symptoms, clinical findings and examinations should be considered when forming future jSS criteria.

For *Paper III,* we included consecutive pSS patients with a clinical diagnosis of SS, regardless of whether they did or did not fulfill the AECG (10) or ACR/EULAR (17) classification criteria for pSS. The patient characteristics per se most likely did not influence the evaluation of images and videos, but the number of patients with pathological SGUS changes were lower in *Paper III* (sensitivity 43.8%) than in *Paper I-II* (sensitivity 52.6-61.2%). It is possible that this lower frequency of pathological findings (grade 2-3), and thus a higher frequency of normal findings or slight/early changes (grade 0-1) may have influenced the evaluation of images and videos.

Table 5. Application of modified jSS criteria in the cohort from *Paper II*.

	Criteria item	**N**
1	Recurrent parotitis or parotid enlargement	37/58
2	Recurrent conjunctivitis (non-allergic and non-infectious), or keratoconjunctivitis sicca	42/67
2[+]	Xerostomia	48/66
3	Recurrent vaginitis	Md
4	Systemic: fever of unknown origin, arthralgias, hypokalemic paralysis, or abdominal pain	24/59
5	Ocular dryness (ocular staining or Schirmer I-test)	34/64
6	Abnormal sialography	1/3
6[#]	Salivary gland involvement/Hyposalivation/UWS*	20/60
7	Elevated serum amylase	Md
8	Leukopenia or elevated ESR	Md
9	Hyperimmunoglobulinaemia (polyclonal)	Md
10	Renal tubular acidosis	Md
11	At least one of: anti-Ro/SSA, anti-La/SSB, high titer ANA (speckled pattern), RF	63/67
12	Lymphocytic infiltration of minor labial salivary glands or other organs	28/34
	SGUS	41/67
	AECG criteria	35
	ACR/EULAR criteria	39
	jSS criteria	30
	jSS criteria xerostomia, UWS and SGUS	50

[+]Xerostomia added to item 2, [#]UWS added to item 6, *UWS based on reference values for adults

12.3 Statistics

In *Paper I,* statistical methods were applied as described in section 10.7. In *Paper II* we applied Benjamin-Hochberg adjustment to reduce the false discovery rate (FDR) caused by multiple comparisons. Multiple comparisons increase the risk of significant findings being by chance and thus representing false positives. In *Paper III,* we used inter- and intracorrelation coefficient (ICC), obtaining intervals of numbers describing how strongly units within the same group, or between groups, resemble each other.

12.4 Data collection

Data collection for *Paper I* at the time of inclusion encompassed results from clinical examination including SGUS of both parotid and submandibular glands, unstimulated and stimulated sialometry, Schirmer's I-test, blood samples/serology, and the patients up-dated medical history. For the patients with a previous diagnosis of pSS (n=82) retrospective data such as autoantibody profile (Ro/SSA, La/SSAB, RF) and minor salivary gland focus score were obtained from the patients charts. For the newly diagnosed patients (n=15), autoantibody profiles were determined at inclusion, and minor salivary gland biopsies performed in cases where the diagnosis otherwise could not be determined.

Paper II was an explorative cross-sectional study including children and adolescents. Therefore, we wanted to minimize the number of invasive procedures such as blood tests, minor salivary gland biopsies and other imaging modalities, unless they were part of the clinical evaluation or follow-up of the individual patient. Historical laboratory tests, minor salivary gland biopsy results, and imaging findings from the diagnostic work-up (sialograpy and sialoscintigraphy) were retrospectively collected

from the patients' medical journals. Patients had been evaluated at different hospitals in different cities, countries and continents, with differences in health care systems, diagnostic policies and treatment strategies. During plotting and analysis of data, some regional differences surfaced, which could have, had the data been interpreted separately, provided different results than the ones obtained for the cohort as a whole. SGUS findings were considerably more frequent in jSS patients recruited from Europe and the USA compared to Brazil. This important observation confirms how essential international collaborations are, in order to secure inclusion of a sizeable and representative cohort of patients and reduce confounding factor caused by regional differences when studying rare conditions such as jSS.

Clinical examination including SGUS examination and evaluation, unstimulated sialometry and Schirmer's I-test were performed upon inclusion. Other experts than the main investigators (DH, MVJ) examined the patients from the Netherlands and the USA.

Paper III was a cross-sectional study and all clinical data and material, except for minor salivary gland biopsy results, were collected upon inclusion. When planning *Paper III* in 2017, the most recent ACR/EULAR classification criteria (17) had recently been published and we chose to apply these criteria in favor of the previously applied AECG classification criteria of 2002 (79). The two sets of classification criteria have been validated and seem comparable in pSS studies (17, 40).

12.5 Clinical considerations

The use of SGUS in pSS was first described in 1988 (87). Considering the number of papers available on the subject, it is safe to say that the interest has gradually increased. In *Paper I,* we showed a significant connection between SGUS and clinical findings such as autoantibodies, unstimulated and stimulated salivary secretion, tear secretion, and sicca symptoms. We also applied a simplified scoring system, focusing on homogeneity and hypoechogenic areas. Since we published *Paper I*, other studies have confirmed that homogeneity and hypoechogenic areas are the most important items to evaluate in SGUS (78) and the connection to clinical findings (75, 77, 95). We demonstrated that patients that were both anti-Ro/SSA and anti-La/SSB positive were more likely to be SGUS+ compared to patients that were only anti-Ro/SSA positive. We also demonstrated that patients that were both anti-Ro/SSA and anti-La/SSB negative were highly unlikely to be SGUS+.

The inverse correlation between SGUS score and age (*Paper I*) lead to our interest in the rare, juvenile form of SS, jSS, affecting children and adolescents. Our working hypothesis was that SGUS changes are more prominent in jSS (*Paper II*). SGUS had previously been investigated in jSS (35, 96, 97), but only in smaller cohorts.

In *Paper II* we have to date the largest cohorts investigating SGUS in jSS. The study was an explorative, cross-sectional study and a first step to investigate the correlation between SGUS, patients' symptoms, clinical findings and existing classification criteria for pSS (10, 17). In this thesis, the suggested criteria for jSS were also applied (34) (Table 3 and 6). In line with findings in pSS, we detected a correlation between autoantibodies and SGUS (95). Earlier observations of parotid swelling/parotitis in jSS (33-35) and systemic manifestations (30, 38) were also confirmed in *Paper II*.

The prevalence of pathological SGUS changes was 61% in jSS (*Paper II*) compared to 53% in pSS (*Paper I*), and in line with published findings in pSS patients (98). Our

initial hypothesis that jSS patients had more SGUS changes than pSS patients could therefore not be confirmed.

In *Paper II,* we found a high degree of EGM, which is slightly higher compared to other studies (30, 38).Systemic treatment was frequent, most commonly HCQ. Interestingly, current HCQ treatment was more common in the SGUS- patients. *Paper II* is a cross-sectional study and not a prospective intervention study, and possibly confounding factors could explain this correlation. Nonetheless, it does invite to speculation whether HCQ could have a protective effect, preventing development of the salivary gland disease if applied early in the disease development.

During SGUS examinations for *Paper II,* we could observe that some of the jSS patients displayed distinct, evenly distributed hypoechogenic areas throughout the whole salivary gland. The changes were clearly visible when scanning the whole gland, and prominent enough to qualify as grade 2. The changes were however, difficult to demonstrate when freezing the image, and thus to document. These observations raised the question of the more suitable storage media for post-examination evaluation, tested out in the pilot project shown in Figure 6 and to *Paper III*.

Previous studies have investigated interrater reliability for SGUS (74, 77). *Paper III* is to our knowledge the first study to compare bedside evaluation to both video and image post-examination evaluations. In *Paper III,* we could see a tendency that longitudinal video of the parotid gland provided results most similar results to bedside evaluation. Nonetheless, we still speculate that a video-recording of a thorough scan throughout the salivary gland provides more information, reduces the possibility for user-dependent errors, and is more likely to correlate with bedside scoring, compared to one image/slide. Being a pilot study, further investigation is warranted.

13. CONCLUSIONS

In line with the aims of this thesis the following conclusions can be drawn:

- Major salivary gland ultrasound findings in pSS patients correlate with several clinical and laboratory findings for both pSS and jSS.
- The prevalence of SGUS+ seems similar in pSS and jSS.
- Extraglandular manifestations and parotitis are common in jSS
- Using a simplified scoring system, both inter- and intrarater correlations of SGUS are excellent for experienced examiners.
- Longitudinal video of the parotid gland US tends to be the most reliable post-examination evaluation, compared to bedside examinations.

14. FUTURE PERSPECTIVES

Salivary gland US represents a potential useful tool in both diagnostic and prognostic evaluation, and monitoring of treatment efficiency (28, 29, 53, 56-58, 72, 75, 99). Pathological SGUS changes such as inhomogeneity and hypoechogenic areas are highly specific for pSS (57, 72). In my experience, learning to perform the SGUS examination on a patient and to identify clearly pathological changes applicable for clinical use only requires brief training, making SGUS a useful diagnostic tool for the salivary gland component in pSS and jSS. However, evaluation of minute details requires more extensive practice and experience. Furthermore, technical complications with anatomical variants, subcutaneous fat, and atrophic or fibrotic salivary glands may complicate image acquisition, as well as interpretation.

Salivary gland US findings are associated with known clinical and laboratory findings typical for pSS such as systemic complications and ESSDAI, as well as skin vasculitis and salivary gland swelling, elevated autoantibody titers and GC-like structures in minor salivary gland biopsies (58, 75, 77, 95). All these factors are indicative of a more severe disease profile and increased risk for complications, most severely the development of lymphoma (6, 8, 100) And in that regard, SGUS represents a useful tool also for prognostic considerations (56).

Some recent studies have demonstrated a slight improvement of SGUS findings following treatment with rituximab (28, 29), but the observations were not convincing enough to determine whether SGUS is a reliable tool to measure local treatment effect. The lack of established and reliable outcome measures is indeed a challenge for the SS research community. However, efforts to establish suitable tools are addressed in in the recently initiated EU H2020 Innovative Medicines Initiative (IMI2-2017-12-03 NECESSITY, Contract #806975) a collaboration between EU, the pharmaceutical industry and leading European research groups.

Before we can use SGUS as a tool for monitoring disease progression or remission, we need to determine the natural development of SGUS changes in SS. Autoantibodies, important biomarkers in pSS occur several years before disease onset (101) and suggest that the inflammatory changes occurring in the major and minor salivary glands also have a pre-clinical phase (Figure 8). In order to predict a possible therapeutic window and reverse disease pathogenesis and salivary gland destruction, it is crucial that we establish when such changes start to occur and the period before inflammatory changes develop into irreversible tissue damage.

Previous studies have attempted to define this moment in disease pathogenesis. To our knowledge, only one study has included SGUS evaluation. Briefly, two SGUS analyses were performed with a mean time of 1.9 years apart (102), but no significant changes could be determined in the assessed parameters. It is possible that the patients included already had developed subclinical disease changes prior to diagnosis. More likely, the first SGUS examination and the follow-up were not long enough apart in time. With regard to results presented in this thesis (*Papers I-III*), it is also possible that jSS patients (*Paper II*) are more likely to have early stage inflammatory changes rather than actual tissue damage. The disease in adult patients often has a longer subclinical phase, with a delay from disease onset to diagnosis, possibly causing more extensive tissue damage. Early or minute changes are difficult to document in the SGUS images. The inverse correlation between SGUS changes and age we found in *Paper I* could suggest that patients diagnosed at an earlier age have a more severe disease profile, and therefore demonstrate a higher degree of pathological SGUS changes. The results from *Paper III* confirm the subjective contribution possibly depending on the clinical investigators training and experience.

In the future, automatic image analysis could be an instrumental tool to detect minute SGUS changes and monitor disease progression – or remission. Long-term studies of patients with early SS-related SGUS changes can provide important information

addressing the natural history of the salivary gland component in SS. A prospective study would ideally examine newly diagnosed SS patients, where the period from first symptom to inclusion is short. Hypothetically, the SGUS changes would then mainly represent reversible inflammatory lesions rather than actual tissue damage. Standardized SGUS examinations performed over time, evaluated using automated image analysis would also provide data crucial for objective studies of treatment outcomes. Targeted treatment of susceptible patients early in their disease could result in remission of the salivary gland disease in SS, which would be possible to objectively evaluate and document by SGUS.

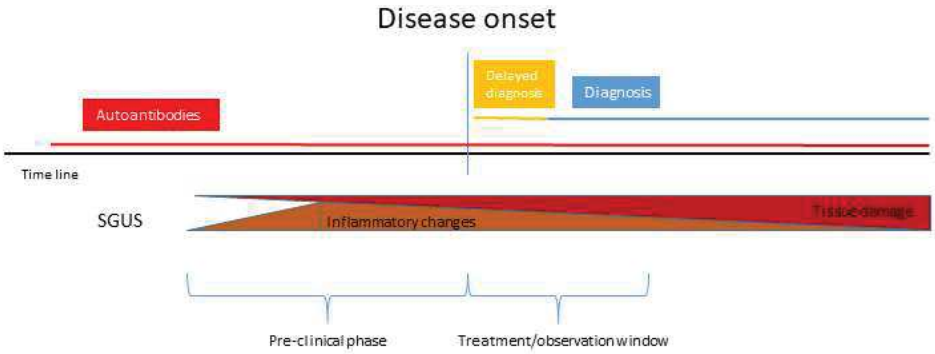

Figure 8. Suggested timeline for development of SGUS changes in pSS/jSS. *Figure by Daniel Hammenfors*.

15. REFERENCES

1. Jonsson R, Bowman SJ, Gordon TP. Sjögren's syndrome. In: Koopman WJ, editor. Arthritis and Allied Conditions. 15th ed. Philadelphia: Lippincott Williams & Wilkins; 2005. p. 1681-705.
2. Mengshoel AM, Norheim KB, Omdal R. Primary Sjögren's syndrome: fatigue is an ever-present, fluctuating, and uncontrollable lack of energy. Arthritis Care Res (Hoboken). 2014;66(8):1227-32.
3. Flores-Chavez A, Kostov B, Solans R, Fraile G, Maure B, Feijoo-Masso C, et al. Severe, life-threatening phenotype of primary Sjögren's syndrome: clinical characterisation and outcomes in 1580 patients (GEAS-SS Registry). Clinical and experimental rheumatology. 2018;36 Suppl 112(3):121-9.
4. Baldini C, Pepe P, Quartuccio L, Priori R, Bartoloni E, Alunno A, et al. Primary Sjögren's syndrome as a multi-organ disease: impact of the serological profile on the clinical presentation of the disease in a large cohort of Italian patients. Rheumatology (Oxford). 2014;53(5):839-44.
5. Ramos-Casals M, Brito-Zeron P, Solans R, Camps MT, Casanovas A, Sopena B, et al. Systemic involvement in primary Sjögren's syndrome evaluated by the EULAR-SS disease activity index: analysis of 921 Spanish patients (GEAS-SS Registry). Rheumatology (Oxford). 2014;53(2):321-31.
6. Johnsen SJ, Brun JG, Göransson LG, Smastuen MC, Johannesen TB, Haldorsen K, et al. Risk of non-Hodgkin's lymphoma in primary Sjögren's syndrome: a population-based study. Arthritis Care Res (Hoboken). 2013;65(5):816-21.
7. Sutcliffe N, Inanc M, Speight P, Isenberg D. Predictors of lymphoma development in primary Sjögren's syndrome. Seminars in arthritis and rheumatism. 1998;28(2):80-7.
8. Theander E, Henriksson G, Ljungberg O, Mandl T, Manthorpe R, Jacobsson LT. Lymphoma and other malignancies in primary Sjögren's syndrome: a cohort study on cancer incidence and lymphoma predictors. Ann Rheum Dis. 2006;65(6):796-803.
9. Delli K, Villa A, Farah CS, Celentano A, Ojeda D, Peterson D, et al. World Workshop on Oral Medicine VII: Biomarkers predicting lymphoma in the salivary glands of patients with Sjögren's syndrome. A systematic review. Oral diseases. 2019.
10. Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, et al. Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Ann Rheum Dis. 2002;61(6):554-8.
11. Salomonsson S, Jonsson MV, Skarstein K, Brokstad KA, Hjelmström P, Wahren-Herlenius M, et al. Cellular basis of ectopic germinal center formation and autoantibody production in the target organ of patients with Sjögren's syndrome. Arthritis Rheum. 2003;48(11):3187-201.
12. Theander E, Vasaitis L, Baecklund E, Nordmark G, Warfvinge G, Liedholm R, et al. Lymphoid organisation in labial salivary gland biopsies is a possible predictor for the development of malignant lymphoma in primary Sjögren's syndrome. Ann Rheum Dis.70(8):1363-8.
13. Sene D, Ismael S, Forien M, Charlotte F, Kaci R, Cacoub P, et al. Ectopic Germinal Center-Like Structures in Minor Salivary Gland Biopsy Tissue Predict Lymphoma

Occurrence in Patients With Primary Sjögren's Syndrome. Arthritis Rheumatol. 2018;70(9):1481-8.
14. Haacke EA, van der Vegt B, Vissink A, Spijkervet FKL, Bootsma H, Kroese FGM. Germinal centres in diagnostic labial gland biopsies of patients with primary Sjögren's syndrome are not predictive for parotid MALT lymphoma development. Ann Rheum Dis. 2017;76(10):1781-4.
15. Delaleu N, Mydel P, Brun JG, Jonsson MV, Alimonti A, Jonsson R. Sjögren's syndrome patients with ectopic germinal centers present with a distinct salivary proteome. Rheumatology (Oxford). 2016;55(6):1127-37.
16. Delaleu N, Mydel P, Kwee I, Brun JG, Jonsson MV, Jonsson R. High fidelity between saliva proteomics and the biologic state of salivary glands defines biomarker signatures for primary Sjögren's syndrome. Arthritis Rheumatol. 2015;67(4):1084-95.
17. Shiboski CH, Shiboski SC, Seror R, Criswell LA, Labetoulle M, Lietman TM, et al. 2016 American College of Rheumatology/European League Against Rheumatism classification criteria for primary Sjögren's syndrome: A consensus and data-driven methodology involving three international patient cohorts. Ann Rheum Dis. 2017;76(1):9-16.
18. Askanase AD, Friedman DM, Copel J, Dische MR, Dubin A, Starc TJ, et al. Spectrum and progression of conduction abnormalities in infants born to mothers with anti-SSA/Ro-SSB/La antibodies. Lupus. 2002;11(3):145-51.
19. Eliasson H, Sonesson SE, Sharland G, Granath F, Simpson JM, Carvalho JS, et al. Isolated atrioventricular block in the fetus: a retrospective, multinational, multicenter study of 175 patients. Circulation. 2011;124(18):1919-26.
20. Miyasato-Isoda M, Waguri M, Yamada Y, Miyano A, Wada Y. Anti-Ro52 antibody level is an important marker of fetal congenital heart block risk in anti-Ro/SSA antibody positive pregnancy. Modern rheumatology. 2018;28(4):690-6.
21. Xue D, Shi H, Smith JD, Chen X, Noe DA, Cedervall T, et al. A lupus-like syndrome develops in mice lacking the Ro 60-kDa protein, a major lupus autoantigen. Proceedings of the National Academy of Sciences of the United States of America. 2003;100(13):7503-8.
22. Gøransson LG, Haldorsen K, Brun JG, Harboe E, Jonsson MV, Skarstein K, et al. The point prevalence of clinically relevant primary Sjögren's syndrome in two Norwegian counties. Scand J Rheumatol. 2011;40(3):221-4.
23. Qin B, Wang J, Yang Z, Yang M, Ma N, Huang F, et al. Epidemiology of primary Sjögren's syndrome: a systematic review and meta-analysis. Ann Rheum Dis. 2015;74(11):1983-9.
24. Saraux A, Pers JO, Devauchelle-Pensec V. Treatment of primary Sjögren syndrome. Nature reviews Rheumatology. 2016;12(8):456-71.
25. Gottenberg JE, Ravaud P, Puechal X, Le Guern V, Sibilia J, Goeb V, et al. Effects of hydroxychloroquine on symptomatic improvement in primary Sjögren syndrome: the JOQUER randomized clinical trial. Jama. 2014;312(3):249-58.
26. Devauchelle-Pensec V, Mariette X, Jousse-Joulin S, Berthelot JM, Perdriger A, Puechal X, et al. Treatment of primary Sjögren syndrome with rituximab: a randomized trial. Annals of internal medicine. 2014;160(4):233-42.
27. Bowman SJ, Everett CC, O'Dwyer JL, Emery P, Pitzalis C, Ng WF, et al. Randomized Controlled Trial of Rituximab and Cost-Effectiveness Analysis in Treating

Fatigue and Oral Dryness in Primary Sjögren's Syndrome. Arthritis Rheumatol. 2017;69(7):1440-50.
28. Fisher BA, Everett CC, Rout J, O'Dwyer JL, Emery P, Pitzalis C, et al. Effect of rituximab on a salivary gland ultrasound score in primary Sjögren's syndrome: results of the TRACTISS randomised double-blind multicentre substudy. Ann Rheum Dis. 2018;77(3):412-6.
29. Jousse-Joulin S, Devauchelle-Pensec V, Cornec D, Marhadour T, Bressollette L, Gestin S, et al. Brief Report: Ultrasonographic Assessment of Salivary Gland Response to Rituximab in Primary Sjögren's Syndrome. Arthritis Rheumatol. 2015;67(6):1623-8.
30. Anaya JM, Ogawa N, Talal N. Sjögren's syndrome in childhood. The Journal of rheumatology. 1995;22(6):1152-8.
31. Lieberman SM. Childhood Sjögren syndrome: insights from adults and animal models. Current opinion in rheumatology. 2013;25(5):651-7.
32. O'Neill EM. Sjögren's syndrome with onset at 10 years of age. Proceedings of the Royal Society of Medicine. 1965;58(9):689-90.
33. Cimaz R, Casadei A, Rose C, Bartunkova J, Sediva A, Falcini F, et al. Primary Sjögren syndrome in the paediatric age: a multicentre survey. European journal of pediatrics. 2003;162(10):661-5.
34. Bartunkova J, Sediva A, Vencovsky J, Tesar V. Primary Sjögren's syndrome in children and adolescents: proposal for diagnostic criteria. Clinical and experimental rheumatology. 1999;17(3):381-6.
35. Fang QG, Liu FY, Sun CF. Recurrent submandibular gland swelling as a first manifestation in a child with primary Sjögren syndrome. The Journal of craniofacial surgery. 2013;24(4):e413-5.
36. Nikitakis NG, Rivera H, Lariccia C, Papadimitriou JC, Sauk JJ. Primary Sjögren syndrome in childhood: report of a case and review of the literature. Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics. 2003;96(1):42-7.
37. Bogdanovic R, Basta-Jovanovic G, Putnik J, Stajic N, Paripovic A. Renal involvement in primary Sjögren syndrome of childhood: case report and literature review. Modern rheumatology. 2013;23(1):182-9.
38. Virdee S, Greenan-Barrett J, Ciurtin C. A systematic review of primary Sjögren's syndrome in male and paediatric populations. Clinical rheumatology. 2017;36(10):2225-36.
39. Shiboski SC, Shiboski CH, Criswell LA, Baer AN, Challacombe S, Lanfranchi H, et al. American College of Rheumatology classification criteria for Sjögren's syndrome: A data-driven, expert consensus approach in the Sjögren's International Collaborative Clinical Alliance Cohort. Arthritis Care Res. 2012;64(4):475–87.
40. Tsuboi H, Hagiwara S, Asashima H, Takahashi H, Hirota T, Noma H, et al. Comparison of performance of the 2016 ACR-EULAR classification criteria for primary Sjögren's syndrome with other sets of criteria in Japanese patients. Ann Rheum Dis. 2017;76(12):1980-5.
41. T. O. Sjögren’s Disease Research Committee review on research reports: annual report of the Ministry of Health and Welfare. Tokyo: Japanese Ministry of Health. 1977.
42. Fox RI, Robinson CA, Curd JG, Kozin F, Howell FV. Sjögren's syndrome. Proposed criteria for classification. Arthritis Rheum. 1986;29(5):577-85.

43. Homma M, Tojo T, Akizuki M, Yamagata H. Criteria for Sjögren's syndrome in Japan. Scandinavian journal of rheumatology Supplement. 1986;61:26-7.
44. Manthorpe R, Oxholm P, Prause JU, Schiodt M. The Copenhagen criteria for Sjögren's syndrome. Scandinavian journal of rheumatology Supplement. 1986;61:19-21.
45. Vitali C, Bombardieri S, Moutsopoulos HM, Balestrieri G, Bencivelli W, Bernstein RM, et al. Preliminary criteria for the classification of Sjögren's syndrome. Results of a prospective concerted action supported by the European Community. Arthritis Rheum. 1993;36(3):340-7.
46. Miyawaki S. [Revised Japan criteria for Sjögren syndrome]. Ryumachi [Rheumatism]. 2000;40(1):48-53.
47. Le Goff M, Cornec D, Jousse-Joulin S, Guellec D, Costa S, Marhadour T, et al. 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. Arthritis Res Ther. 2017;19(1):269.
48. Schuetz C, Prieur AM, Quartier P. Sicca syndrome and salivary gland infiltration in children with autoimmune disorders: when can we diagnose Sjögren syndrome? Clinical and experimental rheumatology. 2010;28(3):434-9.
49. Yokogawa N, Lieberman SM, Alawi F, Bout-Tabaku S, Guttenberg M, Sherry DD, et al. Comparison of labial minor salivary gland biopsies from childhood Sjögren syndrome and age-matched controls. The Journal of rheumatology. 2014;41(6):1178-82.
50. Houghton K, Malleson P, Cabral D, Petty R, Tucker L. Primary Sjögren's syndrome in children and adolescents: are proposed diagnostic criteria applicable? The Journal of rheumatology. 2005;32(11):2225-32.
51. Shiboski CH, Shiboski SC, Seror R, Criswell LA, Labetoulle M, Lietman TM, et al. 2016 American College of Rheumatology/European League Against Rheumatism classification criteria for primary Sjögren's syndrome: A consensus and data-driven methodology involving three international patient cohorts. Ann Rheum Dis. 2017;76(1):9-16.
52. Yokogawa N, Lieberman SM, Sherry DD, Vivino FB. Features of childhood Sjögren's syndrome in comparison to adult Sjögren's syndrome: considerations in establishing child-specific diagnostic criteria. Clinical and experimental rheumatology. 2016;34(2):343-51.
53. Zengel P, Schrotzlmair F, Reichel C, Paprottka P, Clevert DA. Sonography: the leading diagnostic tool for diseases of the salivary glands. Semin Ultrasound CT MR. 2013;34(3):196-203.
54. Riente L, Delle Sedie A, Filippucci E, Iagnocco A, Meenagh G, Epis O, et al. Ultrasound imaging for the rheumatologist XIV. Ultrasound imaging in connective tissue diseases. Clinical and experimental rheumatology. 2008;26(2):230-3.
55. Mossel E, Delli K, van Nimwegen JF, Stel AJ, Kroese FGM, Spijkervet FKL, et al. Ultrasonography of major salivary glands compared with parotid and labial gland biopsy and classification criteria in patients with clinically suspected primary Sjögren's syndrome. Ann Rheum Dis. 2017;76(11):1883-9.
56. Fidelix T, Czapkowski A, Azjen S, Andriolo A, Trevisani VFM. Salivary gland ultrasonography as a predictor of clinical activity in Sjögren's syndrome. PloS one. 2017;12(8):e0182287.

57. Baldini C, Luciano N, Tarantini G, Pascale R, Sernissi F, Mosca M, et al. Salivary gland ultrasonography: a highly specific tool for the early diagnosis of primary Sjögren's syndrome. Arthritis Res Ther. 2015;17:146.
58. Theander E, Mandl T. Primary Sjögren's syndrome: diagnostic and prognostic value of salivary gland ultrasonography using a simplified scoring system. Arthritis Care Res (Hoboken). 2014;66(7):1102-7.
59. Jousse-Joulin S, Gatineau F, Baldini C, Baer AN, Barone F, Bootsma H, et al. Modification of the Classification Criteria for Primary Sjögren Syndrome: An International Vignette Survey. ACR 2017. 2017;ACR abstract number: 877.
60. Takagi Y, Nakamura H, Sumi M, Shimizu T, Hirai Y, Horai Y, et al. Combined classification system based on ACR/EULAR and ultrasonographic scores for improving the diagnosis of Sjögren's syndrome. PloS one. 2018;13(4):e0195113.
61. Salaffi F, Carotti M, Iagnocco A, Luccioli F, Ramonda R, Sabatini E, et al. Ultrasonography of salivary glands in primary Sjögren's syndrome: a comparison with contrast sialography and scintigraphy. Rheumatology (Oxford). 2008;47(8):1244-9.
62. El Miedany YM, Ahmed I, Mourad HG, Mehanna AN, Aty SA, Gamal HM, et al. Quantitative ultrasonography and magnetic resonance imaging of the parotid gland: can they replace the histopathologic studies in patients with Sjögren's syndrome? Joint Bone Spine. 2004;71(1):29-38.
63. Jousse-Joulin S, Devauchelle-Pensec V, Morvan J, Guias B, Pennec Y, Pers JO, et al. Ultrasound assessment of salivary glands in patients with primary Sjögren's syndrome treated with rituximab: Quantitative and Doppler waveform analysis. Biologics. 2007;1(3):311-9.
64. Shimizu M, Okamura K, Yoshiura K, Ohyama Y, Nakamura S. Sonographic diagnosis of Sjögren syndrome: evaluation of parotid gland vascularity as a diagnostic tool. Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics. 2008;106(4):587-94.
65. Ophir J, Cespedes I, Ponnekanti H, Yazdi Y, Li X. Elastography: a quantitative method for imaging the elasticity of biological tissues. Ultrasonic imaging. 1991;13(2):111-34.
66. Samier-Guerin A, Saraux A, Gestin S, Cornec D, Marhadour T, Devauchelle-Pensec V, et al. Can ARFI elastometry of the salivary glands contribute to the diagnosis of Sjögren's syndrome? Joint Bone Spine. 2016;83(3):301-6.
67. Zhang S, Zhu J, Zhang X, He J, Li J. Assessment of the Stiffness of Major Salivary Glands in Primary Sjögren's Syndrome through Quantitative Acoustic Radiation Force Impulse Imaging. Ultrasound in medicine & biology. 2016;42(3):645-53.
68. Jousse-Joulin S, Devauchelle-Pensec V, Morvan J, Guias B, Pennec Y, Pers JO, et al. Ultrasound assessment of salivary glands in patients with primary Sjögren's syndrome treated with rituximab: Quantitative and Doppler waveform analysis. Biologics. 2007;1(3):311-9.
69. Delli K, Haacke EA, Kroese FG, Pollard RP, Ihrler S, van der Vegt B, et al. Towards personalised treatment in primary Sjögren's syndrome: baseline parotid histopathology predicts responsiveness to rituximab treatment. Ann Rheum Dis. 2016;75(11):1933-8.
70. Delli K, Haacke EA, Kroese FG, Pollard RP, Ihrler S, van der Vegt B, et al. In primary Sjögren's syndrome high absolute numbers and proportions of B cells in parotid

glands predict responsiveness to rituximab as defined by ESSDAI, but not by SSRI. Ann Rheum Dis. 2016;75(6):e34.
71. Pijpe J, Meijer JM, Bootsma H, van der Wal JE, Spijkervet FK, Kallenberg CG, et al. Clinical and histologic evidence of salivary gland restoration supports the efficacy of rituximab treatment in Sjögren's syndrome. Arthritis Rheum. 2009;60(11):3251-6.
72. Hocevar A, Ambrozic A, Rozman B, Kveder T, Tomsic M. Ultrasonographic changes of major salivary glands in primary Sjögren's syndrome. Diagnostic value of a novel scoring system. Rheumatology (Oxford). 2005;44(6):768-72.
73. Zhou M, Song S, Wu S, Duan T, Chen L, Ye J, et al. Diagnostic accuracy of salivary gland ultrasonography with different scoring systems in Sjögren's syndrome: a systematic review and meta-analysis. Scientific reports. 2018;8(1):17128.
74. Jousse-Joulin S, Nowak E, Cornec D, Brown J, Carr A, Carotti M, et al. Salivary gland ultrasound abnormalities in primary Sjögren's syndrome: consensual US-SG core items definition and reliability. RMD Open. 2017;3(1):e000364.
75. Delli K, Dijkstra PU, Stel AJ, Bootsma H, Vissink A, Spijkervet FK. Diagnostic properties of ultrasound of major salivary glands in Sjögren's syndrome: a meta-analysis. Oral diseases. 2015;21(6):792-800.
76. De Vita S, Lorenzon G, Rossi G, Sabella M, Fossaluzza V. Salivary gland echography in primary and secondary Sjögren's syndrome. Clinical and experimental rheumatology. 1992;10(4):351-6.
77. Delli K, Arends S, van Nimwegen JF, Dijkstra PU, Stel AJ, Spijkervet FKL, et al. Ultrasound of the Major Salivary Glands is a Reliable Imaging Technique in Patients with Clinically Suspected Primary Sjögren's Syndrome. Ultraschall in der Medizin (Stuttgart, Germany : 1980). 2018;39(3):328-33.
78. Mossel E, Arends S, van Nimwegen JF, Delli K, Stel AJ, Kroese FGM, et al. Scoring hypoechogenic areas in one parotid and one submandibular gland increases feasibility of ultrasound in primary Sjögren's syndrome. Ann Rheum Dis. 2018;77(4):556-62.
79. Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, et al. Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Ann Rheum Dis. 2002;61(6):554-8.
80. Jonsson MV DN, Marthinussen MC, Jonsson R. Oral and dental manifestations of Sjögren's syndrome – Current approaches to diagnostics and therapy. Sjögren's syndrome: Pathogenesis and therapy New York, Dordrecht, Heidelberg, London: Springer2011. p. 221-42.
81. Jonsson MV RT, Delaleu ND, Marthinussen MC. Diagnostikk av munntørrhet og bruk av saliva som diagnostikk verktøy. Den Norske Tannlegeforenings Tidende 2011;2011;121(14):908–13.
82. Vissink A WA, Vermann ECI. Saliva Collectors. In: Wong DT, editor. Salivary Diagnostics2008.
83. Jonsson R, Bolstad AI, Brokstad KA, Brun JG. Sjögren's syndrome--a plethora of clinical and immunological phenotypes with a complex genetic background. Annals of the New York Academy of Sciences. 2007;1108:433-47.
84. Lee J, Koh JH, Kwok SK, Park SH. The EULAR Sjögren's Syndrome Patient-Reported Index is an independent determinant of health-related utility values of Korean

patients with primary Sjögren's syndrome. Clinical and experimental rheumatology. 2016;34(4):663-7.
85. Seror R, Bowman SJ, Brito-Zeron P, Theander E, Bootsma H, Tzioufas A, et al. EULAR Sjögren's syndrome disease activity index (ESSDAI): a user guide. RMD Open. 2015;1(1):e000022.
86. Cicchetti DV. Guidelines, criteria, and rules of thumb for evaluating normed and standardized assessment instruments in psychology. Psychological Assessment. 1994(6 (4)):284–90.
87. Bradus RJ, Hybarger P, Gooding GA. Parotid gland: US findings in Sjögren syndrome. Work in progress. Radiology. 1988;169(3):749-51.
88. Hammenfors DS VV, Pasoto SG, Lilleby V, Nieto JC, Silva C, Pereira RMR, Bica B, Brun JG, Jonsson R, Jonsson MV. Major Salivary Gland Ultrasonography Bed-Side and Still Image Scoring – a Pilot Comparison in Juvenile Sjögren's Syndrome [abstract]. Arthritis Rheumatol. 2017;2017; 69 (suppl 10). https://acrabstracts.org/abstract/major-salivary-gland-ultrasonography-bed-side-and-still-image-scoring-a-pilot-comparison-in-juvenile-Sjögrens-syndrome/.
89. Jousse-Joulin S, Nowak E, Cornec D, Brown J, Carr A, Carotti M, et al. Salivary gland ultrasound abnormalities in primary Sjögren's syndrome: consensual US-SG core items definition and reliability. RMD Open. 2017;3(1):e000364.
90. Brito-Zeron P, Acar-Denizli N, Zeher M, Rasmussen A, Seror R, Theander E, et al. Influence of geolocation and ethnicity on the phenotypic expression of primary Sjögren's syndrome at diagnosis in 8310 patients: a cross-sectional study from the Big Data Sjögren Project Consortium. Ann Rheum Dis. 2017;76(6):1042-50.
91. Brito-Zeron P, Acar-Denizli N, Ng WF, Zeher M, Rasmussen A, Mandl T, et al. How immunological profile drives clinical phenotype of primary Sjögren's syndrome at diagnosis: analysis of 10,500 patients (Sjögren Big Data Project). Clinical and experimental rheumatology. 2018;36 Suppl 112(3):102-12.
92. Narayan AK, Baer A, Fradin J. Sonographic findings of IgG4-related disease of the salivary glands: Case report and review of the literature. Journal of clinical ultrasound : JCU. 2018;46(1):73-7.
93. Bretz WA, do Valle EV, Jacobson JJ, Marchi F, Mendes S, Nor JE, et al. Unstimulated salivary flow rates of young children. Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics. 2001;91(5):541-5.
94. Torres SR, Nucci M, Milanos E, Pereira RP, Massaud A, Munhoz T. Variations of salivary flow rates in Brazilian school children. Brazilian oral research. 2006;20(1):8-12.
95. Nieto-Gonzalez JC, Ovalles-Bonilla JG, Estrada E, Serrano-Benavente B, Martinez-Barrio J, Gonzalez-Fernandez CM, et al. Salivary gland ultrasound is linked to the autoimmunity profile in patients with primary Sjögren's syndrome. The Journal of international medical research. 2018:300060518767031.
96. Nieto-Gonzalez JC, Monteagudo I, Bello N, Martinez-Estupinan L, Naredo E, Carreno L. Salivary gland ultrasound in children: a useful tool in the diagnosis of juvenile Sjögren's syndrome. Clin Exp Rheumatol. 2014;32(4):578-80.
97. Civilibal M, Canpolat N, Yurt A, Kurugoglu S, Erdamar S, Bagci O, et al. A child with primary Sjögren syndrome and a review of the literature. Clin Pediatr (Phila). 2007;46(8):738-42.

98. Baldini C, Zabotti A, Filipovic N, Vukicevic A, Luciano N, Ferro F, et al. Imaging in primary Sjögren's syndrome: the 'obsolete and the new'. Clin Exp Rheumatol. 2018;36 Suppl 112(3):215-21.
99. Martire MV, Santiago ML, Cazenave T, Gutierrez M. Latest Advances in Ultrasound Assessment of Salivary Glands in Sjögren Syndrome. Journal of clinical rheumatology : practical reports on rheumatic & musculoskeletal diseases. 2018;24(4):218-23.
100. Brito-Zeron P, Kostov B, Fraile G, Caravia-Duran D, Maure B, Rascon FJ, et al. Characterization and risk estimate of cancer in patients with primary Sjögren syndrome. Journal of hematology & oncology. 2017;10(1):90.
101. Theander E, Jonsson R, Sjostrom B, Brokstad K, Olsson P, Henriksson G. Prediction of Sjögren's Syndrome Years Before Diagnosis and Identification of Patients With Early Onset and Severe Disease Course by Autoantibody Profiling. Arthritis Rheumatol. 2015;67(9):2427-36.
102. Gazeau P, Cornec D, Jousse-Joulin S, Guellec D, Saraux A, Devauchelle-Pensec V. Time-course of ultrasound abnormalities of major salivary glands in suspected Sjögren's syndrome. Joint Bone Spine. 2018;85(2):227-32.

16. APPENDIX

ESSPRI

1) How severe has your dryness been during the last 2 weeks ?

No dryness	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10	Maximal imaginable dryness

2) How severe has your fatigue been during the last 2 weeks ?

No fatigue	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10	Maximal imaginable fatigue

3) How severe has your pain (joint or muscular pains in your arms or legs) been during the last 2 weeks ?

No pain	☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10	Maximal imaginable pain

ESSDAI

Constitutional domain [3] Please be careful of not rating constitutional symptoms not related to the disease (such as fever of infectious origin, voluntary weight loss)		
No activity	Absence of the following symptoms	
Low activity	Mild or intermittent fever (37.5°-38.5°C) / night sweats Involuntary weight loss of 5 to 10% of body weight	
Moderate activity	Severe Fever (>38.5°C) / night sweats Involuntary weight loss of >10% of body weight	

Lymphadenopathy domain [4]		
No activity	Absence of the following features	
Low activity	- Lymphadenopathy ≥ 1cm in any nodal region or ≥ 2cm in inguinal region	
Moderate activity	- Lymphadenopathy ≥ 2cm in any nodal region or ≥ 3cm in inguinal region, - and/or splenomegaly (clinically palpable or assessed by imaging)	
High activity	- Current malignant B-cell proliferative disorder	

Glandular domain [2] Please be careful of not rating glandular swelling not related to the disease (such as stone or infection)		
No activity	Absence of glandular swelling	
Low activity	Small glandular swelling with: - enlarged parotid (≤ 3cm), - or limited submandibular or lachrymal swelling[1]	
Moderate activity	Major glandular swelling with: - enlarged parotid (>3cm) - or important submandibular or lachrymal swelling[1]	

[1]Distinction between limited and important submandibular or lachrymal swelling is left to the physician judgment

Articular domain [2] Please be careful of not rating articular involvement not related to the disease, such as osteoarthritis		
No activity	Absence of currently active articular involvement	
Low activity	Arthralgias in hands, wrists, ankles and feet accompanied by morning stiffness (>30 min)	
Moderate activity	1 to 5 synovitis among a 28 count	
High activity	≥ 6 synovitis among a 28 count	

Cutaneous domain [3] Please be careful of rating as "No activity" stable long lasting features that are related to damage rather than disease activity, or cutaneous involvement not related to the disease		
No activity	Absence of currently active cutaneous involvement	
Low activity	Erythema multiforme	
Moderate activity	Limited cutaneous vasculitis, including urticarial vasculitis[2], or purpura limited to feet and ankle, or subacute cutaneous lupus	
High activity	Diffuse cutaneous vasculitis, including urticarial vasculitis[2], or diffuse purpura or ulcers related to vasculitis	

[2] Limited cutaneous vasculitis involve <18% body surface area; Diffuse Cutaneous vasculitis involve >18% body surface area

Body surface area (BSA) is defined using the rules of nines (used to assess extent of burns) as follows: Palm (excluding fingers) = 1% BSA; each lower limb = 18% BSA; each upper limb = 9% BSA; torso (front) = 18% BSA; torso (back) = 18% BSA

Pulmonary domain [5] Please be careful of rating as "No activity" stable long lasting features that are related to damage rather than disease activity, or respiratory involvement not related to the disease, (tobacco…)		
No activity	Absence of currently active pulmonary involvement	
Low activity	Persistent cough or bronchial involvement with no radiographic abnormalities on X-ray Or radiological or HRCT evidence of interstitial lung disease[3] with: • No breathlessness, • And normal lung function test.	
Moderate activity	Moderately active pulmonary involvement, such as interstitial lung disease proven by HRCT[3] with • shortness of breath on exercise (NHYA II) • or abnormal lung function tests restricted to: – 70% >DL_{CO} ≥ 40% and/or 80% > FVC ≥ 60%	
High activity	Highly active pulmonary involvement, such as interstitial lung disease proven by HRCT[3] with : • shortness of breath at rest (NHYA III, IV) • or with abnormal lung function tests: - DL_{CO} < 40% and/or FVC < 60%	

[3]For diagnosis of interstitial lung disease HRCT or radiography is required and must have been performed in the last 2 years

DLCO= diffusing CO capacity; FVC= forced vital capacity; HRCT= high-resolution computed tomography; NHYA= New York heart association classification

Renal domain [5] Please be careful of rating as "No activity" stable long lasting features that are related to damage rather than disease activity, and renal involvement not related to the disease. If biopsy has been performed, please rate activity based on **histological** features first.		
No activity	Absence of currently active renal involvement: - Proteinuria< 0.5g/d, no hematuria, no leucocyturia, no acidosis. - Or long lasting stable proteinuria due to damage	
Low activity	Evidence of specific active renal involvement, limited to: • Tubular acidosis without renal failure (GFR[4] ≥60ml/min) • Glomerular involvement - with proteinuria (between 0.5 and 1 g/d) - without hematuria or renal failure (GFR4 ≥60ml/min)	
Moderate activity	Moderately active renal involvement, such as: • Tubular acidosis with renal failure (GFR[4] < 60 ml/min) • Glomerular involvement - with proteinuria between 1 and 1.5g/d - without hematuria or renal failure (GFR4 ≥ 60ml/min) • or histological evidence of - Extra-membranous glomerulonephritis - Important interstitial lymphoid infiltrate	
High activity	Highly active renal involvement, such as: • Glomerular involvement - with proteinuria > 1.5 g/d - or haematuria - or renal failure (GFR[4] < 60 ml/min) • or histological evidence of - proliferative glomerulonephritis - cryoglobulinemia related renal involvement	

[4] Glomerular filtration rate (GFR) estimated with MDRD formula

Muscular domain [6] Please be careful of not rating muscular involvement not related to the disease, such as weakness due to corticosteroids…		
No activity	Absence of currently active muscular involvement	
Low activity	Active myositis proven by abnormal EMG or biopsy with: - no weakness - and creatinine kinase ($N < CK \leq 2N$)	
Moderate activity	Moderately active myositis proven by abnormal EMG or biopsy with: - weakness (maximal deficit of 4/5), - or elevated creatinine kinase ($2N < CK \leq 4N$),	
High activity	Highly active myositis proven by abnormal EMG or biopsy with: - weakness (deficit $\leq 3/5$) - or elevated creatinine kinase ($> 4N$)	

EMG= electromyogram

Peripheral nervous system domain [5] Please be careful of rating as "No activity" stable long lasting features that are related to damage rather than activity, or PNS involvement not related to the disease		
No activity	Absence of currently active PNS involvement	
Low activity	Evidence of active peripheral nervous system involvement, such as: - - Pure sensory axonal polyneuropathy proven by NCS - - Trigeminal (V) neuralgia	
Moderate activity	Evidence of moderately active peripheral nervous system involvement shown by NCS, such as: - Axonal sensory-motor neuropathy with maximal motor deficit of 4/5, - Pure sensory neuropathy with presence of cryoglobulinemic vasculitis, - Ganglionopathy[5] with symptoms restricted to mild/moderate ataxia, - Inflammatory demyelinating polyneuropathy[6] (CIDP) with mild Functional impairment (maximal motor deficit of 4/5or mild ataxia), Or cranial nerve involvement of peripheral origin (except trigeminal (V) neuralgia)	
High activity	Evidence of highly active peripheral nervous system involvement shown by NCS, such as: - Axonal sensory-motor neuropathy with motor deficit ≤ 3/5 - Peripheral nerve involvement due to vasculitis (mononeuritis multiplex, etc…) - Severe ataxia due to ganglionopathy[5] - Inflammatory demyelinating polyneuropathy[6] (CIDP) with severe functional impairment: motor deficit ≤ 3/5 or severe ataxia	

NCS: Nerve conduction studies.

[5] Pure sensory impairment with ataxia and diffuse impairment or abolition of sensitive potential on NCS

[6] Polyradiculoneuropathy with suggestive clinical symptoms (4 limbs sensorimotor deficit, proximal motor deficit, generalised areflexia, initial sensory symptoms affecting the upper limbs, and/or associated involvement of cranial nerves), increased protein level and/or supportive abnormal NCS (prolonged motor distal latency, reduced nerve conduction velocity, prolonged F wave latency, conduction block and/or temporal dispersion)

Central nervous system domain [5] Please be careful of rating as “No activity” stable long lasting features that are related to damage rather than disease activity, or CNS involvement not related to the disease		
No activity	Absence of currently active CNS involvement	
Moderate activity	Moderately active CNS features, such as: - Cranial nerve involvement of central origin - Optic neuritis - Multiple sclerosis-like syndrome with symptoms restricted to pure sensory impairment or proven cognitive impairment	
High activity	Highly active CNS features, such as: - Cerebral vasculitis with cerebrovascular accident or transient ischemic attack - Seizures - Transverse myelitis. - Lymphocytic meningitis - Multiple sclerosis-like syndrome with motor deficit	

Haematological domain [2] Please be careful : - considering anemia[7], thrombopenia[8] and neutropenia[9], only auto-immune cytopenia must be considered - not rating cytopenia not related to the disease (such as vitamin or iron deficiency, drug-induced cytopenia, as for example lymphocytopenia associated with cyclophosphamide)		
No activity	Absence of auto-immune cytopenia	
Low activity	Cytopenia of auto-immune origin with: - neutropenia (1000 < neutrophils < 1500/mm3) - or anemia (10 < Hb < 12g/dl) - or thrombocytopenia (100,000 < Plt < 150,000/mm3) Or lymphopenia (500<lymphocytes<1000/mm3)	
Moderate activity	Cytopenia of auto-immune origin with: - neutropenia (500 ≤ neutrophils ≤ 1000/mm3), - or anaemia (8 ≤ Hb ≤ 10g/dl) - or thrombocytopenia (50,000 ≤ Plt ≤ 100,000/mm3) Or lymphopenia (≤500/mm3)	
High activity	Cytopenia of auto-immune origin with: - neutropenia (neutrophils < 500/mm3), - or anemia (Hb < 8 g/dl) - or thrombocytopenia (Plt < 50,000/mm3),	

[7] Anaemia with positive Coombs testing and increase reticulocyte count

[8] Thrombopenia of peripheral origin with no other aetiology found, or in case of difficulty with anti-platelet autoantibodies and/or presence of megakaryocyte on bone marrow aspirate and/or associated autoimmune anaemia.

[9] Neutropenia with no other etiology found

Biological domain [1]		
No activity	Absence of any of the following biological features	
Low activity	- Clonal component - or hypocomplementemia (low C4 or C3 or CH50) - or hypergammaglobulinemia or IgG level between 16 and 20g/L	
Moderate activity	- presence of cryoglobulinemia - or hypergammaglobulinemia or high IgG level > 20g/L - or recent onset[10] hypogammaglobulinemia or recent decrease of IgG level (<5g/L)	

[10] In the last 6 months

SCORING METHODOLOGY:

- The score of each domain is the product of the weight of the domain by the level of activity. The domain weights are indicated in bracket for each domain.
- Activity levels should be scored as follows:
    - No activity = 0
    - Low activity = 1
    - Moderate activity = 2
    - High activity = 3
- The total score is the sum of the score of all domains.

17. PAPERS I-III

II

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Article type : Original Article

Juvenile Sjögren's syndrome: clinical characteristics with focus on salivary gland ultrasonography

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ABSTRACT

Background/objectives: Juvenile Sjögren's syndrome (jSS) is a rare, poorly defined and possibly underdiagnosed condition affecting children/adolescents. The aim of this study was to characterize symptoms and clinical findings of jSS, and to explore the clinical application of major salivary gland ultrasonography (SGUS) in patients with jSS.

Methods: A cross-sectional multicenter study recruited patients with disease onset until 18 years of age (n=67). Disease characteristics were recorded, unstimulated whole sialometry and SGUS examination of the parotid and submandibular salivary glands was performed.

Results: Female:male ratio was 58:9 (6.4:1). Mean age at first symptom was 10.2 years, 12.1 years at diagnosis. Ocular and oral symptoms were noted in 42/67 (63%) and 53/66 (80%) patients, respectively. AECG or ACR-EULAR classification criteria for primary SS (pSS) was fulfilled by 42/67 (63 %) patients. Pathological SGUS findings (SGUS+) were observed in 41/67 (61%) patients; 26/41 (63%) SGUS+ fulfilled pSS criteria. Salivary gland enlargements/parotitis was noted in 37/58 patients and non-significantly associated with SGUS+ (p=0.066). Mean levels of saliva was 5.6 ml/15 minutes in SGUS- patients compared to 3.3 ml/15 minutes in the SGUS+ patients (p=0.049). 36/41 (88%) SGUS+ patients were anti-Ro/La+ compared to 14/26 (54%) SGUS- patients (p=0.001). 24/39 (62%) SGUS+ patients were RF+, whereas only 5/25 (20%) SGUS- patients were RF+ (p=0.001).

Conclusions: Juvenile SS is characterized by a large spectrum of clinical symptoms and findings. Several glandular and extraglandular parameters such as hyposalivation, swollen salivary glands and autoantibodies are associated with pathological SGUS findings.

Significance and innovation:

Interest for jSS is increasing and international collaborations are emerging. To date, this is the largest cohort world-wide characterizing juvenile Sjögren's syndrome and also the first large study investigating salivary gland ultrasonography in this patient group.

INTRODUCTION

Primary Sjögren's syndrome (pSS) is a systemic autoimmune disorder. Patients with pSS experience oral and ocular dryness and extraglandular manifestations (EGM) such as fatigue, arthralgia and arthritis (1). In addition to subjective and objective findings of salivary and/or lacrimal gland involvement, the pSS classification is based on either the presence of autoantibodies against Ro/SSA and/or La/SSB, and/or focal mononuclear cell inflammation with a focus score ≥ 1 in a minor labial salivary gland biopsy (2). Serum autoantibodies have been presented as early markers of pSS (3).

Juvenile Sjögren's syndrome (jSS) is a rare, poorly defined and possibly underdiagnosed condition (4, 5). Mean age at time of diagnosis is around 10 years (6). A common initial symptom is swelling of the major salivary glands (6, 7). Several organ systems may be affected, resulting in neurological, dermatological, musculoskeletal, vascular, gastrointestinal, respiratory, renal, and haematological manifestations (8, 9). Extraglandular manifestations occur in approximately 50 % of children with jSS (4). Criteria for jSS are not available in current literature (10-12) and neither the American-European Consensus Group (AECG) criteria (2) nor the American College of Rheumatology/European League Against Rheumatism (ACR-EULAR) criteria (13) have been validated in a juvenile population. In addition, the AECG criteria may not be applicable due to diverse clinical manifestations in children compared to adults (5).

Interest regarding ultrasonography examination of the major salivary glands (SGUS) (14) as a diagnostic tool for pSS is increasing (15-19). Salivary gland US may serve as a supplement, or even alternative, to minor salivary gland biopsy (11, 17). Development of a non-invasive, diagnostic method for evaluation of the salivary gland component, to aid the diagnosis of jSS is especially important in the younger population, both with regard to the in some patients late onset of sicca-symptoms, and current lack of diagnostic criteria. Previous studies indicate that SGUS may be of value in establishing a jSS diagnosis (7, 20, 21). However, current reports include small numbers of patients and SGUS application remains to be evaluated in a larger cohort. Further studies on jSS are needed and to our knowledge our study is to date the largest cohort for this patient group. The aim of this study was to characterize symptoms and clinical findings in patients with jSS and to investigate SGUS as a diagnostic tool for jSS.

MATERIALS AND METHODS

Patients

The study design is a cross-sectional multicentre study. Patients were recruited from Haukeland University Hospital in Bergen, Norway, Oslo University Hospital, Norway, Hospital General Universitario Gregorio Marañón in Madrid, Spain, Hospital Universitário Cassiano Antônio Moraes of Federal University of Espírito Santo in Vitória Brazil, Hospital Universitário Clementino Fraga Filho, Federal University of Rio de Janeiro in Brazil and Hospital das Clinicas, Faculdade de Medicina da Universidade de Sao Paulo (HCFMUSP) in Brazil, University Medical Center Groningen, the Netherlands, and Department of Pediatrics, University of Florida, Gainesville, Florida. Patients had previously received the diagnosis jSS, by a specialist in rheumatology or paediatric medicine, at the age of 18 years or younger. At

the time of inclusion, all patients were younger than 25 years. Identification of patients at each clinic was performed by the local specialist in rheumatology or paediatric medicine. Clinical examination, sialometry and SGUS were performed in all patients. Upon inclusion, patients were asked for EGM, and medical history was collected from medical charts, including information regarding autoantibodies, biopsy results and current/previous treatment.

Patients recruited in Norway (n=11), Spain (n=5) and Brazil (n=40) were examined and included by the primary investigators, SDH/MVJ, with the local specialist. Patients recruited from the Netherlands (n=8) and USA (n=3) were examined in collaboration with local experts and included retrospectively.

Being a cross-sectional study, not all clinical examinations and tests had been performed or were available in all patients.

Subjective symptoms and EULAR Sjögren's Syndrome Patient Reported Index (ESSPRI)

EULAR Sjögren's Syndrome Patient Reported Index (ESSPRI) (22) was determined upon inclusion. Children were aided by an accompanying parent/guardian, when necessary.

EULAR Sjögren's syndrome disease activity index (ESSDAI)

EULAR Sjögren's syndrome disease activity index (ESSDAI) (23) was registered upon inclusion. The haematological and biological domains were determined from the most recent blood samples available.

Tear secretion

Tear secretion was evaluated by the Schirmers-I test. Wetting of the strip was recorded in mm, with levels of 5 mm or less wetting of the paper strip considered as pathologically reduced tear secretion.

Sialometry

Salivary gland functional capacity was evaluated by unstimulated sialometry measured in ml/15 minutes, with the patients fasting 90 minutes prior to examination. The volume of saliva was determined by weighing, with 1 gram corresponding to 1 ml. Saliva secretion levels ≤ 1.5 ml/15 minutes were considered pathologically reduced.

Salivary gland ultrasonography

The SGUS examination of the parotid and submandibular glands was performed using linear high-frequency transducers (6-15 MHz) and a simplified scoring system. Glandular homogeneity and presence of hypoechogenic areas were evaluated and graded (0-3). Grades 0-1 were considered to correspond to normal/non-specific changes and grades 2-3 to correspond to pathological changes (Figure 1). The SGUS was performed and scored bed-side by local experts in USA and the Netherlands, and by SDH/MVJ in Norway, Spain and Brazil.

Ethical considerations

This study was performed in accord with the Regional Medical and Health Research Ethics regulations, and necessary applications were approved by the regional Committees in the

participating centers/countries (Norway: 145/96-44.96, 242.06 2009/686; Brazil: 44787015447870154478701544787015.433.660/201644787015.6.2001.0068.) Informed consent was obtained from all participants and from parents of patients < 16 years old, according to the Declaration of Helsinki. Parents/legal guardians of under-aged patients were present at inclusion (clinical examination and SGUS imaging). In Spain, the Netherlands and USA, the non-invasive study design and number of patients included were in accordance with regional ethical guidelines and did not need a specific project approval.

Statistical analysis

The Student's t-test with Welch's correction was used to study differences between groups and Pearson correlation for the relationship between two variables. Correlations within ranges 0.0-0.2 were considered as poor, 0.2-0.4 as fair, 0.4-0.6 as moderate, 0.6-0.8 as good and 0.8-1.0 as excellent.

For categorical data Chi-square analysis was employed. To adjust for type 1 error due to multiple comparisons Benjamin-Hochberg adjustment was applied. All analyses were performed using IBM SPSS Statistics version 19.0 (Armonk, NY, IBM Corp).

RESULTS

Patient characteristics

Our cohort consists of 67 patients. The female:male ratio was 58:9. Mean age at first symptom was 10.2 years (range 1-17), 12.1 years (range 4-18) at diagnosis and 16.3 years (range 6-25 years) at inclusion (Table 1).

Out of the 67 patients, 35 (52%) and 39 (58%) patients fulfilled the AECG (2) and ACR-EULAR (13) classification criteria, respectively. When combining the criteria, 32 (48%) patients

fulfilled both criteria and 42 (63%) fulfilled either the AECG or the ACR/EULAR criteria. One patient had mixed connective tissue disease (MCTD) and seven patients were diagnosed with systemic lupus erythematosus (SLE) (Table 1).

In the patients with MCTD/SLE (n=8), 7/8 patients presented with autoantibodies, and fulfilled the AECG and the ACR-EULAR classification criteria. In this subgroup, 6/8 had ocular symptoms and 5/8 had ocular signs. All patients had experienced major salivary gland swelling and 6/8 had salivary gland involvement by sialometry (n=2), scintigraphy (n=2), sialometry and scintigraphy (n=1), or sialometry, scintigraphy and sialography (n=1). Five had available minor salivary gland biopsy; the three patients lacking biopsy did have objective evidence of salivary gland involvement, either by a positive scintigraphy (n=1), sialometry and scintigraphy (n=1), or sialometry, scintigraphy and sialography (n=1). All five biopsies were positive, i.e with a focus score of ≥ 1, corresponding to one or more chronic inflammatory cell foci consisting of 50 or more cells per $4mm^2$ of otherwise normal-appearing minor salivary gland tissue. In comparison, for the remaining patients (n=59) not diagnosed with an additional connective tissue disease, information on minor salivary gland biopsy was available from 29 patients, with a positive biopsy in 23 cases. Among these, the accurate focus score was available from 16 patients; 15/16 biopsies were positive (focus score ≥ 1). Interestingly, all MCTD/SLE patients had extraglandular manifestations and three had renal affection, findings which were not registered in any of the other patients.

Subjective symptoms

Subjective ocular and oral dryness symptoms were noted in 42/67 (63%) and 53/66 (80%) patients, respectively (Table 2). Only 8/66 (12%) reported no dryness symptoms. Ocular signs were noted in 26/40 (65%) patients with ocular symptoms (p=0.004).

Subjective oral symptoms were not associated with a positive minor salivary gland biopsy or objective salivary gland involvement (sialometry, sialography or scintigraphy). Sialoscintigraphy and/or sialography had been performed in thirty-one patients (Table 2), though interestingly only in the Brazilian part of the cohort.

ESSPRI for dryness correlated non-significantly with age at inclusion (p=0.062, n=58). Mean ESSPRI for patients (n=25) not fulfilling classification criteria was 2.7 compared to 4.0 for patients (n=40) fulfilling either AECG and/or ACR-EULAR classification criteria for pSS (p=0.04).

Major salivary gland imaging (SGUS) and glandular characteristics

Pathological SGUS findings (Figure 1, C-D) were observed in 41/67 patients. Mode SGUS score was 2 for all glands. The sum of the SGUS score (0-12) correlated with the ACR-EULAR points (r=0.321, p=0.016, n=56) (Figure 2). Interestingly, SGUS+ findings were observed in 26/27 of the European and North American patients as compared to 15/40 of the Brazilian patients (p=0.001).

Subjective sicca symptoms were not associated with pathological SGUS findings; ocular symptoms were noted only in 19/41 (46%) SGUS+ patients compared to 23/26 (88%) SGUS- patients (p=0.001). Ocular signs were noted in 17/41 (41%) SGUS+ patients compared to

16/24 (67%) SGUS- patients (p=0.050). Oral symptoms were noted in 30/40 (75%) patients with SGUS+ compared to 18/26 (69%) with SGUS- (p=0.607).

Salivary gland involvement (sialometry, sialoscintigraphy and sialography) was noted in 41/61 (67 %) patients; 20/35 (57 %) SGUS+ and 21/26 (81 % %) with SGUS- (p=0.052) (Table 2). When considering the individual items for salivary gland involvement, unstimulated whole saliva levels (ml/15 min) correlated inversely with the sum of the SGUS score (0-12) for all four glands (r=-0.324, p=0.015, n=56) (Figure 2), and with the SGUS score (0-3) for both submandibular glands (r=-0.372, p=0.005, n=56) and (r=-0.301, p=0.024, n= 56), right and left gland, respectively. A correlation was also observed in the right parotid gland (r=-0.285, p=0.033, n=56). Mean level of saliva was 5.6 ml/15 minutes in SGUS- patients compared to 3.3 ml/15 minutes in the SGUS+ patients (p=0.049), but when comparing saliva cut-off levels (≤ 1.5 ml/15 minutes) SGUS+ patients were below threshold in 11/34 (32%) cases, compared to 9/26 (35%) SGUS- patients (p=0.854). Scintigraphy findings coincided with both normal and pathological SGUS findings (p=0.350) (Table 2).

Information regarding labial minor labial salivary gland biopsy was available in 34 patients. Among these, 28/34 (82%) patients had a focus score ≥ 1, 21/26 (81%) SGUS+ compared to 7/8 (88%) SGUS- (p=0.662) (Table 2). For 20 patients the precise focus score was available, ranging from 0 to 8 with a mean of 1.7. Mean focus score was 1.9 in the SGUS+ patients (n=16) compared to 1.0 in the SGUS- patients (n=4), but the difference was not statistically significant (p=0.368), most likely due to low numbers. This probably also influenced the lack of correlation between precise focus score and total SGUS score, both when considering the glands separately, and for all glands (Figure 2).

In total, 37/58 (64%) patients had experienced salivary gland enlargements/parotitis (Table 2) and although not significant, a trend was noted with regard to pathological SGUS findings; 25/34 (74 %) patients with SGUS+ had experienced salivary gland swelling, compared to 12/24 (50 %) SGUS- patients (p=0.066).

Autoantibodies

The majority of patients, 50/67 (75%), presented with anti-Ro/SSA and/or anti-La/SSB. In the group with SGUS+ findings, 36/41 (88%) patients were anti-Ro/SSA positive, whereas in the SGUS- group only 14/26 (54%) patients were anti-Ro/SSA positive (p=0.002). Similarly, SGUS+ was associated with anti-La/SSB; 23/41 (56%) of the SGUS+ patients were anti-La/SSB+, whereas only 4/26 (15%) of the SGUS- patients were anti-La/SSB+ (p=0.001), and 24/39 (62%) of the SGUS+ patients were positive for RF, whereas only 5/25 (20%) of the SGUS- patients were RF+ (p=0.001) (Table 2).

Interestingly, 11/36 (31%) SGUS+ patients with positive anti-Ro/SSA did not fulfil the current classification criteria for pSS (2, 13). Out of these eleven patients, all did not fulfil or were lacking one or several items; ocular symptoms (n=11, 3 positive), oral symptoms (n=10, 7 positive), ocular signs (n=11, all negative), minor salivary gland biopsy (n=3, all negative), salivary gland involvement (n=6, 2 positive), sialoscintigraphy (n=1 positive), sialometry (n=6, all negative), OSS (n=6, one positive). Six patients had experienced salivary gland swelling; two of these had also experienced lacrimal gland swelling.

Taking a closer look at patients not fulfilling classification criteria for pSS (n=25), 11/25 (44 %) presented with anti-Ro/SSA and SGUS+, whereas the remaining (n=14) were either SGUS+ but lacked anti-Ro/SSA (n=4) or were anti-Ro/SSA- and had a normal-appearing SGUS (n=10). Among the SGUS+/anti-Ro/SSA- patients, 2/4 were indeed ANA+. For the SGUS-

/SSA- patients, all had pathological findings by sialoscintigraphy, 9/10 (90%) were ANA+, 3/10 (30%) had pathological levels of UWS, whereof one also had a positive sialography. One of the SGUS-/anti-Ro/SSA- patients was diagnosed with SLE.

Extraglandular manifestations

Various EGM at some time-point had been noted in 56/62 (90 %) patients (Table 3). Mean ESSPRI total was 4.0 for patients (n=40) fulfilling classification criteria for pSS (2, 13) compared to 2.7 for the remaining patients (n=25) (p=0.040). ESSPRI pain was also increased in patients fulfilling the AECG and/or ACR-EULAR classification criteria for pSS, with mean levels 3.5 compared to 1.9 (p=0.05).

Mean ESSDAI was non-significantly higher in the SGUS+ compared to for the SGUS- patients (p=0.138) (Table 3).

Treatment

Symptomatic treatment was observed in 29/59 (49%) patients (Table 4). Symptomatic treatment was registered in 16/34 (47 %) SGUS+ patients compared to 17/25 (68 %) with SGUS- (p=0.109). Only 5/59 (8%) patients had received salivary substitutes; four of these patients had SGUS+. Three patients had received Pilocarpine, none had received Cevimeline. A higher number of patients, 30/59 (51%), had received or were using lacrimal substitutes, where of the majority, 18/30(72%) had normal SGUS (p=0.005) (Table 4). Mean ESSPRI for dryness was 4.1 for patients who had received symptomatic treatment compared to 2.4 for those without symptomatic treatment (p=0.028).

Current or previous use of NSAIDs was noted in 16/34 (47 %) SGUS+ patients compared to 9/25 (36 %) SGUS- patients (p=0.396). Out of 59 patients, 54 were currently (n=40) or previously (n=14) treated with hydroxychloroquine. Interestingly, 29/34 (85 %) SGUS+ patients had hydroxychloroquine experience, whereas 25/25 (100 %) SGUS- patients had used or were using hydroxychloroquine (p=0.045). When instead comparing never users (n=5) and previous users (n=14) to current users (n=40), 18/34 (53%) SGUS+ and 22/25 (88 %) SGUS- patients were on current treatment (p=0.004) (Table 4).

Interestingly, systemic treatment was noted in 56/59 (95 %) patients, and was associated with EGM in 52/53 patients (p=0.001). In ten patients systemic treatment was ceased, and 42/53 patients with EGM were currently receiving systemic treatment (p=0.481). In 40/46 (87 %) the systemic treatment was current hydroxychloroquine (p=0.001).

DISCUSSION

To date, this is the largest study investigating clinical characteristics of patients with jSS in combination with SGUS. The female:male ratio was approximately 6.5:1, i.e. slightly altered with more male patients as compared to 9:1 for adults with pSS (24-27). The mean time from symptom debut to diagnosis was 1.8 years, naturally shorter than in adult pSS patients, where the time from debut of symptoms to diagnosis spans up to 11-14 years (26).

Nearly 2/3 of the patients fulfilled the current classifications criteria for pSS, and there were no significant differences in the application of the 2002 AECG (2) and the ACR-EULAR criteria (13). Due to different practices between regions and the fact that not all examinations in the classification criteria are routinely performed in all paediatric patients, we cannot be certain

that patients not fulfilling the classification criteria in this study would still not fulfil criteria, were all necessary examinations performed.

Major salivary gland ultrasonography findings were associated with anti-Ro/SSA and anti-La/SSB, similar to previous findings in adult pSS (28). Out of the 36 SGUS+ patients with positive anti-Ro/SSA, 11 patients did not fulfil the AECG or ACR-EULAR classification criteria, due to lack of objective salivary or lacrimal gland involvement such as reduced salivary and/or lacrimal secretion. Out of these 11 patients, 6/8 had registered salivary gland enlargement.

When considering the patients not fulfilling classification criteria for pSS (n=25), 11 (44 %) were anti-Ro/SSA+ and SGUS+, 4 (16 %) were SGUS+ and anti-Ro/SSA-, and 10 (40 %) were anti-Ro/SSA- and SGUS-. Among the anti-Ro/SSA- patients 11/14 (78.6 %) were ANA+. In addition, all the anti-Ro/SSA- and SGUS- patients had previously documented salivary gland involvement by sialoscintigraphy. Our findings thus support the notion that the existing criteria for pSS are not optimal for clinical evaluation and research of jSS (10, 12, 29). Of note, among the individuals fulfilling either the AECG or the ACR-EULAR classification criteria, 1/42 SGUS+ patients was ANA+ but anti-SSA/Ro-. Concerning the individuals fulfilling both AECG and ACR-EULAR criteria, all 19/19 SGUS+ patients were anti-Ro/SSA+ compared to 11/13 of the SGUS- patients (p=0.077). Interestingly, other relevant autoantibodies such as anti-La/SSB and RF were also associated with SGUS findings, further supporting the link between autoantibodies and salivary gland involvement in jSS.

In this cohort of patients with jSS, pathological SGUS changes (61 %) were slightly more common compared to adults (30) and non-significantly associated with salivary gland swelling, a clinical feature generally associated with jSS. Similar to findings in adult pSS, a

higher SGUS score was associated with lower levels of unstimulated whole saliva (28), non-significantly associated with oral dryness symptoms. Treatment of oral dryness symptoms was rare, but linked to SGUS+ in 4/5 cases. Ocular sicca symptoms and symptomatic treatment of dry eye was more common in SGUS- patients. Interestingly, ESSPRI dryness showed a trend to increase with age at inclusion (p=0.062). One might speculate whether this is due to actual later onset of sicca symptoms, or that oral dryness is not perceived as a problem by the younger patients.

Although mean focus score was higher in SGUS+ compared to SGUS-, and imply that focus score can mirror the degree of damage visualized by SGUS, comparison of findings in the major and the minor glands were limited due to lack of data on minor salivary gland focus score.

Similar to earlier reports in both pSS and jSS, the patients in this cohort display a high degree of EGM (30, 31). Both mean ESSPRI and mean ESSPRI pain was higher in the patients fulfilling AECG and ACR-EULAR classification criteria, and mean ESSDAI was non-significantly higher in the SGUS+ compared to the SGUS- patients.

Eight patients with EGM differed from their jSS peers, in several aspects. All eight had at some point been diagnosed with SLE or MCTD and three had renal affection, an EGM with a varied prevalence of 2 to 67 percent in pSS (32-34). Kidney involvement was not observed in any other patients in the cohort. Although 7/8 patients did fulfill the classification criteria for pSS, a possible misdiagnosis cannot be ruled out as differentiation between various connective tissue diseases can be difficult. Nonetheless, 3/8 SLE/MCTD patients presented with SS-like SGUS changes such as hypoechogenic areas and inhomogeneity (27), consistent with the frequency of imaging findings in this subgroup of the cohort.

In line with EGM, previous or current systemic treatment was also quite frequent, especially in patients with EGM. For the majority of patients, the current systemic treatment was hydroxychloroquine. Interestingly, only 18/40 of patients with current hydroxychloroquine treatment displayed SGUS+ changes, whereas 16/19 without current treatment showed changes. These results should however be interpreted with care, as there is both a higher proportion of patients with EGM (39/40 vs 14/19, p=0.005), current systemic treatment (37/40 vs 9/19, p=0.001) and a lower degree of SGUS+ in a subgroup of the cohort consisting of patients included in Brazil compared to Europe/USA (15/40 vs 26/27, p=0.000) and a possible confounding factor cannot be ruled out. Patients in this subgroup were also older when included, and the mean time from diagnosis to inclusion was higher.

Studying the current group of patients revealed variation in subjective symptoms, clinical findings, application of diagnostic tests/methods, and treatment regimes. More invasive diagnostic methods (such as sialoscintigraphy and sialography, compared to sialometry) and systemic treatment (Azathioprine and high dose corticosteroids/prednisone) was observed in the subgroup of the cohort consisting of patients included in Brazil, possibly due to a higher incidence of EGM.

The data was a combination of newly acquired and retrospective data. In consequence, shortcomings include lack of new blood tests and incomplete clinical data, limiting the application of AECG and ACR/EULAR classification criteria. Different ultrasonography machines and probes were used for the SGUS examination, possibly affecting the results obtained. To move forward, a prospective study with more rigorous design, a more standardized regime for data collection, and international collaborations are necessary to

characterize this rare condition and establish adequate criteria for diagnosis and classification.

In conclusion, findings in the jSS patients studied indicate an association between SGUS findings, hyposalivation and autoantibodies. In many patients, SGUS was also associated with salivary gland swelling, a previously known and common clinical finding in jSS. SGUS is an interesting diagnostic tool for identifying patients with jSS.

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REFERENCES

1. Jonsson R, Bolstad AI, Brokstad KA, Brun JG. Sjogren's syndrome--a plethora of clinical and immunological phenotypes with a complex genetic background. Annals of the New York Academy of Sciences. 2007;1108:433-47.
2. Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, et al. Classification criteria for Sjogren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Ann Rheum Dis. 2002;61(6):554-8.
3. Johnsen SJ, Brun JG, Göransson LG, Smastuen MC, Johannesen TB, Haldorsen K, et al. Risk of non-Hodgkin's lymphoma in primary Sjögren's syndrome: a population-based study. Arthritis Care Res (Hoboken). 2013;65(5):816-21.
4. Anaya JM, Ogawa N, Talal N. Sjogren's syndrome in childhood. The Journal of rheumatology. 1995;22(6):1152-8.
5. Lieberman SM. Childhood Sjogren syndrome: insights from adults and animal models. Current opinion in rheumatology. 2013;25(5):651-7.
6. Cimaz R, Casadei A, Rose C, Bartunkova J, Sediva A, Falcini F, et al. Primary Sjogren syndrome in the paediatric age: a multicentre survey. European journal of pediatrics. 2003;162(10):661-5.
7. Fang QG, Liu FY, Sun CF. Recurrent submandibular gland swelling as a first manifestation in a child with primary Sjogren syndrome. The Journal of craniofacial surgery. 2013;24(4):e413-5.
8. Nikitakis NG, Rivera H, Lariccia C, Papadimitriou JC, Sauk JJ. Primary Sjogren syndrome in childhood: report of a case and review of the literature. Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics. 2003;96(1):42-7.
9. Bogdanovic R, Basta-Jovanovic G, Putnik J, Stajic N, Paripovic A. Renal involvement in primary Sjogren syndrome of childhood: case report and literature review. Modern rheumatology. 2013;23(1):182-9.
10. Schuetz C, Prieur AM, Quartier P. Sicca syndrome and salivary gland infiltration in children with autoimmune disorders: when can we diagnose Sjogren syndrome? Clinical and experimental rheumatology. 2010;28(3):434-9.
11. Yokogawa N, Lieberman SM, Alawi F, Bout-Tabaku S, Guttenberg M, Sherry DD, et al. Comparison of labial minor salivary gland biopsies from childhood Sjogren syndrome and age-matched controls. The Journal of rheumatology. 2014;41(6):1178-82.
12. Houghton K, Malleson P, Cabral D, Petty R, Tucker L. Primary Sjogren's syndrome in children and adolescents: are proposed diagnostic criteria applicable? The Journal of rheumatology. 2005;32(11):2225-32.
13. Shiboski CH, Shiboski SC, Seror R, Criswell LA, Labetoulle M, Lietman TM, et al. 2016 American College of Rheumatology/European League Against Rheumatism classification criteria for primary Sjogren's syndrome: A consensus and data-driven methodology involving three international patient cohorts. Ann Rheum Dis. 2017;76(1):9-16.
14. De Vita S, Lorenzon G, Rossi G, Sabella M, Fossaluzza V. Salivary gland echography in primary and secondary Sjogren's syndrome. Clinical and experimental rheumatology. 1992;10(4):351-6.
15. Obinata K, Sato T, Ohmori K, Shindo M, Nakamura M. A comparison of diagnostic tools for Sjogren syndrome, with emphasis on sialography, histopathology, and ultrasonography. Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics. 2010;109(1):129-34.
16. Milic VD, Petrovic RR, Boricic IV, Marinkovic-Eric J, Radunovic GL, Jeremic PD, et al. Diagnostic value of salivary gland ultrasonographic scoring system in primary Sjogren's syndrome: a comparison with scintigraphy and biopsy. The Journal of rheumatology. 2009;36(7):1495-500.
17. Salaffi F, Argalia G, Carotti M, Giannini FB, Palombi C. Salivary gland ultrasonography in the evaluation of primary Sjögren's syndrome. Comparison with minor salivary gland biopsy. The Journal of rheumatology. 2000;27(5):1229-36.

18. Cornec D, Jousse-Joulin S, Marhadour T, Pers JO, Boisrame-Gastrin S, Renaudineau Y, et al. Salivary gland ultrasonography improves the diagnostic performance of the 2012 American College of Rheumatology classification criteria for Sjogren's syndrome. Rheumatology. 2014.
19. Carotti M, Ciapetti A, Jousse-Joulin S, Salaffi F. Ultrasonography of the salivary glands: the role of grey-scale and colour/power Doppler. Clinical and experimental rheumatology. 2014;32(1 Suppl 80):S61-70.
20. Nieto-Gonzalez JC, Monteagudo I, Bello N, Martinez-Estupinan L, Naredo E, Carreno L. Salivary gland ultrasound in children: a useful tool in the diagnosis of juvenile Sjogren's syndrome. Clinical and experimental rheumatology. 2014;32(4):578-80.
21. Civilibal M, Canpolat N, Yurt A, Kurugoglu S, Erdamar S, Bagci O, et al. A child with primary Sjogren syndrome and a review of the literature. Clinical pediatrics. 2007;46(8):738-42.
22. Lee J, Koh JH, Kwok SK, Park SH. The EULAR Sjogren's Syndrome Patient-Reported Index is an independent determinant of health-related utility values of Korean patients with primary Sjogren's syndrome. Clinical and experimental rheumatology. 2016;34(4):663-7.
23. Seror R, Bowman SJ, Brito-Zeron P, Theander E, Bootsma H, Tzioufas A, et al. EULAR Sjogren's syndrome disease activity index (ESSDAI): a user guide. RMD Open. 2015;1(1):e000022.
24. Haugen AJ, Peen E, Hulten B, Johannessen AC, Brun JG, Halse AK, et al. Estimation of the prevalence of primary Sjogren's syndrome in two age-different community-based populations using two sets of classification criteria: the Hordaland Health Study. Scandinavian journal of rheumatology. 2008;37(1):30-4.
25. Virdee S, Greenan-Barrett J, Ciurtin C. A systematic review of primary Sjogren's syndrome in male and paediatric populations. Clinical rheumatology. 2017.
26. Ramirez Sepulveda JI, Kvarnstrom M, Eriksson P, Mandl T, Norheim KB, Johnsen SJ, et al. Long-term follow-up in primary Sjogren's syndrome reveals differences in clinical presentation between female and male patients. Biology of sex differences. 2017;8(1):25.
27. Goransson LG, Haldorsen K, Brun JG, Harboe E, Jonsson MV, Skarstein K, et al. The point prevalence of clinically relevant primary Sjogren's syndrome in two Norwegian counties. Scandinavian journal of rheumatology. 2011;40(3):221-4.
28. Hammenfors DS, Brun JG, Jonsson R, Jonsson MV. Diagnostic utility of major salivary gland ultrasonography in primary Sjogren's syndrome. Clinical and experimental rheumatology. 2015;33(1):56-62.
29. Yokogawa N, Lieberman SM, Sherry DD, Vivino FB. Features of childhood Sjogren's syndrome in comparison to adult Sjogren's syndrome: considerations in establishing child-specific diagnostic criteria. Clinical and experimental rheumatology. 2016;34(2):343-51.
30. Both T, Dalm VA, van Hagen PM, van Daele PL. Reviewing primary Sjogren's syndrome: beyond the dryness - From pathophysiology to diagnosis and treatment. International journal of medical sciences. 2017;14(3):191-200.
31. Virdee S, Greenan-Barrett J, Ciurtin C. A systematic review of primary Sjogren's syndrome in male and paediatric populations. Clinical rheumatology. 2017;36(10):2225-36.
32. Bossini N, Savoldi S, Franceschini F, Mombelloni S, Baronio M, Cavazzana I, et al. Clinical and morphological features of kidney involvement in primary Sjogren's syndrome. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2001;16(12):2328-36.
33. Pertovaara M, Korpela M, Pasternack A. Factors predictive of renal involvement in patients with primary Sjogren's syndrome. Clinical nephrology. 2001;56(1):10-8.
34. Vitali C, Bombardieri S, Moutsopoulos HM, Balestrieri G, Bencivelli W, Bernstein RM, et al. Preliminary criteria for the classification of Sjogren's syndrome. Results of a prospective concerted action supported by the European Community. Arthritis Rheum. 1993;36(3):340-7.

Table 1. Patient demographics and associated diseases in all included patients (n=67) with a clinical diagnosis of juvenile Sjögren's syndrome (jSS). Values presented as mean (SD) or the number of positive observations from the number of available observations. The p-values represents comparison of SGUS+ and SGUS- patients.

	N	mean, years (SD)	SGUS-	SGUS+	p-value	Benjamin-Hochberg adjustment
Age at inclusion	67	16.3* (4.7)	17.3 (5.7)	15.6 (3.9)	0.146	ns
Age at first symptom	64#	10.2 (3.7)	10.5 (4.5)	10.1 (3.2)	0.675	ns
Age at diagnosis	61#	12.1 (3.8)	12.0 (4.5)	12.2 (3.3)	0.875	ns
Time from symptom onset to diagnosis	60#	1.8 (1.9)	1.5 (1.5)	1.9 (2.0)	0.355	ns
Time from diagnosis to inclusion	61#	4.1* (4.4)	5.5 (5.3)	3.3 (3.5)	0.062	ns
Female:male ratio	58:9		25:1	33:8	0.067	ns
Hypothyroidism	5/59 (8%)		3/25 (12%)	2/34 (6%)	0.404	ns
SLE	7/59 (12%)		4/25 (16%)	3/34 (9%)	0.400	ns
MCTD	1/59 (2%)		1/25 (4%)	0/34 (0%)	0.240	ns

SLE: Systemic Lupus Erythematosus; MCTD: Mixed Connective Tissue Disease

* Regional differences (Patients included in Brazil compared to patients included in Europe/USA)# Data not available from all patients

Table 2. Summary of subjective ocular and oral symptoms, objective signs of impaired tear secretion, salivary gland inflammation and hypofunction, serum autoantibodies and fulfilment of classification criteria in all included patients (n=67) with juvenile Sjögren's syndrome (jSS), presented as number of patients with positive findings/patients with available information. Roman numerals indicate the corresponding items of the AECG criteria. Values presented as the number of positive observations from the number of available observations, or mean (SD) when applicable. The p-values represents comparison of SGUS+ and SGUS- patients.

	N		SGUS-	SGUS+	p-value	Benjamin-Hochberg adjustment
Ocular symptoms (I)	42/67* (63%)		23/26 (88%)	19/41 (46%)	0.001	sig
Oral symptoms (II)	53/66 (80%)		18/26 (69%)	30/40 (75%)	0.607	ns
Salivary gland enlargement	*37/58[§] (64%)*		*12/24 (50%)*	*25/34 (74%)*	*0.066*	*ns*
Ocular signs (III)	33/65* (51%)		16/24 (67%)	17/41 (41%)	0.05	ns
Schirmer I-test (ever)	27/64[%] (42%)		13/25 (52%)	14/39 (36%)	0.203	ns
OSS	20/37 (54%)		12/18 (67%)	8/19 (42%)	0.134	ns
Focus score ≥ 1 (IV)	28/34 (82%)		7/8 (88%)	21/26 (81%)	0.662	ns
Mean focus score (SD)	20	1.7 (1.7)	1.0 (0)	1.9 (1.9)	0.368	ns
Salivary gland involvement (V)	41/61*[#] (67%)		21/26 (81%)	20/35 (57%)	0.052	ns
UWS ≤1.5 ml/min	*20/60[%] (33%)*		*9/26 (35%)*	*11/34 (32%)*	*0.854*	*ns*
Mean UWS ml/15 min (SD)	*60*	*4.3 (4.6)*	*5.6 (5.8)*	*3.3 (3.2)*	*0.049*	*ns*
Sialography	*1/3*[$] (33%)*		*1/2 (50%)*	*0/1 (0%)*	*0.386*	*ns*
Sialoscintigraphy	*29/31*[%] (94%)*		*20/22 (91%)*	*9/9 (100%)*	*0.350*	*ns*
SGUS	*41/67*[%%] (61%)*		-	-	-	
Autoantibodies (VI)	50/67* (75%)		14/26 (54%)	36/41 (88%)	0.002	sig
ANA	*62/67[§] (93%)*		*23/26 (88%)*	*39/41 (95%)*	*0.312*	*ns*
Anti-Ro/SSA	*50/67[π]* (75%)*		*14/26 (54%)*	*36/41* (88%)	*0.002*	*sig*
Anti- La/SSB	*27/67*[%%] (40%)*		*4/26 (15%)*	*23/41 (56%)*	*0.001*	*sig*
Anti-Ro/SSA and anti-La/SSB	*27/67*[%%] (40%)*		*4/26 (15%)*	*23/41 (56%)*	*0.001*	*sig*

Anti-Ro/SSA or anti-La/SSB	*50/67*π(75%)*	*14/26 (54%)*	*36/41 (88%)*	*0.002*	*sig*
RF	*29/64*(45%)*	*5/25 (20%)*	*24/39 (62%)*	*0.001*	*sig*
AECG criteria	35/67 (52%)	14/26 (54%)	21/41 (51%)	0.834	ns
ACR-EULAR criteria	39/67 (58%)	15/26 (58%)	24/41 (59%)	0.946	ns
ACR-EULAR and AECG	32/67 (48%)	13/26 (50%)	19/41 (46%)	0.770	ns
ACR-EULAR or AECG	42/67 (63%)	16/26 (62%)	26/41 (63%)	0.877	ns

OSS: Ocular Staining Score; AECG: American-European Consensus Group; ACR: American College of Rheumatology EULAR: European League Against Rheumatism.

* Regional differences (Patients included in Brasil compared to patients included in Europe/USA)

[§] Eight patients with SLE/MCTD, [π]seven patients with SLE/MCTD, [#] six patients with SLE/MCTD, [&] five patients with SLE/MCTD, [%] four patients with SLE/MCTD, [%%] three patients with SLE/MCTD, [$]one patient with SLE/MCTD

Table 3. Extra-glandular manifestations in the included patients with juvenile Sjögren's syndrome (jSS), presented as number of patients with positive findings/patients with available information, mean ESSPRI scores, and total and clinical ESSDAI score (SD). The p-values represents comparison of SGUS+ and SGUS- patients.

	n		SGUS-	SGUS+	p-value	Benjamin-Hochberg adjustment
Extraglandular manifestations	56/62*§ (90%)		24/25 (96%)	32/37 (86%)	0.214	ns
Constitutional	24/59*& (41%)		12/25 (48%)	12/34 (35%)	0.326	ns
Cutaneous	17/59& (29%)		8/25 (32%)	9/34 (26%)	0.643	ns
Lymphadenopathy	35/59*% (59%)		17/25 (68%)	18/34 (53%)	0.245	ns
Articular	34/59§ (58%)		17/25 (68%)	17/34 (50%)	0.167	ns
Muscular	5/59 (8%)		1/25 (4%)	4/34 (12%)	0.290	ns
Pulmonary	1/59 (2%)		1/25 (4%)	0/34 (0%)	0.240	ns
Renal	3/59% (5%)		1/25 (4%)	2/34 (6%)	0.745	ns
PNS	1/59 (2%)		1/25 (4%)	0/34 (0%)	0.240	ns
CNS	3/59 (5%)		3/25 (12%)	0/34 (0%)	0.038	ns
Hematological domain	16/58& (28%)		6/24 (25%)	10/34 (29%)	0.711	ns
Biological domain	21/58# (36%)		9/24 (38%)	12/34 (35%)	0.863	ns
Mean ESSPRI score (SD)	65	3.5 (2.5)	3.2 (2.8)	3.7 (2.3)	0.368	ns

Mean ESSPRI Dryness (SD)	65	3.4 (3.0)	3.4 (2.9)	3.5 (3.1)	0.919	ns
Mean ESSPRI Fatigue (SD)	65	4.3* (3.4)	3.8 (3.9)	4.6 (3.0)	0.314	ns
Mean ESSPRI Pain (SD)	65	2.9 (3.2)	2.4 (3.4)	3.2 (3.2)	0.362	ns
Mean total ESSDAI (SD)	62	4.7 (5.4)	3.4 (2.8)	5.5 (6.5)	0.138	ns
Mean clinical ESSDAI (SD)	59	3.2 (4.1)	2.7 (3.0)	3.6 (4.8)	0.443	ns

ESSPRI: EULAR Sjögren's Syndrome Patients Reported Index; ESSDAI: EULAR primary Sjögren's Syndrome Disease Activity Index

PNS: Peripheral nervous system; CNS: Central nervous system

* Regional differences (Patients included in Brazil compared to patients included in Europe/USA)

[§] Eight patients with SLE/MCTD, [#] six patients with SLE/MCTD, [&] five patients with SLE/MCTD, [%] three patients with SLE/MCTD

Table 4. Treatment in all included patients with juvenile Sjögren's syndrome (jSS), presented as number of patients with positive findings/patients with available information. The p-values represents comparison of SGUS+ and SGUS- patients.

	n	SGUS-	SGUS+	p-value	Benjamin-Hochberg adjustment
Current Symptomatic treatment	29/59*# (49%)	17/25 (68%)	16/34 (47%)	0.109	ns
Symptomatic treatment ever	47/59# (80%)	22/25 (88%)	25/34 (74%)	0.172	ns
Current Salivary substitutes	4/59 (7%)	1/25 (4%)	3/34 (9%)	0.466	ns
Salivary substitutes ever	5/59 (8%)	1/25 (4%)	4/34 (12%)	0.466	ns
Current Lacrimal substitutes	*25/59&* (42%)*	16/25 (64%)	9/34 (26%)	0.004	sig
Lacrimal substitutes ever	*30/59&* (51%)*	18/25 (72%)	12/34 (35%)	0.005	sig
Current NSAIDS	9/59 (15%)	1/25 (4%)	8/34 (24%)	0.039	ns
NSAIDs ever	25/59$ (42%)	9/25 (36%)	16/34 (47%)	0.396	ns
Systemic treatment ever	56/59§ (95%)	25/25 (100%)	31/34 (91%)	0.127	ns
Current systemic treatment	46/59*§ (78%)	23/25 (92%)	23/34 (68%)	0.026	ns
Hydroxycholoquine ever	*54/59§ (92%)*	25/25 (100%)	29/34 (85%)	0.045	ns
Hydroxycholoquine current	*40/59§ (68%)*	22/25 (88%)	18/34 (53%)	0.004	sig
MTX, ever	*22/57$(39%)*	9/25 (36%)	13/32 (41%)	0.722	ns
MTX, current	*14/57$ (25%)*	6/25 (24%)	8/32 (25%)	0.931	ns
Azathioprine, ever	*12/58*% (21%)*	6/25 (24%)	6/33 (18%)	0.588	ns
Azathioprine, current	*10/58% (17%)*	5/25 (20%)	5/33 (15%)	0.628	ns

Low prednisone, ever	*31/57[#] (54%)*	11/24 (46%)	20/33 (61%)	0.269	ns
Low prednisone, current	*12/57[&] (21%)*	4/24 (17%)	8/33 (24%)	0.489	ns
High prednisone, ever	*13/59*[#] (22%)*	6/25 (24%)	7/34 (21%)	0.755	ns
High prednisone, current	*5/59[%] (8%)*	2/25 (8%)	3/34 (9%)	0.911	ns

* Regional differences (Patients included in Brazil compared to patients included in Europe/USA) [§] Eight patients with SLE/MCTD, [#] six patients with SLE/MCTD, [&] five patients with SLE/MCTD, [%] four patients with SLE/MCTD, [$]one patient with SLE/MCTD

FIGURES

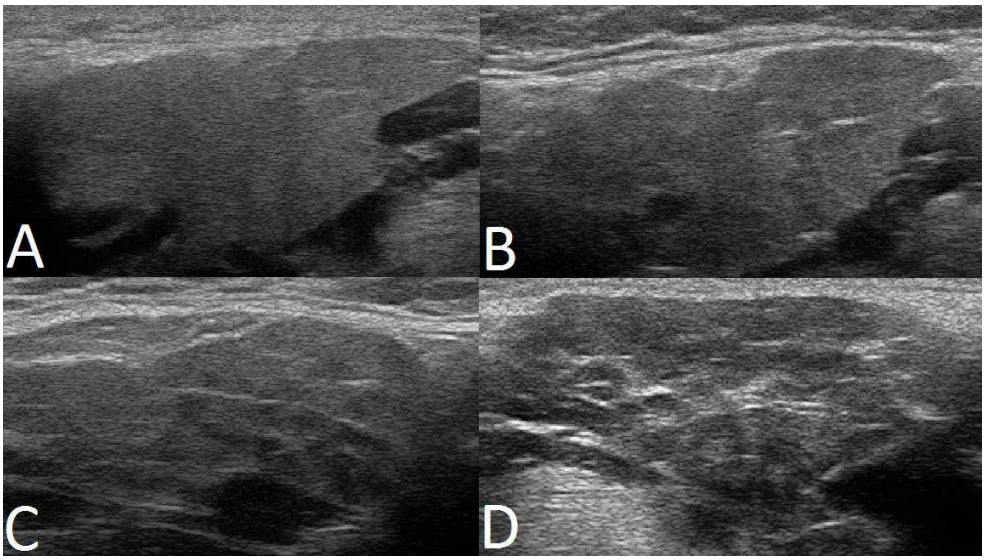

Figure 1: Representative major salivary gland ultrasonography (SGUS) images of submandibular glands illustrating grade 0 (A), grade 1 (B), grade 2 (C) and grade 3 (D), with grades 0-1 (A-B) corresponding to normal-appearing morphology, and grades 2-3 corresponding to pathological changes in the submandibular and parotid glands of patients with clinical symptoms of jSS.

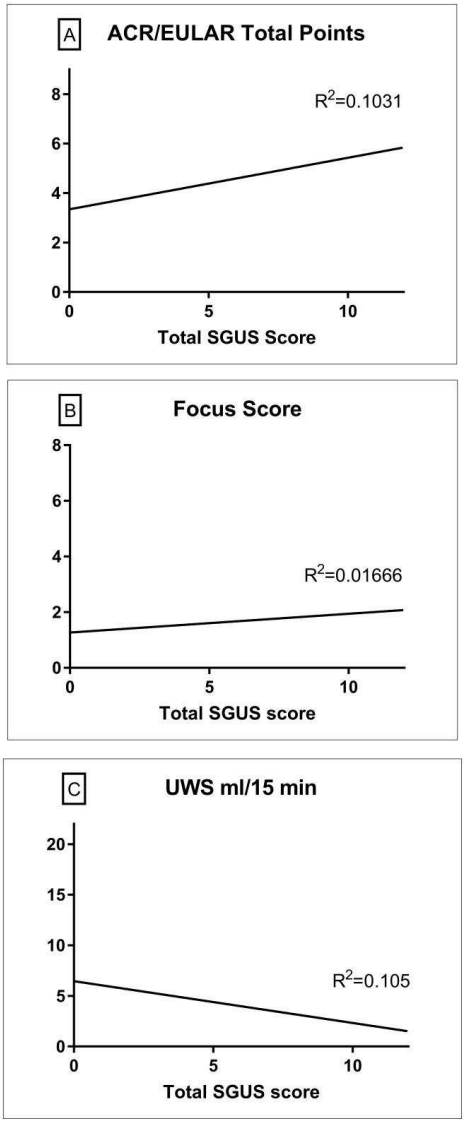

Figure 2: Total SGUS score (0-12) for all four glands correlated with the total sum of the ACR/EULAR classification criteria points (0-9) (p=0.016) (A), and unstimulated whole saliva flow (0-21.36 ml/15 min) (p=0.015) (C). The correlations were not significant after Benjamin-Hochberg adjustment.

We did not find a correlation with focus score (0-8) (B).

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