Diagnostic Performance of Serology Against Histologic Assessment to Diagnose Sjögren's Syndrome

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

Medical Sciences - Oral Medicine

University of Alberta

Abstract

Many non-communicable chronic diseases are not prevented, controlled or cured because of disease misdiagnosis or over diagnosis. In both situations, the patients will not benefit from the diagnostic process, instead, the consequences of maltreatment may harm them. For instance, Autoimmune Diseases (AD) are a major public health problem, with high prevalence and impact on individuals' general health and well-being. AD patients affected by diagnosis issues face drug side effects, unnecessary tests, extra personal and public costs, with obvious significant impact on the health system. Sjögren's syndrome (SS) is a good example of an AD, chronic and worldwide prevalent misdiagnosed/undiagnosed disease, for which the diagnostic criteria are still a matter of debate, with many patients going untested and not receiving a confirmed diagnosis. SS is characterized primarily by dry mucous membranes, mainly in oral and ocular membranes, due to the decrease or absence of glandular secretions (Sicca syndrome). SS can also present with extra-glandular involvement, with cutaneous, pulmonary, musculoskeletal, renal, or neurological manifestations. SS shows an estimated prevalence of 1% and a 9:1 female predominance. It is more commonly seen between the ages of 45 to 75. SS patients present a lower quality of life, sometimes similar to that observed in other rheumatic diseases, such as rheumatoid arthritis and systemic lupus. The oral component of SS shows glandular dysfunction (hyposalivation) and sensation of dryness (Xerostomia). A dry mouth is at a higher risk of periodontal disease and caries, in unusual locations including root and incisal surfaces. The oral soft tissues also are affected by the condition, sometimes with burning sensation on the tongue or on the oral mucosa, and different forms of candidiasis and mucositis.

The complexity of the clinical approach demands an accurate SS diagnosis. American College of Rheumatology and the European League Against Rheumatism (ACR/EULAR) new

diagnostic criteria indicate that either salivary gland (SG) biopsy or anti-SSA must be positive, corroborating their central role in the diagnostic process. However, some authors and centres have suggested that the SG biopsy could be circumvented, and that only clinical and serological features are required to define the diagnosis Subtle changes in the ACR/EULAR criteria have been explored in an attempt to improve accuracy, such as increasing the threshold score from 4 to 5 out of 9 possible points. Nevertheless, in the clinical setting, some rheumatologists remain reluctant to always accept the objective numerical score and subjective "expert opinion" or "clinical diagnosis" continues to often guide treatment decisions. Well-designed research in the diagnostic accuracy in SS is needed, in order to improve the diagnostic process.

We performed an accuracy study of the main items of the current SS diagnostic criteria in a sample of the public health system in Alberta, Canada We compared the performance of the two principal items in the ACR/EULAR criteria, anti-SSA and histopathology of labial minor salivary gland biopsy. Our analysis was approached using the standard ACR/EULAR criteria but also using a separate modification of the ACR/EULAR criteria, the latter incorporating a consideration of the clinical diagnosis and a higher anti-SSA threshold. Our results show that anti-SSA can be the main test in SS diagnostic process, but salivary gland biopsy is useful in many cases, due to its great specificity. Nevertheless, fine adjustments in both serological and histological assessments can improve the diagnostic performance of both items observed in the SS ACR/EULAR criteria.

Keywords: Sjögren's syndrome, Rheumatologic diseases, Salivary glands diseases, Sicca syndrome, accuracy study, Anti-SSA, serology, Focus Score.

Preface

This thesis is an original work by Luiz Claudio Viegas Costa. The research project “Diagnostic performance of serology against the histologic assessment to diagnose Sjogren's syndrome”, of which this thesis is a part, received research ethics approval number Pro00094361from the University of Alberta Research Ethics Board on February 12, 2020.

The research protocol was elaborated by myself, with the assistance of Dr. W.T. McGaw. The data collection and analysis are my original work, as well as the literature review in chapter 2. The statistical analysis, in chapter 4, was done by me, under the supervision of Dr. H. Lai. Chapter 5 of this thesis presents a published peer-reviewed article as “Viegas-Costa LC, Friesen R, Flores-Mir C, McGaw T. Diagnostic performance of serology against histologic assessment to diagnose Sjogren's syndrome: a systematic review. Clinical rheumatology. 2021.” I was responsible for the conception, data collection and analysis as well as the manuscript composition. Dr. W.T. McGaw assisted with the data collection and all co-authors contributed to manuscript edits.

Aos meus pais, meu filho Thales e minha amada Michelle. Vocês nunca deixaram de me apoiar em todas as necessidades, carências e momentos de dificuldade. O carinho e amor que sempre me deram tem sido a propulsão que movem meus projetos. Dedico esta conquista a vocês, de todo meu coração, e com a minha total gratidão. Muito obrigado!

Acknowledgments

I want to thank Dr. McGaw, who was the first person to believe in me and opened the doors of the UofA, at the very beginning. You have been much more than a supervisor, instructor and professor. I consider you a friend that life has kindly provided to me. I have no words to describe my gratitude.

My deepest appreciation to my research committee members, Dr. Lai and Dr. Friesen, as well as to Dr. Gibson, for all the contributions you've provided to me and to this project, in general. Much obliged!

I also want to thank Dr. Ivonne Hernández, who also trusted in me from the beginning and for so many times supported me and my projects. Thank you for everything!

My appreciation to all my instructors and professors is simply enormous. You have been essential for my training and I'll take all the lessons and learnings to the rest of my life.

I want to say thanks to my colleagues and the staff at the University of Alberta. My life became much happier and brighter with your presence in it. Thanks so much!

My special thanks to Michelle, for the help in the hard task of English editing. I hope you had at least a little fun, even though I know it's not easy for whom is not in the field. Anyhow, thanks so very much.

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List of abbreviations

µ3R: µ3-muscarinic receptor
µ3R: muscarinic 3 acetylcholine receptor
ACA: antiphospholipid antibodies anticentromere
Ach: acetylcholine
ACR/EULAR: American College of Rheumatology and the European League Against Rheumatism
AI: autoimmune
ANA: antinuclear antibodies
ANCA: anti-neutrophil cytoplasmic antibodies
Anti-CA II: Anti-carbonic anhydrase II antibodies
APCs: antigen presenting cells
AUC: Area under the (ROC) curve
BAFF: B-cell activating factor
BAFFR: BAFF receptor
BCR: B-cell receptor
BLK: B lymphoid tyrosine kinase
CGRP: calcitonin gene-related peptide
CHRM3: cholinergic muscarinic receptor
CMV: cytomegalovirus
COP: cryptogenic organizing pneumonia
CXCL13: CXC ligand 13 protein
DC: dendritic cells
DES: dry eye syndrome
DHEA: dehydroepiandrosterone
DLBCL: diffuse large B-cell lymphoma
DMARDs: disease-modifying antirheumatic drugs
EBV: Epstein–Barr virus
ECM: extracellular matrix
EDA-A2: ectodysplasin
EGF: Epidermal growth factor
ENAs: extractable nuclear antigens
ER: estrogen receptor
ESSDAI: EULAR SS disease activity index
ESSPRI: EULAR Sjögren's Syndrome Patient Reported Index
EWASs: Epigenome-Wide association studies
fDC: follicular DC
FLS: focal lymphocytic sialadenitis
FS: focus score
GCs: germinal centres
GWAS: genome-wide association studies
HLA: Human Leukocyte Antigen
HRQoL: health-related quality of live
HTLV-1: human T-lymphotropic virus type-1
ICAM: intercellular adhesion molecule

ICOS: Inducible T-cell COStimulator
iDC: interdigitating DCs
IFN: interferon
IFNAR: IFN-α receptor
IFNGR: IFN-γ receptor
IHC: immunohistochemistry
IL: interleukin
ILD: Interstitial lung disease
IP3: inositol 1,4,5-triphosphate
IRF5: interferon regulatory factor 5
KCS: keratoconjunctivitis Sicca
LFA: lymphocyte function associated antigen
LFU: lacrimal functional unit
LIP: lymphocytic interstitial pneumonitis
LMSG: labial minor salivary glands
MALT: mucosal-associated lymphoid tissue
MCP: metacarpophalangeal
MFU: mean fluorescence units
MGD: Meibomian gland dysfunction
MHC: Major Histocompatibility Complex
miRNAs: micro-RNAs
MMPs: matrix metalloproteinases
Mø: macrophages
MRI: magnetic resonance imaging
MS: Multiple sclerosis
ncRNAs: non-coding RNAs
NK: Natural killer
NPV: Negative Predictive Value
NPY: neuropeptide Y
NSAIDs: non-steroidal anti-inflammatory drugs
OAS-1: 2′-5′ oligoadenylate synthetase 1
OSDI: ocular surface disease index
PBMC: peripheral blood mononuclear cells
pDCs: plasmocytoid dendritic cells
PIP: proximal interphalangeal
PPV: Positive Predictive Value
pSS: primary Sjögren’s syndrome
RF: Rheumatoid factor
RNP: Ribonucleoprotein
ROC: Receiver operating characteristic
SAD: systemic autoimmune diseases
SARD: Systemic autoimmune rheumatic diseases
SGEC salivary gland epithelial cells
SLE: systemic lupus erythematosus
SNPs: Single nucleotide polymorphisms
SS: Sjögren's syndrome

SSc: systemic sclerosis
STAT4: signal transducer and activator of transcription 4
Tfh: T follicular helper cells
Tfr: T follicular regulatory cells
TLRs: Toll-like-receptors
TLS: tertiary or ectopic lymphoid structures
TNF: tumor necrosis factor
TNFAIP3: tumor necrosis factor-alpha-induced protein 3
TNFSF4: tumor necrosis factor super family member 4
TNIP1: TNFAIP3-interacting protein
Tph: peripheral-helper T-cells
UIP: usual interstitial pneumonitis
US: ultrasound
VCAM: vascular cell adhesion molecule
VD: Vitamin D
VIP: vasointestinal peptide

Chapter 1 - Introduction

The results of patient-oriented research can be translated into clinical decisions that increase the effectiveness of health care. Although patient-oriented research and evidence-based medicine has been publicized during last decades, evidence based-decision making is still a difficult task and a complex issue for many clinicians (1, 2). Many patients are symptomatic for years with misdiagnoses before the clinician is able to distinguish overlapping clinical and laboratory features accurately (3). Proper and timely diagnosis is an essential part of the process of clinical decision-making in evidence-informed health care, following clear clinical guidelines (1, 2).

Many non-communicable chronic diseases, including autoimmune (AI) conditions, have multifactorial etiology and are not effectively prevented, controlled or cured because of disease misdiagnosis or over-diagnosis. In both situations, the patient will not benefit from the diagnosis, instead, they may also be harmed because of the consequences of the maltreatment. In fact, the patients can be burdened with drug side effects, unnecessary paraclinical tests, such as serology and histopathology, over- and under-utilization of health care, and its associated personal and collective costs (4-8).

Sjögren's syndrome (SS) is a chronic, AI disease characterized primarily by dry mucous membranes, mainly of the oral (xerostomia/hyposalivation) and in the ocular (xerophthalmia/hypolachrymation) membranes, due to the decrease or absence of glandular secretions (Sicca syndrome). SS can also present with extra-glandular involvement, with musculoskeletal, cutaneous, renal, pulmonary, or neurological manifestations. SS is a good example of a worldwide prevalent misdiagnosed/undiagnosed disease, for which the question of diagnosis criteria is still a matter of debate, with many patients going untested and not receiving a confirmed diagnosis (9-12).

The etiology of SS is unknown, but genetic, immunological, environmental and hormonal factors are thought to participate in the inflammatory process affecting the exocrine glands, generating tissue dysfunction and destruction (13-15). Glandular hyposecretion is the final result of

both innate and adaptive immune system interactions, with infiltration of lymphocytes and activation of plasma cells in the affected glands. A plethora of autoantibodies and soluble inflammatory mediators is involved in the initiation and perpetuation of the immune response (15, 16).

Consideration of epidemiological aspects of SS are essential for understanding the burden to the society and the health system, as well as for shedding light on etiology. Globally, SS has an estimated prevalence of 1% (0.1 – 4.8%). Like most AI diseases, SS shows a female predominance, with a female:male ratio of about 9:1. It is more commonly seen between the ages of 45 to 75, but a peak incidence is in the 40–55 year age group. It is noticeable that the improvement of diagnosis processes - and the increased awareness of the disease have demonstrated an expansion of the demographics of SS, which have included women of all racial and ethnic backgrounds; more men; and even well documented cases in children (10, 12, 17-19).

Autoimmune diseases are one of the major public health problems because of their high prevalence and their impact on individuals' general health and well-being, as well as their cost burden of the health system. Different studies have documented poor quality of life in SS patients compared to healthy controls, sometimes similar to that observed in other rheumatic diseases such as rheumatoid arthritis, fibromyalgia and even compared to systemic lupus. Ocular and oral pathological conditions, pruritus, chronic fatigue and pain, sleep and sexual disorders, autonomic dysfunction, higher risk of adverse maternal and neonatal outcomes, psychological disorders, and general physical function impairments are the most impacting factors in SS patients' quality of life (12, 20, 21).

Patients who have keratoconjunctivitis Sicca (KCS) feel a recurrent gritty-burning sensation due to the persistent dry eyes, and commonly report the feeling of sand in the eyes. These features can be associated with redness, photophobia, and fluctuating blurry vision exacerbated by prolonged visual effort or a low-humidity environment. Other severe vision-threatening ophthalmic complications include corneal melts, uveitis, scleritis, and optic neuritis (10, 15, 16).

The oral component of the SS is characterized by a sensation of dryness accompanied by thirst. Patients may initially note intermittent daily or nocturnal dryness that gradually becomes more prominent during the day. Xerostomia affects 90% of patients with SS, with patients reporting

difficulties upon talking for a prolonged time and while chewing dry food. Dry mouth can also be accompanied by changes in the voice, which can become hoarse and/or weak. Swallowing may also be difficult. Sometimes the sharpness of taste can be diminished or changed (hypogeusia / dysgeusia), which may or may not be associated with the alteration of smell (dysosmia). When there is dryness, there is a higher possibility of developing caries, in unusual locations including root and incisal surfaces, and periodontal disease (10, 15). The soft tissues of the oral cavity are affected by the condition present in the SS, which can lead to a burning sensation of the tongue (glossodynia) or of different areas of the oral mucosa (stomatopyrosis). Chronic erythematous candidiasis is commonly observed as angular cheilitis, atrophy of the filiform papillae and erythema of the tongue or oral mucosa. Pseudomembranous candidiasis occurs less frequently (10).

It is vitally important that a diagnosis is made early to avoid long-term local and systemic complications that threaten the patient's overall health. The establishment of effective communication between dentists, rheumatologists, family physicians, ophthalmologists, ENT specialists and pathologists is the key to optimal care for the patient with SS (15).

With such complex framework, treatment targeting the etiology of this disease is lacking, and supportive treatment is currently given to alleviate the symptoms of dryness, rather than addressing the underlying disease process. Although immunomodulatory drugs are available for treatment, SS has to be definitively diagnosed if these are to be used, otherwise a personal burden, as well as to the whole healthcare system, can occur (11, 12, 22).

According to the literature, one of the characteristics of autoimmunity in SS is the presence of autoantibodies. The majority (85%) of the patients with SS present antinuclear antibodies (ANA) in the serum. Rheumatoid factor (RF) is also observed in between 50 to 70% of patients, irrespective of whether it is primary or secondary SS. Patients with suspected SS who present with positive ANA should be investigated for anti-SSA and anti-SSB antibodies (16, 23).

Although there are several tests available to aid in the diagnosis of SS, none of them have shown a high enough sensitivity and specificity to be used alone. This therefore necessitates a comprehensive assessment of multiple parameters including a careful examination of the eyes, measurement of tear and saliva production, as well as the clinical and histologic examination of the salivary glands. Histologic examination of the salivary glands aims to determine the presence of an

inflammatory infiltrate, which plays an integral role in the diagnosis of SS. This last examination, in the opinion of some experts, plays an integral role in the diagnosis of primary SS and is the main diagnostic criterion, as long as its implementation and interpretation are adequate (10, 14, 15, 24). The biopsy of minor salivary glands, especially labial minor salivary glands, is performed on an outpatient basis and allows differentiation between age-related glandular involution, and other pathologic conditions that infiltrate salivary glands such as SS, sarcoidosis, and even neoplasms. Its diagnostic yield depends on both adequate gland sampling and appropriate analysis of the biopsy sample. The main histological finding in SS is focal lymphocytic sialadenitis, defined as the presence of one or more dense aggregates of 50 or more lymphocytes, located in periacinar or periductal distribution. The focus score (FS) is assigned by evaluating the number of infiltrates per glandular area of four mm2. A focus score ≥ 1.0 is deemed to support a diagnosis of SS. (10, 14, 15, 24, 25).

Regarding the correlation between the auto-antibodies and histological features, some studies have demonstrated an association between seropositivity for anti-SSA (26) and for both anti-SSA/SSB and FS (25, 27-29). However, there are inconsistencies in the literature, with some studies reporting showing more significant lymphocytic infiltration in anti-SSA/SSB patients versus those only positive for anti-SSA, or in patients negative for both antibodies (30). Also, even if a consistent correlation could be confirmed between FS and serological results, some studies have concluded that labial salivary gland biopsy remains useful in clinical practice due to a higher sensibility, specificity, positive and negative predictive values for diagnosis of primary SS (31-34).

In order to determine the procedures for diagnosing SS, new criteria set has been approved by the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR), indicating that either salivary gland biopsy or anti-SSA in order to confirm diagnosis of SS (35). However, some authors and centres have suggested that the labial minor salivary glands (LMSG) biopsy could be circumvented, and only clinical and serological features are required to define the diagnosis of SS.

The purpose of this research was to assess the diagnostic performance of the two main items of the 2016 SS ACR/EULAR criteria, and upon that, validate and / or suggest amendments in order to improve the overall accuracy of the current guidelines.

Chapter 2 – Literature Review and State of the Art

2.1 – Historical facts of Sjögren's syndrome

From the late 19th century eighteen hundreds to the beginning of the 20th century, several patients with xerostomia (dry mouth) and xerophthalmia (dry eyes) had been described. In 1888, Mikulics reported a case of a 42-year-old man who presented with an increase in the size of the salivary glands in which an infiltration of lymphocytes was present in the salivary gland biopsy, without concurrent lymphadenopathy. Since then, there have been other reports of the association between salivary glands dysfunction and enlargement, sometimes with other organs affected. However, in 1926, Henri Gougerot first described these features as part of a syndromic condition (36-40). Subsequently, the extensive research, done from 1933 to 1955 by Henrik Sjögren, described and classified this complex and intricate disease. Dr. Sjögren's extensive pathologic studies, and his recognition that SS was not only a localized condition but a generalized systemic disorder, led to the disease being named after him. made Dr. Sjögren worldwide appreciated, and the disease was named after him. However, it is important to highlight that Dr. Sjögren was not who first described SS, but the first to comprehensively characterize it in a larger group of patients. He also discerned SS from other causes of keratoconjunctivitis Sicca, such as TB and vitamin-A deficiency (10, 40-42).

In the following decades, several studies demonstrated different aspects of the condition, including clinical manifestations and classification of SS, serological aspects, diagnostic methods and association to other autoimmune diseases (43-45). Subsequently, the up to 44 times increased risk of developing lymphoma compared with the general population was firstly cited in the literature, after identifying a subgroup of SS patients with marked lymphoid reactivity (46).

Interestingly, the clinical management also has a long history, with a medicinal remedy first encountered in the Amazon Forest, the Jaborandi. It was brought to Paris in 1875, by a Brazilian physician - Symphronio Coutinho, who observed a significant salivation increase upon chewing its leaves. In one of the initial case discussions about the oral and ocular dryness, it was demonstrated that Jaborandi provided relief to a patient with xerostomia and, about a century later, the drug

component (pilocarpine) was approved for clinical use (40, 47). Likewise, a traditional quinin remedy from South American plants, known for treating Malaria, was introduced in Europe for the treatment of SS and Lupus Erythematosus in the end of the 19th

century. By the middle of the 20th century, the drugs Chloroquine and Hydroxychloroquine were synthesized and accepted for SS management due to their parasympathetic effects to improve salivary and tear production during early stages of the disease (47). During the 1970's there were great advances in the understanding of the etiopathogenesis of SS and the many associated immunological abnormalities, comprising hypergammaglobulinemia, monoclonal gammopathy and lymphoid pathologies, such as lymphomas. In addition, a novel approach using LMSG biopsies as a main diagnostic tool was developed. New immunomodulation and suppressive therapies, such as corticosteroids, were incorporated into the management of SS. Also, the association with HLA-B8 and DR3 was established, and the first animal models for SS were elaborated, opening new windows for research and treatment (10). The understanding of the disease was further refined in the 1980's, with immunological studies demonstrating the role of abnormal B cells and T cells of the adaptive immune system in generating autoreactive antibodies and direct cytotoxicity. Other developments during the 1980's included the recognition of a correlation between SS and chronic fatigue; new protocols with cholinergic agents used to increase salivary gland flow; and the association of SS with anti-SSA and anti-SSB antibodies. The new millennium has heralded further studies of SS that have clarified the role of other cell types, including the salivary gland epithelial cells (SGEC); improved understanding of the involvement of the innate immune system; and an appreciation of the contribution of genetic factors. Also, the improvement of animal models and novel therapeutic trials have led to the development of new classes of drugs, named "biological immunosuppressant agents" (such as Rituximab and Belimumab) and their incorporation into the treatment of SS, especially cases of SS complicated by the development of lymphoma (10, 47).

Although there have been successive efforts to create worldwide consensual classification criteria for SS, the variety of phenotypes; the complexity of clinical presentations; and the array of diagnostic approaches, have complicated the task of elaborating a unified classification of SS and unified diagnostic criteria. Since the late 1970s, until the beginning of the 21st century, the discussion about these criteria remains an open field. A significant advance in consensus was

achieved when both the European and American Rheumatology societies unified their diagnostic criteria in 2016 (35, 48, 49).

2.2 – Epidemiology

Understanding the incidence of a disease, which is a measure of the risk of developing new cases of the disease in a period of time, and the prevalence of a disease, which measures the proportion of a population observed to have the disease, is fundamental to describing the burden of the disease and better understanding its etiology. In the case of published SS research, several sets of diagnostic criteria have been proposed around the world in different times and this creates special challenges in the accurate analysis of SS epidemiology. This is further complicated by inconsistencies in case-finding and case-ascertainment approaches and differences in the populations under study. Some of the published SS studies are characterized by selection bias and misclassification bias, making the data interpretation more difficult. Further refinement of the diagnosis and reporting of SS is essential not only for a better epidemiologic picture of the disease, but also to optimized the individual and collective approach to the disease, meaning adequate diagnosis and treatment, and better health policy making processes (17, 50).

The pooled Incidence Rate (IR) of SS has been reported to be 6.57 per 100 000 person-years in Asia; 3.9 to 5.3 in Europe; and ~4 in the U.S.A. (where SS is considered the second most common autoimmune rheumatic disease after rheumatoid arthritis (10, 12, 17).

The pooled Prevalence Rate (PR) of SS has been reported to be 71.22 per 100 000 inhabitants in Europe; 44.85 per 100, 000 inhabitants in Asia; and in South America a higher PR of 170. Worldwide, SS presents an estimated prevalence of 1% of adult population, ranging from 0.1 to 4.8% (10, 17).

Even though an accurate picture of the incidence and prevalence of SS in Canada is not available, a nationwide estimation about systemic autoimmune rheumatic diseases (SARD), comprising complex autoantibody-associated chronic inflammatory disorders, was performed in 7/10 Canadian provinces, including Alberta between 1993 and 2007. The conditions analyzed were systemic lupus erythematosus (SLE), systemic sclerosis (SSc), primary Sjögren's syndrome (pSS), and polymyositis-dermatomyositis. As a whole, these conditions presented a greater prevalence in urban-versus-rural areas, with an overall prevalence of between 2–5 cases per 1000 Canadians, which is very consistent with the general prevalence of North American data, more specifically for SLE, SSc, pSS, and inflammatory myopathies. In this study, a higher prevalence in females vs males

and with older age has been observed, and the occurrence of SARD in older women approached or exceeded 1 in 100. This is particularly observed in the prevalence of primary SS, affecting up to 1% of older women (51).

Like most autoimmune diseases, SS shows a female predominance, with a female/male ratio of about 9:1, with this ratio being up to 14:1. SS is more commonly seen between the ages of 45 to 75, but a peak incidence is in the 40–55 year age group. The overall prevalence of SS in the elderly population seems to be five to eight times higher than in young adults, depending on the age used to define those groups. Current estimations suggest that ~4 million Americans suffer from SS and that, in 50% of these individuals, SS occurs in association with another autoimmune disease (10). It is notable that the improvement of diagnosis processes, and the increased awareness of the disease, have expanded the demographics of SS to include women of all racial and ethnic backgrounds, more men, and even well documented cases of SS in children (10, 12, 17, 50).

SS is rare in children, with a mean age of 9.84 years (range 9.5–10.7 years), and a female to male ratio ranging from 5-8:1 (19, 50). Within the pediatric population, SS affects parotid gland more commonly despite being recognised that, with the exception of mumps parotitis, all other causes of parotitis in children are rare. Other clinical features of pediatric SS, such as constitutional symptoms and lymphadenopathy, and less frequent occurrence of oral and ocular dryness, which could potentially explains the more frequent use of less conservative diagnostic methods in children suspected of possible SS, including scintigraphy, sialometry and biopsy in children, making the pediatric diagnostic process more arduous (19).

2.3 – Sjögren's syndrome: Burden of illness and psychosocial impact

Autoimmune diseases are one of the most significant public health problems because of their high prevalence and their impact on individuals' general health and well-being, as well as their cost burden on the health system. Ocular and oral pathological conditions, pruritus, chronic fatigue and pain, sleep and sexual disorders, autonomic dysfunction, higher risk of maternal and neonatal outcomes, psychological disorders, and general physical function impairments are the most impacting factors in SS patients' quality of life (12, 20, 21).

SS is associated with substantial direct and indirect costs. Among the direct costs, substantial resources are devoted to SS diagnosis, treatment and rehabilitation. In the U.K., the mean annual total direct cost per SS patient has been comparable to those of patients with rheumatoid arthritis (£2188 vs. £2693) and were estimated to be more than twice the annual health care costs for community controls (£949) (12, 20, 38). The same can be observed if oral healthcare is taken into account, costing three times more than healthy patients, due to significantly increased number of dental visits, more decayed teeth and more dental restorations. Oral lesions (erythema, ulceration), burning symptoms, and fungal infections are also common reasons for seeking out oral healthcare, and these additional oral manifestations represent an important source of physical and psychological distress (20, 38, 52).

Regarding annual indirect costs, these include loss of economic productivity related to the impact of SS, including labour but also other activities such as housework and childcare. The estimated loss of economic productivity for SS patients in the U.K. is over £7677, which is eight times greater than seen in the control sample (20, 38). In Canada, SS patients will suffer an additional $4,357 to $5,554 in loss of economic productivity each year, mainly from employment absenteeism (53).

2.4 – Etiology and pathogenesis of Sjögren's syndrome: still a gap in knowledge

SS etiology is incompletely understood and it is unlikely that a single etiological agent, gene or a simple combined mechanism could explain various aspects of this erratic disease, wherein two major biologic events characterize the autoimmune nature of this condition: 1): the periepithelial lymphocytic infiltration of the affected tissues; and 2): the B-cell hyperactivity. These different biological events lead to the loss of the immune balance and the extensive infiltration of the exocrine glands, generating tissue dysfunction and destruction. Incessant activation, defective regulation, or inherent defects of the immune system may all participate (13-15, 54, 55). Apparently, SGECs also play an important role, suggesting that SS could be considered an "autoimmune epithelitis". In addition to immune mechanisms, several non-immune factors may be involved in pathogenesis of SS glandular hyposecretion (54, 55).

Identification of an etiological agent still seems elusive, though there is some evidence for role of virus infection in lymphoproliferation. Wider gene studies highlighted the role played by the so-called interferon (IFN) pathway, NFkB pathway, Th subsets and B cell stimulation, even if the odds ratios of these genetic associations might be low. This may argue for a greater role of environmental and epigenetic factors in SS pathogenesis. The final effect is the production of a plethora of autoantibodies and soluble inflammatory mediators, which are involved in the initiation of the disease and perpetuation of the immune response (15, 16, 54). In summary, three steps seem to be involved in the development of SS – some environmental trigger factor or factors acting upon a particular genetic background generate an exacerbated autoimmune response, influenced by aberrant immune regulatory mechanisms, leading to a chronic inflammatory process which causes lymphoepithelial lesion and tissue damage (55).

2.4.1 – Environmental and possible etiologic factors

Autoimmune diseases can, theoretically, be triggered by environmental factors, such as infections, when the genetic background predisposes an individual. In SS, a possible role has been attributed to viruses, suggesting an antigen driven response, due to the restricted clonality of T cell receptor repertoire and B cell hypermutation. Autoantigens, such as Ro52, Ro60 (SSA) and even complexes of La (SSB) are induced by IFNs, usually produced at high level during viral infection, and an autoimmune response can be triggered by exaggerated autoantigen expression in tissues. The

hypothesis of the primary tissue injury being triggered by a viral infection of the lacrimal and salivary glands has been present in the literature for decades. It is known that viral antigens are frequently recognized by Toll-like-receptors (TLRs), triggering IFN production, upregulation of adhesion molecules and apoptosis. Epstein–Barr virus (EBV), KS-virus (HHV-8), cytomegalovirus (CMV), hepatitis-C virus, human T-lymphotropic virus type-1 (HTLV-1), and Coxsackie virus have been implicated in the etiopathogenesis of SS. However, whether there are actual etiological factors, or confounding factors instead, remains as a question to be answered, given the common presence in humans, sometimes innocuously, of those viruses (10, 54).

EBV, for instance, is a pervasive virus that causes chronic infection and is believed to induce autoimmunity. EBV has relative B cell tropism causing chronic lymphoproliferation and has also been prominently found in saliva and in salivary gland and lacrimal gland biopsies in SS patients, as compared to controls. Studies have indicated a possible role of chronic infection of salivary epithelium in the pathogenesis of SS, but a causality role could not yet be demonstrated (16, 54).

Studies from both endemic and non-endemic regions have shown some possible role of HTLV-1 in SS pathogenesis, with antibodies to HTLV-1 detected in patients with SS. Also, studies with HTLV-1 tax transgenic mice revealed SS-like aspects of autoimmune exocrine glands disease. On the other hand, in Asian Indian patients these correlations could not be confirmed, casting doubt on a causal association of this virus to SS (54).

The possible activation of autoimmune responses by a cross reaction with antibodies to major epitopes of Ro60 kD autoantigen of SS has been cited, and a homologous peptide of Coxsackie virus 2B protein was found to be capable of that effect. Coxsackie 2B viral RNA has been demonstrated in salivary gland biopsied tissue from SS patients. Nevertheless, these findings have not shown solid evidence hitherto (54).

Other viruses, such as hepatitis-C virus, which is associated with initiating SS like symptoms upon infection, and CMV, which has caused glandular chronic T-cells inflammation that continued even after the infection clearance, and high anti-Ro titers in a mouse model, points out an additional possible etiology for SS, but this has not been consistently demonstrated. SS has shown a robust IFN signature, and IFN-α secreted by plasmocytoid dendritic cells (pDCs) in response to viral agonists in salivary tissues in SS indeed supports the theory of viruses as an environmental trigger

for its onset. Nonetheless, the existence of a definite model of viral etiology for SS is still an open question (10, 16, 54).

Lastly, researchers have also focused on the role of intestinal microbiota in SS etiopathogenesis. Even though the literature is limited, it has demonstrated that when compared with healthy individuals, SS patients have higher abundances of Pseudobutyrivibrio, Escherichia/Shigella, Blautia and Streptococcus, and decreased numbers of Bacteroides, Parabacteroides, Faecalibacterium and Prevotella. Moreover, studies have suggested that dysbiotic intestinal microbiome, determined by reduced profusion of commensal bacteria and an augmented abundance of pathogenetic genera, is a hallmark of SS. An association of the severity of ocular and systemic manifestations with an inversely proportional microbial diversity has also been suggested, but not conclusively (16).

2.4.2 – Genetics and pathogenesis of SS

SS is considered a complex genetic disorder, but its heritability and relative genetic risk is unknown. The low number of twin studies in SS, usually case reports and small studies, do not provide strong evidence but show that the predictable concordance rate for SS is low. Similarly, the sibling prevalence is low, suggesting that the heritability of SS is low and environmental factors might be more significant. Even though familial aggregation of different autoimmune diseases has been observed in SS, the percentages are not very high (30–35%), mostly among first degree relatives with autoimmune thyroid disease, Multiple sclerosis (MS), RA and SLE, with well-established genetic associations for dozens of genetic loci (38, 54, 55).

Understanding of the role of genetics in SS has advanced with the advent of genome-wide association studies (GWAS), which offer the ability to simultaneously screen hundreds of thousands of regions of DNA to identify loci associated with a certain disease phenotype. In SS, two GWASs involving European and Han Chinese populations analyzed the Major Histocompatibility Complex (MHC), located in the short arm of chromosome 6 (6p21). MHC contains genes whose proteins participate in the immune response, mainly components of the human leukocyte antigen - HLA, which present both exogenous and endogenous antigens to T-lymphocytes and are highly known as a risk factor for the development of autoimmune diseases. These genes are organized into two categories HLA class I (A, B, C), HLA class II (DR, DQ, DP), these two are mainly associated with

autoimmunity, and HLA class III (complement, TNF, etc.). The major genetic contribution to SS is from the human leukocyte antigen (HLA) region, particularly the HLA-DR3. Those studies have confirmed previously recognized associations with HLA-DR and -DQ alleles as well as established strong associations with multiple new genetic risk loci (9, 10, 36, 54, 56). The association of HLA alleles with SS is not specific, and the ancestral haplotype 8.1 (HLA-A1, -B8, -DR3, -DQ2) is also related to other autoimmune diseases such as Type-1 Diabetes mellitus, celiac disease, SLE, myasthenia gravis, Addison's disease, among others (36). Susceptibility to SS is influenced by genes outside the MHC locus. Single nucleotide polymorphisms (SNPs) have been identified in other risk genes, such as those involved in IFN production, lymphocyte migration, cytokine and cytokine receptor functions and other intracellular signaling pathways, both in innate and adaptive immune responses. The modest odds ratios for these associations suggest that dysregulation of multiple pathways, involving numerous SS risk variants in multiple immune cell subsets, is necessary to precipitate the disease (9, 10).

It is fundamental to understand that numerous interactions between diverse inflammatory pathways have been implicated in the pathogenesis of SS, and these processes sustain the inflammatory status characteristic of SS (9) In addition to the plethora of genetic and environmental factors, Epigenetic changes, which are stable and inherited alterations in gene function that do not implicate a change in DNA sequence, have been identified in SS. These modifications generally comprise posttranslational modifications of amino acids on the amino-terminal tail of histones, covalent modifications of DNA bases and non-coding RNAs (ncRNAs) (56).

The importance of Epigenetics has been demonstrated in the pathophysiology of different autoimmune diseases by monitoring gene expression during the cell cycle, in response to environmental or biological variations, which partly explains their trigger mechanisms in autoimmune conditions. Epigenetic processes lead to modifications that can silence regions of the genome by impeding melting processes, thus influencing the etiological complexity of SS. Three main key epigenetic points are recognized: histone adjustments, DNA methylation and interference RNA (microRNA, lncRNA). The most studied epigenetic mechanisms in SS are the DNA methylation and interference RNA (54, 57) Advanced approaches, such as Epigenome-Wide association studies (EWASs), have shown hypomethylation at IFN-induced genes, detected in diverse cell types and shown to be more evident in patients with particularly high levels of anti-SSA

and/or anti-SSB antibodies. In general, DNA methylation reflects the epigenetic status at a certain time point that might correlate with disease activity or specific disease manifestations, and also reflects a connection between SS susceptibility loci and epigenetic regulation. Also, studies involving ncRNAs have been performed, mainly with miRNAs. These are a class of evolutionary highly conserved 19-25 nucleotides single-stranded RNA molecules that can bind to target mRNA transcripts and interfere with translation. These have been demonstrated in many autoimmune conditions, including SS (54, 56).

Upregulated expression of miR-146a/b in peripheral blood mononuclear cells (PBMC) from SS patients has been consistently found in several studies, suggesting that the mechanism for miR-146-dependent regulation of immune responses is via negative feedback mechanisms targeting TLR signalling. Thus, dysregulated miR-146 expression may promote excess inflammation, leading to autoimmune responses. Even though the literature is scarce, some studies point out a correlation of clinical manifestations, laboratory parameters and even the risk of lymphoma development with excessive miRNA expression (54, 56).

Genetic and epigenetic research reveals the complexity of SS and the array of the disease mechanisms underlying the diverse clinical manifestations. A deeper assessment of the epigenetics in SS is out of the scope of this review. Nevertheless, genetic and epigenetic mechanisms contribute to switch-on and switch-off the expression of genes related to inflammatory pathways, which could be targets to explore in future studies of biomarkers and potential management approaches in SS (54, 56).

2.4.2.1 - Antigen presentation and epithelial cell function

The genetic predisposition to SS is driven by the MHC region that encodes the HLA proteins, and associations have been identified with different Class-II alleles. These associations differ by population and serological status, but -DR2 and -DR3 alleles at HLA-DRB1 have consistently been found in Caucasian SS populations (10, 54-56). SNPs in the transcription factor interferon regulatory factor 5 (IRF5), in the signal transducer and activator of transcription 4 (STAT4), and BLK genetic loci, as well as in cholinergic muscarinic receptor (CHRM3) and in novel susceptible loci at IL-12A and CXCR5 regions have been reproducibly associated with SS. It is important to highlight that IRF5 is activated by TLR ligation and promotes the production of IFN-α and

proinflammatory cytokines. Associations with HLA-DRB1/HLA-DQA1 and HLA-DPB1 have been established in Asian SS patients. In a Chinese specific SS population, GTF2IRD1-GTF2I has been demonstrated as a new genetic risk factor for SS. In both European and Asian SS population studies, the associations with TNFAIP3 and TNIP3 implicate a role for NFkB signalling in SS (10, 54, 56). When serological findings are taken into consideration, studies have demonstrated remarkably strong associations of anti-SSA and/or anti-SSB production with DRB1*03 and DQB1*02 alleles or with heterozygosity for DQw1 and DQw2 alleles (10). Curiously, SS patients with high levels of both anti-SSA and anti-SSB antibodies have a very high probability of being HLA DR3 DQ2 positive, whereas SS patients who have high levels of the anti-SSA only and are negative for anti-SSB antibodies have an increased occurrence of DR2 and DQ6 (38).

Another interesting finding is that an MHC-linked locus that encompasses the Class I polypeptide related sequence A (MICA) gene has been identified as an associated SS risk, expressed in epithelial cells. MICA encodes a stress-induced glycoprotein recognized by the natural killer group 2D (NKG2D) receptor expressed on numerous subsets of CD4+T, CD8+T and natural killer (NK) cells. The risk allele is associated with augmented levels of stress-inducible MICA protein in serum and may contribute to mutual pathogenic pathways involved in reduced protection against cancer and autoimmunity (10).

2.4.2.2 - Interferon responses and other innate immunity factors

Innate immunity in SS begins with the activation of IFN pathways, followed by cytokine and chemokine production by monocytes/macrophages (Mø) and dendritic cells (DC). Then, these antigen presenting cells (APCs) present antigen to lymphocytes, which initiates adaptive responses that ultimately lead to autoantibody production. Microarray studies have consistently shown an Interferon signature in peripheral blood and in salivary gland of individuals with SS, which is a hallmark of innate immunity dysregulation. Type I IFN is produced by all nucleated cells, and type II IFN (IFN-γ) which is produced by activated T and natural killer cells, are differentially expressed in SS glandular tissue and have marked pathogenetic roles. SS patients show augmented expression of type 1 and type 2 IFN regulated genes, which are involved in innate immune response in both peripheral blood and salivary tissue (9, 10, 16, 54, 55). A mechanistic link between antibody responses and the IFN signature seen in tissues can be postulated, based upon the augmentation of

type I IFN–producing pDCs in SS tissues and also the evidence that immune complexes containing self-antigens can stimulate type I IFN production by pDCs via TLR ligation (9). Further evidence for association with genes involved in transcription of IFN-inducible genes has been reported in SS, remarkably including IRF-5, STAT- 4, interleukin 12A (IL-12A) and 2′-5′ oligoadenylate synthetase 1 (OAS-1). The products of these genes have been strongly associated with high disease activity, higher anti-SSA and anti-SSB serological titres and B-cell activating factor (BAFF) gene expression as well, the last being crucial for B cell maturation. BAFF is usually produced by monocytes, Mø and DC, and is crucial to the crosstalk between innate immunity and stimulation of autoreactive B cells (10, 54-56).

Indeed, IFN will serve as the link between innate and adaptive immune responses in SS. Both IFN-α and INF-γ activate and mediate T and B lymphocytic infiltration of salivary glands, induce expression of MHC and costimulatory molecules on SGEC, stimulate BAFF, as well as promote apoptosis. Thus, BAFF protein is produced by T and B cells, as well as by SGEC, and its levels in serum of SS patients are highly related with disease activity and titre of circulating autoantibodies (10, 54, 56). In addition, IFNs have the ability to promote B-cell differentiation and antibody production, enhance T-cell survival, and induce the maturation of antigen- presenting cells. IFN-γ can also drive the formation of ectopic germinal centres (GCs). If left uncontrolled, the physiologic effects of IFN may drive the immune dysregulation seen in SS (9). INFγ is mainly produced by T cells and Natural killer (NK) cells, and production of IFN-γ along with IL-12, an immunomodulatory cytokine primarily secreted by DCs and monocytes, causes further differentiation of CD4+ T cells into Th1 lymphocytes. Also, mice model studies have demonstrated the role played by IFN-γ in pre-immune phase the development of SS and increased acinar cell apoptosis, hyposalivation and abnormal salivary protein expression (10, 54).

NK lymphocytes play an essential role in innate immunity and have also been involved in SS pathogenesis. Based on animal models of sialadenitis, a gene of a natural killer (NK)-specific activating receptor that controls the cross talk between NK cell and DC, directly influencing type-II IFN secretion (NCR3/NKp30), was studied and it showed an association of SS with promoter SNPs. Furthermore, the distribution of NK cells was similar to that of DC in minor salivary glands. Moreover, this distribution revealed a positive correlation with the FS in LMSG biopsy specimens. Interaction between NKp30 and its ligand, B7- H6 is crucial for NK-DC and NK-SGEC cross talk,

and it was noticed that NK cells from SS patients expressed higher amount of the NK cell activating receptor, NCR3/NKp30, and that both DCs and SGEC present B7-H6. Upon binding SGEC via the ligand B7-H6, NK cells also release INF-γ, which has a key role in salivary gland dysfunction in SS as mentioned above. These findings support a potential role for NK cells through promoting an NKp30-dependent inflammatory state in salivary glands (10, 54, 57).

2.4.2.3 - T and B - Lymphocyte functions

Several genes acting in adaptive immunity, predominantly in T and B cells, and the consequent dysregulation of adaptive immune responses noticeably contributes to the pathogenesis of SS. This is seen in the characteristic histological finding, which is the infiltration of lymphoid cells, predominantly T cells and also B cells. It is important, though, to mention that the salivary gland infiltrates' composition varies from mild, where T-cells and DCs predominate, to severe lesions, where typically B-cells and Mø tend to be more present (10, 54).

SS has been considered to be a Th-1 predominant disease, but with an important participation of Th- 17 pathway as well. The robust association of SS with specific MHC alleles implies a significant role of T cells in the development of the disease. The majority of infiltrating T cells are CD4+, and T-cell infiltrates can be oligoclonal, which is suggestive of an antigen-driven immune response. A recent genetic association of SS with a gene relevant to T cell functions, the tumor necrosis factor super family member 4 (TNFSF4/OX40L), has been demonstrated. Multiple cells express TNFSF4 on their surface, including pDCs, B cells, NK cells, and endothelial cells. The interaction TNFSF4-TNFSF4/OX40L drives the Th1 T cell response and production of Type I IFN. This ligand is involved in signal transduction leading to T cell proliferation and cytokine production, ultimately inhibiting production of regulatory T cells that produce IL-10. Studies have shown that IL-10 participates in maintenance of peripheral tolerance and can inhibit the development of autoimmune disease. It has been shown that clinically inactive SS patients have an increased frequency of IL-10 producing B cells (9, 55, 58). The role of B and T lymphocytes, according to the cytokine environment and consequent type of immune response (Th1, Th2, and Th17) can be extensively reviewed in the literature (55).

T-cell functions are driven by Th17 pathway as well, and proinflammatory IL-17–expressing CD4+ T cells or Th17 cells have been identified in SS glands (9). Studies have shown that STAT4

is activated by type I IFN, interleukin-12 (IL-12), and IL-23, and stimulates the production of IFN-γ and IL-17. The IRF5 and STAT4 risk alleles have an additive effect on susceptibility to SS, thus highlighting a potential for IFN- or type 17 helper T cell (Th17)–mediated pathogenesis. Increased IL-17 expression has been detected in plasma, as well as its mRNA and protein expression in LMSG biopsies, correlating with FS and formation of germinal centres. Further evidence for a significant role of Th17 immune response in SS pathogenesis was the increased levels of IL-6 and IL-23 in blood and in the salivary tissue, as well as IL-22, a cytokine downstream of IL-23 in the Th17 pathway, overexpression in LMSG of SS patients. It has been shown that IL-22 acting along with IL-17 elicit an intense inflammatory response in SS, and serum levels of the later was correlated with salivary hypofunction, as well as with anti-SSA, anti-SSB and RF serology in SS (9, 54).

It has been demonstrated that T follicular helper cells (Tfh) cells have an important role in B cell activation and formation of tertiary or ectopic lymphoid structures (TLS). These lymphoid aggregates of T and B cells, formed in non-lymphoid organs in response to chronic inflammation, have been shown to be associated with worse disease progression in SS. Apparently, the formation and growth of TLS seem to be dependent on IL-22 signalling, in absence of IL-27. In spite of the fact that SS patients express IL-27 locally, it is unable to inhibit Th17 differentiation, leading to a significant INF-γ response. In vitro research has also pointed that IL- 17 and IL-22 might to play a major role in the epithelial-to-mesenchymal shift in SGEC. This inflammatory milieu that prompts and sustains TLS in SS salivary glands has been associated with salivary gland fibrosis in SS. This process might also drive the epithelial to mesenchymal transition and be involved in premature aging of SGSC, which will ultimately decrease the regenerative capacity of the gland and contribute to the development of hypofunction of the gland (54, 59). Hence, Tfh cells facilitate T cell-dependent B cell responses in GC-like structures, mainly by secretion of other Th17 related key cytokines, such as IL-21 and IL-6. These 2 cytokines drive the differentiation of activated B cells towards plasma cells. Tfh cells, and the recently described pathogenic peripheral-helper T-cells (Tph), are the main source of IL-21, expression of which was demonstrated in LMSG biopsies and in serum of SS patients. The levels of this cytokine were also correlated with serum immunoglobulin levels and lymphocytic infiltration, further supporting the role of T cells, especially Th17, in the pathogenesis of SS (9, 54, 59).

Studies have shown that SGEC also induce differentiation of T helper cells into Tfh cells by secretion of IL6 and Inducible T-cell COStimulator (ICOS) ligand (54). Remarkably, the blockade of ICOS decreased the production of IL-21, IL-6, IL-8 and TNF-α in ex-vivo studies. Conversely, a Tfh cell regulatory counterpart, the T follicular regulatory cells (Tfr) cells, exerts an immunosuppressive effect on Tfh and B cell proliferation and activation in secondary lymphoid tissues. A possible mechanism to counteract the inflammation is seen in a higher number of Tfr cells seen in SS LMSG, which was inversely correlated to their presence in peripheral blood. However, when the cytokine milieu favours Th17, the balance is tipped in favour of Th17 cells, increasing inflammation. This variation in the presence of different sub-sets of CD4+ T cells has been correlated to different histological features, such as the severity of the glandular lesions, FS, inflammation grade and certain risk factors for lymphoma development (54, 55, 59, 60). In addition, Tfr and Tfh cell ratio was altered in blood and salivary gland biopsies of SS patients, when compared with healthy controls, suggesting an important role of this imbalance between pro-inflammatory and immunoregulatory pathways in the pathogenesis of SS. Once Tfh cells that are CXCR5 positive prevail and secrete IL21, B cell migration, proliferation and maturation is enhanced. This is an important step towards lymphoid follicle formation and ectopic germinal centre formation in salivary gland of SS patients. CXCR5, which is located on B cells as well as Tfh, is the receptor for the chemokine CXC ligand 13 protein (CXCL13), also known as B-cell-attracting chemokine-1 or B-lymphocyte chemoattractant. CXCL13 guides B cell entry into the follicles, and the lymphoid organisation seen in LSMG biopsies of SS is credited to CXCL13 acting along with CXCR5 (54, 59).

Indeed, the importance of B cell disturbances and hyperactivity in SS is demonstrated by the distinctive immunological features of SS. These are represented by hypergammaglobulinemia, cryoglobulinemia, circulating immune complexes seen in small vessel vasculitis in some patients, the plethora of autoantibodies, along with the altered distribution of peripheral B cell subpopulations, the oligoclonal B cell expansion, and GC formation. These structures present distinct T- and B-cell zones, follicular dendritic cell networks, and high endothelial venules typical of secondary lymphoid tissues (9, 54, 55). At this point, IgG- and IgM-producing plasma cells predominate, while IgA is the main type found in normal salivary glands. This B cell infiltration escalates with an increase in severity of inflammation in the LMSG, and ectopic germinal centres are seen in the LMSG biopsies of up to a quarter of SS patients. Increased apoptotic activity as well

as antibody production are remarkable in germinal centers, and their presence is risk factor for lymphomagenesis. Their presence suggests that local salivary gland antigens drive the hyperactive B-cell response and formation of high-affinity autoantibodies. Activation-induced cytidine deaminase, an enzyme required for Ig heavy-chain class-switch recombination and affinity maturation, is expressed in ectopic GC-like structures, corroborating that the glandular tissues are generating autoantibodies (9, 54, 55, 59).

As mentioned previously, B cell abnormalities are seen in both salivary gland tissues and peripheral blood of SS patients, and the distribution of peripheral blood B cell subsets is helpful in distinguishing SS from other rheumatic conditions. For instance, activated naive and CD27- memory B-cells cells are over-represented in the peripheral blood of SS patients, whereas a decreased number CD27+ memory B-cells is observed. Another observation is that higher plasma cells counts in peripheral blood were noted in SS patients with higher FS (54). Another finding, corroborating the central role of B-cells, is the elevated salivary and serum levels of CXCL13 in patients with SS, which may become a biomarker for the stage of the condition. As mentioned above, the result from CXCL13 acting together with CXCR5 is the lymphoid reaction appreciated in LMSG biopsies of SS, and its blockade by a neutralising monoclonal antibody revokes inflammation in salivary glands. Targeting the CXCR5-CXCL13 axis is a promising novel therapeutic approach in SS (54, 56).

Furthermore, B cell hyperactivity, maturation, class switching, survival and proliferation especially in advanced disease is directly influenced by abnormalities of BAFF. As previously shown, it is induced by IFN, and those effects are more pronounced in autoreactive B cells, even though it is also produced by SGEC, DC, Mø, and activated T cells. BAFF is expressed in large amounts by B and T cells infiltrating SS salivary glands and its levels are increased in SS serum and saliva and correlate with autoantibody titres, as well as with the lymphocytic infiltration and presence of GC-like structures (9, 54).

The genetic background involved in B cell function is quite vast and has been slowly unveiled. Changes in expression levels of Early B-cell factor 1 (EBF1), a key factor involved in enhancement of transcriptional activity during B cell development, can lead to impairment of this process, and interfere with Ig production as well. B cell function in SS is also thought to be affected

by a dual transcriptional control in the region comprising two genes, FAM167A and the B lymphoid tyrosine kinase (BLK) locus. FAM167A and BLK are transcribed possibly from common promoter elements, but in opposite directions, and expression levels are inversely correlated. The function of FAM167A remains unclear but may have a potential role in pulmonary involvement. BLK is expressed and plays a major role in B cell signaling that results in activation of multiple nuclear transcription factors. Reduced expression of BLK is hypothesized to lead to a breakdown in tolerance by allowing autoreactive cells to escape deletion. These polymorphisms predispose also SLE, RA, and Systemic Sclerosis (10, 36, 55).

The phenotypic observations described above can be correlated to genetic findings, such as upregulated BAFF gene expression in labial salivary gland tissue. Disease susceptibility for anti-SSA- and anti- SB positive SS has been associated with the CTAT haplotype of 4 SNPs located in the 5' regulatory region of the BAFF gene, while the TTTT haplotype has been associated with elevated BAFF levels in SS. In addition, dysregulated expression of the coding region of CXCR5 has been correlated with decreased expression of CXCR5 in peripheral blood B cells, low numbers of CXCR5+ peripheral blood B cells, and gathering of CXCR5+ B cells in LMSG. Also, two genes that regulate the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) protein complex have been associated with SS. Chronically active NF-kB signaling has a central role in rapid regulation of transcriptional responses to injury and is seen in numerous inflammatory diseases. Other risk variants have been identified in the form of tumor necrosis factor-alpha-induced protein 3 (TNFAIP3) which, in cooperation with TNFAIP3-interacting protein (TNIP1), suppresses TLR-induced apoptosis by negatively regulating NF-kB signaling in SS. These processes are unclear but likely operate through pathogenic alterations in TNF-induced apoptosis, TLR activation, or cytokine production, and some of those factors have been associated with increased risk of lymphoma in SS (10, 36, 55). In summary, among the susceptibility factors for the development of autoimmune diseases and especially SS, the HLA alleles are mainly DRB1 * 03: 01, DQA1 * 05: 01, DQB1 * 02: 01. The presence of variants in the IFR5, STAT4, CXCR5, TNPI1, IL12A, BLK and PTPN22 genes have shown the greatest association with an increased risk of development of SS. The complexity of genotypic and phenotypic interactions is reflected in the pathogenesis, physiopathology and clinical presentations of SS (9, 36).

2.4.2.4 – Role of Salivary gland epithelial cell

Hyposalivation and hypolacrimation are the striking clinical manifestations of the exocrine gland pathology in SS. Cholinergic and muscarinic receptors, and numerous other molecules, such as the vasointestinal peptide (VIP) or neuropeptide Y (NPY), are located in the exocrine glands. Unmyelinated afferent nerve fibers provide signals from the lacrimatory and salivatory nuclei of the midbrain to the exocrine glands. Salivary production and secretion, for instance, involves neuronal signals from CNS, which affect the epithelial and stromal cells. The interaction of the neurotransmitters with their receptors and other neurotransmitters located on the surface of these cells, and the subsequent interaction of many proteins, causes cytosolic levels of calcium to increase in acinar cells. This activates ion channels that generate an osmotic gradient and drives the inflow of water through specific channels (10, 55). In the past, SGEC were seen as passive spectators, or even victims of the complex inflammatory process in SS. Nonetheless, regardless of the obvious role for inflammation in SS pathophysiology, the fact that sometimes a low glandular inflammation is observed, despite a concurrent significant loss of gland activity, demonstrates a low level of correlation between salivary flow and tissue infiltration/damage. Early intrinsic epithelial activation and the subsequent altered glandular homeostasis seem to precede lymphocytic infiltration in SS, showing that SGEC are active players in SS pathogenesis, giving rise to the theory of "autoimmune epithelitis". This brings up the idea that, since the primary autoantigens in SS are pervasive proteins but are targeted specifically in the exocrine glands, it is possible that the tissues supply the antigens and play an active role in driving the disease process (9, 54, 55).

SGEC express many of the molecules for antigen presentation and immune activation, including MHC class I and II, releasing pro-inflammatory cytokines, chemokines, and co-stimulatory molecules, such as B7, PD-L1, CD80, CD86, intercellular adhesion molecule (ICAM) -1, vascular cell adhesion molecule (VCAM) -1 and CD40, enhancing their interaction with immune cells (55).The functional expression of immunoreactive molecules indicates that SGEC are likely able to mediate the presentation of antigenic peptides and the transmission of activation signals to T-cells. Another aspect to be analyzed is that, serving as a physical barrier is one of the key functions of the salivary epithelia, and loss of tight junction function is also connected to the development of chronic immune conditions. Loss of barrier function, besides leading to introduction of neoantigens, also causes redistribution of the apical and basolateral cell components, disrupting the salivary

glands' secretory machinery. This can be initiated as a response to cytokines such as tumor necrosis factor (TNF) and IFN, and studies of the inflamed salivary gland tissues of SS patients have indicated that the ductal and acinar SGEC display a number of other immunoreactive molecules that are involved in homing, activation, differentiation, and proliferation of immune cells. SGEC also show salivary gland antigens, salivary gland protein-1, carbonic anhydrase-6 and parotid secretory protein, as well as an aberrant redistribution of the Ro/SSA and La/SSB autoantigens in the cytoplasm, even before clinical disease (9, 10, 54, 55).

The interaction between glandular epithelia and the immune system is complex and, overall, SGEC can produce cytokines that are crucial for both innate and adaptive immunities. This includes IFNs and other cytokines involved in Th1, Th17, T follicular helper cell response and B cell stimulation. When compared to healthy controls, patients with SS present increased mRNA expression in SGEC of IL-1, IL-6 and TNF-α, as well as IL-7, IL-18, adiponectin, BAFF and IL-22, which are crucial cytokines for adaptive immune response and T and B cell activation. Of these, IL-18 and IL-7 regulate the Th1 response, and IL-22 has been reported in SS SGEC, with one of its crucial receptors (IL22-R1) was found expressed exclusively by epithelial cells (54, 55).

Another important aspect of the interplay between SGEC and immune system cells is the production of chemokines such as CXCL13, CCL17, CCL19, CCL21 and CCL22 by SGEC, resulting in DC infiltration. Secondarily, CXCL10 and CXCL9 contribute to homing of T cells into salivary gland from blood and CXCL13 directs B cell migration into salivary gland and further formation of lymphoid structures within the salivary gland (54).

In addition, the constitutive expression of functional Toll-like receptors (TLRs; TLR-1, TLR-2, TLR-3, TLR-4, TLR-7, and TLR-9) and CD91 molecules by cultured salivary gland epithelial cells suggests that they are involved in the initiation of local innate immunity. Cytokine production resulting from TLR3 ligation could be mediated by IRF and NFkB (nuclear factor kappa-light-chain-enhancer of activated B cells) pathways. Overall, TLR signalling in SGEC can also upregulate MHC, costimulatory molecules and adhesion molecules, CD54/ICAM-1, CD40, and CD95/Fas proteins expression, thus linking the innate and adaptive immune responses, and promoting apoptosis in SGEC. The tissue damage and epithelial cell destruction and/or dysfunction in SS are caused by different mechanisms, including the cytotoxic effect of T cells or autoantibodies,

as seen above, and the cell matrix degradation. Cell–matrix interactions are crucial for cellular functions of the SGEC, including response to growth factor signals, proliferation, and ability for cellular regeneration. Proteases, such as matrix metalloproteinases (MMPs) and lysosomal cysteine proteinases (cathepsins) are key during normal embryonic development of glandular tissue, and they also mediate cellular migration and differentiation, via the controlled degradation of extracellular matrix (ECM). However, destruction and atrophy of exocrine tissues are also caused by persisting action of MMPs and collagenases, resulting in Sicca symptoms. SGEC in SS patients overexpress a family of specific receptors including integrins to these matrix proteins. Furthermore, another key factor for the decreased glandular production is the induction of apoptosis. Both the Fas/Fas-Ligand (FasL) pathway, and the cytotoxic effect of proteases, such as perforin and granzymes, and/or cytokines, such as IFNγ, are implicated in SS glandular lesions (36, 54, 55).

Other mechanisms, in addition to those mentioned above, have been involved in the pathogenesis of SS. For instance, a new molecule - ectodysplasin (EDA-A2) has been shown to trigger apoptosis of SGEC through caspase activation. Also, stress in the endoplasmic reticulum, which mediates the development of autophagy and apoptosis, acts as an inducer of apoptosis that leads to the expression on the cell surface, and in apoptotic bodies, of Ro/SSA and La/SSB autoantigen and this has been to contribute to further immune reaction and inflammatory response (36).

Finally, in addition to apoptosis, epithelial cells seem to participate in autoantigen release via an alternative pathway for the presentation of intracellular self-components to the immune system. This pathway involves small vesicles (30–100 nm) of endosomal origin, called exosomes. Exosomes, which are distinct from apoptotic bodies, participate in physiological processes such as the exclusion of obsolete proteins and membranes, the exchange of cellular material, and intercellular communication. Exosomes are thought to represent an extracellular mechanism for the transfer of antigens to antigen-presenting cells and the stimulation or inactivation of T cells, directly or indirectly by the transfer of antigens to DC (54, 55).

Even though the research is less copious about the role of the DC, both pDCs and classic DCs participate in SS pathogenesis. Important alterations include the periductal presence of mature and activated DC, generating significant stimulation of the LT cells, resulting from the constant

activation by the SGEC. Furthermore, the pDCs, which are predominantly IFNα-producing cells, are present in the lymphocytic foci of salivary glands in patients with SS but not in healthy controls. SGEC self-nucleic acids released during apoptosis and viral nucleic acids are recognised by TLR 7 and TLR 9 of the pDCs, resulting in IFN production. The clustering of DCs around epithelial cells also has been noted in salivary gland of SS patients. Decreased peripheral blood levels of pDC and some other types of DCs could also suggest augmented migration of these cells from blood to salivary gland. Interestingly, DC infiltrates are mainly observed in early stages of the disease, suggesting that DC and innate immune response are mostly involved in the induction and initial phases of SS, but not in the maintenance and long-term consequences (36, 54).

2.4.3 – Other etiopathogenetic aspects

As noted, a model that explains the physiopathology of SS, based on the alteration of the SGEC by apoptosis and inflammation as explained above, does not cover the neurogenic processes involved in salivary productions and secretion. Exocrine secretions depend on the parasympathetic nervous system, mediated by acetylcholine (ACh) action on the muscarinic receptor type 3, and the subsequent activation of inositol 1,4,5-triphosphate (IP3) and increased intracytoplasmic calcium (Ca2 +), as mentioned previously. Apparently, not only do SS patients have less functional SGEC, but their functions seem to be affected as well. Studies demonstrate that SS isolated acinar cells present a reduced sensitivity to muscarinic stimulation and that, in SS, aquaporin-5 has an abnormal distribution in SGEC, instead of the apical region. This alteration has also been induced by autoantibodies directed against the anti-M3 muscarinic receptor. The interplay between the immune system and neurogenic secretory processes that lead to glandular hypofunction can be summarized as (36, 61):

1. Inhibition of the release of Cytokine-induced ACh

2. Increase in degradation of ACh due to an increase in cholinesterase

3. Muscarinic receptor blockade by anti-M3 antibodies

4. Altered synthesis of nitric oxide

5. Disturbance of the release of calcium

6. Altered distribution of aquaporin 5

These mechanisms have important implications for better understand the pathophysiology of SS (Figure 1) and for the development of new treatments for SS.

Figure 1: “This figure was removed because of copyright restrictions. It depicted the alteration of normal glandular function in SS” Original source: Fox RI. Sjögren's syndrome. Lancet (London, England). 2005;366(9482):321-31.

A discussion of etiopathogenesis of SS is incomplete without reviewing the role of the sex hormones and of chromosome X, as well as the influence of Vitamin D (VD) on immunomodulation. Taking into consideration the robust female predominance seen in SS, mostly in the peri/post-menopausal period of life, it appears that both androgens and estrogen seem to protect SGECs from apoptosis. Studies have shown that the estrogen receptor (ER) and its mRNA have been detected in the salivary glands, showing the effect of estrogens in maintaining exocrine secretions. Deficiency of estrogens induces the over-expression of the retinoblastoma-associated protein 48 (RbAP48), a transcription factor that causes apoptosis of SGECs, promotes lymphocytic infiltrates in glands exocrine and promotes autoantibody formation (anti-Ro, anti-La, anti-α fodrin). Additional evidence for the role of estrogens is observed in mice deficient in aromatase, a necessary enzyme for estrogen production. These animals developed significant B cell hyperplasia. Other mouse models have shown that estrogen suppresses the development of SS, whereas ovariectomy leads to a condition similar to SS. Furthermore, estrogen supplementation has been shown to regulate T-cell recruitment in salivary glands and even prevent cell apoptosis in lacrimal glands, as well as preventing the development of sialadenitis (36, 54, 55). These results indicate that long-term estrogen deficiency may cause autoimmune exocrinopathy, but further investigation is needed to obtain a more precise view of human disease.

Interestingly, some studies of the role of sex hormones in SS pathogenesis suggest that is a change in the androgen/estrogen ratio, rather than actual lower levels of estrogen, that is the most significant factor in SS risk. r. Defective androgen influence could result in impaired extracellular matrix remodelling and acinar atrophy in SS patients as compared to healthy controls, according to SGEC culture studies. Also, when compared to healthy controls, both serum and salivary levels of

dehydroepiandrosterone (DHEA), testosterone and dihydrotestosterone were found to be low in SS patients. Converting DHEA to testosterone, but not dihydrotestosterone, in salivary glands is due to the expression and abnormal localization of key enzymes in steroidogenesis. The local and systemic androgen deficiency hypothesis is also supported by the demonstration of decreased serum and salivary levels of DHEA metabolites in patients with SS. All these findings help to explain why women are particularly vulnerable to developing SS, since the local production of dihydrotestosterone is completely dependent upon the local conversion of DHEA, while in men, naturally- produced systemic androgens can meet the requirements from the local environment (36, 54).

Other potential explanations for the remarkable gender disparity in SS would include a role for the number of X chromosomes, which influence the likelihood of developing SS, possibly through a gene dosage mechanism. It has been shown that men with Klinefelter´s syndrome (47, XXY) have a similar risk of developing SS as normal 46, XX women. In addition, another genetic alteration (47, XXX) has been found in excess among women with SS. Moreover, the estimated prevalence of SS in women with 47, XXX is approximately 2.9 times higher than that in those with 46, XX, while SS is extremely rare in f Turner syndrome (45, X). The identification of structural chromosomal aberrations resulting in partial triplications of the X chromosome (Xp11.4:pter), in a few patients with SS, indicates that dosage-sensitive risk genes may be situated within this chromosomal interval. Numerous other genes with a role in immunological processes serve as interesting candidates, however the causal gene(s) that mediate the female predominance in SS are thus far unclear (10, 54, 56).

Finally, vitamin D (VD) apparently plays a role in the transcription of 913 genes, controlling up to 3% of the endocrine genome, as well as a role in immunomodulation of SS. The physiological role of VD in calcium and phosphorus metabolism and in glandular secretion is well appreciated. Also, its receptor (VD-R) acts in the production of predominantly anti-inflammatory cytokines in both the innate and acquired immune system, contributing to immunologic tolerance towards self-antigens. Overall, VD inhibits pro-inflammatory processes by suppressing the activity of lymphocyte subtypes of T-CD4 + (Th1, Th2 and Th17) and causing a decrease in the production of inflammatory cytokines IL-2, IFN-γ and TNF-α. On the other hand, studies have shown that VD deficiency, and most likely dysfunction of VD-R, increase the activity of various autoimmune

diseases, such as SS, rheumatoid arthritis and systemic sclerosis. VD deficiency, dysfunction of the VD-R, or both, can modify the course of clinical activity of SS. Although there is some evidence that lower serum levels of VD alter the clinical and para-clinical behaviour, well designed prospective studies are necessary to prove the influence of VD in SS prevalence and whether any change in VD levels could decrease the intensity of the disease (36).

A summarized overview of SS etiopathogenesis is shown in the figure 2 (9).

Figure 2: “This figure was removed because of copyright restrictions. It depicted the summarized proposed model of SS pathogenesis” Original source: Hochberg MC. Rheumatology. Seventh edition. ed. Philadelphia, PA: Elsevier, Inc.; 2019. 1837 p – Figure 147.12

2.5 – Clinical evaluation and diagnosis

Considering the complexity of SS, its diagnosis depends on both subjective symptoms and objective clinical, serological and histological findings, and typically requires a multidisciplinary approach including rheumatologists, ophthalmologists, family physicians and dentists. The clinical features can vary significantly from mild to severe Sicca symptoms as an isolated phenomenon (primary SS - pSS) or in conjunction with another AI disease (secondary SS - sSS), especially systemic lupus erythematosus (15–36%), rheumatoid arthritis (20–32%), or systemic sclerosis (11–24%) (10, 15). This clinical heterogeneity could be explained by the multifactorial etiopathogenesis, as discussed previously.

As a consequence of such heterogeneity of signs and symptoms, estimated disease incidence and prevalence can vary significantly depending on the classification criteria and diagnostic criteria applied. This can also result in misdiagnosis or delayed diagnosis of SS. In this chapter, we will approach SS clinical spectrum, which ranges from a local benign exocrine condition to a non-exocrine multi-system disease, including severe organ involvement and the development of malignancy, which could lead to fatal outcome in some patients (9, 10, 15, 16, 36, 62).

Overall, between 80% to 96% of SS patients present with Sicca symptoms, mostly dry eyes followed by dry mouth, describing them as a burning or foreign body sensation, itching, inability to tear, photophobia, mild visual disturbances, increased need of drinking water, difficulties chewing dry food, and speech difficulties. The remaining 14-20% of SS patients will experience minimal Sicca symptoms and considered atypical cases. Therefore, even in the absence of classic Sicca syndrome, an SS diagnosis should still be considered when the patient presents inflammatory joint and muscle pain, chronic fatigue, swollen salivary glands, demyelinating disease, Raynaud phenomenon, esophageal dysfunction, neuropathies or abnormal lab values (12, 55, 62).

It is crucial to keep in mind the broad differential diagnosis of Sicca symptoms. This includes the impact of a wide variety of drugs associated with anticholinergic effects (antihypertensives, antidepressants, muscle relaxants, sedative/hypnotic agents, opioid analgesics, antihistamines, etc.) Differential diagnosis also includes hepatitis C, HIV, Vitamin A deficiency, SOX syndrome (sialadenitis, osteoarthritis, xerostomia), and IgG4 syndrome, all of which can mimic some of the SS signs and symptoms (12).

2.5.1 – Ophthalmic manifestations and management

2.5.1.1 – Structural and functional aspects of lacrimation and eye protection

The ocular surface consists of the entire epithelial surface bounded by the skin on the upper and lower lid margin. Histologically, it comprises two main areas, the cornea and the conjunctiva, both presenting great specialization and marked environmental exposure. The outer tunica of the eye is composed by the sclera and the cornea, the latter being located in its anterior portion, with a convex and spherical shape and representing approximately one sixth of the tunica. Its optical characteristics are given by regularity of the surface, the refractive index and the transparency, which is due to its avascularity and the relationship of the collagen fibrils with the ECM. Cornea refractive index is in average 1.3375, which, together with the refractive indices of air, tear film and aqueous humor (1,000, 1,336 and 1,336 respectively) and their radius of curvature, give a refractive power to the central cornea of 43 diopters. The regularity of the surface arises from the epithelial uniformity and by the tear film, which is considered a third component of this indivisible functional complex, the so-called ocular surface. These characteristics require permanent protection, to avoid drying out and consequent deterioration, in order to maintain adequate corneal transparency (10, 36). In summary, for the epithelium of the ocular surface to be healthy, an adequate tear film is required and to have a healthy tear film, a healthy surface epithelium is essential (36).

The tear film is a dynamic and active structure with approximately 10 μ of thickness. While some authors consider it to have only two layers, an external lipid surface layer, and a second layer of a hydrated gel composed of mucins, proteins and aqueous component (36) – Figure 3, others divide it in three main components: the aqueous layer, lipid layer, and mucin layer (10). The aqueous layer comprises the largest component and is produced by both the primary lacrimal gland and accessory lacrimal glands. The mucins in this gel are a mixture of soluble and gel-shaped mucins produced by the lacrimal glands, and the corneal and conjunctival mucinous cells, as well as goblet cells within the conjunctival epithelium. This gel is firmly bound with the mucins of the epithelial cell membrane by means of chemical bonds. The mucin layer is a glycocalyx formed primarily by high molecular weight glycosylated membrane-associated mucins such as MUC1, MUC2, MUC4, and MUC16. These mucins interact with galectin-3, an important signaling protein that helps prevent pathogen entry into the eye. Another important function of the mucins is to decrease friction

of the ocular surface during blinking. Finally, the Meibomian glands, located in both the upper and lower eyelids, produce the outer lipid layer, which stabilizes the tear film and limits the evaporation of tears. Abnormalities in the any of these three layers can lead to an unstable tear film and to the debilitating morbid condition called the dry eye syndrome (DES) (10, 36, 55, 63).

Many of the proteins present in the tear film are secreted by the lacrimal gland, although some pass through the conjunctiva by diffusion from the serum. A healthy and stable tear film is crucial for high quality vision since it is the primary refracting surface of the eye. The main functions of the tear film are (36, 55):

• Optical: forms the first interface, air-tear, and regulates the corneal surface;

• Metabolic: Provides oxygen and some nutrients to the corneal epithelium through enzymes, such as lacrimal β-amylase, which helps in glucose metabolism in epithelial cells, as well as growth factors (epidermal growth factors);

• Antimicrobial: through enzymes (lysozyme, lactoferrin, transferrin, peroxidase), secretory immunoglobulins (IgA, IgM, IgG, IgE), cystatins, defensins and other antibacterial agents;

• Sweeping: together with blinking, it is used to remove cellular debris and foreign substances from the ocular surface.

Figure 3: “This figure was removed because of copyright restrictions. It depicted the tear film composition” Original source: Anaya J, Sarmiento-Monroy, J, García-Carrasco, M. Síndrome de Sjögren. Segunda edición. 2nd ed. Bogotá D.C.2017. 578 p. – Figure 5, page 74.

The main lacrimal gland is located deep in the anteriorly in the superior temporal orbit, within the lacrimal fossa, supported against the frontal bone and divided into an orbital and an eyelid portion by the levator aponeurosis. The accessory lacrimal glands are located along the orbital border of the tarsal plate and also participate of the exocrine secretion. Krause's glands are found in the stroma of the conjunctival fornix, although there are some in the lower portion and to a lesser extent in the caruncle. Wolfring's glands are seen in the tarsal conjunctiva, on its posterior edge,

being more numerous in the upper eyelid than in the lower one. The main and accessory lacrimal glands, along with the ocular surface (cornea, conjunctiva and the Meibomian gland) and the associated sensory and motor nerves comprise the lacrimal functional unit (LFU) - Figure 4. SS causes profound dysfunction of multiple components of the LFU, eventually resulting in severe DES (36, 55, 63).

The lacrimal gland is a tubulo-acinar exocrine gland formed by a secretory columnar epithelium arranged in a lobular pattern, enveloped by myoepithelial cells that contract and squeeze them to drain their secretions into confluent ducts, then onto the ocular surface. The stroma is composed by fibroblasts, collagen and matrix of interstitial spaces, mast cells, as well as B and T lymphocytes and plasma cells. Both sympathetic and parasympathetic fibers are responsible for its innervation, with predominance of cholinergic fibers, which release acetylcholine that binds to the muscarinic 3 acetylcholine receptor (μ3R) and vasoactive intestinal peptide types I and II located on the basolateral cell membranes of lacrimal gland secretory epithelia. Sympathetic nerves also innervate the lacrimal gland, and the binding of norepinephrine to α1- and β-adrenergic receptors increases Ca + flux into the cytosol. In addition, the neurotransmitters substance P and calcitonin gene-related peptide (CGRP) are released by sensory nerves in the lacrimal glands. The lacrimal gland also secretes proteins, such as lysozyme, lactoferrin, and lipocalin. Finally, the mucosal-associated lymphoid tissue (MALT) is observed within the lacrimal gland, which contains T and B lymphoid follicles and IgA-producing plasma cells surrounding the acini, also seen in the conjunctiva (55, 63).

The conjunctiva covers the major ocular surface and serves as a major support system for this surface, by producing tear components and supplying immune and inflammatory cells. In order to offer adequate protection against infections, the conjunctiva relies upon on the palpebral sweep, on anti-infectious substances in the tears, and on the migration of inflammatory cells and antibodies from the deep stroma towards the surface. The conjunctiva forms a continuous mucosal surface of three topographic zones: bulbar (anterior globe), palpebral (lines the inner surface of the eyelids), and forniceal (connect palpebral and bulbar). The conjunctiva is innervated by the first division (ophthalmic) of the Trigeminal nerve, parasympathetic fibers of the Facial nerve, and a minor sympathetic innervation with unclear function. The conjunctiva is composed of a stratified nonkeratinized secretory epithelium, mucin producing goblet cells and the underlying highly

vascularized stroma, where numerous bone marrow-derived cells are also present. Finally, the cornea, a highly specialized tissue with the highest density of sensory nerve endings of the body, the Meibomian glands, the upper and lower eyelids and a delicate lymphatic system complete the structure of the LFU (36, 55, 63).

Figure 4: "This figure was removed because of copyright restrictions. It depicted the lacrimal functional unit - LFU" Original source: Tiwari S, Ali MJ, Vemuganti GK. Human lacrimal gland regeneration: Perspectives and review of literature. Saudi journal of ophthalmology: official journal of the Saudi Ophthalmological Society. 2014;28(1):12-8.

2.5.1.2 – Ocular involvement

DES affects around 5-34% of the global population and, beyond the decreased tear production itself, it is considered a multifactorial condition. Both intrinsic, such as abnormalities of the eyelids and Meibomian gland dysfunction, and extrinsic factors, including eye surface irregularities, allergies, environmental effects and use of contact lenses play a role in this condition. In addition, other behavioral facts can contribute to the symptoms. As examples, we observe that smoking degrades the tear lipid film, a prolonged lid opening leads to increased evaporation of the tear film, increased air flow and temperature and low humidity are all co-factors associated with symptoms of dry eye. Likewise, hyperosmolarity, resulting from an increased evaporation or reduction of watery secretion, tear film instability and inflammation are all mechanisms of injury of the ocular surface. The increase in the protein and electrolytes concentration causes a reduction in the tear volume that causes subsequent inflammation and damage. It is estimated that SS patients represent around 11% of all DES cases, and DES represents the second most frequent reason for consulting an ophthalmologist (9, 36, 55).

A comprehensive assessment is necessary to determine the cause(s) of the dry eye. Lacrimal gland secretory dysfunction in SS is clinically recognized by symptoms of ocular irritation, dryness and a foreign body sensation, typically exacerbated upon visual effort or exposure to dry or drafty environmental conditions. In addition, inability to produce tears reflexively in response to emotional stimuli, wind or cold is also reported. Due to technical challenges and risk of complications, histopathological studies of lacrimal glands in SS patients are rare and tend to follow the same grading systems used for LMSG biopsies when describing lacrimal gland histology. When lacrimal

gland biopsy is done, usually it is from the palpebral part of the gland under local anesthesia, and perioperative bleeding may occur, as well as a significant risk of injury to the secretory ducts. Overall, progressive periductal lymphocytic infiltration develops in the orbital and accessory lacrimal glands, leading to a variety of reversible or permanent mechanisms causing lacrimal gland secretory dysfunction (Figure 5). In the conjunctiva, deleterious changes can include squamous metaplasia, loss of goblet cells, and chronic stromal inflammation (9, 36, 55, 62, 64).

Figure 5: “This figure was removed because of copyright restrictions. It depicted the histopathology of a lacrimal gland with focal infiltrations of mononuclear cells in glandular parenchyma” Original source: Bjordal O, Norheim KB, Rødahl E, Jonsson R, Omdal R. Primary Sjögren's syndrome and the eye. Survey of ophthalmology. 2020;65(2):119-32.

Also, it has been demonstrated that neural dysfunction plays a role in the DES association with SS. The sensory nerve fibers have decreased density, and their function seems to be reduced, in SS compared to healthy controls, and a functional deficiency of corneal nerves may decrease eye symptoms from DES, but concurrently eliminates the so important tear reflex, thus diminishing tear production even more (64). Moreover, there is evidence that Meibomian gland dysfunction (MGD) also plays a role. An evaporative dry eye, in which the evaporation of tear film is abnormally high, has been reported in SS as an adjunctive mechanism. Classically in SS, aqueous-deficient dry eye represents the main mechanism associated with reduced tear production, leading to the typical condition of keratoconjunctivitis Sicca (KCS) (9, 36, 55, 62).

KCS is a condition associated with different AI diseases, especially SS, and is manifested by factors influencing the balance of the ocular surface. This causes inflammation and damage, leading to quantitative and qualitative changes in the tear film. The most common symptoms are frequent gritty and/or burning eyes sensation, constant dry eyes and regular need for tear substitutes, intolerance to contact lenses, photophobia, and fluctuating blurry vision aggravated by prolonged visual effort or a low-humidity environment. An average of a 10-year history of dry eyes is typically present before an SS diagnosis is rendered and about 10% of all subjects with clinically significant dry eye receive a subsequent diagnosis of SS (10, 55, 62). Other conditions affecting the cornea, due to the eye dryness, are seen in SS. Keratitis is a corneal inflammation, manifested as a red eye and pain, increased discharge and blurred or decreased vision. Its complications can range from

corneal ulceration, opacification, thinning, to frank corneal perforation. In addition, filamentary keratitis presents as strands of small filaments formed by devitalized cells and mucus attached to the superficial portion of the corneal epithelium, which can produce the sensation of having a foreign body on the surface of the eye (9, 10, 36).

It is, however, important to note that, in the presence of slight changes of KCS, the differential diagnosis must include blepharitis (irritation and low-grade infection of the Meibomian glands), herpetic keratitis, viral and bacterial conjunctivitis, blepharospasm (neural alteration that leads to uncontrolled blinking), and anterior uveitis. It also needs to be remembered that slight symptoms and signs of dry eyes are aggravated by anxiety, depression, and/or medications (10, 61). The clinical assessment of SS patients usually shows a lack the correlation between the eye symptoms described and the objective findings during the examination. Sometimes, severe symptom complaints occur in the context of very mild objective changes on the ocular surface. Paradoxically, when the severity of DES reaches advanced stages, the reported symptoms diminish as a consequence of the decrease in corneal sensitivity that parallels advanced disease. Decreased corneal sensitivity has also been documented as a normal change associated with age and as a consequence of the use of contact lenses (10, 36, 64).

Approximately 25% of SS patients may experience extra-glandular ocular involvement, including vision-threatening diseases, such as uveitis, episcleritis / scleritis, optic neuropathy, retinal vasculitis, corneal scarring, cicatrizing conjunctivitis, conjunctival chemosis, sterile corneal ulcer / infiltration, corneal melt / perforation, and orbital inflammation. Even though some studies claimed that men with SS are more vulnerable to diverse ocular complications and extra-glandular systemic manifestations compared with women, this is not yet a consensus (36, 64).

Decreased aqueous tear production can be detected on clinical exam using the Schirmer test, originally described in 1903. Although it is sometimes briefly bothersome for the patient, it is safe, inexpensive, and easy to perform. A result of less than 10 mm/5 minutes indicates decreased tear production. There is a poor correlation between DES symptoms and Schirmer test results. There is also some controversy regarding its repeatability, especially for the higher scores. Nevertheless, the Schirmer test has the advantage of assessing a functionality that is lost early in the course of SS, which is the eyes reflexively tearing in response to sensory stimulation. Ocular surface disease in

SS is assessed by staining the ocular surface with diagnostic vital dyes. While diffuse punctate staining, including the central cornea, is typically observed in SS upon fluorescein technique, the use of lissamine green and rose bengal have greater sensitivity to detect compromised epithelium of the conjunctiva (10, 55, 62). Different tests have been used in ophthalmologic assessment of SS patients, with variable sensitivity and specificity performances, respectively, such as Schirmer test (76% and 72%), Rose Bengal staining (78% and 67%) and tear osmolarity (94% and 97%), among others (55).

2.5.1.2 – Management of the ocular manifestations

Since a cure for KCS is not possible yet, its management is heavily focused on mitigating symptoms, ocular surface rehabilitation, and monitoring for complications or for the development of vision-threatening ocular conditions, such as corneal melts, uveitis, scleritis, and optic neuritis. Strategically, taking care of the classical cause of tear production or aqueous tear-deficient dry eye is fundamental, as it is with regards to blepharitis, or MGD, which also leads to evaporative dry eye. A distinction between these causes is crucial for an adequate clinical approach, currently including the use of tear substitutes, topical and systemic immunosuppressive therapies, immunomodulation, and other interventions, such as lacrimal punctal occlusion (10, 12, 36).

As in almost every chronic disease, emphasis on patient education on the nature of the whole condition, aggravating factors, self-care and therapeutic aims is vital for an optimal compliance and subsequent outcome. Adaptation measures, such as directing heating and cooling vents away from the eyes, eye protection with wraparound sunglasses or moisture chamber goggles, and using an ambient humidifier are general recommendations, when deemed necessary. In mild-to-moderate cases for both types of dry eyes, management measures usually involve the use of topical tear substitutes, nighttime gels and ointments, and anti-inflammatory drops. The objective is to increase moisture and achieve a lubricating effect on the ocular surface and thus improve the symptoms. Recently, the possibility of using preservative-free artificial tears in single unit applicators have shown greater benefit in all clinical situations in which the regimen requires more frequent application, up to 4 times a day, to prevent ocular surface irritation caused by overuse of preserved tears (9, 12, 36, 40). Additionally, options for longer lasting relief include ocular gels or ointments

utilized at bedtime and the insertion inside the lower eyelid of body heat-dissolvable pellets, which release tear substitute onto the ocular surface throughout the day (10).

With the increase of the severity to a moderate to severe levels, patients experience frequent or constant irritation and additional therapies are required. These include the use of anti-inflammatory drops and/or oral prescription secretagogues. Topical corticosteroids for short-term relief of KCS symptoms may be employed to achieve a degree of local immunosuppression. However, such use of topical corticosteroid for long periods can cause potentially harmful side effects to the eye such as increased intraocular pressure, cataract formation and infection. For this reason, the use of topical corticosteroids is recommended for short cycles of no more than two weeks. Topical cyclosporine 0.05%, refrigerated to avoid stinging upon instillation, is an option to decrease inflammation by several mechanisms, such as inhibition of T-lymphocyte activation, apoptosis, reduction of proinflammatory cytokines and even an effect on the density of the goblet and epithelial cells of the conjunctiva (9, 10, 36).

A recent new topical treatment has shown effective results against SS eye dryness. Lifitegrast © is an integrin antagonist which blocks lymphocyte function associated antigen (LFA)-1/ICAM-1 interaction preventing T-cell activation, recruitment and release of inflammatory mediators (10). In severe or refractory cases, topical application of autologous serum has been used with success. (10, 36) The autologous serum provides a source of vitamins and growth factors, such as epidermal growth factor (EGF), and fibronectin, which promotes proliferation, maturation and differentiation of the corneal epithelial cells. In addition, the serum albumin in autologous serum causes suppression of apoptosis in conjunctival cells. The combined us of topical autologous serum together with tear substitutes has shown better results than either of these alone and this combination seems to be a safe and effective topical option in long-term treatment of dry eye in patients with SS (36).

Local care for patients with blepharitis, or MGD, is based on daily judicious eyelid hygiene using warm compresses, eyelid massage and gentle scrubs twice a day to encourage flow from the Meibomian glands. For moderate MGD, additional measures comprise the use of lipid-based tear drops, topical antibiotics, such as erythromycin or azithromycin, as well as oral antibiotics such as doxycycline. Patients with severe or refractory MGD may need a more advanced approach, such as

Meibomian gland probing, intense pulsed light, or continuous controlled thermal compression, along with the general measures to treat aqueous tear deficiency as mentioned above (10).

Finally, lacrimal punctal occlusion is an adjuvant procedure performed in a stepwise fashion beginning with the inferior lacrimal canaliculi. It is based on the placement of a lacrimal plug that blocks the natural anatomical drainage of the tear, with a therapeutic aim of accumulation of tear fluid and improved lubrication of the ocular surface. Non-absorbable silicone plugs have been used for a long time but complications, such as extrusion, granuloma formation, displacement, canaliculitis and infections may require the removal of the punctum plug. An alternative is a collagen absorbable tear plug that solidifies at body temperature and achieves an effective obstruction of the lacrimal duct. There is evidence that this type of occlusion results in a decrease in the necessary frequency of artificial tear use and improvement in different tests, such as Schirmer, Fluorescein, Rose Bengal and even the ocular surface disease index (OSDI) (36, 40, 55). An irreversible procedure can also be done with the thermal cauterization of the lacrimal punctum, recommended for the patients with refractory disease or significant side effects from the removable plugs (10, 12, 36, 40, 55). The use of neuromodulator local injections or surgical methods aiming partial closure of the interpalpebral fissure and decreasing evaporation from the ocular surface is also an option for severe dry eye patients. Patients with filamentary keratitis may benefit from topical N-acetylcysteine drops, as well (40).

Beyond the local measures and treatments, there is evidence that oral secretagogues, such as pilocarpine and cevimeline, may be effective in relieving dry eye symptoms. Nevertheless, these oral secretagogues seem to have a greater beneficial impact on dry mouth symptoms. Furthermore, their use has sometimes been associated with increased perspiration (36, 40).

The use of nutritional supplementation with omega-3 essential fatty acid has been recommended by some authoris for management of the dry eye (9, 40). However, recent study has shown no benefit of such supplements over placebo when used for management of dry eye (10).

Newer avenues have been attempted in the treatment of the DES with the use of biologic drugs, which show improvement of dry eye symptoms and tear production. Among them, patients have benefited from rituximab (RTX) (a chimeric monoclonal antibody against CD20 protein),

Belimumab (a monoclonal antibody which inhibits BAFF), and Abatacept (a fusion protein composed of the Fc region of immunoglobulin IgG1 that prevents activation of the T-cells) (36).

In conclusion, a comprehensive management from both ophthalmologists and rheumatologists is essential for the management of ophthalmic aspects of SS. It is important understand that DES is widely prevalent in SS patients, and that it leads to significant disabilities and costs for the patient and the health system. It is sensible to take a stepwise approach while managing SS related DES and tailor therapy to each individual patient (36, 40). The use of clinical algorithms may help clinicians, such as ophthalmologists and rheumatologists to make evidence-based information when making treatment-decisions regarding SS-related DES (40).

2.5.2 – Orofacial involvement

Orofacial manifestations of SS are a major aspect of the disease, and a major factor of quality of life deterioration for those affected by them, making their management a priority. These manifestations involve xerostomia and hyposalivation, autoimmune sialadenitis and salivary gland swelling, and secondary conditions, such as dental caries and tooth loss, oral candidiasis, periodontal disease, bacterial sialadenitis, oral malodour, oral ulcers and pain. The initial and foremost oral component of SS is characterized by dry mouth, which could be a real hyposalivation, or a subjective sensation of oral dryness, also known as xerostomia. In the clinical arena, the salivary flow can be measured by sialometry, and the intensity of the perceived mouth dryness, xerostomia, by using a visual analog scale. Patients may initially note intermittent daily or nocturnal dryness that gradually becomes more prominent during the day (36, 55, 65).

Overall, xerostomia is a symptom present in 30% of the population, more often in women, and increasing with age. However, differences are not always shown in salivary flow among different age groups. The most important causes of xerostomia, in addition to senile glandular atrophy, are dehydration, malnutrition, use of anticholinergic or sympathomimetic medications (xerogenic drugs), chemotherapy, head and neck radiotherapy, SS and infection by sialotropic viruses. Other comorbidities, such as anxiety/depression, diabetes mellitus, sarcoidosis, alcoholism, smoking, HIV, hepatitis-C, and IgG4-related disease may cause or aggravate this condition. Dry mouth is the initial complaint of around 40% of diagnosed SS patients, and xerostomia affects 90% of these individuals. Oral Sicca symptoms have been significantly associated with decreased quality

of life in SS patients, with an impact on general health and social functioning, lowered energy levels and greater fatigue levels (36, 40, 55, 66, 67).

In addition to the glandular changes of actual lower production of saliva, the sensation of dryness (xerostomia) depends on a neuronal circuit that starts at the mucosal surface, travels through the unmyelinated nerves of the oral mucosa, and is connected to specific areas of the midbrain (salivatory nuclei) via afferent nerves. Efferent adrenergic and cholinergic nerves regulate gland secretion, and the involvement of cholinergic pathways in the brain might explain the presence of Sicca symptoms in patients with different conditions, such as fibromyalgia, multiple sclerosis, and Alzheimer's disease. This also explains why patients with salivation within normal limits sometimes complain of a dry sensation in their mouths. As mentioned previously, salivary production implicates neuro-exocrine processes, which will involve the interaction of neurotransmitters with parenchymal salivary cells, and final saliva secretion. Thus, both neural and glandular malfunction are involved in the symptoms reported by SS patients (10, 55, 61).

2.5.2.1 – Structural and functional aspects of salivation

The salivary glands are eccrine exocrine glands that release saliva by exocytosis from secretory cells into an epithelial walled duct or ducts and thereafter onto the oral cavity. The major salivary glands, parotids (serous), as well as submandibular, and sublingual (both mucous-serous) are found in pairs, and produce 90% of total saliva, with the minor salivary glands producing the rest. The capacity of fragmentation and initial degradation of food, providing the first steps for proper absorption at the GI tract is the classic function of the saliva. However, it takes numerous chemical modifications of the acinar content to formulate the basic elements of saliva, allowing it to accomplish its functions, both digestive and non-digestive. A series of transmembrane ion exchange processes, autonomic nervous system activity, vascular level changes, and intrinsic factors influence the final composition of the saliva, which varies according to countless conditions, from hydration and nutrition, to hormonal factors and physiological impact of aging (10, 36).

The basic functional unit of the salivary gland is the acinus, which is composed of mucinous and/or serous cells, according to the type of secretion. When the acini present both cell types, it is called mixed. Histologically, the mucinous cells present a clear cytoplasm, due to the water-soluble glycosylated proteins and anionic oligosaccharides. On the other hand, serous cells show an

eosinophilic stained cytoplasm due to proteins that have not undergone the glycosylation process. In addition to the parenchyma, other vascular, neural, muscular, and ductal components complete the intricate features of these organs (Figure 6) (36).

Saliva composition is 99% water and 1% proteins, minerals, and enzymes. It is a colorless chemically complex fluid, which has over 2000 proteins and glycoproteins, lipids, electrolytes, small molecules, immunoglobulins, and hormones. It has a density between 1.002 and 1.020, and due to the presence of buffers, a pH range between 5.3 and 7.8, which is a favorable environment for enzymes, especially amylase which is responsible for the initial carbohydrate digestion function. Daily production of saliva ranges between 800 ml to 1500 ml, presenting peaks of secretion during food intake (10, 36). The functions of the saliva are wide and in recent years these have been amplified, revealing that saliva and minor salivary glands are important sources for novel diagnostic biomarkers by applying high-throughput proteomic analysis, mass spectrometry and novel genomic technology (68).

Overall, saliva maintains oral homeostasis, protects and preserves teeth, and constrains microorganisms' growth, through mechanical cleaning and the action of enzymes, antimicrobial proteins (lysozyme, histatin, lactoferrin), and immunoglobulins (IgA). Statherin, proline-rich proteins, calcium, and phosphate promote remineralization of the dentition, as well. Saliva also plays a role in the lubrication and protection of the oral soft tissues (mucin, EGF – epithelial turnover), taste sensation (contact with taste receptors), as well as in mastication, deglutition, and speech. In addition, it has also been postulated that alterations in oral microbiota associated with salivary dysfunction may contribute to creating so-called dysbiosis, which may play a role in the pathogenesis of autoimmune diseases. Therefore, the oral consequences of SS encompasses far more than mere dryness of the mouth (36, 40).

Figure 6: "This figure was removed because of copyright restrictions. It depicted the histophysiology of the salivary gland" Original source: Anaya J, Sarmiento-Monroy, J, García-Carrasco, M. Síndrome de Sjögren. Segunda edición. 2nd ed. Bogotá D.C.2017. 578 p. – Figure 1, page 35.

2.5.2.2 – Oral and facial manifestations, assessment and management

2.5.2.2.1 – Oral and facial clinical features

Hyposalivation and Xerostomia: The salivary flow rate varies widely from person to person, which makes it difficult to accurately determine the level of reduction in salivary flow in each individual. Standardized measurement is done by the unstimulated salivary flow rate (USFR) of whole saliva, collecting it for 5 min at rest. Hyposalivation is when the saliva collected is less or equal to 0.1 mL/min. The salivary gland damage seen in most SS biopsy samples is not sufficient to explain the complete dryness of the mouth in those patients. The residual glandular structure appears to be dysfunctional even though they maintain their neural innervation and upregulation of their muscarinic receptors, as we discoursed in the etiopathogenesis. Furthermore, the reported symptom (xerostomia) can be due to actual decreased production or increased consumption of saliva, or can be due to altered neural sensation. Even though xerostomia is not in itself a disease, it can alter people's quality of life and can be an indicator of the presence of a systemic disease, such as SS. It tends to receive little attention, and physicians generally do not always appreciate the impact of oral symptoms on quality of life. Often the patient does not reveal a decreased quality of life until asked directly, or sometimes not until 50% of the initial volume of saliva has been lost (36, 61).

Hyposalivation causes more than a general discomfort of oral soft tissues, and a variety of mucosal lesions may occur in SS patients, affecting oral and general health, and jeopardizing the patient’s quality of life. Due to the decreased lubrication and increased friction of the oral tissues against each other, chronic atrophic mucositis and ulcerations, along with sharp pain, burning sensation (stomatopyrosis), and erythema are observed. Often, food residues are seen and the whole ingestion process is difficult (dysphagia), leading to dehydration and affecting the patient’s nutritional status. Dry mouth also causes cracked dry lips (cheilosis) and difficulties in controlling dentures and speaking, which is recognized by a clicking quality of sound as the tongue sticks to the palate (67, 69). In addition, burning pain on the tongue (glossodynia), which becomes erythematous and fissured, as well as atrophic papillae, are often associated with Candida infection (Figure 7) (67, 69, 70).

Figure 7: “This figure was removed because of copyright restrictions. It depicted the oral mucosal manifestations. A: Dry mouth - food residues; B: Lobulated tongue histophysiology of the salivary gland” Original source: Scully. C; Georgakopoulou EA. Oral Involvement. In: al. MR-Ce, editor. Sjögren’s Syndrome. London: Springer-Verlag; 2012. p. 85-106.

Candida albicans is the most common type of Candida spp. in the oral environment, existing normally as a saprophyte, and in both symptomatic and asymptomatic SS patients, a higher than normal total and CFU count of this fungus has been detected. Lower pH rate and salivary flow, as well as other qualitative alterations, such as decreased histatin, chromogranin A, and immunoglobulins favor candidal growth in susceptible patients, as seen in SS (67, 69). Chronic erythematous candidiasis is commonly associated with atrophic changes on the oral mucosa, as mentioned above, and pseudomembranous candidiasis occurs less frequently in SS patients. This infection is also observed as angular cheilitis, where Candida glabrata has also been found at the affected commissures (10, 65, 70). In patients wearing dentures, redness, irritation, and pain under the prosthesis can lead to a diagnosis of candidiasis, which can also affect the palate and gingiva. In severe conditions the tonsils and esophagus may also be involved (Figure 8) (69).

Figure 8: “This figure was removed because of copyright restrictions. It depicted the types of oral candidiasis in SS patients: (A) Erythematous (B) Pseudomembranous (C) Atrophic - under removable dentures (D) Angular cheilitis” Original source: López-Pintor RM, Fernández Castro M, Hernández G. Oral involvement in patients with primary Sjögren's syndrome. Multidisciplinary care by dentists and rheumatologists. Reumatologia clinica. 2015;11(6):387-94.

Dry mouth can also be accompanied by chemosensory dysfunction, manifested as reduction, distortion, or absence of the senses of taste and/or smell, as well as burning sensation or numbness, which profoundly impacts patients’ quality of life. In general, the taste acuity can be diminished or unpleasantly changed (hypogeusia / dysgeusia) or even absent (ageusia), which may or may not be associated with the alteration of smell (dysosmia/hyposmia/anosmia). There is still debate about the causes of smell and taste impairments, but hyposalivation, neurogenic issues and even inflammatory response may interfere with normal signal transduction and taste-bud cell turnover (10, 15, 65, 71). In addition, hoarseness and dysphonia are late manifestations of SS and usually are related to chronic mucosal dryness or persistent mucus that coats the vocal cords, as well as secondary lesions on their surfaces (55).

Oral lesions of an autoimmune nature have also been described in SS subjects, mostly lichen planus, aphthae and chronic ulcerative stomatitis, affecting more often the buccal mucosa, the tongue, or gingiva. The diagnosis of the gingival lesions needs distinction between the lichenoid lesions, candidal infection, or even common gingivitis. The last two of correlate with hyposalivation, however the lichenoid lesions do not (55, 65, 69).

When there is oral dryness, there is a higher accumulation of dental plaque, compromising the health of the teeth and of the periodontal apparatus. The oral environmental circumstances promote a greater gingival index (65%), plaque bacterial index (75%), and increased bleeding gingival on probing (gingivitis) in SS patients compared with healthy controls (70). On the other hand, impact on health of the deeper periodontium in SS patients is not clear, according to some authors (65, 67). Augmented accumulation of dental plaque and calculus on dental surfaces contributes to destruction of supporting tissues, and it has been found that SS patients do present greater dental loss due to periodontal causes. However, no difference has been found regarding periodontopathogenic microorganisms, making weak the correlation between SS and periodontitis-related premature loss of teeth (65, 70).

A stronger correlation, though, is demonstrated between SS and the development of caries, in unusual locations and ages. Due to an autoclysis (natural dental cleaning while chewing) deficit and a diminished mechanical cleaning process, in SS patients, dental surface sucrose elimination is more difficult, which facilitates the colonization with Streptococcus spp., and Lactobacillus spp. A common finding is the development of rampant caries on tooth surfaces that are normally fairly caries-resistant, such as incisal edges, smooth buccal or lingual surfaces, and root aspects (Figure 9). Dental caries in SS patients is usually seen in rapid progression, presenting in weeks or months compared to years as typically occurs. The lack of saliva's protective functions, including pH imbalance, leads to non-carious tooth surface loss, such as enamel erosion, abrasion, and attrition. These amplify dental hypersensitivity, which can cause discomfort upon consumption of more acidic foods, as well as hot and cold drinks (Figure 16) (10, 15, 55, 65, 69, 70).

Other orofacial conditions have been observed in SS subjects. At least one third of SS patients present salivary gland enlargement, or sialomegaly. Unilateral or bilateral swelling is seen on the major salivary glands, usually parotid, where a milking maneuver may elicit a low rate of

thick saliva. This procedure is important during the assessment of major salivary gland ducts (Stenson's - parotid / Wharton - submandibular), evaluating consistency and texture of the gland, as well as the quantity and color and quality of the secretion, if there is any. The presence of a cloudy or purulent discharge from the salivary ducts may indicate infection of the major salivary glands (65, 67, 69, 70).

It is known that many AI diseases are predisposed to the development of neoplasms. In SS, the more prominent malignancy is the non-Hodgkin B-cell monoclonal lymphoma, and 80% of them are MALT lymphomas. The presence of persistent, painless enlargement of the salivary glands, especially along with splenomegaly, lymphadenopathy, cutaneous vasculitis, hypocomplementemia, cryoglobulinemia, anemia, lymphopenia, and neutropenia, prompts concern for this malignancy (10, 23, 46, 72).

It has been estimated that SS patients are 44 times more prone to salivary gland enlargement that the rest of the population, possibly related to the stimulation of cytokines, environmental factors, viral infections, and vitamins deficiencies (70). The clinician must pay attention to the diverse differential diagnosis for a painless bilateral swelling, which includes chronic inflammation and/or fatty infiltration (sialadenosis), sarcoidosis, IgG4- related diseases, and relation to other systemic conditions, such as diabetes, hyperlipidemia, or metabolic conditions. Sialolithiasis (salivary stones) and bacterial sialadenitis are also seen in SS patients, due to the low salivary flow and retrograde infections. If untreated, these conditions can lead to severe pain, swelling, trismus, fever, as well as abscess formation (65, 67, 69, 70).

Figure 9: "This figure was removed because of copyright restrictions. It depicted a patient with SS-related hyposalivation and a great amount of caries in unusual locations" Original source: López-Pintor RM, Fernández Castro M, Hernández G. Oral involvement in patients with primary Sjögren's syndrome. Multidisciplinary care by dentists and rheumatologists. Reumatologia clinica. 2015;11(6):387-94. (65).

2.5.2.2.2 – Clinical assessment of dry mouth in SS

The salivary flow rate varies widely from person to person. A proper diagnosis is a fundamental step for an adequate clinical practice, especially in populations affected by chronic

conditions presenting long pre- clinical stage (8). To collect and measure the whole unstimulated saliva production using sialometry, the patient is seated and after a previous swallowing, the patient places the saliva that spontaneously accumulates in the mouth into a graduated container. It is considered normal if the salivary flow is > 1.5 ml in 15 minutes (≤0.1mL/min), with this test presenting 56% of sensitivity and 81% of specificity. Higher sensitivity is seen in individual gland sialometry, instead of whole mouth salivary flow, since submandibular and sublingual tend to be involved prior to the parotids in early SS. Nonetheless, due to the need for specialized collection devices and technical issues, sialometry of individual glands is never performed in clinical practice, being reduced to only research scenarios (10, 36, 55, 73).

Xerostomia is generally related to altered quantitative rather than qualitative properties of saliva. However, sialochemistry examines electrolytes and proteins in saliva, and a huge spectrum of biomarkers has been described and correlated to SS clinical features. For instance, decreased levels of sulfated oligosaccharides within mucinous acini may contribute to the dry symptom. Other qualitative changes in the saliva of SS patients are higher levels of sodium, chloride, lactoferrin, β-2 microglobulin, IgA, IgG, lysozyme C, and cystatin C, albumin, salivary kallikrein, inflammatory mediators (eicosanoids, PGE2, thromboxane B2), as well as diminished salivary amylase, carbonic anhydrase, and phosphate. Apparently, both quantitative and qualitative analysis of the saliva are promising instruments, not only for the diagnosis, but for monitoring the evolution and prognosis of the disease (55, 68).

Other diagnostic tests used in clinical and research settings present both advantages and caveats. Salivary gland scintigraphy with 99mTc-technetium pertechnetate (Tc-99) is a non-invasive and very sensitive procedure utilized for studying the dynamic function of salivary glands. However, only the parotid and submandibular salivary glands can be sufficiently displayed, as well as the thyroid gland and the level of the oral and nasal mucosa - Figure 10 (36, 73-75). Performed on a fasting person, to avoid the powerful stimulation of chewing, after an intravenous dose of Tc99, sequential imaging is obtained at 5, 10, 15, 30, 45 and 60 minutes post-injection, which allows for the assessment of vascularity and the capacity to concentrate the tracer. To evaluate the excretory phase, lemon juice is usually administered, revealing three phases (36, 73):

1. Vascular flow phase: reveals bilateral and symmetrical flow to the salivary glands;

2. Concentration phase: characterized by a marked, gradual, and symmetrical increase of activity at the parotid and submandibular (5-15 minutes); and

3. Secretory phase: occurring 15 to 30 minutes post-injection, while the intraglandular activity decreases, and Tc99 is transported to saliva followed by the mouth, which shows activity at 60 minutes greater than in the glands.

Most scintigraphic abnormalities can be seen in a variety of diseases associated with salivary gland dysfunction and are not specific for SS. Regardless, a grading system has been proposed (Schall's classification) and originally, each gland was classified separately, but to simplify the process, the score is based on the highest value of both parotids and both submandibular glands together. Four grades can be used to summarize salivary gland functional impairment. From grade 1 (normal) to grade 4 (severe), the latter representing the complete absence of tracer uptake, with mild and moderate levels in the middle (36, 73-76). The improvement and standardization of quantitative indices that reflect glandular function more accurately may represent an important advance for the use of this diagnostic method in SS since its main limitation is the inter-observer variation (75).

Figure 10: "This figure was removed because of copyright restrictions. It depicted the salivary scintigraphy in grade II (mild) disease. Adequate uptake is observed with slowed glandular excretion" Original source: Anaya J, Sarmiento-Monroy, J, García-Carrasco, M. Síndrome de Sjögren. Segunda edición. 2nd ed. Bogotá D.C.2017. 578 p. – Figure 2, page 44.

Amongst imaging methods, sialography is a radiological technique in which the use of water-soluble contrast allows visualization of the duct system of the salivary gland, providing information about the duct as well as the glandular parenchyma. It is especially useful to diagnose obstructive conditions of glands and ducts, though its value in patients with xerostomia is questionable, and the discomfort for the patient and the possible complications have made its use very uncommon. The major advantage of sialography is to proceed with minimally invasive interventions to remove sialoliths and repair ductal stenoses (73, 76).

The ultrasound (US) study can also guide the diagnosis and longitudinal follow-up of salivary gland involvement in SS. A bilateral glandular inhomogeneous pattern with hypoechoic

and cystic areas is compatible with SS, helping with the distinction from other diseases, such as sarcoidosis, chronic sialadenitis, sclerosing sialadenitis (Küttner tumor), abscesses, sialolithiasis, among others. The reproducibility, low cost, lack of radiation exposure, and lack of complications of this non-invasive technique have popularized its use. Although its sensitivity and specificity are not great, the use of US of major salivary glands in SS patients has been added to the main diagnostic tools in SS, which means salivary gland function assessment, LMSG biopsy, and serology (73, 76, 77). A grading system has been proposed and parenchymal features seen on ultrasound are scored from 0 to 4 in parotid and submandibular glands: "0" if the salivary gland showed homogeneity; "1" when slight inhomogeneity with small hypoechoic spots, no echogenic bands, regular or increased glandular volume, and an ill-defined posterior glandular border are seen; "2" if mild inhomogeneity with multiple scattered hypoechoic areas (< 2 mm), no echogenic bands, and regular or increased glandular volume are present, with an ill-defined posterior glandular border; "3" indicates evident inhomogeneity with multiple hypoechoic areas (2–6 mm), echogenic bands, regular or decreased glandular volume, and no visible posterior glandular border; and "4" when a gross inhomogeneity with multiple hypoechoic areas (> 6 mm), echogenic bands, severe damage to the glandular architecture, decreased glandular volume, and no visible posterior glandular borders are observed (76, 77).

The comparison between the sensitivity of the US and magnetic resonance imaging (MRI) in SS has shown some advantage to the later, 78% and 81%, respectively. Nevertheless, US presents benefits, such as lower cost and wider availability than MRI, and all salivary glands can easily be evaluated at the same time (78). Thus, even if considered a golden standard for many soft tissue assessments, MRI has not been largely applied in salivary gland analysis. Sometimes, however, MRI use is necessary for the diagnostic evaluation of patients with xerostomia, as well as when a heterogeneous pattern has been described as characteristic of the SS. Still, further research should be conducted, to endorse its diagnostic significance and cost-effectiveness in SS, before its inclusion in the diagnostic criteria of SS (73, 78, 79). Recent studies have shown that MRI could be advantageous both in early stages of SS while diffuse glandular enlargement occurs, and late stages of the disease with the lobular destruction of the gland and associated fat and fibrous tissue deposition. The most characteristic SS MRI findings are an inhomogeneous internal pattern on both T1 and T2 sequences, described as a "salt and pepper" or "honeycomb", which represents areas of increased fat and decreased undamaged lobules (76, 79). Another aspect of SS imaging that should

be considered besides diagnosing and assessing the stage and activity of the disease, is the suspicion of neoplasm development within the glands, such as lymphomas. In general, though, MRI features are non-specific for benign or malignant lesions, and in most cases biopsy and histological assessment is required (79).

Finally, the biopsy of the salivary glands has been the principal and classic diagnostic method in SS. Its main disadvantages are associated with local pain, morbidity, especially to the facial nerve, and scarring. Major salivary gland biopsy is not recommended in the assessment of patients with xerostomia, and only has a place if there is a suspected local pathology. Overall, abnormal LMSG biopsy is a hallmark of SS diagnosis. It is performed under local anesthesia on an outpatient basis and allows distinction between age-related salivary gland involution versus pathologic conditions, such as SS, sarcoidosis, amyloidosis, and neoplasms. Most commonly, the so-called Daniel's technique is performed in the middle part between the commissure and the midline, on clinically intact mucosa, after 2% xylocaine local anesthesia. Subsequently, a 1.5 to 2 cm horizontal incision and a blunt dissection is performed while avoiding any contact of the sensory nerves, to expose the glands. The specimens must include five or more lobes, to be immediately immersed and fixed in 10% neutral formaldehyde, and sent for histopathology processing and examination. Finally, the mucosa is sutured with 3 separate stitches. Although LMSG is considered an invasive technique, it has few side effects, of which local discomfort and sensory changes are most common (73, 76, 80). Minor innovations in the technique, such as the use of "S" forceps, seems to help with the ergonomics, surgical stabilization, bleeding control, and visualization, as well as better selection of the samples with a potential lower morbidity (Figure 11) (24). The main pitfalls of the LMSG biopsy include insufficient glandular tissue for histologic analysis, which can lead to overestimation of the FS, histological misinterpretation of the local inflammatory infiltrate, and inadequate definition or lack of focus score quantification (10, 73).

Figure 11: "This figure was removed because of copyright restrictions. It depicted the excision of a minor salivary gland with the aid of an "S" forceps" Original source: Varela-Centelles P, Seoane-Romero JM, Sanchez-Sanchez M, Gonzalez-Mosquera A, Diz-Dios P, Seoane J. Minor salivary gland biopsy in Sjogren's syndrome: a review and introduction of a new tool to ease the procedure. Medicina oral, patologia oral y cirugia bucal. 2014;19(1):e20-3.

2.5.2.2.3 – Orofacial clinical management in SS

Hyposalivation and xerostomia are the original and main issues of the complex clinical scenario seen within SS patients and goes far beyond the simple difficulty in eating certain foods or the higher frequency of dental problems. The management approach requires not only knowledge and technical skills, but also a holistic perspective since the impact of the condition in each patient's quality of life is tremendous. Having a chronic illness like SS may lead to anxiety and depression, and the psychosocial aspects of care are essential to manage its emotional and physical challenges (81, 82). For an accurate diagnosis and proper management of the SS patient, a close relationship and adequate communication between the rheumatologist and the dentist is critical. The measures needed to cope with dry mouth conditions and promote oral health, as well as prevent the potential drastic consequences involve a combination of environmental changes (high room humidification, for instance), habits and self-care education, local mitigating actions and, eventually, systemic medication. First of all, the clinician should assess the patient's use of any xerogenic drug, such as antihypertensives, diuretics, anti-depressants, opioid analgesics, sleeping aids, anti-convulsants, and neuropathic medications. Then, an assessment of local and systemic conditions that interfere with salivation and the existence of subjective symptoms (xerostomia) is necessary. Subsequently, the objective basal salivary flow needs to be measured, and repeated if needed. From that standpoint, it is important to keep in mind that the goal of the treatment is to attain adequate levels of saliva and control the symptoms, prevent caries and infections, and enable patients to speak, eat, and swallow properly. Different therapies are utilized according to the degree of oral dryness (Figure 12) to improve patient quality of life and avoid false expectations (65, 69, 82).

Many environmental measures may be recommended, such as to avoid low humidity conditions (air- conditioning, forced air heat) and to use ambient ultrasonic cool mist humidifiers, particularly at night. Controlling environmental allergens and maintaining a proper nasal hygiene, with open upper airways to avoid mouth breathing is also important, as well as avoiding smoking, alcohol and astringent mouthwashes, and toothpastes (65, 69, 82).

The patient needs to be educated about keeping meticulous oral hygiene and regular dental care, avoiding high in sugar and "soft" foods, as well as careful chewing of food before swallowing since it stimulates salivation and facilitates deglutition. To maintain oral comfort, additional

behavioral changes can be adopted. Patients should eat more frequently and in smaller portions to stimulate saliva flow, as well as with frequent small sips of water just to keep the mucosa wet. It is fundamental to maintain proper hydration by regularly consuming water or noncarbonated, sugar-free, nonacidic liquids throughout the day. It is important to avoid drinking water during the night to prevent nocturia, which contributes to low sleep quality and the associated fatigue (67, 69, 82, 83).

Grade	Characteristics	Therapeutic recommendations
1	Mild, sporadic discomfort on eating dry or course foods No signs of oral dryness Unstimulated salivary flow ≥ 0.25 ml/min Stimulated salivary flow ≥ 1 ml/min	Education and modification of diet Elimination, if possible, of xerostomia-related medication Avoidance of alcohol and tobacco use Hydration
2	Mild intermittent discomfort not only when eating No evidence of oral dryness Unstimulated salivary flow <0.25 and > 0.1 ml/min Stimulated salivary flow <1 and >0.7 ml/min	Add to grade 1: Use of sugarless chewing gum and sugarless lemon-flavored hard candy Use of saliva substitutes: gel and spray
3	Frequent constant discomfort not only when eating Difficulty in eating, swallowing and speaking Signs of oral dryness can be observed Unstimulated salivary flow ≤ 0.1 ml/min Stimulated salivary flow ≤ 0.7 ml/min	Add to grades 1 and 2: Use of saliva substitute If there is no improvement with saliva substitute, use of systemic stimulants (after salivary gland biopsy to determine whether there is a certain degree of salivary function)
4	Permanent discomfort Great difficulty in eating, swallowing and speaking Advanced signs of oral dryness Unstimulated salivary flow ≤ 0.05 ml/min Stimulated salivary flow ≤ 0.3 ml/min	Add to grades 1, 2 and 3: Consider the use of intraoral devices and saliva substitute

Figure 12: Therapeutic strategies to increase salivary flow according to severity of oral dryness in patients with SS (65).

Management strategies for dry mouth and the available products can be summarized as follows (65, 69, 83):

- Local salivary stimulants (gustatory or masticatory stimulus): sugarless chewing gum to raise the pH and boost the buffering capacity; sugarless hard candy (lemon-flavored) to stimulate the taste buds, and consequently the output of saliva. An alternative is a new intraoral electrostimulation device that stimulates the residual secretory capacity through modulation of the autonomic reflex arc that regulates salivation.

- Systemic salivary stimulants (sialogogues): cholinergic drugs have been used to treat xerostomia and xerophthalmia in patients with residual salivary gland function. It is recommended to start this treatment at the lowest possible dose after meals (pilocarpine, 5 mg, or cevimeline, 30 mg) and tapper up the dosage as tolerated and needed, up to the daily maximal divided dose. An improvement in oral dryness with these agents is present in up to 60% to70% of the patients although contraindications and side effects limit its use.

- Saliva substitutes: a wide variety of products available in mouthwash, gel and spray form that contain substances with an aqueous component enhanced with calcium, phosphate, enzymes, proteins and fluoride ions. Additionally, coconut oil, sesame oil, and olive oil can be used topically on the oral mucosa twice a day, after meals and at bedtime to alleviate oral symptoms.

The clinical management of hyposalivation and xerostomia tends to contribute to the control of the secondary pathologic oral conditions, such as dental decay, candidiasis and mucositis. Nonetheless, adjuvant measures might be necessary to achieve an optimal result. A synthesis of the main steps to make the clinical approach more suitable and comprehensive are presented in Figure 13 (15, 65, 67, 69, 83, 84).

A thorough assessment of the literature and the level of evidence is out of the scope of this manuscript, and a portrait of currently available data with specific regard to caries prevention in SS patients can be viewed in clinical practice guidelines for oral management of SS (84).

Condition	Therapeutic recommendations
Dental demineralization and caries	• Dietary control of sucrose intake • Avoid tooth abrasives • Control extrinsic acid exposure • Daily use of fluoride toothpastes (5000 ppm) • Remineralizing mouthwash • Additional fluoride applications • Topical amorphous calcium phosphate (ACP) • Triclosan containing toothpastes • Probiotics • Frequent dental visits for early detection of caries
Gingival changes	• Strict oral hygiene, including daily flossing • Professional dental cleaning every 3 months • Use of topical (rinse) chlorhexidine gluconate • Topical lactoperoxidase gel • Topical antibiotics (retard gingival recession)
Candidosis	• Denture cleansing and disinfection • Use of topical (rinse) chlorhexidine gluconate • Brushing the palate • Physical or manual cleaning with a tongue brush, scraper, or powered toothbrush • Topical miconazole gel, nystatin pastilles, clotrimazole troches dissolved in the mouth – avoid antifungal agents containing sugar • Probiotics • Systemic amphotericin, itraconazole, posaconazole or fluconazole (if needed)
Sialadenitis	• Hydration • Stimulation of salivation • Culture of the exudate / antibiotic therapy • Regular manual manipulation (milking) of the salivary glands • Surgical drainage (if needed) • Lactoperoxidase gel • Antibacterial agents (0.12% or 0.2% chlorhexidine gluconate mouth rinse and xylitol)

Figure 13: Therapeutic strategies to manage oral comorbidities in patients with SS (15, 65, 67, 69, 82, 83).

2.5.3 – Extra-glandular abnormalities

Involvement of exocrine glands is the main clinical framework seen in SS and goes beyond the ocular and oral dry signs and symptoms. Less commonly, but also observed, are the respiratory, gastrointestinal and genital tracts, as well as cutaneous issues. Since exocrine glands exist all over the body other xeroses and / or complications can occur. Among these, SS patients may develop xeroderma, chronic pruritus, xeromycteria, nasal crusting, epistaxis, recurrent sinusitis, hoarseness, dry throat, chronic non-productive cough (xerotrachea), atrophic esophageal mucosa, atrophic gastritis, constipation. In addition, in women it can result in Vaginitis Sicca, with increased susceptibility to infections and dyspareunia, which leads to sexual dysfunction and pain. (10, 15, 85).

The architectural deterioration and functional impairment observed in the salivary and lacrimal glands of SS patients can be associated with systemic damage as well, with many other organs and systems affected during the course of the disease. Approximately 50% to 60% of SS patients present extra-glandular abnormalities and there is a 5% incidence of lymphoma in SS patients (12, 16, 86).

At times, these systemic features prevail at onset and become the presenting manifestations of SS along with dry symptoms. One of the most frequent constitutional symptoms in SS is abnormal fatigue, which is noticeable in nearly 70% to 80% of patients and is often related to labor disability. Even though there is a lack of reliable instruments for the evaluation of fatigue, it seems that the physical and somatic aspects are more frequently affected in SS patients, instead of mental exhaustion. The etiopathogenesis of fatigue in SS is unclear, leading to a significant challenge to manage, and often fatigue in SS patients tends to persist over time (10, 85, 86).

Other non-specific symptoms, and closely associated with fatigue, are sleep disorders, anxiety, and depression, affecting around 15%, 20% and 40%, respectively. In addition, low-grade fevers may also be present in 6-13% of SS patients, reported either as an early or later in the course of the disease. Occasionally, fevers are more severe and concomitant with malaise, asthenia, flu-like complaint, and when they become persistent, can be associated with weight loss and/or night sweats, which requires prompt assessment for a possible lymphoma (10, 85, 86).

Other musculoskeletal manifestations are very common in SS patients, especially chronic pain associated with articular conditions and myalgia, reported in 50% to 75% of them. The most common pattern observed is symmetric non-erosive / non-deforming polyarthritis, although monoarthritis has also been observed. The most commonly affected joints are the proximal interphalangeal (PIP), metacarpophalangeal (MCP) joints of the hands, and the wrist, although other joints can be involved too. Arthralgia is frequently observed, and frank synovitis may occur in 15% to 35%, even though subclinical synovitis is often observed by musculoskeletal ultrasonography in patients with SS. Concomitant fibromyalgia and/or coincidental age related osteoarthritis can further make the evaluation of musculoskeletal pain in SS patients more difficult. Muscle involvement has been documented in around 27% of patients with SS. Myalgia is common, while myositis, once diagnosed, is rarely related to SS myopathy (10, 12, 85, 86).

Clinically significant respiratory manifestations are present in 10% to 20% of SS patients, mainly in the airways. Therefore, obstructive lung disease, such as bronchiectasis, is more frequent than interstitial lung disease. Nevertheless, usual interstitial pneumonitis (UIP), cryptogenic organizing pneumonia (COP) and lymphocytic interstitial pneumonitis (LIP) may also occur, the later occasionally progressing to lymphoma. Recurrent respiratory infections caused by dryness in the airways may also occur, due to compromised muco- ciliary clearance (12, 85).

The hematologic manifestations of SS can be separated into cellular and humoral components. The main cellular abnormalities, such as autoimmune hemolytic anemia and severe thrombocytopenia occur in less than 5% of SS patient but can even be the presenting feature. Humoral manifestations include hypergammaglobulinemia, hypogammaglobulinemia, monoclonal gammopathy, cryoglobulinemia, and elevations in autoantibodies. Although the hematologic manifestations and lymphoproliferative disorders found in SS are typically mild and clinically silent, several are prognostic markers for the development of lymphoma and increased disease mortality. Furthermore, in patients with hypergammaglobulinaemia and cryoglobulinemia, cutaneous vasculitis can be seen, such as flat purpura and palpable purpura. Overall, about 10% of SS patients have skin lesions, mostly some form of a vasculitis with involvement of small and medium vessels of the lower limbs. Other less common skin manifestations may occur in SS patients, such as annular erythema and urticarial vasculitis (10, 15, 85, 86).

Also, of clinical relevance is the renal involvement occurs in roughly 5% of SS patients, usually associated with tubule-interstitial changes, along with distal renal tubular acidosis with hypokalemic muscular hypotonia. The peripheral nervous system is also affected, especially later stages of the disease, typically manifested as sensory neuropathy in 10% to 25% of those individuals. CNS manifestations are rare and difficult to diagnose, so that distinguishing multifocal CNS lesions between multiple sclerosis lesions and SS lesions on MRI is a complicated task (15).

A summary of the main extra-glandular manifestations of SS is presented by Vivino, 2017 (12).

2.5.4 – Pathology of Sjögren's syndrome

The functional deterioration and architectural damage of the lacrimal and salivary glands, that causes persistent dryness of the eyes and mouth, is the hallmark of the autoimmune epithelitis seen in SS patients. The LMSG biopsy is considered one of the most important criteria in the identification of this disease, and standardizing its procedure and analysis is the key in the diagnosis of SS. The first requirement for a precise histological assessment remains an adequate number of glands collected, between 3 and 5 in total, which allows sufficient glandular surface for evaluation. Furthermore, since there are issues regarding the concordance between both oral and general surgical pathologists, there is a consensus that the histopathological interpretation should be carried out by an experienced pathologist, to prevent inadequate interpretation and false diagnoses (86, 87).

Even though studies have shown a relatively good reliability of this analysis, both inter- and intra- observer inconsistencies are present in the literature. In one series, authors found errors in the application of the grading system in up to 45% of the SS LMSG biopsies, resulting in 10% of misdiagnoses, and 34% of non- diagnoses. Even when the grading system was properly applied, the authors detected 36% of false-positive cases (34). Another study assessing the observers' agreements showed an intra-observer κ value of 0.80 and inter-observer κ values of 0.71 for FS in LMSG biopsies (88). Moreover, the re-evaluation of 60 SS biopsies showed that more than half of them presented clinical-pathologic discordance and failed to endorse the initial histologic diagnosis. The non-application of the FS system is likely the most common cause for non-agreement between the final histologic diagnosis and the actual clinical scenario, resulting in significant diagnostic discrepancies in SS patients (89). Hence, training, calibration and consistent application of the current histologic grading system is crucial to avoiding misdiagnosis and guide clinical decisions (34, 88, 89).

Interestingly, the histological grading most commonly use was established in 1974, when Greenspan, et al modified a previously introduced system by Chisholm and Mason, in 1968, which graded according to the presence of lymphocytes and plasma cells per 4mm2. Within this system, it was determined grades: 0 – absent; 1 – slight infiltrate; 2 – moderate infiltrate; 3 – one focus; and 4 – more than one focus. The alteration adopted by Greenspan is the number of foci per 4mm2 of section, representing an extension of the grade 4 seen in the previous classification. Also, a FS of

10 was arbitrarily defined as the highest that could usually be counted, and an FS of 12 corresponds to those cases in which the foci were so numerous that they became confluent (90).

Overall, the main histological finding in SS is the focal lymphocytic sialadenitis (FLS), defined as the presence of one or more dense aggregates of 50 or more lymphocytes, located in periacinar, perivascular or periductal areas - Figure 24. In addition to the presence of FLS, examination of an LMSG biopsy may identify nonspecific chronic sialadenitis, sclerosing chronic sialadenitis, acinar atrophy, interstitial fibrosis and ductal dilation (86).

Different microscopic changes occur throughout the evolution of the disease, with interlobular and periductal chronic inflammatory infiltrate within a normal glandular architecture in the initial stages (Figure 14). With the advancement of the glandular involvement, an architectural alteration is observed, with a reduction of the acinar parenchyma along with ductal hyperplasia. Progressively, there is increased angiogenesis and accumulation of hyaline material in the lumen of ducts and around blood vessels. Finally, acinar depletion, fatty infiltration, and fibrosis are observed in advanced cases, when no more functional glandular parenchyma can be seen (14, 15, 25, 85, 89).

Figure 14: “This figure was removed because of copyright restrictions. It depicted the nodular lymphocytic infiltration in a patient with SS” Original source: Anaya J, Sarmiento-Monroy, J, García-Carrasco, M. Síndrome de Sjögren. Segunda edición. 2nd ed. Bogotá D.C.2017. 578 p. – Figure 1, page 107.

The composition of the inflammatory infiltrate is mainly T-lymphocytes (most CD4+) and B- lymphocytes, Mø and DC, in a proportion that depends on the stage of disease. It has been shown, through immunohistochemistry (IHC) (Figure 25), that T-cells (CD3 +) and its subpopulations CD4 +/CD8 + and Treg cells (Foxp3+), B cells (CD20+), Mø (CD68+), interdigitating DCs (iDC) (S100+), follicular DC (fDC) (Fascin+) and NK cells (CD56+) are the major types. These cells are present in a variable incidence and distribution, according to the disease severity (mild, intermediate and severe) and grade of infiltration. Both CD3+ T cells and CD20+ B cells were frequently found permeating ductal epithelial structures and represented over 90% of the mononuclear infiltrate. CD3+ T cells were present mostly at the periphery of infiltrates of mild lesions, whereas CD20+ B cells were seen centrally, and more evident in severe lesions, as well as in GCs. B-cell incidence

has been associated with clinical disease stage, positive serology, noticeable glandular hypofunction and also extra glandular manifestations (87, 91).

In addition, mast cells have been identified within LMSG specimens of SS patients and they may play an important role in chronic inflammation, being linked with angiogenesis. IHC studies have shown that a more advanced mononuclear infiltration, with intense CD20+ B-cell population, present a positive correlation with CD68+ cells (Mø), even though these cells are present in varying proportions in different stages of SS (Figure 15). Overall, CD68+ cells are seen dispersed all over the glandular parenchyma or grouped in lymphoepithelial lesions near the salivary ducts. However, the incidence of Mø seems to be associated with lesion severity, and a decline in iDC incidence and unchanged fDC infiltration have been reported when the lesion becomes more severe. This finding suggested that the major decay of ductal epithelial structures observed in those lesions could be translated to deficient iDC recruitment in LMSG lesions. The increased presence of Mø have also been associated with lymphoma development, and development of this malignancy is also correlated with GC formation in the SS infiltrates (91, 92).

Figure 15: “This figure was removed because of copyright restrictions. It depicted the IHC of SGs in SS patients” Original source: Dinescu Ş C, ForŢofoiu MC, Bumbea AM, Ciurea PL, Busuioc CJ, Muşetescu AE. Histopathological and immunohistochemical profile in primary Sjögren's syndrome. Romanian journal of morphology and embryology = Revue roumaine de morphologie et embryologie. 2017;58(2):409-17.

The salivary glands, predominantly the parotids, are the most frequent site of MALT lymphomas in patients with SS, although they can also occur in the stomach, lungs and eyes. The progression of a low-grade lymphoma towards a diffuse large B-cell lymphoma (DLBCL) is often the pathologic evolution seen in this malignancy. Histologically, they show a population of poorly differentiated B-cells, with minimal cytoplasm (85, 92).

2.5.5 – Serological features

SS, similarly to SLE, is a prototype of autoimmune disease characterized by a great diversity of autoantibodies, both organ-specific and nonspecific. The major etiopathogenic abnormality of SS is the B- lymphocyte hyper-activation, which results in these autoantibodies production. Some of them help in the diagnostic process (ANA, RF, anti-SSA, anti-SSB), or are recognized as having a possible pathogenic role (anti-SSA, anti-SSB, anti-carbonic anhydrase, anti-muscarinic) (72, 94).

The association of several of these serological factors with clinical manifestations of SS have been reported, but a lack of specificity prevents most of them being used for diagnosis or classification criteria.

ANA are the most prevalent autoantibodies in SS, being found in the serum of up to 85% of the patients with SS, most frequently with fine speckled or nucleolar patterns. Their presence in patients complaining of Sicca symptoms brings up the suspicion of SS in non-specialized clinical settings, such as in primary care (10, 16, 23, 86, 95). ANA have been associated with parotid enlargement, and with various extra-glandular manifestations, including Raynaud's phenomenon, articular involvement, and cutaneous vasculitis. A correlation with other serological features of SS, including hypergammaglobulinemia, elevations in the erythrocyte sedimentation rate, and autoantibodies directed against extractable nuclear antigens (ENAs) has also been reported (95, 96).

RF and cryoglobulins are present in SS patients in 40% to 70% and 10% to 15%, respectively, regardless of the presence of joint manifestations or if it is a case of SS associated with rheumatoid arthritis. Both have been correlated with a diagnosis at an earlier age, as well as systemic activity of SS, especially Raynaud phenomenon, vasculitis, and arthritis. Their positivity is correlated to ANA and anti-SSA, and RF's use to select patients for B-cell-targeted therapies has been cited in the literature (16, 23, 72, 95, 96). Overall, the presence of RF correlates to more severe exocrine gland manifestations and can be used as a prognostic marker in SS. However, it cannot be useful for clinical diagnosis or research classification purposes because it is so commonly found in other AI diseases (97). Likewise, the presence of cryoglobulins is correlated to peripheral neuropathies. Presence of cryoglobulins indicates a clinical subset of SS with poor prognosis, as they have been recognised as risk factors for lymphoma development and death (23, 72). However, like RF, cryoglobulins have not proven useful in the early diagnosis of SS.

Research aims to clarify if the antibodies detected in SS patients' sera are linked to the immunopathogenesis of the disease or are only epiphenomenal in the context of the hyper-reactivity of B cells, which also characterizes SS. It is recognized that anti-SSA and anti-SSB are the typical antibodies in patients with SS. The prevalence of anti-SSA antibodies in sera of patients with primary SS varies between 50% and 70%, and the presence of anti-SSB antibodies varies between 30% and 60%. Ro/SSA antigen is a Ribonucleoprotein (RNP) complex containing hY-RNAs and at least two proteins (Ro 52 kD and Ro 60 kD) that are associated with small molecules of RNA. La/SSB antigen consists of a protein 48 kD, which is involved in variable aspects of RNA metabolism, including the binding of viral-related RNAs (86, 95, 98). It is also known that a specific immunogenetic base is required for the formation of these autoantibodies, which suggests that HLA class II could participate in the initiation and perpetuation of the autoimmune response. In addition, their presence has been associated with glandular and extra-glandular manifestations, as well as alterations in B- cell function (95, 97).

Studies have shown that anti-SSA and anti-SSB antibodies are associated with an earlier onset of the disease. For example, patients <35 years have a higher prevalence of anti-SSA antibodies at the diagnosis than those with a later onset (45% vs. 12%), while when the groups are defined as >70 years and <70 years, the prevalence of antibodies is similar (99). The presence of serum anti-SSA and anti-SSB has been associated with longer disease duration, more severe dysfunction of the exocrine glands, recurrent parotid gland enlargement and ocular dry symptoms, as well as with positive results in the Schirmer test and Bengal Rose staining (97, 100, 101).

Regarding the correlation between these autoantibodies and histological features, some studies have demonstrated an association of seropositivity for anti-SSA (26) and of both anti-SSA/SSB with higher intensity of the lymphocytic infiltrates in the minor salivary glands, as well as with FS (25, 27-29). However, there remains a lack of consistency, with some studies showing more significant lymphocytic infiltration in anti-La/SS- B patients versus those only positive for anti-SSA, or negative for both antibodies (30). A correlation seen between FS and the presence of Ro 52 kD and 48 kD in both saliva and plasma supports the hypothesis that these autoantibodies are produced in the salivary glands, and may indicate that the local autoantibody production is a consequence of local inflammation (102). Similarly, the association of the development of GC with a higher prevalence of RF, anti-SSA and anti-SSB antibodies has also been described (103).

Other autoantibodies exhibiting possible pathogenetic role have been cited in the literature. Anti- carbonic anhydrase II antibodies (Anti-CA II) are detected in 12.5 to 20.8% of SS patients in higher levels among those with distal renal tubular acidosis have than those without renal tubular acidosis. Mouse model studies have shown the development of SS-like autoimmune sialadenitis upon previous injections with those autoantibodies, suggesting their possible participation in the pathogenesis of the human condition. In addition, as described in the pathogenesis of SS, parasympathetic cholinergic neurotransmission to salivary and lacrimal glands is mediated by μ3-muscarinic receptor (μ3R). The prevalence of anti-μ3R autoantibodies varies from 55.8% to 90% of the patients with SS. Apparently, autoantibodies with anti-μ3R are correlated to early onset of the disease, increased levels of cryoglobulins, and presence of cytopenias. Their activity could block the μ3R receptor in SS patients, contributing to the glandular hypofunction and other autonomic manifestations in SS patients (72, 95).

The presence of atypical autoantibodies, such as anti-DNA, antiphospholipid antibodies, anticentromere (ACA), and anti-neutrophil cytoplasmic antibodies (ANCA), in SS patients should alert to the presence of certain clinical phenotypes and to the development of a second organ or non-organ specific autoimmune disease. These autoantibodies may predict the appearance of a second autoimmune disease, such as SLE and limited scleroderma, and help to identify patients with a more serious illness. It has been suggested that a subset of SS patients positive for ACA present a clinical phenotype intermediate between SS and systemic sclerosis (72, 104). There is a group of antibodies in this entity whose significance is still uncertain and that will be the subject of future research. A summary of the most common and important autoantibodies found in SS is seen in the Figure 16.

Figure 16: “This figure was removed because of copyright restrictions. It depicted the characteristics of the major autoantigens in SS IHC of SGs in SS patients” Original source: Tzioufas AG, Tatouli IP, Moutsopoulos HM. Autoantibodies in Sjogren's syndrome: clinical presentation and regulatory mechanisms. Presse medicale (Paris, France: 1983). 2012;41(9 Pt 2): e451-60.

2.5.6 – SS ACR/EULAR Criteria

In order to determine the procedures for diagnosing SS, a combined criteria set has been approved by the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR), which was published in 2016 - Figure 17. The main goal of such criteria sets is to standardize the recruitment of patients for clinical trials, thus the focus of it is on primary rather than secondary SS (35). The current ACR/EULAR criteria added the presence of Sicca symptoms or a EULAR SS disease activity index (ESSDAI) of ≥1 as an entry criterion. In the 2016 ACR/EULAR criteria, anti-SSB, ANA and RF positivity were not adopted, and the presence of anti-SSA antibodies is the only positive serology criterion. The ocular staining score (OSS) score was also added to the ACR/EULAR criteria with a cut-off of ≥5, but sialography and scintigraphy were not included and some updates were made in the exclusion criteria for classification as primary SS (35, 105).

Item	Weight/score
Labial salivary gland with focal lymphocytic sialadenitis and focus score of ≥1 foci/4 mm2	3
Anti-SSA/Ro positive	3
Ocular Staining Score ≥5 (or van Bijsterveld score ≥4) in at least 1 eye	1
Schirmer's test ≤5 mm/5 minutes in at least 1 eye	1
Unstimulated whole saliva flow rate ≤0.1 ml/minute	1

* These inclusion criteria are applicable to any patient with at least 1 symptom of ocular or oral dryness, defined as a positive response to at least 1 of the following questions: 1) Have you had daily, persistent, troublesome dry eyes for more than 3 months? 2) Do you have a recurrent sensation of sand or gravel in the eyes? 3) Do you use tear substitutes more than 3 times a day? 4) Have you had a daily feeling of dry mouth for more than 3 months? 5) Do you frequently drink liquids to aid in swallowing dry food?, or in whom there is suspicion of SS from the European League Against Rheumatism SS Disease Activity Index questionnaire (at least 1 domain with a positive item). † Exclusion criteria include prior diagnosis of any of the following conditions, which would exclude diagnosis of SS and participation in SS studies or therapeutic trials because of overlapping clinical features or interference with criteria tests: 1) history of head and neck radiation treatment, 2) active hepatitis C infection (with confirmation by polymerase chain reaction, 3) AIDS, 4) sarcoidosis, 5) amyloidosis, 6) graft-versus-host disease, 7) IgG4-related disease.

Figure 17: American College of Rheumatology/European League Against Rheumatism classification criteria for primary SS: The classification of primary SS applies to any individual who meets the inclusion criteria,* does not have any of the conditions listed as exclusion criteria, † and has a score of ≥4 when the weights from the 5 criteria items below are summed (35).

Studies have been performed to validate the ACR/EULAR criteria for SS using its classification according to expert opinion as the gold standard. Apparently, the ACR/EULAR

criteria has shown great diagnostic accuracy in general, with greater accuracy when using parotid gland biopsies. However, the addition of new items and the validity of Schirmer's test and sialometry has been questioned since their accuracy was poor, and possible positive results in many non-SS patients. The ACR/EULAR criteria have important advantages compared with other criteria sets, and have been endorsed by both the ACR and EULAR, allowing for international consensus regarding the classification of SS (35, 105).

2.5.7 – SS management – an overview

Regardless of the many advances in understanding of the pathogenesis of SS, a specific treatment for the disease is not yet available, and its therapy remains empiric, symptomatic, and focused on mitigating Sicca symptoms. As mentioned in previous chapters, the use of eye lubricants, saliva and tear substitutes, as well as glandular secretion stimulation strategies are the foundations of therapy in SS. Despite the fact that frequently the manifestations are mild or limited at the time of diagnosis, around 40% to 70% of SS patients will present extra-glandular or systemic autoimmune diseases (SAD) at some point, requiring some intervention with medications that may carry significant risk of adverse effects. The chronic course of SS requires a daily, long-term use of therapies and it is therefore important to focus upon therapies with the least frequent/significant adverse effects, with topical therapies being preferred when feasible (9, 10, 52, 55, 65, 106).

Clinical practice guidelines for management of the ocular, oral and rheumatologic/systemic manifestations of SS have been recently published (106-108). However, in the clinical scenario, the management must be tailored to each individual, according to the disease activity and the presence and extent of extra-glandular manifestations. The EULAR Sjögren's Syndrome Patient Reported Index (ESSPRI) and ESSDAI have been used to assess the severity of the disease and includes assessments of the many parameters, such as Blood cell counts, Complement / Cryoglobulin / IgG and serum gammaglobulin levels, as well as serum monoclonal components and muscle enzyme levels. SS may be an ominous systemic condition, with heavy impact on the health-related quality of live (HRQoL) due to dryness, fatigue and pain, as well as the involvement of internal organs and increased mortality caused by cancer (lymphoma) (36, 106).

A recent set of recommendations for the management of patients with SS has been published under the auspices of the EULAR and recognizes that SS patients should be managed in and around

centres of expertise, including professionals with solid clinical experience in assessing patients with SAD. In general, an interdisciplinary team, including family physicians, rheumatologists, ophthalmologists, ENT specialists, oral medicine specialists, and dentists, as well as other specialists (gynecologists, pulmonologist, neurologists, etc.) may be required to provide adequate treatment. Two main lines of therapy have been used in the treatment of patients with extra-glandular manifestations, depending on the disease activity and the organ or system involved: a "traditional" therapy based on the use of the Disease-modifying therapy; and a "novel" therapy, which utilizes biologic agents that target pathophysiological mechanisms (15, 36, 86). Overall, the management of the systemic aspects in SS should follow a two-stage sequential regimen, similar to what is used in other SAD. A first intensive immunosuppressive approach targeting to restore organ function and induce disease remission promptly, followed by a second therapeutic course aimed at the maintenance of remission (106).

The conventional therapy is based on disease-modifying antirheumatic drugs (DMARDs), as well as with non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids and other immunosuppressants with systemic effects, according to experiences in the management of other AI and related rheumatic diseases, such as SLE. The main goal of this kind of treatment is to manage dry symptoms, constitutional and musculoskeletal symptoms, pain and fatigue, since these are the main and most frequent group of manifestations. The use of corticosteroids, mainly prednisone in low-dose is recommended for the treatment of arthritis and cutaneous symptoms. Higher doses of prednisone have shown benefit in renal, lung and CNS features, though the long-term use of corticosteroids is associated with serious effects, including osteoporosis, diabetes, weight gain and dyslipidemia (86, 109).

Although the use of hydroxychloroquine in treating Sicca symptoms is not a consensus, it is an agent with a satisfactory side-effect profile, and a fair choice for numerous mild to moderate systemic manifestations. It seems to be beneficial for musculoskeletal complaints such as arthralgia, myalgia, fibromyalgia-like features, and non-erosive polyarthropathy associated with SS, as well as for cutaneous lesions, and fatigue. Hydroxychloroquine inhibits TLR signaling pathways, affecting the innate immune response by reducing the production of pro inflammatory cytokines, such as type-I IFNs, and other serological parameters, such as IL-6, IgG, erythrocyte sedimentation rate and rheumatoid factor (15, 55, 86). Hydroxychloroquine is associated with a risk of retinopathy, which

increases significantly with its long-term use or greater doses. Other systemic medication options include methotrexate (either alone or in addition to hydroxychloroquine), and leflunomide, sulfasalazine and azathioprine, which were deemed to have similar efficacy in managing the inflammatory musculoskeletal pain, as steroid sparing agents. Nonetheless, in situations with evidence of other major organ involvement, such as Interstitial lung disease (ILD), azathioprine is preferred to treat multi-system disease (10, 109). A great emphasis has been placed on avoiding opioids for chronic musculoskeletal pain. Such musculoskeletal pain may be more appropriately managed with antidepressants and anticonvulsants, while chronic neuropathic pain may improve with use of pregabalin, gabapentin or amitriptyline, unless any exacerbation of dryness symptoms occur (106).

When the systemic compromise results in significant morbidity and mortality, such as glomerulonephritis, vasculitis, central and/or peripheral neuropathies, and interstitial lung injury, immunosuppressive management with high doses of glucocorticoids, or with cyclophosphamide, azathioprine, mycophenolate, or cyclosporine can be helpful. In the case of vasculitis, the use of corticosteroids in pulsed high doses intravenously is recommended if the patient is organ-compromised. In less serious presentations of vasculitis, oral administration of corticosteroids may suffice, sometimes combined with other immunosuppressants, especially cyclophosphamide. Immunoglobulins seem to be an adequate therapeutic option for patients with compromised peripheral nervous system pain that does not respond to other pharmacological approaches. On the other hand, complications in the airways, and tubulo-interstitial renal or liver conditions usually have a more stable and chronic course and may respond to less aggressive therapy, with azathioprine or cyclosporine (10, 106, 109).

Considering the current understanding about the pathogenesis of SS and its immunological alterations in T and B-lymphocytes, plasmacytoid dendritic cells and overexpression of various inflammatory molecules, various potential targets for the treatment of the disease have emerged. The use of monoclonal antibodies against TNF-α has shown controversial results. Some trials with infliximab demonstrated a significant improvement of clinical and functional parameters, such as visual analog scale, ESR, saliva and tear secretion, arthralgia and fatigue. However, these results were reassessed in other clinical trials, with only partial or no benefit from that medication for the same parameters. No efficacy was observed with etanercept either, and based on these results, the

use of this class of drugs has not been recommended for patients with SS. Tocilizumab is a monoclonal antibody which blocks the IL-6 receptor, and even though there are isolated reports of possible benefit in a SS patient complicated with optic neuromyelitis, its use is not yet recommended in this disease. Preliminary results have suggested a possible efficacy of belimumab, a human monoclonal antibody that inhibits B-cell activating factor (B-lymphocyte stimulator) in SS. Although an improvement in Sicca symptoms, parotid growth, systemic activity and levels of some biomarkers have been shown, its potential use in SS depends on well design clinical trials (10, 109).

A better therapeutic benefit has been attributed to rituximab, a chimeric monoclonal antibody that induces the death of B cells via apoptosis, antibody-dependent cell-mediated cytotoxicity or complement-dependent cytotoxicity, by binding to the CD20 surface marker expressed on those cells. This causes a transient depletion of B cells in the circulation, with a variable duration among individuals. It has shown good efficacy against clonal B cells expressing CD20, such as in some types of lymphoma. In SS patients, open studies and some clinical trials have suggested that late-stage patients showed no significant response. However, it showed efficacy in reducing Sicca symptoms, extra-glandular symptoms and fatigue, as well as improvement in histopathologic parameters, saliva production and quality of life, especially in early age- diagnosed patients (10, 86, 109). A recent paper recommended the use of rituximab as an option in SS patients with severe, refractory systemic disease. Furthermore, this paper suggested that the best indication for this drug would be for symptoms linked to cryoglobulinemic-associated vasculitis, mentioning the use of belimumab as a possible rescue therapy. A more complete review is available in the EULAR recommendations for the management of SS with topical and systemic therapies (106).

Chapter 3 – Material and Methods

Our literature review led to the conclusion that there is a need to carry out studies to corroborate practice guidelines for clinicians to prevent misdiagnosis and mistreatment for one of the most prevalent autoimmune diseases in adults, especially women.

The literature review showed that:

1. As a multisystem condition, with a heterogeneous presentation, course, and outcome, it has not yet reached a rigid consensus in terms of the diagnostic process. There is not yet a 'gold standard' test, and the closest to it is still labial salivary gland histopathological evaluation;

2. Seropositivity for SS-A/SS-B is associated to several different auto-immune diseases, such as idiopathic inflammatory myopathy, rheumatoid arthritis, systemic lupus erythematosus, and systemic sclerosis, among others, including erratic results to primary SS;

3. None of the papers assessed performed an adequate research to directly compare the diagnostic performance of both serology and histology in SS;

4. There are a scarce number of diagnostic accuracy studies for SS, and the few we found were not designed in a manner that could compare accuracy, costs, and potential burden of the tests analyzed;

5. The only accuracy study found in the literature so far utilizes the 'expert opinion' or the 'clinical diagnosis' as the gold standard for comparison between each analysed criterion, which maintains a certain level of subjectivity during the diagnostic process.

Therefore, more well-designed studies in diagnostic accuracy of SS are needed, ensuring recruiting adequate spectrums of patients, making direct appraisals between methods, applying rigorous approaches, and clearly reporting results.

The information from this research will be used to support the practice of guidelines and standard of care for almost a half million of persons affected by this overwhelming condition. The

information will help clinicians in their clinical decision making and will protect the individuals from misdiagnosis and mistreatment.

3.1 – What is the principal research question to be addressed?

We addressed the following research question: *"In patients with a confirmed diagnosis of Sjögren's syndrome through the ACR/EULAR criteria, what is the diagnostic performance of the main tests, such as Focus Score, anti-SSA?"*

3.2 –Specific objectives

1. To assess the diagnostic capability of histologic features versus serologic findings as a diagnostic tool for patients with Sjögren's syndrome;
2. To clarify the association between histopathological features of the minor salivary glands and serological tests currently used to diagnose Sjögren's syndrome;
3. To identify potential improvements to the current Sjögren's syndrome diagnostic criteria (ACR/EULAR).

Primary hypothesis

"A comprehensive histological assessment of the minor salivary glands is still an essential tool in the diagnostic of Sjögren's syndrome when compared with the serological assessment."

3.3 – Study design

This is a retrospective chart review study - Database Review (paper charts, electronic health records, or administrative health data).

3.4 – Eligibility criteria for participants and the settings where the data was collected

Inclusion and exclusion criteria:

Cases eligible for inclusion in this research were adults [18-80 years old] with suspected SS based on the revised rules of the American-European Consensus Group Criteria (ACR/EULAR) utilized to diagnose primary Sjögren's syndrome. Only individuals who underwent serologic tests for Anti-SSA [index test] and LMSG biopsy [index test] in Alberta Health Services care in the metropolitan area of Edmonton, Alberta, Canada, were included in the review. The SS ACR/EULAR criteria was used as reference standard. This study enrolled a consecutive sample from Dr. Tim McGaw's referred oral medicine practice from 2009-2020.

The number of records accessed was 140 patient charts and patient records from Dr. McGaw's database, including the reports generated by him at the Oral Pathology Laboratory – UofA. Only the eligible cases were considered for analysis. The cases diagnosed with the use of only one of the main tests, i.e. either serology OR biopsy alone (FS or Anti-SSA) assessed, such that comparison between their accuracy was not possible, were excluded from our analysis.

While collecting the clinical data, we observed that some patients presented low serology titration for Anti-SSA, and among this group, a small subset ~~group~~ had negative FS. In examining this subset of cases more carefully, we identified notes from the rheumatologists casting doubt on the "positivity" of the cases, with the patients considered "non-diseased" by the rheumatologist. Thus, we decided to create a second "reference standard", based on the expert opinion and working clinical diagnosis (Modified-ACR/EULAR 'ClinDx'). In order to envision an objective parameter and try to circumvent the subjectivity of the "expert opinion", and observing that all cases where the working clinical diagnosis that disagreed with the ACR/EULAR criteria had their titration below 200 MFU, we established this threshold as the cut-off line for serology. We subsequently therefore undertook our analysis for both reference standards, ACR/EULAR and separately for our proposed Modified-ACR/EULAR 'ClinDx'.

Specimens from biopsies of LMSG performed in patients under investigation for Sjögren's syndrome registered in the Oral Pathology Laboratory, School of Dentistry, University of Alberta, from 2009 to 2020 were reviewed, according to the following criteria: number of salivary gland

lobules; focus score, assessing the number of foci/4mm^2 (aggregate including at least 50 mononuclear cells); absence or presence of ductal dilatation, acinar atrophy and sclerosis of the glandular connective tissue; acinar/ductal ratio (normal ratio, similar to normal glands; reduced ratio, with increased ductal component); and presence of germinal centers (GC).

Schirmer test was retrieved from referrals and notes, when available, and salivary flow (sialometry) - whole mouth unstimulated salivary flow rate was measured in the dental office, asking the patients to expectorate the whole saliva over 10 minutes, and dividing by the collection time. A score of ≤0.1mL/min met inclusion for the 2016 ACR/EULAR Classification Criteria.

Database:

Clinical and laboratory information was obtained from records (charts) and on the referral forms sent to Dr. McGaw by the rheumatologists in charge of the cases. The data was obtained from a deep search in both Netcare and Connectcare platforms. We scrutinized all pertinent chart record, including clinician's notes, letters, referrals and lab results. The University of Alberta's ethics approval for the entire protocol of this research was "Pro 00094361".

3.5 – Statistical analysis

We described the number of participants with and without SS, according to two criteria settings (ACR/EULAR, Modified-ACR/EULAR 'ClinDx'), and the sensitivity and specificity / positive and negative predictive values. False positives are the cases where the test found a positive result, but the final diagnosis presented negative, according the corresponding criteria. On the other hand, true positive represents the cases where both the test (FS or AntiSSA) and the final diagnosis from the used criteria were positives.

Sensitivity = True positives / (True positives +False negatives)

Specificity = True negatives / (True negatives + False positives)

Positive Predictive Value (PPV) = (True positives / True positives +False positives)

Negative Predictive Value (NPV) = (True negatives / True negatives + False negatives)

Descriptive analyses for all variables were performed and presented as mean and standard deviation (SD) for continuous variables and numbers and percentages (%) for categorical variables.

An *ad hoc* cut-off line was proceeded in the numerical values for Anti-SSA, and only cases with serological titration ≥ 200 were considered positive for diagnostic accuracy analysis of this parameter. This followed the analysis of the charts and information obtained from the rheumatologists involved in the diagnostic process of those cases, with a final decision as "non-diseased" cases.

We proceeded with the bivariate analysis, with Pearson's chi-square test for to verify the significance of the association between the categorical variables.

Since there was inconsistency in the presentation of Anti-SSA lab results, and 27 cases showed only categorical results (positive or negative), without numerical information of the criterion, we estimated those numbers based on the means of positive and negative cases within the cases where we could find that information (total of 60 cases), so that we could utilize all 87 cases in our analyses.

A direct comparison between the accuracy of the tests were also done assessing the sensitivity, specificity, as well as predictive values, demonstrating them in a Receiver operating characteristic (ROC) curve plot graphics, for both models (ACR/EULAR and ClinDx)

These analyses have the objective of clarifying whether one or more predictors could be circumvented from the diagnostic process of suspected cases of SS, and how the current ACR/EULAR criteria could reflect the daily clinical practice with regards to diagnostic approach and individually-guided care.

Chapter 5 – Published Results and Further Contribution to Research

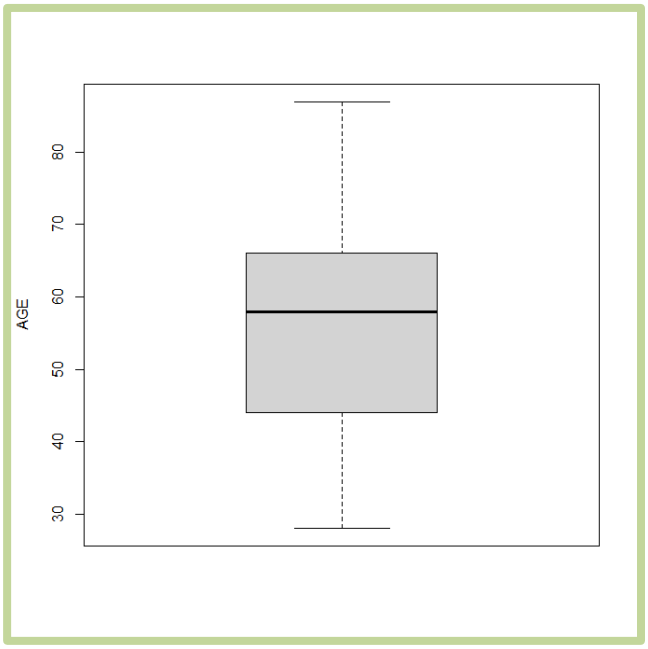
An intriguing diagnostic framework is present in SS patients' lives, which significantly increments the hurdles that clinicians face while managing this complex disease. We performed a systematic review on the accuracy of the two major parameters of the ACR/EULAR criteria, FS and Anti/SSA. The results show a lack of adequately designed studies on that topic, and the controversy about the performance of each criterion remains on air (108).

5.1 – Study results

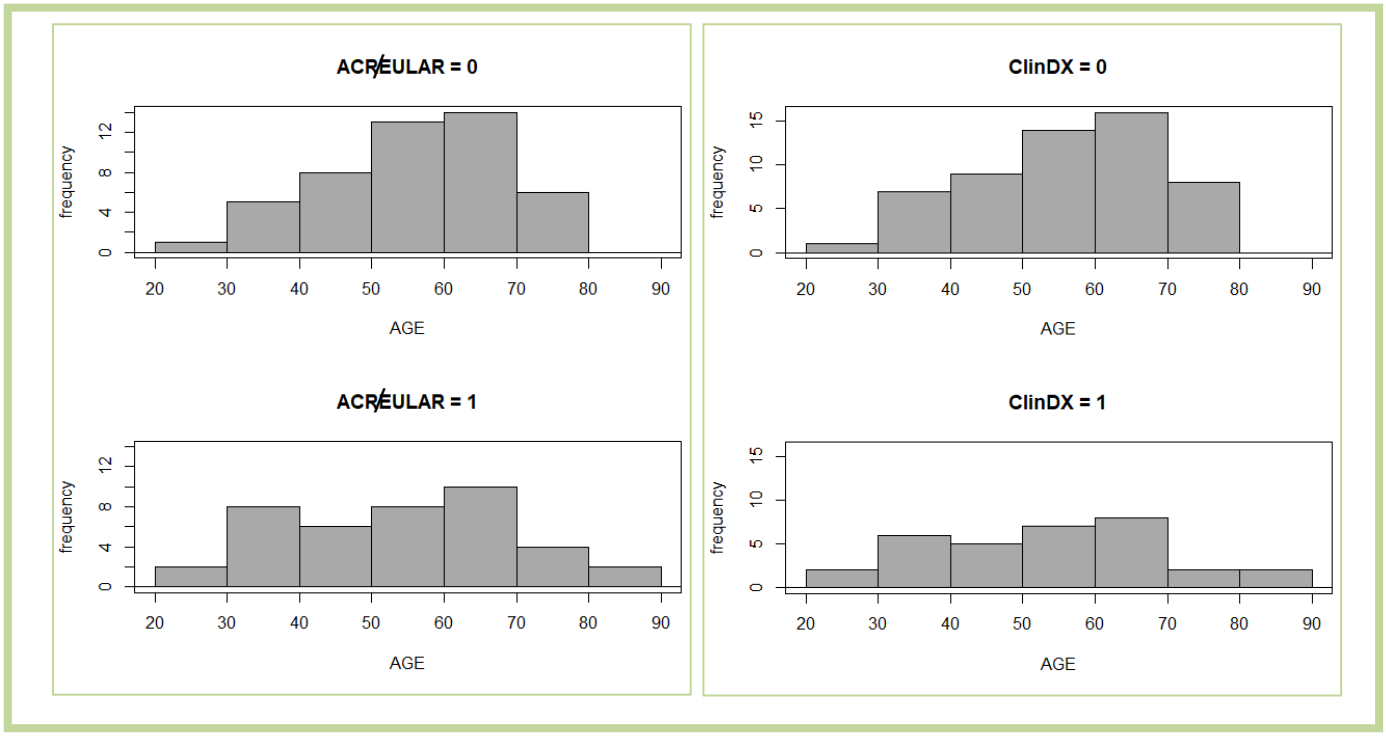
From the initial group of patients (140), a total of 87 subjects fulfilled the eligibility criteria. The data were descriptively analyzed using absolute frequencies and percentages for categorical variables and mean and standard deviation for the numerical variable age. The demographic data of the subjects studied in this project showed a majority of females (77/87), representing 88.5% of the sample, and the mean age 55.8 years old, range 28-87 (SD=14.23 years), and a median of 58.00 years – Table 1 and Graphics 1 and 2. The margin of error used to define the statistical tests was 5% and the intervals were obtained with 95% confidence.

Table 1: **Results overview**

Demographics	• Females = 77 (88.5%) • Males = 10 (11.5%)
• Age range: 28-87 • Mean age: 55.8 • Median: 58.00	• < 60 years: 50 (57,5%) • ≥ 60 years: 37 (42,5%)



Graph 1: Boxplot of Age distribution



Graph 2: Histograms of the ages observed among diseased (1) and non-diseased (0) in both models ACR/EULAR and ClinDx.

Ophthalmologic assessment to patients in this sample showed it was mainly directed to prevention and control of secondary consequences from the disease and its treatment, such as ocular damage by certain groups of medication (data not shown). Even with an extensive search of existing files, the Schirmer test was registered in only a small sample of patients (seven). Five patients presented positive, and two patients negative for the Schirmer tests.

Detailed descriptions of the primary outcomes, including the correlation between major criteria tests (FS, Anti-SSA) with each response (SS ACR/EULAR and ClinDx) can be observed in Table 2. We noticed that when strictly using the ACR/EULAR criteria, among the 87 patients analyzed, 47 cases presented negative, and 40 cases were considered diseased. Nevertheless, when the "final clinical SS diagnosis - ClinDx" is applied, 55 patients are reclassified as non-diseased, leaving 32 considered as SS patients. Eight patients were clinically reclassified as non-diseased and also had their Anti-SSA <200 (MFU).

Table 2: **Diagnostic information, absolute and percentual, and according to both criteria models**

Test		**FS** Positive = 24 (27,6 %) Negative = 63 (72,4 %)		**SSAcat1** Positive = 36 (41,4 %) Negative = 51 (58,6 %)		**SSAcat2** Positive = 25 (28,7 %) Negative = 62 (71,3 %)		
Criteria		+	-	+	-	+	-	Total
ACR/EULAR	+	24	16	35	5	25	15	40
	-	0	47	1	46	0	47	47
ClinDx	+	24	8	27	5	25	7	32
	-	0	55	9	46	0	55	55

In order to assess the association between the categorical variables, Pearson's Chi-square test was used. Table 3 shows the results of the association between the response variable ACR/EULAR

and the variables: FS, SScat1 and SScat2. This table emphasizes that, among the cases classified as positive for FS and SSAcat2, all patients were considered diseased; all those classified as positive by SSAcat1 were considered positive, except for one patient. In summary, table 3 shows that the percentage of sick patients was much higher among patients positive than negative for FS (100.0% / 25.4%), for SScat1 (97.2% / 9.8%) and SScat2 (100.0% / 24.2%) and the association is significant ($p < 0.001$), which was expected since they are items of both criteria settings.

Table 3: Bivariate analysis of SS ACR/EULAR according to the variables FS, SSAcat1, SSAcat2

	ACR/EULAR		
Variable	Diseased (n = 40)	Non-diseased (n = 47)	P value [1]
	(%)	(%)	
FS			p [1] < 0.001*
Positive	24 (100.0)	-	
Negative	16 (25.4)	47 (74.6)	
SSAcat1			p [1] < 0.001*
Positive (≥ 120)	35 (97.2)	1 (2.8)	
Negative (< 120)	5 (9.8)	46 (75.8)	
SSAcat2			p [1] < 0.001*
Positive (≥ 200)	32 (100.0)	-	
Negative (< 200)	8 (24.2)	47 (85.5)	

(*) Significant association: 5%
(1) Pearson's chi-square test

Table 4 presents the results of the association between the response variable ClinDx and the variables: FS, SScat1 and SScat2. Table 4 shows that the percentage of sick patients was much higher among patients positive than negative for FS (100.0% / 12.7%), for SScat1 (75.0% / 9.8%) and SScat2 (100.0% / 11.3%) and the association is significant ($p < 0.001$).

Table 4: Bivariate analysis of ClinDx according to the variables FS, SSAcat1, SSAcat2

	ClinDx		
Variable	Diseased (n = 40)	Non-diseased (n = 47)	P value (1)
	(%)	(%)	
FS			p (1) < 0.001*
Positive	24 (100.0)	-	
Negative	8 (12,7)	55 (87,3)	
SSAcat1			p (1) < 0.001*
Positive (≥ 120)	27 (75,0)	9 (25.0)	
Negative (< 120)	5 (9.8)	46 (90.2)	
SSAcat2			p (1) < 0.001*
Positive (≥ 200)	25 (100.0)	-	
Negative (< 200)	7 (11,3)	55 (88,7)	

(*) Significant association: 5%
(1) Pearson's chi-square test

The accuracy performance of each major criterion, within each criteria model (ACR/EULAR and ClinDx) can be visualized in table 5 (A-F). We can observe that FS is 100% specific in any criteria, and that SSAcat2 also achieves excellence in accuracy when the cut-off threshold of Anti-SSA ≥ 200 is used.

Tables 5 A-C (ACR/EULAR) and D-F (ClinDx): Accuracy of each major item within the criteria settings analyzed.

A)

		ACR/EULAR		
		Positive	Negative	
FS	Positive	24 (TP)	0 (FP)	24
	Negative	16 (FN)	47 (TN)	63
		40 (46%)	47 (54%)	

ACR/EULAR
FS Sensitivity = (24/24+16) = 60%
FS Specificity = (47/47+0) = 100%
FS PPV = (24/24+0) = 100%
FS NPV = (47/47+16) = 75%

B)

		ACR/EULAR		
		Positive	Negative	
SSAcat1	Positive	35 (TP)	1 (FP)	36
	Negative	5 (FN)	46 (TN)	51
		40 (46%)	47 (54%)	

ACR/EULAR
SSAcat1 Sensitivity = (35/35+5) = 88%
SSAcat1 Specificity = (46/46+1) = 98%
SSAcat1 PPV = (35/35+1) = 97%
SSAcat1 NPV = (46/46+5) = 90%

C)

		ACR/EULAR		
		Positive	Negative	
SSAcat2	Positive	25 (TP)	0 (FP)	25
	Negative	15 (FN)	47 (TN)	62
		40 (46%)	47 (54%)	

ACR/EULAR
SSAcat2 Sensitivity = (25/25+15) = 63%
SSAcat2 Specificity = (47/47+0) = 100%
SSAcat2 PPV = (25/25+0) = 100%
SSAcat2 NPV = (47/47+15) = 76%

D)

		ClinDx		
		Positive	Negative	
FS	Positive	24 (TP)	0 (FP)	24
	Negative	8 (FN)	55(TN)	63
		32 (36.8%)	55 (63.2%)	

ClinDx
FS Sensitivity = (24/24+8) = 75%
FS Specificity = (47/47+0) = 100%
FS PPV = (24/24+0) = 100%
FS NPV = (55/55+8) = 87%

E)

		ClinDx		
		Positive	Negative	
SSAcat1	Positive	27 (TP)	9 (FP)	36
	Negative	5 (FN)	46(TN)	51
		32 (36.8%)	55 (63.2%)	

ClinDx
SSAcat1 Sensitivity = (27/27+5) = 84%
SSAcat1 Specificity = (46/46+9) = 84%
SSAcat1 PPV = (27/27+9) = 75%
SSAcat1 NPV = (46/46+5) = 90%

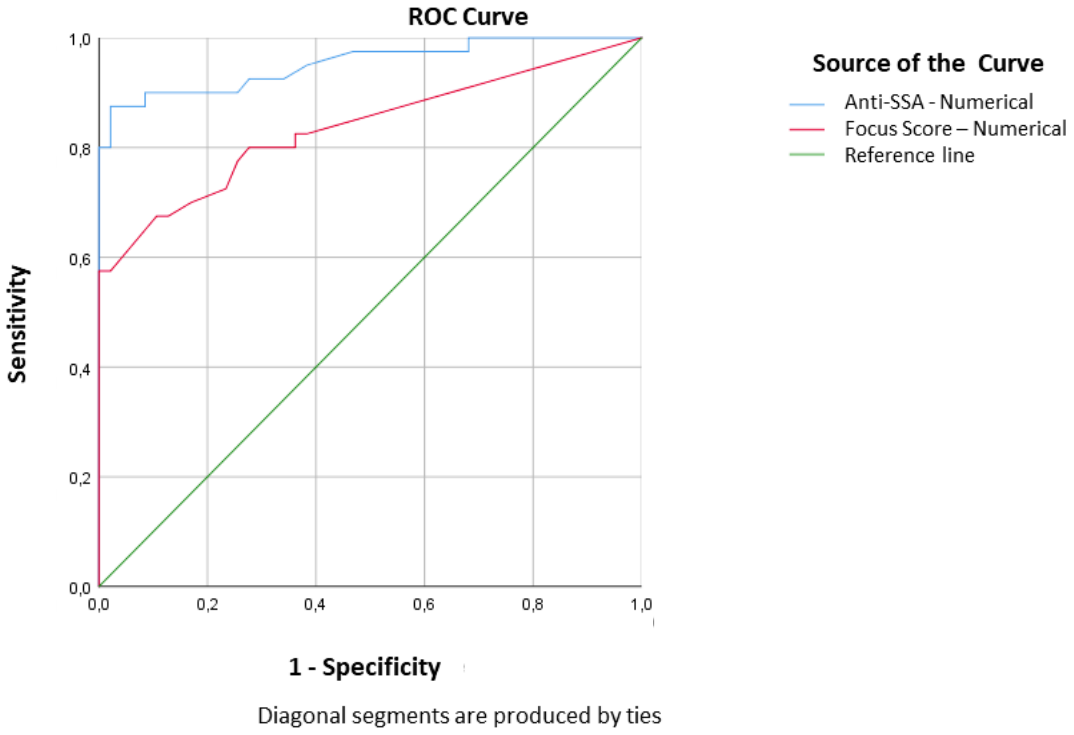
F)

		ClinDx		
		Positive	Negative	
SSAcat2	Positive	32 (TP)	0 (FP)	32
	Negative	0 (FN)	55(TN)	55
		32 (36.8%)	55 (63.2%)	

ClinDx
SSAcat2 Sensitivity = 100%
SSAcat2 Specificity = 100%
SSAcat2 PPV = 100%%
SSAcat2 NPV = 100%

For obvious reasons (clinical diagnosis used to establish the positive cut-off line at Anti-SSA ≥ 200), this variable (SSAcat2) presented perfect correlation with the criteria.

The SSnum and FSnum numerical variables were used to assess the discriminatory power of the ACR/EULAR and ClinDx criteria (response variables), known *a priori* through the ROC curve statistical technique. The technique obtained: area under the curve, significance and confidence interval. The areas under the ROC curves for both models, ACR/EULAR and ClinDx, are respectively seen in the graphics 3 and 4, and tables 6 and 7.

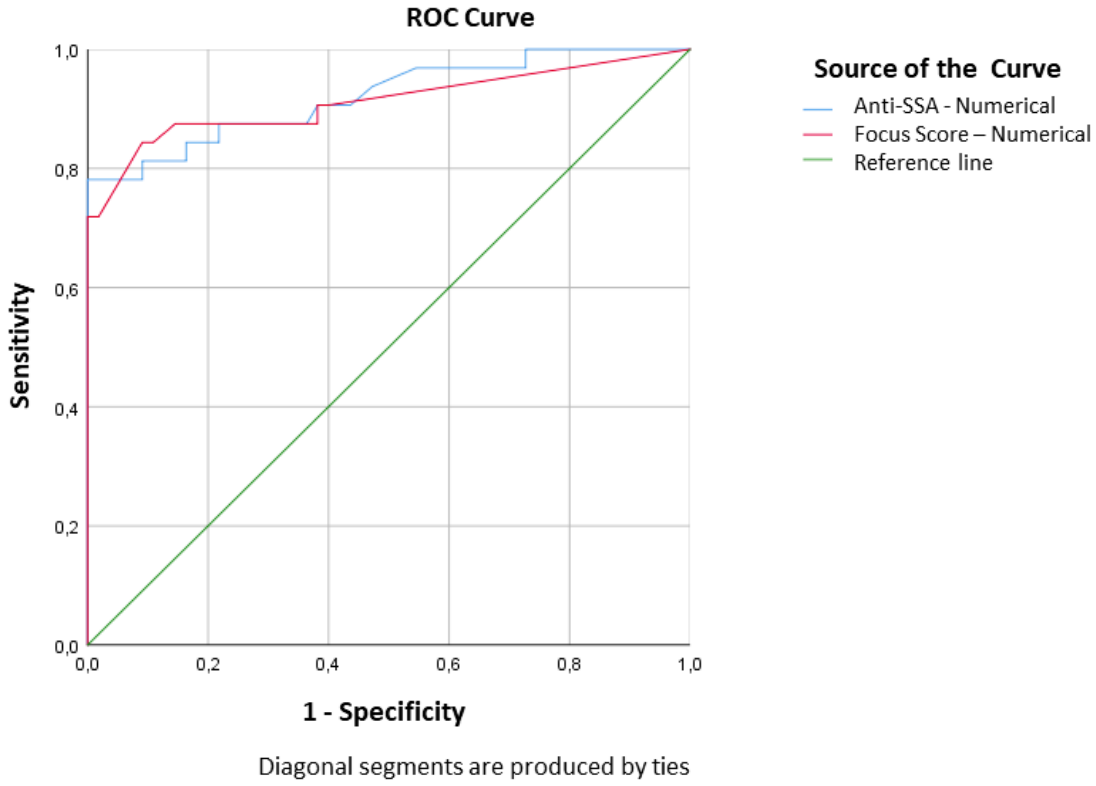


Graph 3: ROC curve for **ACR/EULAR** model, according to the numerical values of Anti-SSA and FS

Table 6: – Areas under the ROC curve (AUC) for **ACR/EULAR** model, according to the numerical values of Anti-SSA and FS

Variable	Area under the curve	P value	AUC CI
Anti-SSA numerical	0.95	p < 0.001*	0.91 to 1.00
FS numerical	0.84	p < 0.001*	0.75 to 0.93

(*) Significant at .05



Graph 4: ROC curve for **ClinDx** model, according to the numerical values of Anti-SSA and FS

Table 7: – Areas under the ROC curve for **ClinDx** model, according to the numerical values of Anti-SSA and FS

Variable	Area under the curve	P value	AUC CI
Anti-SSA numerical	0.92	p < 0.001*	0.86 a 0.99
FS numerical	0.91	p < 0.001*	0.84 a 0.99

(*) Significant at .05

We observe that when the higher serologic threshold (Anti-SSA ≥ 200) is applied, the ClinDx model seems to be more adjusted. The accuracy of Anti-SSA numerical does not change significantly (0.95 to 0.92 - excellent), while FS numerical goes from 0.84 to 0.91 - good to excellent.

5.2 – Peer-reviewed published article

Clinical Rheumatology
https://doi.org/10.1007/s10067-021-05813-5

REVIEW ARTICLE

Diagnostic performance of serology against histologic assessment to diagnose Sjogren's syndrome: a systematic review

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Received: 18 February 2021 / Revised: 20 May 2021 / Accepted: 6 June 2021

Abstract
The objective of this review was to assess and evaluate whether the published diagnostic accuracy studies provide evidence to sustain the current diagnostic guidelines put forth by ACR/EULAR used for patients with suspected Sjögren's syndrome (SS). Literature databases, including Medline, Embase, and EBM Reviews, were searched for relevant studies on the correlation between ACR/EULAR criteria, particularly those with a direct comparison between their accuracy in diagnosing Sjögren's syndrome. We followed Cochrane, QUADAS-2, and STARD guidelines and the four-phase flow diagram by the PRISMA Statement. Reports in several languages, but only human studies were considered. Three studies assessed the accuracy of the current diagnostic tests, and these did not present adequate designs that would allow a well-supported conclusion with a high level of certainty. Due to significant clinical and methodological heterogeneity, a meta-analysis was not performed. A qualitative review of the papers was undertaken. Neither the comparative nor the non-comparative study designs permit conclusive recommendations regarding an alternative diagnostic pathway for SS. Well-designed studies of the diagnostic accuracy of SS tests are needed to validate current guidelines or to suggest changes to the current guidelines.

Keywords ACR/EULAR criteria · Diagnosis accuracy · Diagnostic performance · Sjogren's syndrome · Sicca syndrome

Introduction

Sjögren's syndrome (SS) is a chronic, autoimmune disease characterized primarily by inflammation of exocrine glands, especially salivary and lacrimal, resulting in dry mucous membranes, mainly oral and ocular mucosa. SS can also present with musculoskeletal, cutaneous, renal, pulmonary, or neurological manifestations [1, 2].

The etiology of SS is unknown, but genetic, immunological, environmental, and hormonal factors are thought to participate in the related inflammatory process [3–5]. The observed glandular hyposecretion results from immune system interactions with the infiltration of lymphocytes and plasma cells' activation. The effect of autoantibodies and soluble inflammatory mediators is also involved in initiating and perpetuating the immune response against the epithelial cells of salivary and lacrimal glands [2, 5, 6].

SS has an estimated prevalence of 1% (0.1–4.8%), with a female:male ratio of about 9:1 and more commonly occurring between 45 and 75 years of age, but recent studies have shed light on how SS affects men and children as well. The clinical features can vary from mild sicca symptoms, as an isolated phenomenon (primary SS, pSS), or in conjunction with another autoimmune disease (secondary SS, sSS). The main complication of SS is potential malignant lymphomatous transformation, mainly non-Hodgkin lymphoma, affecting about 5% of the patients [1, 5–11].

The oral component of SS is characterized by xerostomia, found in 90% of patients, accompanied by thirst [1, 5]. A dry mouth can also be accompanied by voice changes, which can become hoarse and/or weak. Swallowing difficulties, diminished (hypogeusia), or altered (dysgeusia) taste can be seen. Patients with SS experience a higher incidence of dental caries and periodontal disease [1, 5]. The oral cavity's soft tissues can be affected, with a burning sensation on the tongue (glossodynia) or other areas of the oral mucosa (stomatopyrosis). Chronic erythematous candidiasis is commonly

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Published online: 17 June 2021

observed, presenting clinically as angular cheilitis, atrophy of the filiform papillae, and erythema of the mucosa. Pseudomembranous candidiasis occurs less frequently [1, 12].

The presence of autoantibodies characterizes SS. The majority (85%) of patients with SS present antinuclear antibodies (ANA) in the serum. Rheumatoid factor (RF) is observed between 50 and 70% of patients. Patients with suspected SS who present ANA should be investigated for anti-SSA and anti-SSB antibodies [6, 13]. The prevalence of anti-SSA antibodies in sera of patients with pSS varies between 50 and 70% and anti-SSB antibodies between 30 and 60% of the cases [14]. However, these antibodies are not specific to SS and may be identified in other autoimmune conditions [15–17].

None of the available tests exhibits adequate sensitivity and specificity to diagnose SS. Therefore, this necessitates a comprehensive assessment of multiple parameters, including examining the eyes, measurement of tear and saliva production, and a clinical and histologic examination of the salivary glands. Histologic examination of the salivary glands aims to determine the presence of an inflammatory infiltrate. The biopsy of labial minor salivary glands allows the differentiation of SS from simple age-related glandular involution and differentiation from other pathological processes such as sarcoidosis or lymphoma [1, 4, 5, 8, 18, 19].

The main histological finding in SS is focal lymphocytic sialadenitis, defined as the presence of one or more dense aggregates of 50 or more mononuclear inflammatory cells located in periductal areas. The focus score (FS) is assigned by evaluating the infiltrates per 4 mm^2 glandular areas [1, 4, 5, 8, 18]. This test has been considered the most accurate in SS diagnosis and may play a role in prognosis appraisal [16, 20–22].

Regarding the correlation between the auto-antibodies and histological features, some studies have suggested an association between histological FS and seropositivity for anti-SSA [23] and/or anti-SSB [18, 24–26]. However, reported papers lack consistency, with some showing more significant lymphocytic infiltration in anti-SS-B/anti-SS-A patients versus those only positive for anti-SSA or those negative for both antibodies [27]. Consideration of these inconsistencies in serological results has led to the widely held conclusion that labial salivary gland biopsy is still valuable for clinical practice due to a higher sensitivity, specificity, and positive and negative predictive values for SS diagnosis [22, 28–31].

To validate the procedures for diagnosing SS, a new set of criteria has been approved by the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR). These criteria emphasize that either labial minor salivary gland (LMSG) biopsy or anti-SSA serology must be positive, underscoring their central role in diagnosing SS [32]. However, some authors and centers have suggested that the LMSG biopsy (an invasive procedure that is not readily available at all clinical centers) could be circumvented and that only clinical and serological features are required to define the diagnosis. These authors have argued that LMSG biopsy should be restricted to suspected SS cases for which anti-SSA serology is negative [16]. Thus, some authors consider that the lack of a "gold standard" diagnostic method for SS opens an avenue for novel criteria, which could utilize new antibodies and images to do so [2].

This systematic review is intended to shed light on the accuracy of the diagnostic tests currently used when SS is suspected. To support an advocated replacement of histological assessment by serology alone, it is essential to provide scientific evidence. Only the demonstration of equivalent or superior diagnostic accuracy of serology could justify such a change in current guidelines. The addressed focused question (PICO) was: "In patients with a confirmed diagnosis of SS through FS (LMSG biopsy), is the diagnostic performance of serological tests, such as anti-SSA and anti-SSB alone when compared with serological tests and biopsy equally performant?".

Materials and methods

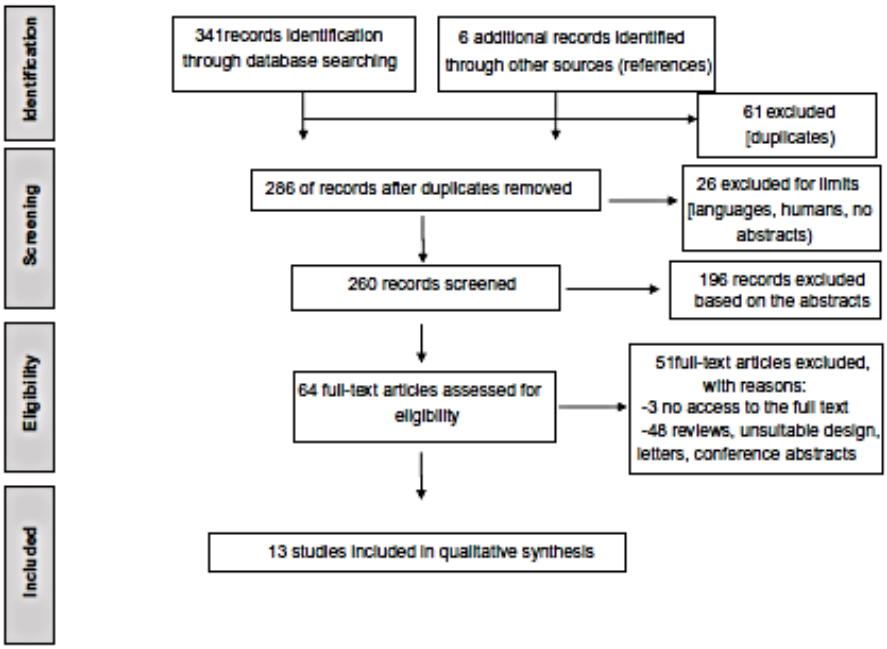
The current protocol delineates an approach informed by the guidelines of The Cochrane Collaboration (Cochrane Handbook for Systematic Reviews [33] and Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy [34]). Synthesis of the evidence was undertaken using A Guide to Knowledge Synthesis [35], QUADAS-2 (Quality Assessment of Diagnostic Accuracy Studies—2) [36], and STARD (Standards for Reporting of Diagnostic Accuracy) 2015 guidelines [37]. The strategy for this systematic review followed the four-phase flow diagram (Fig. 1) put forth by the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) Statement [38]. A pre-specified protocol was set-up on April 17, 2019. No registration was completed.

Study inclusion criteria

Design

Only studies involving human beings, specifically adults diagnosed with SS, were included. The selected studies needed to provide the diagnostic value of anti-SSA or anti-SSB against a confirmed SS through FS (accuracy diagnostic studies) to be considered in this systematic review. To provide a broader scope of the analysis, we also included studies that compared and presented any correlation between these diagnostic parameters (FS and anti-SSA), with the final diagnosis of SS as the outcome.

Fig. 1 PRISMA flow diagram



Outcomes

Our primary outcome is the 'histologic grading and serologic parameters' diagnostic performance, usually assessed in patients with suspected SS. The revised rules of the American-European Consensus Group Criteria (ACR-EULAR [32]) were utilized to diagnose primary and secondary SS.

Information sources and literature search

The literature search strategy was implemented by the research team (LCVC, WTM, RF). With proper truncation and word combination, an electronic search was initially executed on April 24th, 2019, with an expert librarian's aid. We used the databases included in Ovid–SP, which comprise Medline, Embase, and EBM Reviews. We repeated the same strategy on May 1st, 2019, and revised it on April 6th, 2020, amplifying the platform's resources, which substantially increased the number of papers retrieved. The electronic search was undertaken by applying a comprehensive combination of MeSH (Medical Subject Heading) terms ('Sjogren's Syndrome' OR 'Sicca Syndrome' OR 'Sjögren's' OR 'Sjogren' OR 'keratoconjunctivitis sicca' OR 'xerostomia' AND 'serology' AND 'salivary gland' OR 'histopathology' OR 'histology' OR 'biopsy'). Filters for bibliographic research included languages, including English, French, Spanish, Italian, and Portuguese articles, conditional on an English abstract. All searches were limited to "humans" and the availability of abstracts.

Study selection procedure

Screening and quality assessment

After a calibration process on the inclusion and exclusion criteria, the inter-observer agreement score (kappa score) was calculated upon the analysis of 10% of randomly chosen abstracts, and a final score of 0.82 was achieved. The authors (LCVC, WTM) checked the titles and abstracts of the identified studies independently. Non-relevant studies were rejected. The relevant studies were chosen, and those that fulfilled the inclusion criteria were selected for this systematic review. If the two authors carrying out the selection process did not agree, the opinion of a third author (RF) was taken into account to reach an agreement. We combined all the scientific articles and reports retrieved in a complete file through the identification phase and then extracted duplicates. After excluding records not relevant to the systematic review, full texts of selected abstracts were extracted systematically for further eligibility analysis.

Eligibility

The full-text screening was carried out independently by the reviewers (LCVC, WTM) using the standardized form with explicit inclusion and exclusion criteria. Discrepancies were resolved by discussion between the two reviewers, and persisting disagreements were resolved through discussions with a third experienced researcher (RF).

Original investigations that used both FS and serology for anti-SSA and anti-SSB for SS diagnosis were included. We considered every article that mentioned the correlation, association, or comparison between the two diagnostic tests.

Exclusion criteria

Case reports, abstracts, oral presentations, and literature reviews were considered ineligible. Studies in any language different from English, Portuguese, Spanish, French, and Italian were excluded. Duplicates were removed, and abstracts were analyzed utilizing the software RefWorks.

Articles involving other conditions related to xerostomia and or dry mouth, not diagnosed as SS were excluded: 1, generic dry mouth; 2, generic xerostomia; 3, use of anticholinergic drugs; 4, previous head and neck radiation treatment; 5, hepatitis C infection; 6, GVHD; 7, AIDS; 8, pre-existing lymphoma; and 9, sarcoidosis were not considered in our review.

Risk of bias of the included studies

The quality appraisal of individual studies was provided via the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) checklist, following a Cochrane Collaboration recommendation [34]. To avoid other potential sources of bias, which are more likely to occur in correlational studies, only diagnostic accuracy studies were assessed for risk of bias. We selected thirteen criteria in this checklist and considered the fulfilling of six to eight criteria ("yes" answers in the first twelve questions, and "no" in the thirteenth question = "positive" criteria) as the median cut-off point for defining good studies, and the 75% cut-off point ≥ 9 "positive" criteria – as the definition of high-quality studies.

It has been advocated that a tailored combination of both QUADAS-2 and STARD for scoring the items of specific diagnostic accuracy studies would optimize the use of these tools and consequently improve the assessment of validation studies [39–41]. Hence, to complete the papers' quality assessment, we added three items from STARD criteria, which were not covered by QUADAS-2 and connected to the study sample's representativeness and accuracy. The remaining STARD items are directly or indirectly comprised in QUADAS-2. The criteria considered were as follows: "#5 – The sampling process is described?"; "#21 – Sensitivity and specificity results are reported with their respective confidence intervals (CI)?"; and "#15 – Clinical and demographic characteristics of patients are reported?". Good-quality studies should fulfill all three STARD criteria.

Synthesis of results

Among the included studies [28–30], there was significant clinical and methodological heterogeneity. The three principal sources of heterogeneity in a meta-analysis are the clinical heterogeneity between subjects from different studies, the statistical heterogeneity, which may or may not be calculated depending on the design of each study, and the heterogeneity from other sources mostly related to the study designs and the disparity between the research questions [42]. The statistical heterogeneity was not calculated due to the clinical and methodological heterogeneity. In this systematic review, one study only performed accuracy assessment for histology and considered "expert opinion" as the reference test [28], one study assessed the correlation between the tests against the diagnostic criteria [29], and one study used histology as a reference test when evaluating the accuracy of the serological tests [30]. In general, we observed the absence of a standard and adequate "reference test" and lack of actual accuracy assessment within the selected studies because of the inadequate design of the studies and the impossibility of forming pooled estimates for accuracy. In addition, the presented sensitivities and specificities could not be plotted against each other, and a desirable overall summary of the diagnostic test's accuracy performed through the area under a ROC curve was not feasible [43]. Therefore, a meta-analysis was not justified.

Risk of bias across the included studies

The risk of bias across studies was not completed as there is no validated tool for the SRs of intervention studies (GRADE assessment tool).

Results

Study selection

Upon a first search, 341 citations were retrieved, and six new articles were added from reference citations and cross references of the consulted literature. Sixty-one duplicates were removed via "Refworks citation manager" software, using the tool "exact duplicates" and "close duplicates". After removing the duplicates and previously defined limits, 260 articles were screened, and 196 were later excluded based on the abstracts due to lack of inclusion criteria fulfilment, presence of exclusion criteria, and methodological or design inadequacy. Search in the gray literature was attempted and resulted in mainly academic and conference citations and abstracts, which did not provide access to the full content. No further information was acquired from theses and dissertations, research and committee reports, nor government

reports regarding the subject of this research. Consequent analysis of the full content of the articles excluded 47 papers, mainly due to their design, which did not compare both tests considered in this review. Two studies were also excluded since they did not follow the ACR/EULAR histological diagnostic criteria/classification for FS, which prevented any comparison between the tests [23, 25]. One recent prospective cohort study provided a thorough assessment of all items listed in ACR/EULAR criteria, using expert classification as the "diagnostic gold standard", and compared its accuracy of each criterion with that. Even though the results were presented in a ROC curves manner, which is appropriate to this kind of study, the utilization of a subjective factor (expert opinion) impeded the standardization of the analysis among different studies, hampering the broad view the conclusions. The study also lacked a direct comparison between serology and histology accuracy. Therefore, this paper was excluded from the review due to an unsuitable design [44] (Fig. 1).

Only three articles presented a definite assessment of the diagnostic tests' accuracy. Still, none of them showed an exact comparison between serology for anti-SSA/anti-SSB antibodies and FS [28–30]. Nevertheless, we considered papers that used statistical correlation tests, such as Spearman and Pearson tests. The purpose was to extract any information that could corroborate potential evidence of further statements or conclusions in terms of the usefulness of the index test as a replacement, triage, or add-on test [18, 26, 28, 31, 45–50]. To gather the current knowledge regarding whether the surgical procedure could be avoided and still achieve a reliable serological method of diagnosing SS, 13 articles were finally assessed in the present systematic review. Different sources funded these studies, such as the US National Institutes of Health [18], Norwegian Council of Research [50], Corporación para Investigaciones Biológicas [45], diverse Norway organizations [47–49], the University of Florida Center for Autoimmune Diseases [26], and several Danish organizations [51]. No financial support information was provided in five assessed papers [28–31, 46].

Study characteristics

The included studies analyzed 2810 patients, with an age range of 20–90 years old. A remarkable difference was noted between the sample sizes, ranging from 7 to 1787 patients analyzed. The participants were assessed in multiple countries, with a multicenter study in Argentina, China, Denmark, Japan, UK, USA [18], Norway [48, 50], USA [26, 31, 46], Brazil [28], Scotland [45], Denmark [51], Argentina [30], and Israel [29].

ELISA (enzyme-linked immunosorbent assay) was the method used to detect anti-SSA and anti-SSB in the serum of the participants in eight studies [29, 30, 45–49, 51], while immunodiffusion was utilized in one study [50]. The laboratory method was not described in three papers. Nevertheless, we did not exclude these papers to analyze their perspectives on the correlation between serology and histology [18, 26, 31]. With hematoxylin–eosin stain and microscopic assessment by experts, routine histology was the method of analysis for the FS in all studies [18, 26, 28–31, 45–51]. Table 1 shows a summary of the studies assessed in this review.

Risk of bias within studies' characteristics

According to the combined QUADAS-2 analysis of the "Risk of Bias"—Table 2, two articles presented good quality (moderate risk of bias), both suggesting the replacement of the LMSG biopsy by serological testing [29, 30]. One study reached the minimum score to be considered a high-quality study (low risk of bias). Contrary to the other diagnostic papers, this author suggests retaining the LMSG biopsy as an essential diagnostic armamentarium method for investigating suspected SS patients [28]. However, when the STARD quality component is observed, none of the articles achieved a good standard since only one of the questions was positive. That establishes a high risk of bias in three diagnostic papers appraised in this review [28–30].

Results of individual studies

Two studies [29, 30] performed an assessment of sensitivity (73%; 71%), specificity (96%; 85%), negative predictive values (86%; 78%), and positive predictive values (92%; 78%) for serology. In both of these studies, the authors advocate that LMSG biopsy and histological analysis should be performed only when clinically suspected SS patient exhibits negative serology for anti-SSA/SSB.

Our review's recurrent finding was the tendency of many studies to present their results as showing a "strong correlation" between FS and anti-SSA/SSB, but without clear statistical support of that conclusion. There was a lack of accurate assessment of sensitivity, specificity, and negative and positive predictive values [18, 26, 46–48].

Another article claimed to present a correlation between FS and anti-SSA (in both serum and salivary gland B-lymphocytes). Nevertheless, no statistical demonstration or any accuracy test was presented to corroborate anti-SSA's hypothesis being a reliable biomarker for SS [47].

FS and SSA/SSB within studies

The use of serology for diagnosing SS instead of LMSG biopsy was recommended by three studies [26, 29, 30]. However, in one study where a correlation between the different tests was shown, serology was recommended mainly because of a supposed lack of consistency of the FS results.

Table 1 Summary of descriptive characteristics of included articles

Study	Sample size	Age, mean, range, SD	Gender	Sample features	Index test	Reference standard	Method of correlation	Results
Daniels et al. 2011—multinational	1787	54 years (21–90)	93% women	Consecutive multicenter	Serology SS-A-B – test not provided	FS—continuous	Bivariate/multivariate analysis	< 0.0001*
Langerman et al. 2007—USA	49	49 (20–85)	Female:male 42:5	Retrospective review	Serology SS-A-B – test not provided	FS—continuous	Unpaired Student's *t* test likelihood ratios	No significance
Peen et al. 2009—Norway	97	57.4 (25–81)	Female:male 90:7	Consecutive – retrospective study	ELISA—IgA-RF	FS—continuous Anti-SS-A/B immunodiff	Spearman's Mann-Whitney test	< 0.0001* r = 0.726
Anaya et al. 2002—Scotland	39	48 ± 14	Not provided	Convenience	ELISA—IL-10	ELISA—Anti-SS-A/B FS – categorical (+−)	Spearman's Mann-Whitney test	0.01* r not provided
Halse et al. 2000—Norway	17	50 (23–72)	100% women	Convenience—Consecutive	ELISA—Anti-SS-A/B (Ro 52 kD, Ro 60 kD and La 48 kD)	FS—categorical (+−)	Spearman's Mann-Whitney test	Anti-SS-A = 0.01* r = 0.69 Anti-SS-B 0.05* r = 0.49
Stewart et al. 2008—USA	37	56.6 ± 15.5	100% women	Consecutive	Serology SS-A-B – test not provided	FS—categorical (+−)	Kappa coefficient Friedman	Unclear
Pedersen et al. 1999—Denmark	16	40–82	Female:male 7:1	Consecutive	ELISA—Anti-SS-A/B	FS—continuous	Spearman's Wilcoxon test	Anti-SS-A 0.05* r not provided
Halse et al. 1999—Sweden	7	Not provided	100% women	Not provided	ELISA—Anti-SS-A/B	FS—continuous	Mann-Whitney test	Unclear
Jonsson et al. 2007—Norway	169	Not provided	Female:male 160:9	Consecutive	ELISA—Anti-SS-A/B	FS—continuous	Spearman's Mann-Whitney test	Anti-SS-A < 0.05 * Anti-SS-B < 0.001* r = 0.359
Atkinson et al. 1992—USA	43	48 ± 12.8	Female:male 41:3	Not provided	ELISA—Anti-SS-A/B	FS—continuous	Spearman's Mann-Whitney	Anti-SS-B < 0.0025* r = 0.477
Kessel et al. 2005—Israel	41	60 ± 15	Female:male 39:2	Consecutive	ELISA—Anti-SS-A/B	FS—categorical (+−)	Fisher's exact test. Sensitivity—specificity—PPV—NPV	Sensitivity 73% Specificity 96% PPV 92%, NPV 86%
Giovelli et al. 2015—Brazil	290	47.7 ± 12.5	83% women	Consecutive – retrospective study	FS – categorical variable (+-)	Specialist's opinion	Kappa coefficient	Sensitivity 86.57% specificity 97.43% PPV 95%, NPV 92.6%
Santiago et al. 2015—Argentina	218	53 years (41–61)	96% female	Cross-sectional	ELISA—Anti-SS-A/B	FS	Mann-Whitney test. Sensitivity—specificity—PPV—NPV	Sensitivity 71% Specificity 85% PPV 78%, NPV 78%

Table 2 Combination of QUADAS-2 and STARD criteria and classification of accuracy studies of serology and histopathology assessments in SS (only applied to the three studies that assessed accuracy)

Criteria	Kessel et al.—2006	Giovelli et al.—2015	Santiago et al.—2014
QUADAS-2 1. Was the spectrum of patients representative of the patients who will receive the test in practice?	Yes	Yes	Yes
2. Were selection criteria clearly described?	Unclear	Yes	Yes
3. Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?	Yes	Yes	Unclear
4. Did the whole sample or a random selection of the sample, receive verification using a reference standard of diagnosis?	Yes	Yes	Yes
5. Did patients receive the same reference standard regardless of the index test result?	Yes	Unclear	Yes
6. Was the execution of the index test described in sufficient detail to permit replication of the test?	No	No	Yes
7. Was the execution of the reference standard described in sufficient detail to permit its replication?	Yes	Yes	No
8. Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear	Unclear	Unclear
9. Were the reference standard results interpreted without knowledge of the results of the index test?	Unclear	Unclear	Unclear
10. Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?	Yes	Yes	Unclear
11. Were uninterpretable/intermediate test results reported?	Unclear	Yes	No
12. Are there concerns that the included patients do not match the review question?	Yes	No	No
13. Could the selection of patients have introduced bias?	Yes	No	Yes
STARD			
14. The sampling process is described	No	No	Unclear
15. Sensitivity and specificity results are presented with their respective confidence intervals	No (lack of CI – reference) Index not assessed	No (lack of CI – reference) Index not assessed	No (reference not presented) Index properly assessed
16. The demographic characteristics of patients are described	Yes	Yes	Yes

However, these authors still believe that LMSG biopsy is a "gold standard" and a "pivotal" diagnostic component of SS [26]. In the two other studies, the authors justified their recommendation for serology based on their consideration of the sensitivity, specificity, PPV, and NPV of the serological tests in their results [29, 30].

Retaining the histological analysis as a required step during the SS's clinical investigation was recommended in 4 articles [18, 28, 31, 46]. One article presented a strong correlation between FS and SS-A/SS-B. In this study, the positive for anti-SS-A/SS-B subjects were shown to be nine times more likely to have a FS of ≥ 1 than those with negative serology. However, the authors advocate that the histological assessment is still the most accurate element of the diagnostic methods used in SS suspected patients [18].

In another series comparing clinical and serological features with salivary glands histology, the authors did not find a correlation between those findings. Also, they could not point out a reliable method to predict the extent of the glandular damage, which is considered a main SS component. The authors considered LMSG biopsy as an invasive but relatively safe method to diagnose the salivary glands' conditions in SS. On the other hand, they claimed the need for further research to improve the ability to correctly diagnose this disease [31]. In contrast, Atkinson et al. found a correlation between serology and FS, but not strong enough to enable the use of serological results to predict histological results accurately, or vice versa. Moreover, instead of suggesting the replacement of the histological assessment, the authors advocated using serology to monitor therapeutic interventions' response. Therefore, these authors suggest a complete analysis, including LMSG biopsy, as necessary for a proper SS diagnostic [46].

The last study that assessed the LMSG biopsy's accuracy suggested that its specificity and PPV were high. Still, that sensitivity was variable depending on the profile of the

patients [28]. However, in this study, the LMSG biopsy was only undertaken to confirm the tentative diagnosis of SS when the serology was negative. In this case, the authors consider the biopsy a complementary tool to fulfill the diagnostic process, which is essential to suspected SS patients' clinical management. Even though they demonstrated that serology presented some positivity level parallel with the histological features, no statistical evidence of this correlation was provided [28].

The remaining papers showed that the correlation between the parameters did not suggest replacing LMSG biopsy by serological testing. There was no clear statement advocating either retaining or replacing histological assessment during the SS diagnostic process [45, 47–51].

In three studies, the authors used some extra parameters, such as IgA-rheumatoid factor [50], interleukin-10 [45], and presence of germinal centers in the salivary gland [49], as a common factor of correlation between FS and seropositivity of SSA/SSB.

Discussion

The challenge of proper diagnostic guidelines for SS has been a focus for researchers and policymakers. As a multisystem condition, with a heterogeneous presentation, clinical course, and outcome, SS's diagnostic process has not yet been a rigid consensus. The closest to a "gold standard" test remains the LMSG biopsy and histopathological evaluation [1, 4, 5, 49, 52, 53]. However, there remains a controversy, especially with concerns regarding the consistent histological assessment and invasiveness of the procedure [31, 51].

The new ACR/EULAR diagnostic criteria [32] still keep LMSG biopsy as the primary diagnostic criterion with the highest multi-criteria decision analysis (MCDA) weight= 0.22. However, anti-SSA/SSB serology was ranked closely behind LMSG biopsy. This has raised the question of whether the serological tests can completely replace LMSG biopsy, which is an invasive procedure and one that is not readily available in all clinical contexts [29, 30]. It has been relatively well defined that autoantibodies to Ro/SSA and La/SSB are the most significant diagnostic serological markers for SS. Nevertheless, depending on the methodology used, the prevalence of seropositivity ranges from 40 to 70% of SS cases [52]. Furthermore, it has been shown that seropositivity for anti-SS-A is associated with several different auto-immune diseases, such as idiopathic inflammatory myopathy, rheumatoid arthritis, systemic lupus erythematosus, and systemic sclerosis, and it is not considered a disease-specific test [54].

The purpose of this systematic review was to evaluate whether the current evidence supports the replacement of LMSG biopsy by serological testing for anti-SSA in the diagnosis of SS. Our findings show several articles attempting to correlate serological and pathological findings [45, 46, 48–51]. Two of them did not provide the r-value, which compromised their statistical analysis, thus precluding any definite conclusion [45, 51]. Among those correlation studies where the r-values were provided, only one demonstrated a strong positive (≥0.7) linear relationship [50], two showed a moderate positive linear relationship (≥ 0.5–0.69) [46, 48], and one showed a weak correlation [49]. Although these studies could exhibit a positive correlation between the two tests, none of them advocated using serology as a substitute for LMSG biopsy. It has to be noted that a statistically significant correlation is not difficult to reach in biology. The key is how high the correlation is. Even in those cases with a strong positive correlation, if the value is just above 0.7, then the determination coefficient is relatively low (around 50%). This means that the independent variable explains only 50% of the dependent variable's variance. Therefore, a good proportion of its variability is unknown.

Among the three papers where such replacement was suggested [26, 29, 30], statistical limitation invalidates this assumption in one study [26]. It was not a correlation analysis or an accuracy assessment but was based on supposed flaws during histological evaluations. The two other articles [29, 30] were accuracy studies with an adequate standard of bias control. However, they did not compare both tests' accuracy in the same sample, which cast doubt on the reported conclusions.

The current use of LMSG biopsy as an essential part of the diagnostic armamentarium in SS is defended by four articles [18, 28, 31, 46] for different reasons. In another study [18], the authors analyzed LMSG biopsy specimens from 1787 patients from different countries in a well-designed multivariate model. They showed an association of SS phenotypic aspects, such as anti-SSA/SSB and RF serology, ANA titers and IgG concentration, presence of keratoconjunctivitis, and low unstimulated whole salivary flow rates with FS in LMSG. The authors suggest that LMSG biopsy can provide information on the extent and nature of the disease process, even though the risk of misinterpretation is always present. However, a standardized protocol can avoid errors for the assessment of LMSGs, bringing more reliability to the diagnostic process [18]. Another study did not find a significant association between LMSG biopsy results and clinical symptoms or serology with a much smaller sample. They stressed the importance of consistent use of a grading system, with accurate recognition of focal sialadenitis, to establish adequate pathologic analysis of the biopsy during SS's diagnostic process [31]. Nevertheless, Atkinson et al. reported the correlation between serological results and LMSG histology. They suggested that the LMSG played

a central role in diagnosing SS but that longitudinal serological assessment is valuable in following the disease's evolution and response to treatment [46].

Among the studies where the LMSG biopsy was still felt to play an essential role in diagnosing SS, Giovelli et al. demonstrated the LMSG biopsy's high accuracy in SS patients. They particularly stressed the important indication for LMSG biopsy when the serology results are negative. However, they did not compare both tests' accuracy. Therefore, their conclusions are not supported by the study results [28].

The ACR/EULAR diagnostic criteria were assessed in a Dutch referral center, employing an expert consensus on their gold standard test, rendering the study of limited use as an international research gauge due to an apparent subjectivity and unfeasibility of calibration. The researchers did not undertake a straight comparison between the objective items of the criteria, and the variability in expert consensus renders the conclusions in this study less unbiased. The study supported both serology and LMSG biopsy's diagnostic accuracy and did not offer any conclusions regarding replacing LMSG biopsy with serological tests [44].

A recent review by Trevisani et al. set a series of recommendations for pSS diagnostic and prognostic approaches, including imaging, histological approaches, and lab tests, including ANA, RF, Anti-Ro, electrophoresis of protein, urinalysis, hemogram, C-reactive protein, complement, and serology for some microorganisms (VDRL, HCV, HIV) when dryness or systemic manifestation is present [16]. It is important to highlight that not rarely a patient with age or menopause-related hypo-lachrymation or xerostomia, detected by Schirmer's test or sialometry, along with a positive anti-SSA caused by other AI diseases, such as RA and SLE, could be misdiagnosed with SS [5, 55]. On the other hand, a patient with mild or no dry symptoms, and a positive anti-SSA, which can be detected in a significant proportion of patients with undifferentiated connective tissue disease, would not receive the diagnosis of SS. Nevertheless, their clinical conditions could evolve and eventually have the diagnosis of SS or SLE confirmed later on. This overlap between distinct AI entities is mainly due to a variation in the sensitivity of assays used to detect anti-Ro antibodies, which creates confusion in the clinical scenario and raises questions on the accuracy of the current diagnostic criteria for SS [56].

Non-invasive validated biomarkers for SS have been sought for a long time as aids to accurate diagnosis, as well as for monitoring of response to therapy. In addition to ANA, RF, and anti-SSA/SSB, newer molecules have appeared in the spectrum of possibilities, such as Muscarinic type-3 receptor, α-Fodrin, and Calprotectin others [52, 57].

Limitations

To modify any diagnostic protocols or guidelines, it is mandatory to have well-designed accuracy studies. When a new test (index test) is presented as an alternative to the reference test in the diagnostic armamentarium, it can be assigned as a replacement, triage, or add-on method for diseases such as SS. Unfortunately, the most common approach has tended to involve studies focused on assessing a single test, usually based on a retrospective database and convenient samples, as seen in our review [58, 59].

For any consideration of replacement of any reference test by a different test, the basic approach is to compare their accuracy within the same population. In a scenario where the index test demonstrates superior accuracy to the reference test or comparable accuracy with other advantages such as reduced invasiveness, the reference test's replacement by the index test is indicated. When the index test is being explored as a triage test, wherein the index test is to be used before the reference test, the index test needs to demonstrate high sensitivity with a minimal proportion of false negatives. The index test's general accuracy may be lower than the gold standard, but some other advantages, such as low cost or less invasiveness, have to be offered by the index test. When the diagnostic pathway's complexity requires extra information, especially to identify false positives or false negatives, and the index test is not more accurate than the existing one, it still offers a complementary role as an add-on test [58].

In addition to the lack of direct comparison of accuracy, all of these studies failed to show the connection between the sensitivity and specificity of the diagnostic tests. No ROC curve was demonstrated in any of the studies. These studies, therefore, all carry the potential for confounding factors, which could be avoided in a study design using paired analyses with the results displayed in a ROC curve [28–30, 58, 59].

Concerning recommendations regarding the continued role of LMSG biopsy in diagnosing SS, three of the reviewed studies advocated replacing LMSG biopsy by serology alone [26, 29, 30]. Four of the studies recommended retaining LMSG biopsy as an essential method to diagnose SS [18, 28, 31, 46]. One of these four studies stressed the value as the triage test status for the serology by one study [28]. However, none of the recommendations in these seven studies offers enough evidence. Six of the studies made no recommendations concerning the current guidelines [45, 47–51].

The most critical challenges that we faced during this review were insufficient diagnostic accuracy studies for SS. The few we found were not designed to compare the tests' accuracy, nor were the costs and potential burden of the tests analyzed. In conclusion, more well-designed studies of the diagnostic accuracy of SS tests are needed, with the recruitment of adequate spectrums of patients and applying a more

rigorous approach to the appraisal of tests and reporting of results and conclusions. Until the time that such studies are conducted, the current ACR/EULAR criteria should remain the diagnostic criteria of choice.

A Risk of Bias across studies assessment was not possible as currently, there is no validated tool to complete such assessment in a SR of diagnostic studies.

Conclusions

None of the included studies directly compared the diagnostic performance of both serology and histology in SS. Even though a correlation between the tests has been observed (one strong, two moderate, and one weak values), these non-comparative study designs do not permit conclusive recommendations regarding any change in SS's established diagnostic pathway (very low certainty level).

Acknowledgements We would like to thank Lisa Tjosvold for the help during the literature search at the J.W. Scott Library.

Declarations

Ethics approval Pro00094361—University of Alberta.

Disclosures None.

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Chapter 6 – Discussion

While advances in understanding of the pathophysiology of some diseases has led to the development of a single laboratory test with sufficient sensitivity and specificity to render a clear diagnosis, this is not the case with SS and other rheumatic diseases, which comprise an overlapping spectrum of clinical entities. (13-15, 54, 55, 111). While the 2016 ACR/EULAR SS diagnostic criteria represents an attempt to establish consensus on the diagnosis of SS, there have been not yet been adequately designed studies that allow a thorough assessment of the accuracy of the items preconized in the ACR/EULAR SS diagnostic criteria (110).

A comprehensive discussion of the existing literature landscape regarding the accuracy of the 2016 ACR/EULAR SS diagnostic criteria can be appreciated in our published paper (108). Overall, there are no adequately designed studies, to date, that allow a thorough assessment of the accuracy of the items that comprise the ACR/EULAR SS diagnostic criteria. New studies to corroborate practice guidelines for clinicians and prevent misdiagnosis and mistreatment in SS were still necessary. It was also observed that there is not yet a 'gold standard' test, and either labial salivary gland histopathological evaluation or 'clinical diagnosis' were used as reference tests (108, 110).

Therefore, more well-designed studies in diagnostic accuracy of SS are required, ensuring recruiting adequate spectrums of patients, making direct appraisals between methods, applying rigorous approaches, and clearly reporting results.

The complex nature of SS is characterized by heterogeneity of its clinical presentation, evolution, and consequences. When dealing with the clinical presentation of a patient with features shared by SS and other rheumatoid disorders such as RA or SLE, a continued caveat with the application of the ACR/EULAR diagnostic criteria relates to limits of the sensitivity and specificity of the various laboratory tests. Borderline cases continue to present a diagnostic challenge. For example, in the case of SLE, only 70% and 30% of the patients will show positivity for Anti-ds DNA and Anti-SM, respectively. Likewise, in the case of RA, ~30% of patients do not show positivity for rheumatoid factor. In the case of SS, the prevalence of anti-SSA antibodies in sera of patients with primary SS varies between 50 and 70%, and the presence of Anti-SSB antibodies varies between 30 and 60%. A clinician faced with the challenge of distinguishing between

overlapping disorders such as RA and SLE and SS must continue to deal with a complex combination of signs, symptoms and complementary laboratory tests to discern between these similar diseases and to exclude other confounding factors (3, 98, 111, 112). Therefore, when we observed that the managing rheumatologists of the studied sample cases usually do not agree with a SS diagnosis in cases where the histopathology is negative, along with a low titration of Anti-SSA (<200), we attempted to envision a new threshold for anti-SSA serology that could provide greater confidence in the diagnostic criteria. With that, we try to offer an objective parameter for the clinicians, in order to make better evidence-based clinical decisions, instead of using an 'expert opinion', as seen in previous studies (105).

Our study aimed to evaluate the accuracy and compare the performance of the two main items in the current SS diagnostic criteria (ACR/EULAR), i.e. anti-SSA serology and LMSG. The study population comprised 89% females with peak age range of 40-65 years, corresponding closely to the demographics reported in the current SS literature (10, 12). All of the patients were experiencing Sicca symptoms and had been referred to the Oral Medicine Clinic for LMSG biopsy. To evaluate the Sicca symptoms, sialometry was performed on all patients. The other measure of Sicca symptoms, stipulated within the ACR/EULAR guidelines, is the Schirmer test. Although many of the patients had been assessed by an ophthalmologist as part of their workup, it became apparent that most of these ophthalmology assessments had been focused upon either symptomatic management of eye symptoms and/or baseline retinal assessment in anticipation of possible use of hydroxychloroquine in their SS management. Only 7 of our 87 patients had undergone Schirmer test. Positive Schirmer test was documented in 5 of these 7 patients.

It is not clear why the Schirmer test was not specifically included in the rheumatologists' diagnostic workup for our 80 study patients who did not undergo this diagnostic test. The value of the Schirmer test has been previously questioned in the SS literature due to its low accuracy. Also, there is controversy regarding its repeatability, especially for higher scores, as well as its poor correlation with DES symptoms in SS (105, 111, 112). However, to clarify why we observed such low usage of Schirmer test in our patient sample, a qualitative research study could be designed with a focus upon the rheumatologists in our community.

The investigation of complex diagnoses using broad diagnostic criteria can be associated with risk of misdiagnoses. The final diagnostic interpretation will often rely on an expert's judgement after consideration of all presenting clinical and laboratory features. The use of expert judgement as a "gold standard" for the diagnostic performance of diagnostic criteria has been explored in different studies and it has not been demonstrated to be a reliable instrument in making a correct diagnosis in various complex clinical disorders (31, 105, 110, 112).

We encountered the above challenge in our study, with a small group of patients being clinically considered "non-diseased" by the expert (managing rheumatologist), in spite of having Sicca symptoms and a "positive" anti-SSA. However, when we separated the "final diagnosis" into two categories, one strictly observing the ACR/EULAR criteria, and another with the "Modified-ACR/EULAR 'ClinDx'", we compared the performances of the two main items, FS and Anti-SSA. We then noticed that, when we correlate the "ClinDx" with the numerical results of Anti-SSA, in all cases where the biopsy was negative, and Anti-SSA was low positive, the clinicians had designated that patient has not having SS at that time. Then, when we did an *Ad hoc* cut-off line in the Anti-SSA titration ≥ 200 to be considered positive, those cases were included in the group of clinically excluded diagnoses. Also, we noticed that upon this change, both items achieved excellence in their level of diagnostic accuracy, and that the AUC of both (Anti-SSA = 0.92, FS = 0.91) were similar to that observed by Van Nimwegen et al, 2018 (Anti-SSA = 0.93, FS = 0.85), but showing an improvement of the performance of FS (105).

We observed a significant inconsistency, in our regional medical laboratory database (Alberta NetCare), related to a lack of standardization in the reporting of serological test result in SS. In 27 out of the 87 analyzed cases, Anti-SSA detection lab tests did not always provide results in numerical formation (MFU), and instead only the categorical screening of "positive" or "negative" was reported. Within this group of 27 patients, three had anti-SSA serology reported as "negative" (no numerical value provided) but these patients had positive FS, implying a false-negative serology result in more than 10% of the cases. A better standardization of laboratory result reporting, with provision of actual numerical values rather than the simply "positive"/"negative" reporting, would have permitted us to analyse the raw data of these 27 patients in our sub-category of data analysis wherein we set the threshold for "positive" anti-SSA numerical value at a titre level

of >200, instead of using statistical estimation, which is also useful to prevent missing a significant part of our study sample.

Despite the above limitation regarding data analysis, we were able to analyse the anti-SSA data in the 87 patients, in the following manner. Specifically, a data analysis using FS value and anti-SSA titre (with titre ≥ 200 being set as our threshold for "positive" anti-SSA, and correlating with our ClinDx model, demonstrate excellent accuracy, with almost 100% of cases detected properly. This *ad hoc* approach of setting the threshold, for "positive" anti-SSA titre result at the higher level of a titre ≥ 200 may open new avenues for research offer potential for improved accuracy of anti-SSA measures in the ACR/EULAR diagnostic criteria for SS. Even though the current criteria is based on points, and the minimal threshold can be reached upon only one of the main items (FS or Anti-SSA) plus one of the minor items (Schirmer test, Sialometry, ocular staining), false positive cases are possible to occur. A positive Anti-SSA can be associated to hyposalivation and still not represent a real case of SS, and improvements in the current diagnostic process would increase the reliability on it. Of course, this needs to be corroborated by further study with larger sample size as well as a qualitative analysis of "expert clinical diagnosis" through validated questionnaires and more in-depth patient chart assessment.

In rheumatology, the sensitivity and specificity of any criteria depends on multiple variables, and when a gold-standard test is available, such as in acute gout or septic arthritis, both sensitivity and specificity can be established. Nevertheless, once the number of variables increase, the specificity in diagnostic and/or classification criteria increases, but sensitivity decreases, and vice versa. This is why the use of the receiver operator curve (ROC) is a statistical and graphical description of this process showing the equilibrium between sensitivity and specificity. The closest to 1 the area under the curve (AUC) is, the more accurate the test is, theoretically. However, in real practice, we observed that rheumatologists may not be convinced by the results offered by the combination of items' points, especially when the clinical features, as well as the biopsy do not corroborate the serology findings, when it comes out a "low positive" case. The ROC curves in our study show that using the current criteria, an excellent accuracy of Anti-SSA is seen, but this could represent a higher sensitivity, including false positives in some instances. On the other hand, an adjustment of the serological threshold was able to keep the accuracy excellence of Anti-SSA, at the same time that improved the accuracy of FS, from good to excellent. Also, when the accuracy

of diagnostic tests are displayed in a ROC curve, the comparison between them is feasible and leads to a lower potential of confounding factors upon paired analyses (110-112).

When the new ACR/EULAR criteria for diagnosis of SS were published in 2016, a loss of specificity was observed, when compared to the previous version of the SS diagnostic criteria. As a consequence, the sensitivity increased, with better PPV. However, while these more "liberal" diagnostic criteria would arguably be able to detect SS patients earlier, there is an associated increased likelihood of selecting and treating false positive cases. Thus, caution is imperative before using these more "liberal" diagnostic criteria, especially when there are now prospects of possible new biologic agents with uncertain safety profile. Applying the ROC curve allowed us to consider the new ACR/EULAR criteria for SS diagnosis in flexible ways and we were able to establish a different sensitivity / specificity ratio capable of greatly reducing the risk of selecting and treating false positive cases. As mentioned above, we obtained, with the modified-ACR/EULAR 'ClinDx' model, excellent accuracy for both items, FS (numerical) and anti-SSA (numerical). Similar results were achieved by simply moving upwards the cut-off ACR/EULAR criteria score to 5 instead of 4, which raised the specificity of the ACR/EULAR criteria for SS (110, 112). Nevertheless, this measure is not able to be applied to those cases where the low positive Anti- SSA titers are derived from another rheumatic condition, where there are also dry eyes and mouth caused by age, or other causes, for instance. The patient would reach the cut-off point of 5, and would still represent a false positive for SS. In this case, the LMSG biopsy would be vital to define the diagnosis. This could be a reason to consider a flowchart that guides the steps of the diagnostic process in SS, where the biopsy could be used to confirm or detect some outlier cases, when deemed necessary.

A critical appraisal of the role of the LMSG biopsy shows the benefits and pitfalls of such a test. It is undeniable that a surgical procedure always represents risks and consequences, even when minor. Its proper use is predicated on careful intra-observer and inter-observer calibration to avoid diagnostic discrepancies. In addition to training, calibration, and consistent application of the current histologic grading system is crucial to avoid misdiagnosis and to guide clinical decisions (34, 88, 89, 112).

We conclude that in the current clinical context, LMSG biopsy is still necessary, not only to confirm the diagnosis when the serology is negative, or considered “low level” (i.e. titre <200), but also to reveal the individual evolution of the disease.

We offer a cautionary note, regarding the use of LMSG biopsy in investigating possible SS, particularly if the patient is at a much later stage of SS. By late-stage SS, there may be little salivary gland parenchyma present, as a result of progressive acinar atrophy, interstitial fibrosis, ductal dilation, and adipose replacement. This “end stage” histological appearance may lack and active inflammatory infiltratory infiltrate and would enhance be assigned an FS=0 in the ACR/EULAR diagnostic criteria for SS. This would represent a false- negative diagnostic result for the patient and decreases the accuracy of the FS test (14, 25, 87, 90).

Future developments may render the LMSG biopsy of even greater diagnostic and prognostic/predictive value. Recent research has shown that optimizing the LMSG biopsy through immuno- histochemical assessment would be advantageous. More sophisticated characterization of the histopathology, including immunohistochemical measures, might serve to identify patients at higher risk of developing more severe extra-glandular conditions, particularly lymphoma (112).

As an example of more sophisticated future use of LMSG biopsy in SS patient, studies have shown that in some SS patient, the LMSG biopsy demonstrates an accumulation of hyper-reactive memory B cells (CD20+ / CD27+), which overexpress immunoglobulins (mainly IgG and IgA) with autoantibody activity. This can include an accumulation of autoantigen-specific Ro52 and Ro60 CD27+ B cells in areas of fatty infiltration in the SG of SS patients, suggesting a chemotactic gradient favouring B cell recruitment in these areas. The accumulation of hyper-reactive memory B cells in the LMSG biopsy of some SS patients (almost 25% of SS patients), is associated with increased autoantibody production and increased apoptotic activity, both of which appear to represent risk factors for the development of lymphoma. Modification of LMSG biopsy in SS patients, to incorporate a qualitative analysis of the hyperactive B-cell response and of the generated autoantibodies could have important prognostic and predictive value (9, 54, 55, 59).

Likewise, salivary gland histopathology has been utilized to stratify patients based on target validation, drug efficacy, and mechanisms of response, relapse, and resistance to therapy. These methods have been used in clinical trials with novel biologics in SS. The impact of an optimized

LMSG histopathology in the clinical diagnosis and management of SS patients requires the development of consensus and standardisation of methodology but is another avenue that could significantly improve the quality of the care (112). The use of histological markers among the other items of the current SS criteria could be targeted in future research.

The new SS ACR/EULAR criteria resulted from international collaboration and was derived from a well-established and validated methodology. They provide the fundamental features defining the disease and represent important grounds to stimulate the development of collaborative studies and policies. Nevertheless, it is not yet enough to be considered entirely reliable. There is a sense that further scrutiny of the causes of delayed, incorrect, or incomplete characterization of each patient, and establishing it into diagnostic and prognostic categories is crucial for improving quality of life and outcomes in SS. The search for a fine-tuning of the current items, as well as the substitution or addition of other items, seems to be an interesting target to reach. For instance, in the development of AIs and especially SS, the presence of peculiar HLA alleles, as well as of variants in genes, such as IFR5, STAT4, CXCR5, have been demonstrated, and could be helpful in the path towards more specific and sensitive clinical investigation, and precise genetic tests (9, 36). This could also include images, distinct serological markers, and even biomarkers, such as proteomic analysis of saliva, all being promising technologies that cannot be ignored in the future, for diverse AI diseases (68, 72, 78, 95, 112, 113).

AI diseases are a major public health problem, and different studies have documented poor quality of life in SS patients compared to healthy controls, sometimes similar to that observed in other rheumatic diseases such as rheumatoid arthritis, fibromyalgia, and even systemic lupus (12, 20, 21). SS is associated with substantial direct and indirect costs. In terms of the resources necessary for diagnosis, treatment, and rehabilitation, SS costs to the health system could represent almost 3 times greater than community control population.

It is estimated that dental health care costs are also three-fold greater for SS patients. In addition to expenses for dental care related to increased rates of dental caries and periodontal disease, SS patients may develop oral mucosal lesions, burning mouth symptoms, and oral fungal infections that represent sources of additional physical and psychological stress and expense (20, 38, 52)

In terms of indirect costs, loss of economic productivity related to SS in Canada approximates over $5000 per SS patient (20, 38, 53). Overall, when direct and indirect costs are taken into consideration, the adverse economic impact of SS approaches 70-80% of the corresponding impact on RA patients (38).

In summary, further refinement of diagnostic approach and criteria for SS is essential to ensure timely initiation of appropriate treatment and, conversely, to prevent over-diagnosis and over-treatment with potentially toxic immunomodulatory medications. Parallel to refinement of the diagnostic algorithm and criteria for SS, continued research should also be directed to the development of new drugs with enhanced risk– benefit profile and potential to improve the long-term prognosis of SS.

Considering the cost and suffering sustained by SS patient, health professionals, academic researchers, and government must recognize the importance of a collective approach to improving understanding, diagnosis and treatment of this chronic disease (50, 112).

LIMITATIONS:

The limitations of this study were:

- A relatively small sample
- Lack of consistency in the ocular assessment / information
- Lack of consistency in the laboratory methodology/informatics with regards to serology results

7 - Conclusions

Despite the limitations in this study, we can conclude that:

- SS ACR/EULAR criteria (2016) offers more "inclusive" criteria with great sensitivity and specificity, but may benefit from future amendments in terms of add-on tests and adjustment of cut-off thresholds;
- Applying a higher threshold for consideration of Anti-SSA titre values to be deemed "positive", i.e. raising the cut-off to 200 (MFU), can provide a higher accuracy to this criterion, and could make this the most accurate test in the SS ACR/EULAR criteria (2016);
- LMSG biopsy will still be necessary in the diagnostic work-up of some patients, particularly those with negative or low-positive serology findings;
- Improvements and enhancements can be envisioned for LMSG histological assessment, including incorporation of immunohistochemical methodology and, hopefully, refinement of its prognostic/predictive value in management of the SS patient.

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