Primary Sjögren's syndrome: Towards a new era in diagnosis, treatment and e-patient education

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Primary Sjögren's Syndrome: Towards a new era

In diagnosis, treatment and e-patient education

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by

Konstantina Delli
born on 23 May 1980
in Chania, Greece

Supervisors
Prof. F.K.L. Spijkervet
Prof. A. Vissink
Prof. H. Bootsma

Assessment Committee
Prof. R.A.J.O. Dierckx
Prof. F. van den Hoogen
Prof. R. Jonsson

Paranimfen
Drs E. Mossel
Drs J.F. van Nimwegen

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To all patients with Sjögren's syndrome

Chapter 1

General introduction

Sjögren's syndrome (SS) is a common systemic disease, second to rheumatoid arthritis (RA), with a prevalence of 60.8 (95% CI: 43.7 to 77.9) cases per 100,000 inhabitants in the total population [1]. SS commonly affects the exocrine glands, in particular the salivary and lacrimal glands, resulting in a sensation of dry eyes (keratoconjunctivitis sicca) and dry mouth (xerostomia) [2]. SS can be distinguished in primary Sjögren's syndrome (pSS), in case no other autoimmune disease is present, and secondary Sjögren's syndrome (sSS), in case additional connective tissue diseases are present, such as RA, systemic lupus erythematosus (SLE), scleroderma and mixed connective tissue disease.

Although the exact pathogenetic mechanism has not been fully elucidated, in patients with SS both the minor and major salivary glands as well as the lacrimal glands are characteristically infiltrated by mononuclear lymphoid cells [3]. The classic glandular lesion is composed of a periductal lymphoid infiltrate of T and B lymphocytes, whose distribution may vary according to lesion severity. The periductal localization of the infiltrate emphasizes the critical role of the epithelium in the development of the disease. In addition to lymphoid cells, also a wide variety of non-lymphoid cells can be found within the infiltrate; in fact, all elements responsible to carry out (auto)immune responses may be present.

A central role in the pathogenesis of SS is attributed to B-cells, which are found to be hyperactive [4]. In line with this finding, patients with pSS have an increased risk of developing lymhoproliferative diseases, which is about 4% during the first 5 years, 10% at 15 years and 18% after 20 years post-diagnosis [5]. Forty eight to 75% of these lymphomas are of mucosa-associated lymphoid tissue (MALT) lymphoma type [6-8]. Most commonly, the MALT lymphomas arise in the parotid glands and are usually accompanied by a persistent unilateral glandular enlargement. Recurrent swelling, however, of the major salivary glands is a very common phenomenon in patients with SS, but contrary to swelling associated with MALT lymphoma, it is usually bilateral, non-painful to slightly tender and intermittent in nature.

Diagnosis

A joint study of the American European Consensus Group (AECG) presented in 2002 the revised AECG classification criteria for SS. To date, the AECG criteria are the most widely used classification criteria (Table 1) [9]. The AECG criteria are composed of subjective symptoms (dry eyes and dry mouth, Criterion I and II), objective signs (keratoconjuctivitis sicca and salivary gland involvement, Criterion III and V) and histopathological (Criterion IV) and serological findings (Criterion VI). Albeit the aforementioned classification criteria were developed to be used as a research tool to define homogenous groups of patients, they are broadly used in clinical practice as a diagnostic tool. Due to the emergence of biologic agents, the American College of Rheumatology (ACR) proposed new classification criteria for SS, based merely on objective tests (Table 2) [11]. Of note, the ACR criteria do not distinguish pSS from sSS, ignoring the difference in pathophysiology underlying in pSS and sSS [12]. In search of widely accepted criteria, the ACR-EULAR criteria have been developed and recently published [13]. These criteria combine features of the ACR and AECG criteria, based on methodology consistent with current ACR and EULAR guidelines (Table 3) [12,13].

Recent discussion has focused on the accuracy of ultrasonography to assess the involvement of the major salivary glands and eventually to diagnose the disease with less invasive diagnostic procedures [17]. Ultrasonography is a non-invasive, inexpensive, widely available, easily accessible and non-irradiating imaging modality [18]. The sensitivity and specificity of ultrasound have been so far primarily compared to the existing criteria and the labial gland biopsy and thus it remains unknown how ultrasound performs compared to parotid gland biopsy. Additionally, the ultrasonographic characteristics of the major salivary glands in patients with pSS have been mainly compared to healthy or non-SS sicca controls. In fact, no proper disease controls for Sjögren's syndrome were studied, i.e., patients diagnosed with a systemic disease that may involve the major salivary glands, may cause dry mouth or may even have similar histopathological features as pSS, like sarcoidosis, amyloidosis, human immunodeficiency virus (HIV) infection and hepatitis C virus (HCV) infection. Currently, well designed studies investigating the reliability and validity of ultrasonography in diagnosing pSS are rather scarce.

Treatment

Until recently, treatment of patients with SS was limited to reducing the feeling of dryness. Thus, substitution therapy as well as salivary and tear stimulation were the main treatment modalities [19]. In case of extra-glandular and systemic manifestations, corticosteroids and biological disease-modifying anti-rheumatic drugs (DMARDS), such as tumor necrosis factor (TNF) inhibitors, interferon-a (IFN-a), B-depletion therapy and modulation of the CD28-mediated T-cell co-stimulation, have been investigated [20,21].

The outcomes between trials are, however, variable [22] and for that, several factors may be held accountable. The way disease activity is defined differs greatly between studies; the European League Against Rheumatism EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) [23] is currently the most frequently used index. Recently, the Sjögren's Syndrome Response Index (SSRI) [24] has been proposed. Additionally, there are no standardized guidelines to assess the histopathological characteristics of the salivary gland tissue of patients with SS. Interestingly, the fact that so far it could not be predicted if a patient will respond or not to medication has made treatment decision more complicated. As a result, there is, so far, no agreement in the treatment of patients with SS.

Table 1: Revised AECG classification criteria for SS [9].

I. Ocular symptoms: a positive response to at least one 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?
II. Oral symptoms: a positive response to at least one of the following questions: 1. Have you had a daily feeling of dry mouth for more than 3 months? 2. Have you had recurrently or persistently swollen salivary glands as an adult? 3. Do you frequently drink liquids to aid in swallowing dry food?
III. Ocular signs—that is, objective evidence of ocular involvement defined as a positive result for at least one of the following two tests: 1. Schirmer's I test, performed without anaesthesia (<5 mm in 5 minutes) 2. Rose bengal score or other ocular dye score (>4 according to van Bijsterveld's scoring system) [10]
IV. Histopathology: In minor salivary glands (obtained through normal-appearing mucosa) focal lymphocytic sialoadenitis, evaluated by an expert histopathologist, with a focus score >1, defined as a number of lymphocytic foci (which are adjacent to normal-appearing mucous acini and contain more than 50 lymphocytes) per 4 mm^2 of glandular tissue
V. Salivary gland involvement: objective evidence of salivary gland involvement defined by a positive result for at least one of the following diagnostic tests: 1. Unstimulated whole salivary flow (<1.5 ml in 15 minutes) 2. Parotid sialography showing the presence of diffuse sialectasias (punctate, cavitary or destructive pattern), without evidence of obstruction in the major ducts 3. Salivary scintigraphy showing delayed uptake, reduced concentration and/or delayed excretion of tracer
VI. Autoantibodies: presence in the serum of the following autoantibodies: Antibodies to Ro(SSA) or La(SSB) antigens, or both
For primary SS In patients without any potentially associated disease, primary SS may be defined as follows: a. The presence of any 4 of the 6 items is indicative of primary SS, as long as either item IV (Histopathology) or VI (Serology) is positive b. The presence of any 3 of the 4 objective criteria items (that is, items III, IV, V, VI) c. The classification tree procedure represents a valid alternative method for classification, although it should be more properly used in clinical-epidemiological survey
For secondary SS In patients with a potentially associated disease (for instance, another well defined connective tissue disease), the presence of item I or item II plus any 2 from among items III, IV, and V may be considered as indicative of secondary SS
Exclusion criteria: Past head and neck radiation treatment Hepatitis C infection Acquired immunodeficiency syndrome (AIDS) Pre-existing lymphoma Sarcoidosis Graft versus host disease Use of anticholinergic drugs (since a time shorter than 4-fold the half life of the drug)

Table 2: The ACR classification criteria for SS [11].

The classification of SS, which applies to individuals with signs/symptoms that may be suggestive of SS, will be met in patients who have at least 2 of the following 3 objective features:
1. Positive serum anti-SSA/Ro and/or anti-SSB/La or (positive rheumatoid factor and ANA titer ≥1:320) 2. Labial salivary gland biopsy exhibiting focal lymphocytic sialadenitis with a focus score ≥1 focus/4 mm^2 3. Keratoconjunctivitis sicca with ocular staining score ≥3 (assuming that individual is not currently using daily eye drops for glaucoma and has not had corneal surgery or cosmetic eyelid surgery in the last 5 years)
Exclusion criteria History of head and neck radiation treatment Hepatitis C infection Acquired immunodeficiency syndrome Sarcoidosis Amyloidosis Graft versus host disease IgG4-related disease

e-Patient education

Persons experiencing xerostomia or persons being diagnosed and treated for pSS, like any other online health information seeker, may be prone to search the Internet for background knowledge. An interview-based research in seven European countries, deduced that 71% of the e-consumers went online for health purposes [25]. Moreover, internet is becoming an increasingly popular source of health information as well as a potential communication channel among e-health seekers [25,26]. Internet is also believed to have the power to modify patient-doctor relationships by encouraging patients in the management of their health through a more shared decision making approach [27]. By gaining knowledge over aetiology and underlying conditions, impact on daily activities, and treatment strategies, patients can become more compliant, and at the same time, more active, keeping pace with the international trend in healthcare field [28]. To date, the quality of the online information about xerostomia and pSS is sparsely studied.

Table 3: American College of Rheumatology/European League Against Rheumatism classification criteria for primary Sjögren's syndrome [13]: The classification of primary Sjögren's syndrome 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 five criteria items below are summed:

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

*These inclusion criteria are applicable to any patient with at least one symptom of ocular or oral dryness, defined as a positive response to at least one 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 three 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 one 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 PCR)
(3) AIDS
(4) sarcoidosis
(5) amyloidosis
(6) graft-versus-host disease
(7) IgG4-related disease

‡ The histopathologic examination should be performed by a pathologist with expertise in the diagnosis of focal lymphocytic sialadenitis and focus score count, using the protocol described by Daniels et al. [14].

§ Patients who are normally taking anticholinergic drugs should be evaluated for objective signs of salivary hypofunction and ocular dryness after a sufficient interval without these medications in order for these components to be a valid measure of oral and ocular dryness.

¶ Ocular Staining Score described by Whitcher et al. [15]; van Bijsterveld score described by van Bijsterveld [10]

** Unstimulated whole saliva flow rate measurement described by Navazesh and Kumar [16].

Aim of the thesis

The overall aim of the research described in this thesis was to assess new challenges and trends in the diagnosis, treatment and e-education of patients with pSS. Specific aims were to:

1. examine the accuracy of ultrasonography of the major salivary glands in diagnosing pSS.
2. assess on a histopathologic level the effect of rituximab treatment in patients with pSS and to identify biomarkers that may predict responsiveness.
3. assess the qualitative standards of the distributed via Internet sites and YouTube information on xerostomia and pSS.

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Chapter 2

Xerostomia

Konstantina Delli[1], Frederik KL Spijkervet[1], Frans GM Kroese[2], Hendrika Bootsma[2], Arjan Vissink[1]

1. Department of Oral and Maxillofacial Surgery

2. Department of Rheumatology and Clinical Immunology

University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

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Summary

Xerostomia is the subjective feeling of oral dryness. Several diseases and conditions have been associated with xerostomia. Three major causes are: Sjögren's syndrome, medication and radiotherapy in the head and neck.

Sjögren's syndrome is a chronic systemic autoimmune disease characterised by infiltration of the exocrine glands, the salivary and lacrimal in particular. The pathogenesis involves systemic B-cell hyperactivity and T-cell lymphocytes targeting glandular epithelial cells. About 7.5% of patients with Sjögren's syndrome develop malignant B cell lymphoma, mostly mucosa associated tissue lymphomas (MALT).

Certain classes of drugs can induce hyposalivation and/or xerostomia by, e.g., targeting neurotransmitters and receptors. As a result, amongst others the production of fluid and electrolytes in salivary glands can be reduced and the salivary composition can change.

During head and neck radiotherapy, the administration of high doses to the major salivary glands, which are located in the periphery of the head, leads to progressive loss of glandular function and a diminished salivary output. In particular, reduction of the dose to and the volume of irradiated salivary glands by advanced radiotherapy techniques can be highly beneficial for patients.

Introduction

Xerostomia is the subjective feeling of oral dryness. The term is derived from the Greek words "xeros'' (ξηρός), meaning dry and "stoma" (στόμα), meaning mouth. The prevalence of xerostomia is difficult to be determined and numerous studies estimate it between 13 and 63% [1]. It is more prominent in women, in elderly and in individuals housed in long-term care facilities. A number of factors may cause or has been associated with transient or persistent xerostomia (Table 1). This chapter will focus on the three most common causes: Sjögren's syndrome, medication and radiotherapy of the head and neck.

Sjögren's syndrome

Sjögren's syndrome (SS), in the form that it is currently defined, was first described by the Swedish ophthalmologist Henrik Sjögren in 1930. It is a chronic inflammatory and lymphoproliferative disorder that is principally characterised by chronic infiltration of the exocrine glands. It commonly affects the salivary and lacrimal glands, resulting in xerostomia and dry eyes (keratoconjunctivitis sicca). These symptoms, known also as sicca symptoms, may be accompanied by extraglandular manifestations, evident in almost any organ. According to the American-European Consensus Group (AECG) classification criteria [2], SS can be distinguished in primary Sjögren's syndrome (pSS), in case no other autoimmune disease is present, and secondary Sjögren's syndrome (sSS), in case additional connective tissue diseases are present, such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), scleroderma and mixed connective tissue disease.

Epidemiology

SS is one of the most common rheumatic diseases, with a prevalence of 0.5-1% in the total population. It affects mainly women (i.e. female to male ratio equals to 9:1). The median age of occurrence is around 50 years, although it can arise in all ages. In RA the prevalence of SS is around 30% and approximately 20% of patients with SLE fulfil the criteria for sSS.

Clinical features

Glandular manifestations

SS primarily affects lacrimal and salivary glands. With respect to the eyes' symptoms, dryness may result in sensation of itching, grittiness, and soreness, notwithstanding the eyes' appearance can be normal. Ocular complaints may include

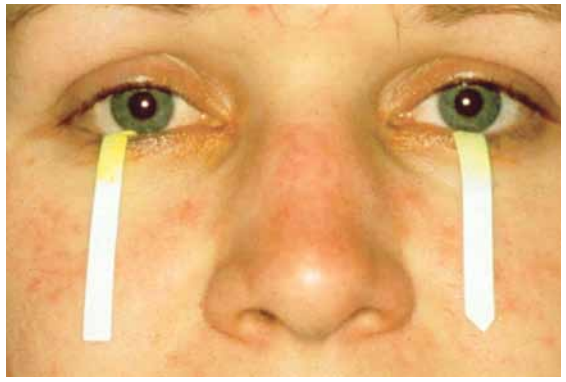


Figure 1: Dry eye due to diminished tear production, examined with Schirmer's test.

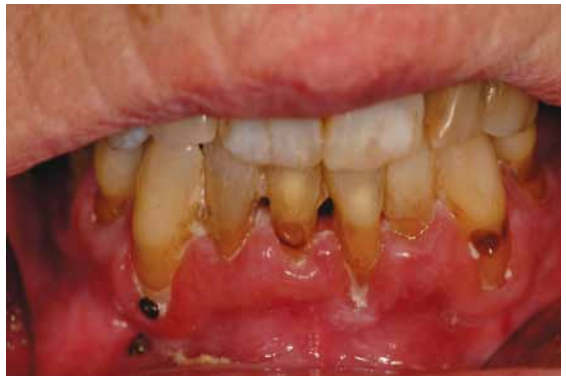


Figure 2: Tender and dry oral mucosa, characteristically forming fine wrinkles.

photosensitivity/photophobia, erythema, eye fatigue, decreased visual acuity, discharge in the eyes, and the sensation of a film across the visual field [3] (Figure 1). Progressive keratitis may result in loss of vision in patients with SS. Ocular complications may include corneal ulceration, vascularisation, opacification, and, rarely, perforation [4].

Another prominent symptom of SS is xerostomia (sensation of dry mouth related to a reduced saliva production). This symptom is often associated with dysgeusia, difficulty in eating dry food (e.g. crackers), problems in speaking for long period of time, burning sensation of the mouth, discomfort while wearing dentures, and increased risk of dental caries, especially cervically (Figure 2), and oral infection, in particular candidiasis. In the onset of SS, mouth appears to be normally moisturised but while the disease progresses, saliva diminishes and becomes foamy, lacking of the usual pooling in the floor of the mouth. In advanced disease, the oral mucosa is tender and dry and characteristically forms fine wrinkles (Figure 3). The tongue, in particular, often becomes fissured and exhibits atrophy of the papillae.

Table 1: Conditions causing xerostomia (modified after Jensen et al., 2010).

	Changed composition		Changed composition
Xerogenic drugs	+	Infectious diseases	
Sjögren's syndrome	+	HIV/AIDS	+/-
Head & neck radiotherapy	+	Hepatitis C virus	?
Chronic inflammatory connective tissue diseases		Metabolic disturbances	
Scleroderma	?	Water and salt imbalance	+
Mixed connective tissue disease	?	Sodium retention syndrome	+
Chronic inflammatory bowel diseases		Malnutrition	+
Autoimmune liver diseases	?	Eating disorders	
Musculoskeletal disorders		Bulimia nervosa	+/-
Fibromyalgia	?	Anorexia nervosa	+
Chronic fatigue syndrome	?	Cancer-associated disturbances	
Amyloidosis	?	Chemotherapy	+/-
Endocrine disorders		GVHD	+
Diabetes mellitus	+/-		
Hypothyroidism	?		
Neurological disorders			
Parkinson's disease	+		
Alzheimer's disease	+		
Holmes-Adie syndrome	?		
Burning mouth syndrome	+		

Enlargement of the salivary glands, especially the parotid and submandibular glands (Figure 4) is a common phenomenon in patients with SS. Swelling of the salivary glands is usually bilateral, may be non-painful to slightly tender and intermittent to persistence in nature. The swelling is generally attributed to the presence of an autoinflammatory process in these glands and stasis of saliva can result in secondary infection, encouraging further swelling. The development of lymphomas, in most cases in the parotid gland, can lead also to more persistent unilateral glandular enlargement. Dryness can occur at mucosal surfaces of upper and lower respiratory tracts resulting in non-productive cough [5]. Dry skin affects about 55% of SS patients while in female patients with SS desiccation of the vagina results in dyspareunia [6].

Table 2: Risk factors for the development of lymphoma.

Clinical	Serological
Persistent enlargement of parotid gland	Monoclonal gammopathy
Persistent lymphadenopathy	Reduced levels of complement C4
Palpable purpura	CD4+ T lymphocytopenia
Vasculitis	Increase in IgG levels
Renal involvement	Cryoglobulinemia
Peripheral neuropathy	

Table 3: Serologic findings in SS patients.

Autoantibody	Frequency in SS patients (%)	Specific for SS
anti-Ro/SSA	70	NO
anti-La/SSB	50	NO
anti-alpha fodrin	30	YES
anti-muscarinic acetylcholine receptor 3	71-90	NO
rheumatoid factor	50	NO

Table 4: Possible factors predisposing to SS.

Endogenous	Immunologic disturbance HLA-DR3/DQ-2 Female gender
Exogenous	Virus infection 1. Epstein Barr 2. Coxsackie 3. Retroviruses (e.g. HTLV-10) 4. HIV 5. Hepatitis C

Lymphoma development

About 7.5% of patients with SS develop malignant B cell lymphoma, 48-75% of which is MALT-type [7,8]. SS patients also have an 18.8 (CI: 9.5-37.3) times increased risk in developing lymphomas [9]. B cell lymphomas are most frequently located in the parotid gland. The conversion from variable to persistent enlargement of the gland is an alarming clinical sign. The presence of palpable purpura (Figure 5), vasculitis (Figure 6), renal involvement and peripheral neuropathy, although not pathognomonic, should raise suspicion, especially when it is combined with monoclonal gammopathy, reduced levels of complement C4, CD+4 T lymphocytopenia, sharp increase in IgG levels, or cryoglobulinemia [7-12] (Table 2).

Serological findings

The most characteristic autoantibodies in SS are the anti-Ro/SSA and anti-La/SSB autoantibodies (Table 3), which are present in 70% and 50% of cases, respectively. Their titers reflect disease activity, while high titers of particularly anti-La/SSB have been associated with extraglandular disease [13]. Despite anti-Ro/SSA and anti-La/SSB are not specific for SS, since they can also occur in, e.g., patients with SLE, their presence should alert the clinician for the possibility of SS-diagnosis.

Diagnostic criteria

A joint study of the AECG presented in 2002 the revised AECG classification criteria for SS and since to date they are the most widely accepted criteria [2]. These criteria successfully combine subjective symptoms, as well as objective signs of keratoconjuctivitis sicca and hyposalvation together with histopathological and serological findings. It must be underlined that SS can be present in a patient who does not completely fulfil these classification criteria and that since anticholinergic drugs are widely used by patients for many conditions, their exclusion should be carefully re-evaluated [1]. Recently, due to the emergence of biologic agents, the American College of Rheumatology (ACR) proposed new classification criteria for SS, based merely on objective tests. The ACR classification criteria were developed from registry data collected with standardized measures and are thought to be more suitable in situations where misclassification may present health risks [14].

Etiopathogenesis

SS is considered to be an autoimmune disorder, but with a pathogenesis that is poorly understood. Undoubtedly, a disturbance of the immune system plays a key role. It is not clarified yet whether this disturbance exists primarily or is a result of an infection, possibly viral or has another cause. Several findings suggest that viruses, such as Epstein Barr, Coxsackie and retroviruses may be implicated. Possibly, their glandular persistence in salivary gland epithelial cells may lead to chronic lymphocytic sialoadenitis with formation of foci around the ducts [15]. Additionally, hepatitis C and HIV infections can produce both symptoms and pathological find-

Figure 3: Red, fissured tongue with atrophy of the papillae.

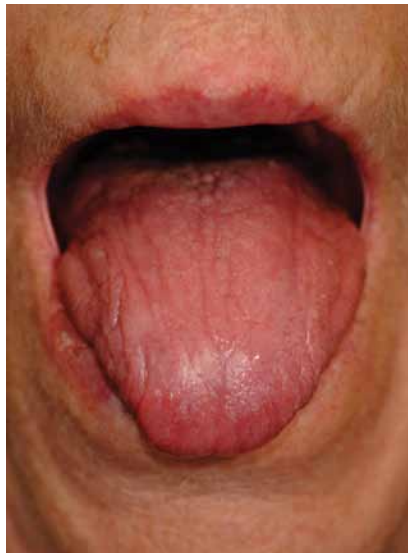
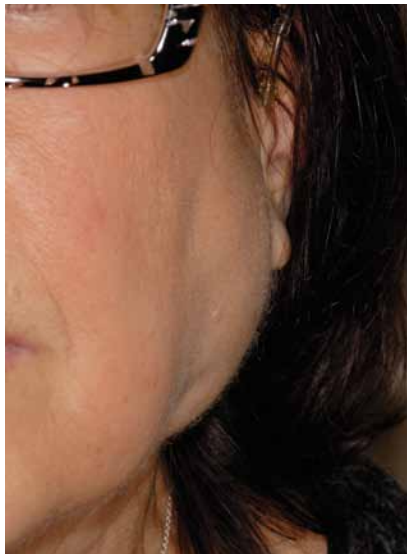


Figure 4: Enlarged parotid salivary gland due to the development of MALT lymphoma.



ings similar to that in SS, but for the time being the presence of these infections is an exclusion criterion for SS.

Apart from these exogenous factors, various endogenous factors may be also involved (Table 4). The strong female preponderance implies possible involvement of hormonal factors, while the extended haplotype HLA-DR3/DQ-2 in combination with the C4null gene being present in 50% of SS patients probably evinces genetic factors, too [16,17].

The complexity of the pathogenetic pathways in SS involves both systemic B-cell hyperactivity and also T-cell lymphocytes targeting glandular epithelial cells:

- Prolonged B-cell survival and B-cell hyperactivity leads to presence of anti-Ro/SSA and anti-La/SSB antibodies, RF, type 2 cryoclobulins and hypergammaglobulinemia in SS patients;
- Ductal epithelial cells are surrounded by activated T-cells, predominantly CD4-positive (70-80%). CD8-positive T-cells constitute around 10% of infiltrating cells in affected labial salivary glands [18].

Histopathology

Biopsy of the minor salivary glands of the lower lip is widely used for the diagnosis of SS and its histopathology is considered as one of the four objective AECG classification criteria of SS as well as one of the three objective ACR criteria [2,4].

Figure 5: Purpura is a common extraglandular manifestation in SS patients.

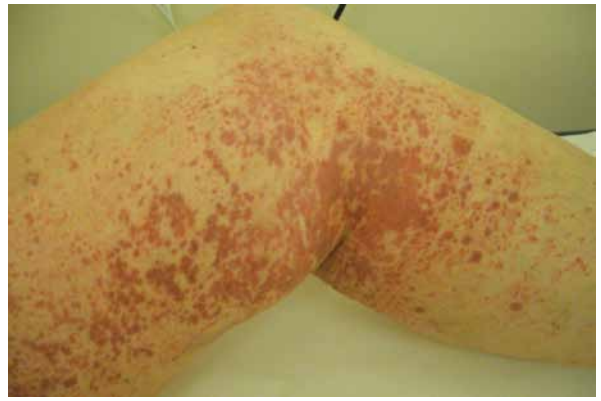
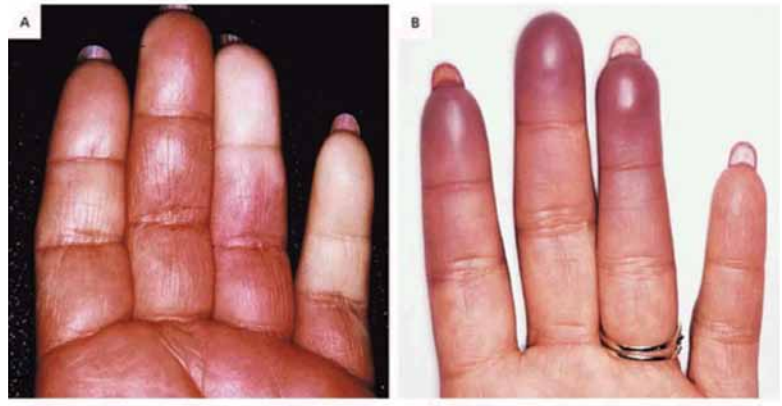


Figure 6: Raynaud's phenomenon.



The procedure is performed under local anaesthesia. A lower lip mucosa incision of approximately 1.5 cm is made and at least seven individual labial glands are collected [19]. Parotid biopsies are increasingly gaining broader acceptance and are used as an alternative to minor salivary gland biopsies. Parotid biopsies are validated for the AECG classification criteria [20], but not yet for the ACR classification criteria. With this technique, also performed under local anaesthesia, parotid tissue is taken from the area around the lower ear lobe. An 1 cm incision is carried out, followed by blunt dissection to the parotid gland and an incisional biopsy. The remaining wound is closed in layers [21] (Figure 7). Several studies show a lower morbidity of a parotid gland biopsy compared to a lip biopsy [20,22] with regard to loss of sensibility and pain. In none of the parotid gland biopsy studies a disturbtion of the facial nerve was observed.

Table 5: Treatment of SS.

SUPPORTIVE		CAUSAL
	1. Local	1. B-cell depletion
	EYE	e.g. Rituximab
a.	Artificial tears	2. Inhibition of costimulation of T-cells
b.	Corticosteroids	e.g. Abatacept
c.	Immunosuppressives	3. anti TNF-a
d.	Sealed glasses	e.g. Infliximab, Etanercept
e.	Blocking the lacrimal punctum	4. IFN
	MOUTH	e.g. Rontalizumab
a.	Artificial saliva	
b.	Antifungal therapy	
c.	Fluoride application	
	2. Systemic	
a.	Pilocarpine	
b.	Cevimeline	

The most prominent microscopic finding in SS is periductal lymphocytic infiltration of salivary glands in combination with destruction of acini (Figure 8A). The infiltrates are composed of both B- and T-lymphocytes as well as non-lymphoid cells and are located around the striated ducts. When these infiltrates are composed of more than 50 cells, they are called focus. The presence of more than one focus per 4 mm^2 area of glandular tissue is regarded as a positive criterion for the diagnosis of SS. Furthermore, if the major glands are enlarged, progression to a lymphoepithelial lesion (LEL) (Figure 8B) can also be present. In major salivary glands, characteristic epimyoepithelial islands in a background of lymphoid stroma are usually seen.

The sensitivity and specificity of the parotid biopsy are comparable with that of labial salivary glands [20] and additionally can provide evidence about LELs and well-formed lymphoid follicles or germinal centers (GC). Theander and colleagues (2011) suggested that the detection of GC-like structures by light microscopy in pSS diagnostic salivary biopsies is a highly predictive and easy-to-obtain marker for non-Hodgkin lymphoma (NHL) development, allowing for risk stratification of patients and the possibility to initiate preventive B-cell-directed therapy [23]. The histopathological results of a parotid biopsy can be indicative of malignant lymphoma, as MALT lymphomas often develop in the parotid gland and rarely in labial glands. Repeated biopsies from the same parotid gland offer information concerning the course of the disease.

Table 6: Top 10 Therapeutic Classes by U.S. Dispensed Prescriptions in 2011 (http://www.imshealth.com).

Ranking	Drug Class	Xerogenic Effect
1	Antidepressants	YES
2	Lipid Regulators	NO
3	Narcotic Analgesics	YES
4	Anti-diabetes agents	NO
5	ACE Inhibitors	YES
6	Beta Blockers	Uncommon
7	Respiratory Agents	YES
8	Anti-ulcerants	YES
9	Diuretics	YES
10	Anti-epileptics	YES

Treatment

Evidence-based therapy for SS is limited and the treatment of patients with SS is mostly mainly supportive (Table 5).

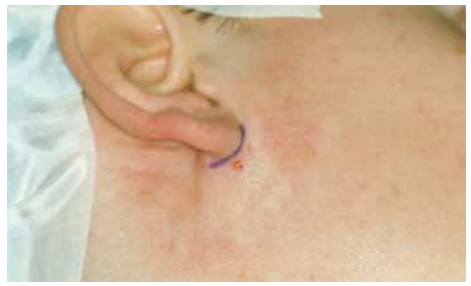
Local treatment for dryness of eyes and mouth is helpful in many cases. Artificial tears lubricate dry eyes, and in case of keratoconjuctivitis local corticosteroids and local immunosuppressive may be used. Sealed glasses are also introduced in an attempt to prevent evaporation of tears and to conserve the tear film. Sealing the lachrymal uncut in the inner margin of the eyelid can also be helpful by blocking the normal drainage to the nose. To treat xerostomia, one first has to estimate whether stimulating salivary secretion by gustatory (sugar-free sweets), mechanical (chewing gum) or sialagogue medication (pilocarpine, cevimeline) results in relief of xerostomia. When stimulation of salivary secretion is uneventful, one can try to treat xerostomia with mouth rinses, artificial saliva and/or oral gels. Antifungal therapy, such as local treatment with nystatin, myconazole or amphotericin B, frequently is needed to treat oral candidiasis. Due to the increased risk of dental caries, a weekly to daily use of topical neutral fluoride applications or mouthrinses is indicated in dentate patients.

During the past two decades, biologicals have become available to target specific cells or cytokines that are fundamental in the immune response. Under this new perspective, inhibitors of TNF-, INF-, B-cell depletion therapies, BAFF-inhibitors and treatments targeting the co-stimulation of T-cells have been also recruited in the treatment of SS [24,25].

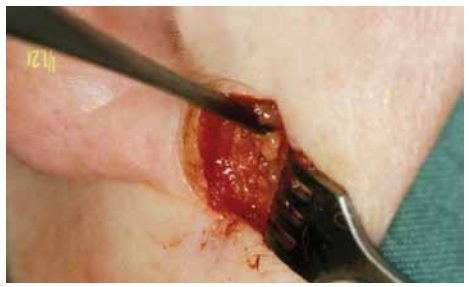
The therapeutic approach to the patients with SS and MALT lymphoma is a matter of debate [26].

Figure 7: Incisional biopsy of the parotid gland.

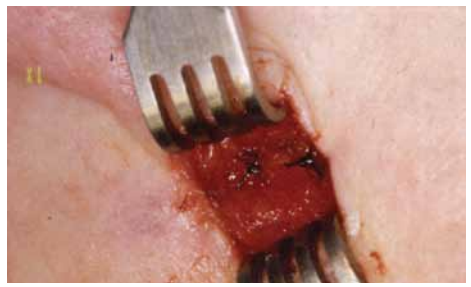
A. The area is anesthetized with local infiltration anesthesia.



B. With a No 15 blade a small 1-2cm incision is made just below and behind the earlobe near the posterior angle of the mandible.



C. The skin is incised and the parotid capsule is exposed by blunt dissection. The capsule of the gland is carefully opened and a small amount of superficial parotid tissue is removed.

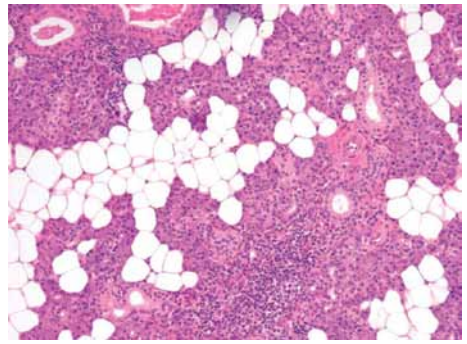


D. The procedure is completed with a 2 to 3-layered closure with 4-0 gauge absorbable sutures (polyglycolic acid), while the skin layer is closed with 5-0 nylon sutures.

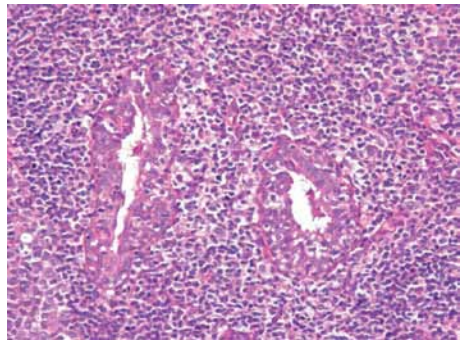
Drugs

Drugs are the most common cause of dry mouth. A review of the 200 most frequently prescribed drugs in the U.S. revealed that the most common side effect was dry mouth (80.5%) followed by dysgeusia (47.5%) and stomatitis (33.9%) [27]. The exact relationship between dry mouth and drugs is variably influenced by many factors, such as type of drug, number of drugs, drug combination, dose, form, time of intake, duration of use, drug interaction and reliability of patient's report. The situation is even more complicated in diseases and disorders that contribute to the problem. Nonetheless, it is generally accepted that the prevalence of dry mouth increases with age and the number of drugs taken per day. Also drug-induced dry mouth is primarily reversible, and an increasing amount of drugs has the capacity to induce oral dryness, while xerostomia can occur in the absence of drugs as well.

Figure 8: Microscopic findings in SS.



(A) Parotid gland tissue showing a periductular lymphocytic infiltrate and deposition of fat between the serous acini (magnification 10x).



(B) Lymphoepithelial lesions surrounded by a lymphocytic infiltrate (magnification 20x) (Pijpe et al., 2007)

Interrelation with age and sex

Studies indicate that the average intake of drugs increases with age. In U.S., for example, the intake of 1-2 drugs/day/person progressively increases from 24% to 87% from the age of 18 to the age of 65 respectively (Figure 9). The prevalence of dry mouth increases with the number of drugs taken per day (Figure 10). In a study that related the prevalence of dry mouth and age, Nederfors et al. not only confirmed the aforementioned data, but also concluded that the presence of drug-induced dryness was greater in women than men [28].

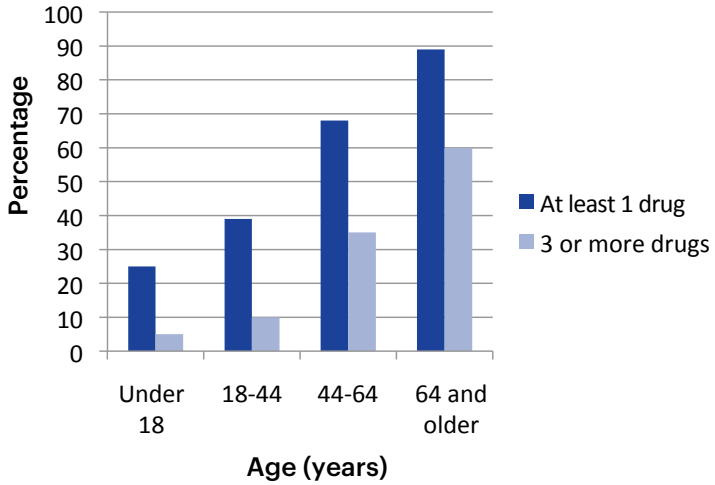
Possible mechanisms

Drugs induce oral dryness by interfering with the production of fluid and electrolytes in salivary glands, by affecting the production of proteins and high molecular weight compounds or by reducing body's content of salt and water. This is mainly the result of their effect on neurotransmitters and receptors. Parasympathetic stimulation produces abundant saliva of low protein concentration while sympathetic stimulation produces little saliva but rich in protein, giving a sensation of dryness [29]. This section will focus on describing the mechanism upon which the most commonly used therapeutic drugs (Table 6) induce xerostomia. More detailed lists about xerogenic medication can be found in 'www.drymouth.info'.

Antidepressants

Antidepressants were the most common drug category prescribed in 2011 in the U.S. Even more notable is the fact that over 60% of patients prescribed antidepressants report taking them for more than 2 years, and 14% for 10 years or more. Antidepressants fall into four different classes, viz. tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase inhibitors

Figure 9: Prescription drug use in U.S., 2001-2004 (modified after: Sreenby and Vissink, 2010).



(MAOIs) and atypical antidepressants. It has been shown that the xerogenic capacity of TCAs is high, and 28% of patients taking TCAs may develop oral dryness [30]. Wilson et al. showed in a meta-analysis of the adverse effects of TCAs versus SSRIs that dry mouth occurred in 28% of patients taking TCAs in comparison to 7% of patients on SSRIs [30].

Narcotic analgesics

Narcotic analgesics (NAs) in general reduce neuronal excitability in the pain carrying pathway by binding to opioid receptors. Morphine and its analogues as well as some synthetic derivatives are classified as NAs and are principally used to alleviate acute and chronic pain. Glare et al. reported a prevalence of 95% of dry mouth among patients who received morphine due to cancer pain. The symptom was persistent with a moderate to severe intensity in 57% of them [31].

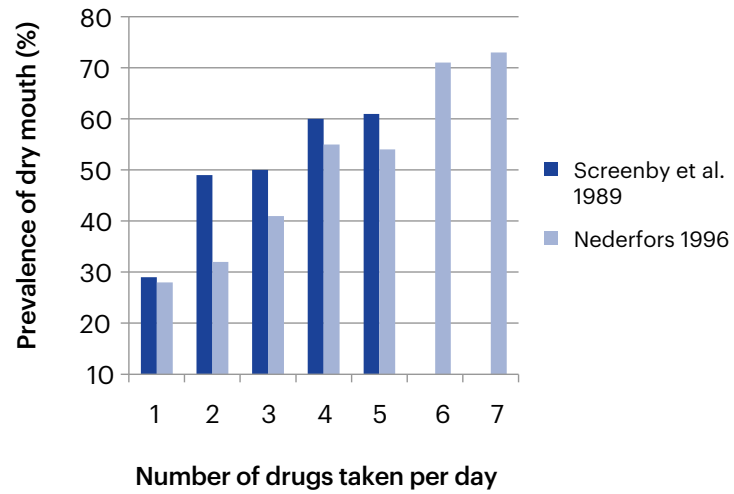
Angiotensin converting enzyme inhibitors (ACE inhibitors)

ACE inhibitors are widely used for the treatment of hypertension. They inhibit the conversion of angiotensin I to angiotensin II. Angiotensin II receptor antagonists (AIIRAs) act by blocking the binding of angiotensin II to the angiotensin receptors located in the vascular smooth muscle. Both ACE inhibitors and AIIRAs present similar actions and side effects. Reports suggest that about 1-13% of patients using these drugs complain of oral dryness [32,33].

Respiratory agents

Smidt et al. (2011) reported that 27% of patients taking respiratory agents experience dry mouth. The main categories of respiratory agents associated to dry

Figure 10: Prevalence of drugs and dry mouth (modified after: Sreenby and Vissink, 2010).



mouth are [34]:

- *drugs for obstructive airway disease* The most commonly reported adverse event in patients on tiotropium (an anti-muscarinic agent) was dry mouth (9.3% versus 1.6% relative to placebo; $p < 0.05$) [35], while overall 6-13% of patients receiving it complain for xerostomia [36,37].

- *cough and cold preparations* Stimulation of α- and ß-adrenergic receptors in the mucous membrane of the respiratory tract by pseudoepinephrine and phenylephrine results in the shrinking of the nasal mucous membranes and relieves nasal obstruction. Studies demonstrated that pseudoepinephrine has the capacity to induce oral dryness in 0.4-11% of patients [38,39].

- *antihistamines* Three antihistamines are commonly used, viz. brompheniramine, clopheniramine and carbinoxamine. Their frequent combination with decongestants as well as breathing through mouth can cause dry mouth [38,39].

Anti-ulcerants

Anti-ulcerants are used as part of the treatment of ulcers. The two basic types of anti-ulcerants are H2-blockers and proton-pump inhibitors. Dry mouth is found in 41% of patients receiving H2-receptor antagonist for eradication of *Helicobacter pylori* [40]. Teare and co-workers reported subnormal parotid or whole saliva flow rates in patients treated with omeprazole, a common proton-pump inhibitor [41].

Diuretics

Diuretics increase the formation and extraction of urine. As a result, there is a decrease in the volume of extracellular water and a consequent reduction in cardiac output and blood pressure. Furthermore, diuretics affect the flow and electrolyte composition of saliva. According to Smidt et al., 17.8% of patients taking diuretics experience dry mouth [34]. Hebbab et al. distinguished the frequency of dry mouth in patients taking thiazide, loop and potassium sparing diuretics in 3, 8 and 16%, respectively [33].

Anti-epileptics

Anti-epileptics are a class of drugs that prevent rapid, repetitive, stimulation of the brain that causes seizure activity. According to Zaccara et al. there is a selective dose-dependent pattern in the onset of dry mouth after the administration of pregabalin, which becomes evident after a dosage of 150mg/day [42]. There are also sporadic reports of transient 'sicca syndrome' during phenobarbital treatment [43] or even phenytoin-induced pseudo-Sjögren's syndrome [44]. Mild xerogenic effect has been attributed to carbamazepine, oxcarbazepine, gabapentin, valproic acid, clonazepam, zonisamide, lamotrigine, and topiramate (www.drymouth.info).

Management of drug-induced xerostomia

Drug induced xerostomia can be prevented or diminished by avoiding xerogenic drugs or minimizing their exposure to them. Substitution of a different agent with similar therapeutic properties can usually relieve the symptoms. If this is not possible, patients should be reassured that in most cases this condition is not permanent and salivary gland function will return to pro-treatment levels after the end of the therapy. In order to support these patients, usage of salivary stimulants or usage of artificial saliva, in particular substitutes with a stimulating additive such as malic acid during daytime and gel type substitutes during night, should be encouraged during their treatment with xerogenic drugs.

Radiotherapy

Radiotherapy plays a fundamental role in the treatment of the majority of patients with head and neck cancer. It can be used as a single modality or in combination with surgery and/or chemotherapy and typically involves administration of high doses to the major salivary glands. It has to be mentioned that ablation therapy of thyroid cancer with radioactive iodine treatment can also result in radiation damage to salivary gland tissue as, besides thyroid glands, salivary glands have high uptake of this agent too.

In most cases, radiation damage to salivary gland tissue results in progressive loss of glandular function and diminished salivary output. Patients complain of oral dryness, impairment of oral functions (speech, chewing, and swallowing) because of insufficient lubrication of mucosal surfaces and of ingested food [45]. The oral mucosa can become dry and atrophic, leading to frequent ulceration and injury. The shift in oral microflora towards cariogenic bacteria in combination with the reduced saliva flow and altered saliva composition may lead in radiation caries [46,47]. It must be noted that the subjective symptom of xerostomia does not always correlate with salivary flow rates; this not only counts to radiation-induced xerostomia, but also to xerostomia from other origins.

Pathophysiology

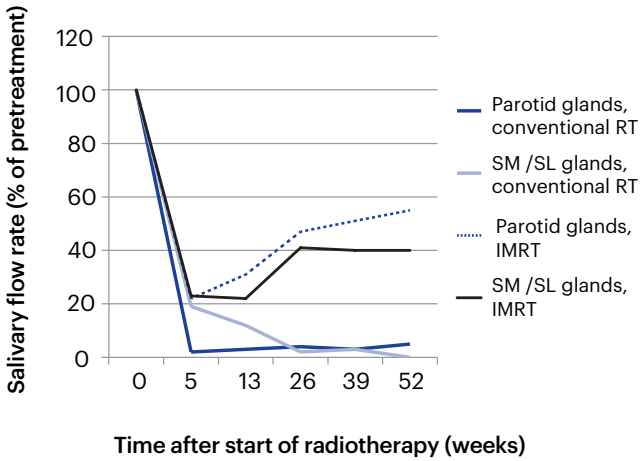
One week after the onset of conventional radiotherapy treatment, when 5-10 Gy are typically delivered, the salivary output declines by 60-90%. The acute phase of xerostomia is characterized by thick and sticky saliva, as a result of the faster decline in the serous, watery content of the saliva, compared to the decline of mucins and proteins. Late recovery is possible in cases of moderate radiation mode [48-50]. More recent studies revealed, however, that the serous parotid and seromucous submandibular glands are probably equally sensitive to ionizing radiation [51].

The mechanism of acute salivary damage is not fully understood and to date several theories have been proposed, amongst others:

1. DNA-damage caused by radiation impairs proper cell division, resulting in cell death or senescence of cells that attempt to divide. Based on the fact that cells of salivary glands have a slow turnover rate (60-120 days), they would be expected to be late responding tissue (>60 days) [52]. However, the changes in quantity and composition of saliva occur shortly after the radiation, indicating that salivary glands respond acutely [51,53]. Radiation injury leads to the loss of saliva producing acinar cells, but ducts, although deprived of function, remain intact [54]. The study of the role of apoptotic cell death after radiotherapy came up with controversial findings. Paardenkooper et al. did not observe a dose related increase in apoptotic cells early after radiation therapy [55], whereas Avila et al. found that early radiation-induced salivary gland dysfunction resulted from p53-dependent apoptosis [56]. Currently, research focuses on very selective blocking certain areas of the parotid gland from radiation injury meanwhile guaranteeing those areas of the parotid gland where the stem cells resided (probably the main excretory ducts);

2. The **leakage of granules** and subsequent lysis of acinar cells have been suggested as an alternative explanation for this phenomenon [57,58]. Nevertheless, studies show no cell loss during the first days after irradiation [51,59-61].

Figure 11: Stimulated parotid and submandibular/sublingual (SM/SL) saliva flow rate after conventional radiotherapy (RT) and parotid sparing intensity-modulated RT (IMRT) (modified after: Vissink et al., 2010).



Management

Reducing the volume of irradiated salivary glands by advanced radiotherapy techniques in combination with salivary protectors and/or stimulators can be highly beneficial for patients.

Advanced radiation delivery techniques

Prevalence rates of xerostomia after radiotherapy with conventional and more advanced techniques are shown in Figure 11, of which 3-dimensional conformal radiotherapy (3-D-CRT) and Intensity modulated radiotherapy (IMRT) are currently most commonly applied.

3-D-CRT is designed to deliver an exact dose of irradiation to a target volume. This is achieved by creating a three dimensional image of the tumor, so that multiple radiation beams can be shaped exactly to the contour of the treatment area. There is evidence that reduced radiotherapy dosages by 3-D-CRT to contralateral parotid glands result in less loss of salivary gland function post-radiotherapy up to 2 years after the completion of radiotherapy [62]. Albeit 3-D-CRT has the potential to decrease the prevalence and severity of xerostomia, xerostomia has been shown to be significantly worse after bilateral compared to unilateral treatment.

IMRT is currently recommended as a standard approach in head-and-neck cancer, as it allows a more accurate distribution of specific radiation dosage and dosage distribution to the tumor and therefore provides better sparing of the surrounding tissues. Since it reduces the dose to salivary glands (parotid, sublingual, submandibular and minor), it can contribute to the maintenance of adequate saliva flow rates and the reduction of xerostomia [63-65]. IMRT compared to 2D-radiotherapy results in a significant decrease of xerostomia (both patient- and observer-rated). However, approximately 40% of patients still complain of dry mouth [66].

A rather new technique that is yet sparsely applied in the clinic is *proton radiotherapy*. This technique uses charged particles (e.g., protons) instead of the currently used photons. The physical and radiobiological properties of protons allow a better dose distribution, compared with photon radiotherapy. Thus, the dose to normal tissues as well as the late side effects are minimized. The existing literature shows that the dose to critical organs can be significantly reduced, especially in patients with tumors located in the pharynx [67,68] and the paranasal sinuses [69,70] as well as in the head-and-neck patients treated with bilateral neck irradiation [71].

Agents for prevention of xerostomia or restoration of lubrication

Pilocarpine

Restoration of lubrication Pilocarpine is a cholinergic parasympathomimetic agent, acting as an agonist at muscarinic receptors. One third to two thirds of patients with post-radiotherapy xerostomia can benefit from the administration of pilocarpine [72,73]. A dose of 5 mg t.i.d. is recommended, and up to 4 weeks might be required before maximum effect is visible. The possible mechanism involves stimulation of the residual function of the major salivary glands as well as the stimulation of the minor salivary glands, especially the ones in the palate, which have been shown to have a greater resistance to irradiation [74].

The results of post-irradiation pilocarpine disappear when patients stop using it. In order to protect salivary glands during radiotherapy and to eliminate the long-term post-irradiation treatment, administration of pilocarpine during radiotherapy is an alternative choice [75,76]. The beneficial effect of pilocarpine is depended on the dose distribution in the parotid glands and when parotid dose exceeds 40 Gy, administration of pilocarpine during radiotherapy can considerably spare parotid flow and reduce patient-rated xerostomia [77].

Amifostine

Direct radioprotection can be achieved by the use of amifostine, a scavenger of free radicals [78]. Although salivary flow is preserved when amifostine is concurrently delivered with radiation, patients continue to experience xerostomia. Intravenous administration is accompanied by several side effects (e.g. nausea, vomiting, hypotension). Furthermore amifostine might also have the undesirable effect of tumor protection. Thus, the debate continues, whether it is safe to use it in cancer patients [79].

Tempol

Tempol is stable nitroxide, providing radioprotection possibly by mimicking superoxide dismutase activity and scavenging free radicals. In a mouse model, tempol has been proven to significantly reduce salivary gland hypofunction [80], by protecting salivary glands, but not tumor tissue [81]. Thus tempol could be considered for human clinical trials.

Keratinocyte growth factor (KGF)

KGF can be administered prior or during radiotherapy. KGF suppresses apoptosis and enhances survival and proliferation of salivary acinar cells [82]. Postirradiation administration of KGF most likely accelerates expansion of the pool of progenitor/stem cells that survived the treatment.

Oral lubricants and saliva substitutes

Symptomatic approach of xerostomia is attempted when stimulation of residual secretion is insufficient or in cases there is a contra-indication in the administration of the aforementioned agents. The most commonly applied and best studied saliva substitutes are based on carboxymethylcellulose [83], mucin [84] or xantham gum [85]. Mucin- and xantham gum-containing substitutes are usally preferred because they have superior rheologic and wetting properties compared to carboxymethylcellulose based saliva substitutes. During night and when daily activities are at a low level, gel-like saliva substitutes are preferred [86].

Adult salivary gland stem cells

Artificial lubricants and sialogogues ameliorate the consequences of hyposalivation, but their effects are at best transient. Such management techniques do not address the source of the problem: a lack of functional saliva-producing acinar cells, resulting from radiation-induced stem cell sterilization. Stem cell replacement therapy may be a good option to treat radiation-induced hyposalivation. Recent identification of salivary gland stem and progenitor cell populations provides a basis for development of a stem cell-based therapy for xerostomia to provide a more durable cure for hyposalivation [87].

Epilogue

Although not essential for the maintenance of life, saliva plays a fundamental role in the function and projection of the human body. Although not much appreciated when it is routinely found abundant in the oral cavity, its diminution leads to the very unpleasant sensation of oral dryness, to an abundance of oral complaints and to a dramatic decrease in the quality of life. Xerostomia is also a common symptom of a wide variety of diseases and conditions and the resulting complaints may point the physician to the underlying disease. While much progress has been achieved so far in the investigation of its mechanisms and treatment options, it is indisputable that there is more to be done as still xerostomia and its related complaints are hard to treat to the patients' satisfaction.

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Chapter 3

Diagnosis

Chapter 3A

Diagnostic properties of ultrasound of major salivary glands in Sjögren's Syndrome: a meta-analysis

Konstantina Delli[1], Pieter U Dijkstra[1,2], Alja J Stel[3], Hendrika Bootsma[3], Arjan Vissink[1], Frederik KL Spijkervet[1]

Best Original Article Award
for 2015 In Oral Diseases

Affiliations:

1. Department of Oral and Maxillofacial Surgery

2. Department of Rehabilitation, Center for Rehabilitation

3. Department of Rheumatology and Clinical Immunology

University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

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Abstract

Objective: To perform a systematic review and meta-analysis on studies examining the properties of ultrasonography of major salivary glands for diagnosing Sjögren's Syndrome.

Materials & Methods: We searched for literature on eight databases. The quality of included articles was assessed with the QUADAS-2 tool. Publication bias, pooled sensitivity, specificity, diagnostic odds ratio and 95% confidence intervals (95%CI) were calculated. Meta-regression analysis was performed.

Results: We identified 37 studies and 33 ultrasonographic scoring systems. High risk of bias was observed in 'patient selection', 'conduct and interpretation of ultrasound' and 'flow of patients and timing of tests' in 78%, 70% and 51% of the studies. We included 29 studies in the meta-analysis. Publication bias was highly probable. Pooled sensitivity was 0.69 (95%CI: 0.67-0.71), specificity 0.92 (95%CI: 0.91-0.93) and diagnostic odds ratio 33.89 (95%CI: 20.75-55.35). Significant heterogeneity was detected between studies. Meta-regression analysis showed that studies with high risk of bias in 'conduct and interpretation of ultrasound' and studies evaluating only parenchymal homogeneity had higher log diagnostic odds ratio (1.09 and 2.49 respectively, $p<0.05$).

Conclusions: The quality of current studies is low thus not allowing to judge the likelihood of salivary gland ultrasonography as a reliable and practical tool in diagnosing Sjögren's Syndrome.

Introduction

Sjögren's syndrome (SS) is one of the most common rheumatic diseases, with a prevalence of 0.05% in the total population. It commonly affects the salivary and lacrimal glands, resulting in a sensation of dry eyes (keratoconjunctivitis sicca) and dry mouth (xerostomia) [1]. Although the exact pathogenic mechanism has not been elucidated, in patients with SS the minor and major salivary glands are characteristically infiltrated by mononuclear lymphoid cells [2]. Enlargement of the major salivary glands, especially the parotid and submandibular is also common. Swelling of the salivary glands is usually bilateral, may be non-painful to slightly tender and intermittent to persistent in nature. The development of lymphomas in SS, in most cases in the parotid gland, can lead also to more persistent unilateral glandular enlargement.

In the assessment of salivary gland involvement in SS, ultrasonography of the major salivary glands merits special attention as a non-invasive, inexpensive, widely available, easily accessible and non-irradiating imaging modality [3]. Ultrasonography enables visualization of deep structures of the body by recording the reflections or echoes of ultrasonic pulses directed into the tissues. Frequencies ranging from 1.6 to 22 MHz are used for diagnostic imaging. B-mode is the most widely used ultrasonography mode; the gray scale of the image consists of pixels whose brightness depends on the intensity of the echo that is received from the corresponding location in the body [4].

Recently, a meta-analysis was published regarding the diagnostic properties, sensitivity, specificity and diagnostic odds ratio of ultrasonography in diagnosing SS [5]. However, the data in the tables in that study did not appear to correspond with the data from the source publications. There seems to be a discrepancy between the data shown in the meta-analysis and the data presented by the source studies [6-11]. Hence, the diagnostic properties of ultrasonography in diagnosing SS remain unclear [12].

The primary objective of this study was to conduct a systematic review and meta-analysis on studies examining the diagnostic properties of ultrasonography of major salivary glands in diagnosing SS in comparison to criteria applied in the classification of SS or in comparison to sialography, scintigraphy, sialometry and histology of the salivary glands. The secondary objective of the study was to evaluate effects of research methodology on diagnostic properties of ultrasound in SS found in the studies.

Materials and methods

Study identification

We conducted a literature search of eight electronic databases (six literature databases and two control trials registries). According to the syntax rules of each database, key words and their combinations were used to identify studies published till June 2014. No restrictions were applied (supplementary Table 1).

Study eligibility

Two observers (K.D. and P.U.D.) independently assessed titles and abstracts identified in the initial search. Inclusion criteria were studies examining the diagnostic properties of ultrasonography of major salivary glands in diagnosing SS in comparison to diagnostic criteria or in comparison to sialography, scintigraphy, sialometry or histology of the salivary glands. Exclusion criteria for titles and abstracts were the following: case reports, case series with fewer than 10 cases, experts' opinions, letters to the editor, review articles, studies that did not report the diagnostic properties of ultrasonography and congress abstracts. If the title and abstract provided limited information or in case of doubt, the studies were moved to the next round (full text assessment). The results of the assessment were compared, and any disagreement was resolved through consensus.

Full texts of the included titles and abstracts were independently assessed according to aforementioned criteria by the same observers. Additionally, the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) tool was used to assess the risk of bias of the included studies [13]. This tool consists of four domains covering 'patient selection', 'index test', 'reference standard', and 'flow of patients through the study and timing of the index test and reference standard'. The observers (K.D. and P.U.D.) independently evaluated the studies. After each stage of selection, inter-observer agreement was calculated as Cohen's kappa and percentage of agreement. Studies written in a language, which the assessors were not proficient, were translated into English by researchers fluent in both that language and English. These studies (4 Italian, 2 Spanish, 1 Russian, 1 Chinese) were assessed for inclusion criteria and risk of bias by one observer (K.D.).

Data extraction was performed (by K.D.) on study and patient characteristics, and on the diagnostic performance of ultrasonography. Information about ultrasonographic criteria for diagnosing Sjögren's syndrome, type of reference standard, ultrasonographic transducer used, and true positive, true negative, false positive, false negative and accuracy results were collected, using a standardized form. If one cell was empty, in the two-by-two tables 0.5 was added to all cells to be able to calculate a diagnostic odds ratio. If a control group was not included in the study, only true positive and false negative results were collected.

The reporting of this study complied with the 'Preferred Reporting Items for Systematic Reviews and Meta-Analyses' (PRISMA) statement [14] and the 'A MeaSurement Tool to Assess systematic Reviews' (AMSTAR) recommendations [15].

Statistical analysis

Inter-observer agreement was calculated with IBM SPSS Statistics 20 (SPSS, Chicago, Illinois, USA). Publication bias was assessed by plotting the log odds ratio against its standard error using the software Comprehensive Meta-Analysis, Version 3 (CMA, Biostat, Englewood, NJ 07631, USA). Pooling of sensitivity, specificity and diagnostic odds ratio was performed with MetaDisc, Version 1.4 (Hospital Universitario Ramon y Cajal, Madrid, Spain). For the pooled diagnostic odds ratio, a random effects model was used. To analyze sources of heterogeneity between studies, a meta-regression analysis (random effects model) was performed with criteria used to diagnose SS, publication year, type of glands examined and ultrasonographic characteristics assessed with ultrasonographic scoring systems, risk of bias in 'patient selection', 'conduct and interpretation of index test', and 'flow of patients and timing of tests' as predictors for differences in diagnostic properties of ultrasound in SS found in the studies (Comprehensive Meta-Analysis, Version 3).

Table 1: Characteristics of the studies included.

AECG: American European Consensus Group Criteria [47]; C: Criteria; CC: Copenhagen criteria [51]; disease: control group with a non-autoimmune salivary gland disease, not related to dry mouth, but potentially causing abnormal ultrasonographic imaging (e.g. salivary gland tumors); ECSG: European Community Study Group Criteria [48]; FC: Fox Criteria [49]; H: Histology; JDC: The Sjögren's Disease research Committee of the Japanese Ministry of Health and Welfare 1977 Diagnostic Criteria for definite Sjögren's Syndrome [52]; ns: not specified; pSS: primary Sjögren's Syndrome; pt: patient; RJDC: Revised Japanese Diagnostic Criteria [50]; SC: scintigraphy; SL: sialography; sSS: secondary Sjögren's Syndrome; TC: Takashima Criteria [43]; y: years

* Carotti et al., 2001 [19] and Salaffi et al., 2000 [40] used the same study population (patient and control group)

Author	Year	Diagnostic Criteria	Reference standard	Ultrsound transducer (MHz)	SS patients (n) included patients (mean age ± SD, range)			Control (n) included subjects (mean age ± SD, range)				Duration of symptoms/ mean ± SD, (range)
					pSS	sSS	ns	sicca	disease	healthy	ns	
Diederich et al.	1987	ns	H,SL				5					
De Clerck et al.	1988	FC	C,SL	7.5			16	26				
Kawamura et al.	1990	JDC	C	7.5	15	9			7	23		
Akin et al.	1991	H	H	5			5					
Corthouts et al.	1991	FC	C	7.5			16	13				
De Vita et al.	1992	CC	C	7.5	27	26		26		64		
Takashima et al.	1992	TC	C	7.5-10	13 (47, 17-63)	17 (47, 17-63)				10 (45, 22-58)		
Ariji et al.	1996	FC	C	7.5			25	19 (55±11, 29-69)		72 (50±18, 17-88)		
Makula	1996	ECSG	C	7			62 (53.2, 29-80) 53 definite 9 probable		44	25		9.8 y
Yoshiura et al.	1997	JDC	C	7.5			24		19	22		
Makula et al.	2000	AECG	C	7			44	11	27	14		
Salaffi et al.	2000	ECSG	C	7.5	30 (54, 29-75)			30 (53, 30-78)				7.6y (0,5-11) 6.9y (8mo-10y)
Carotti et al.*	2001	ECSG	C	7.5	30 (54, 29-75)			30 (53, 30-78)				7.6 y
El Miedany et al.	2004	AECG	C,H	7	47 (54.8,±5.5, 47-66)			20 (55.6±7.3)			20 (55.6±7.3)	
Niemela et al.	2004	AECG	C	9.6-11	27			27		27		
Hocevar et al.	2005	AECG	C	5-12			68				150	
Chikui et al.	2006	RJDC	C	7			91				41	
Decuzzi et al.	2006	ns	C,SC	7.5			20 (52±5, 35-65)					
Shimizu et al.	2006	RJDC	C	6-14			48 (53.5)				32 (53.5)	
Hocevar et al.	2007	AECG	C	5-12			28 (56.6±11.2, 32-84)			29 (56.7±12.3, 32-78)		
Poul et al.	2008	AECG	C	ns	36 (60, 20-85)	9 (60, 20-85)		15 (60, 20-85)				
Salaffi et al.	2008	AECG	C	7.5-10	77 (54±12.1, 30-78)			79 (53±12.3, 24-81)				-pSS: 2.9y (6mo-11y) -sicca non-SS: 2.8y (4mo-12y)
Shimizu et al.	2008	RJDC	C	8			43 (53.1, 17-80)				29 (53.1, 17-80)	
Wernicke et al.	2008	AECG	C	5-10	57	33		78		148		
Chikui et al.	2009	RJDC	SL	7			89 (52.6±16.4)				103 (55.9±16.2)	
Milic et al.	2009	AECG	C	4-10	107 (54.1, 21-78)						28 (54.1, 21-78)	
Milic et al.	2010	AECG	C	4-10	115	44		50		36		
Obinata et al.	2010	RJDC	C	5-12			36 (48, 13-68)	37 (48, 13-68)				
Tagaki et al.	2010	AECG	C	10			188 (56±13)				172 (55±16)	
Milic et al.	2012	AECG	C	4-10	140 (54.5, 21-78)						50 (52.6, 27-70)	
Cornec et al.	2013	AECG	C,H		78 (56.8± 12,7)			80 (56.8± 12.7)				6.7±7 y
Theander et al.	2014	AECG	C	6-18	105 (61±14.9, 20-91)			19 (57±15, 25-91)	20 (57±15, 25-91)			

Results

Study identification and selection

A total of 1245 papers were identified. After excluding duplicates, 742 papers were retrieved and screened by title and abstract (Figure 1). Subsequently, 700 titles and abstracts were excluded (a list of all identified papers and excluded papers not presented in this paper can be requested from the corresponding author). Cohen's Kappa agreement was 0.72 and overall agreement was 93%. We screened the full text of 42 studies. Finally, 37 studies were included for quality assessment (Figure 1) [3,6-9,11,16-46].

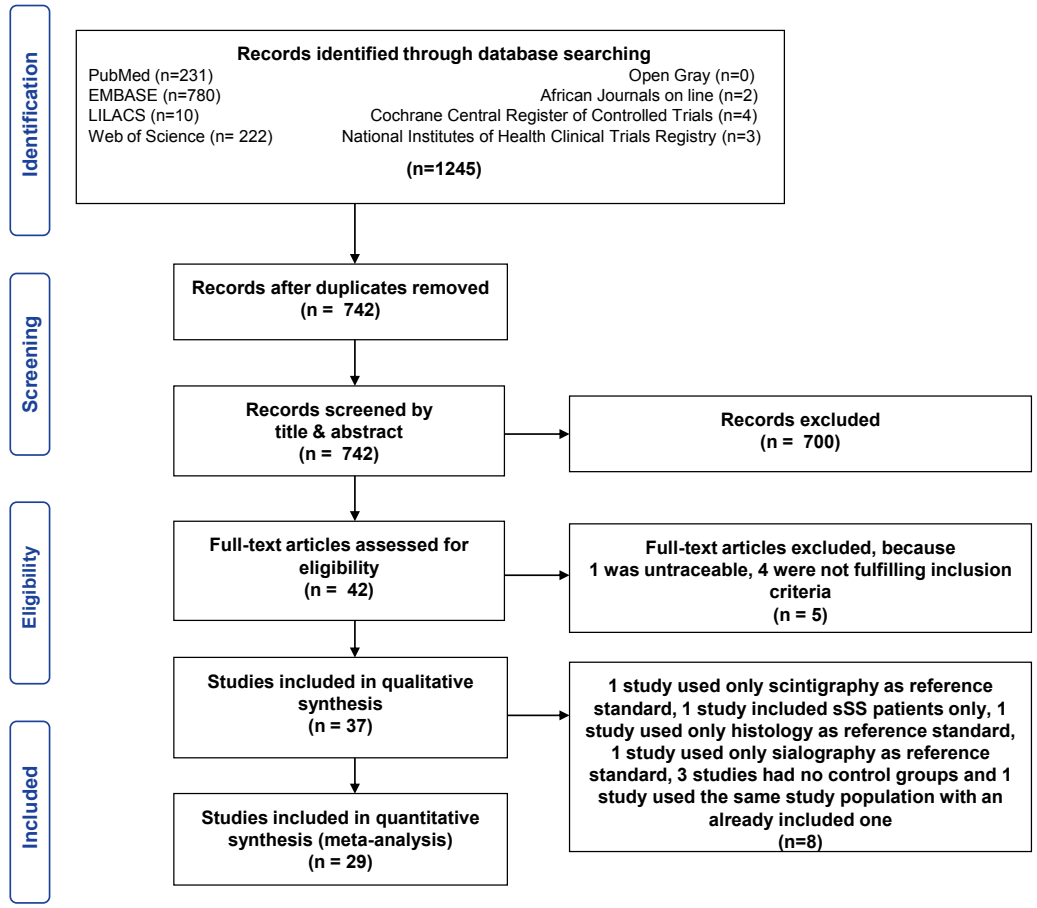
Quality assessment of studies

High risk of bias was observed in 'patient selection', 'conduct and interpretation of index test', and 'flow of patients and timing of tests' (78%, 70% and 51% of the included studies, respectively), while unclear risk of bias was found in the 'conduct and interpretation of the reference test' in 73% of the studies (supplementary Table 2; Figure 2). Kappa and overall agreement at this stage were 0.77 and 86%, respectively. Disagreements were resolved by discussion.

Study characteristics

In the 37 assessed studies, SS was generally diagnosed according to American European Consensus Group Criteria (AECG) [47], European Community Study Group Criteria (ECSG) [48], Fox Criteria (FC) [49], and Revised Japanese Diagnostic Criteria (RJDC) [50]. AECG was used in 15 studies (41%), ECSG in 5 studies (14%), FC in 5 studies (14%) and RJDC in 4 studies (11%). Less common criteria were used in 8 studies (22%) (Table 1) [43,51,52]. The diagnostic properties of ultrasonography were compared to criteria in 30 studies (81%), while in the rest of the studies ultrasonography was compared to sialography, scintigraphy or histology. In total, 2084 patients with SS and 1783 control subjects were included. Nine studies included only primary SS (pSS) patients, 10 studies both pSS and secondary (sSS) patients and 1 study only sSS patients. In the remaining 17 studies the type of the disease (pSS or sSS) was not specified. In 13 studies a healthy control group was included, and in 17 studies a sicca non-SS control group, while in 11 studies no details about the control group were provided. In 6 studies a control group was included with a non-autoimmune salivary gland disease, which was not related to dry mouth, but could cause an abnormal ultrasonography image of the glands (e.g., salivary gland tumors). Overall, 12 studies had multiple control groups and 3 studies no control group.

Figure 1: Study identification and selection progress.



In total 33 ultrasonographic scoring systems were used in 37 studies (supplementary Table 3). This heterogeneity within the scoring systems was related to the type of salivary glands examined, the ultrasonographic characteristics evaluated and the cut-off points applied. Of the scoring systems, 19 were related to both parotid and submandibular salivary glands, 11 were related to only the parotid glands and in 3 systems the type of the examined gland was not clearly mentioned. The main ultrasonographic characteristics assessed were the echogenicity and homogeneity of the glandular parenchyma. Both echogenicity and homogeneity were evaluated in 22 systems, only echogenicity in 8 and only homogeneity in 3 scoring systems. The size of the gland and the clearness of the border were also assessed in 12 and 11 scoring systems, respectively.

Figure 2: Percentage of studies included in the qualitative analysis with low, high, or unclear concern regarding applicability and risk of bias.

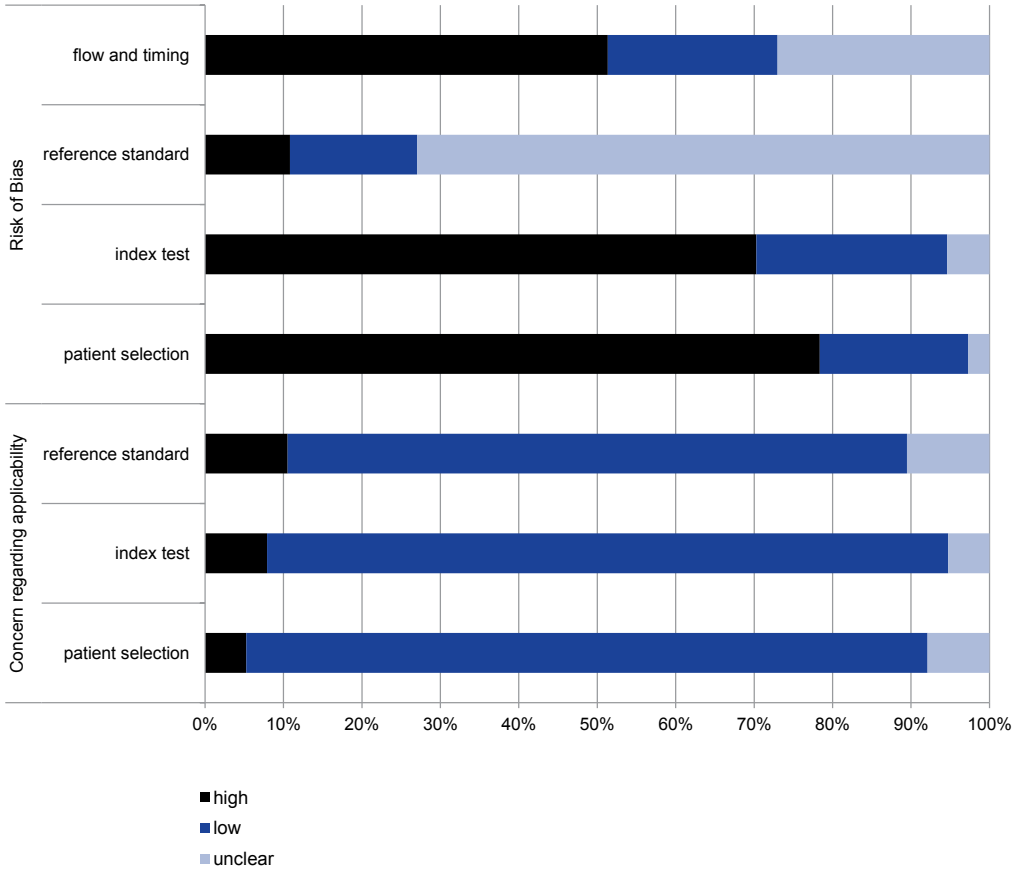
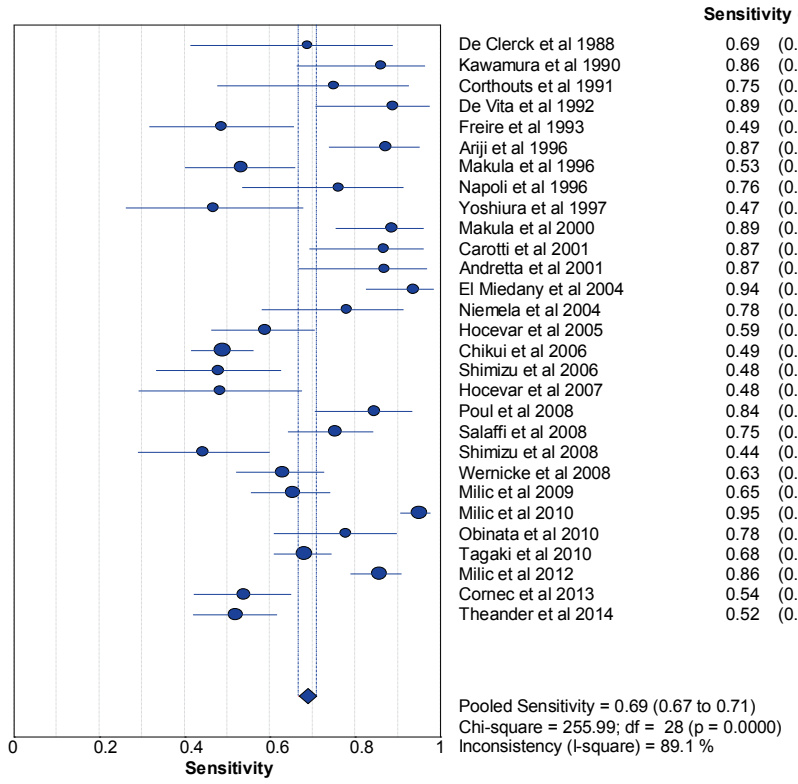


Figure 3: Forest plot showing study-specific and pooled sensitivity of ultrasonography in diagnosing Sjögren's syndrome. The point estimates of sensitivity for each study are shown as solid circles, and the size of each circle indicates the weight that it is given based on the sample size of each study. Error bars are 95% confidence intervals.



Quantitative synthesis

Publication bias

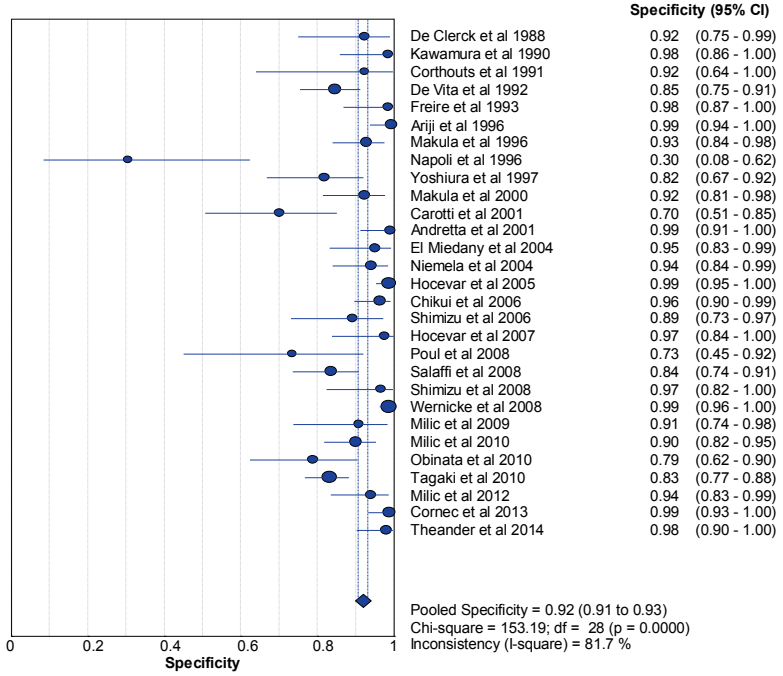
In the funnel plot, no studies were detected in the lower left part of the plot indicating publication bias (supplementary Figure 1).

Diagnostic accuracy of ultrasonography

In the meta-analysis, 29 studies using diagnostic criteria were included. Seven studies were excluded because: one study used only scintigraphy as reference standard [26], one study included sSS patients only [39], one study used only histology and sialography as reference standard [27], one study used only sialography as reference standard [20], and three studies had no control groups [16,44, 46]. Additionally, one study [40] was excluded because in that paper the study population was the same as in another paper [19]. We included only the paper with data on the number of true positives, true negatives, false negatives and false positives [19].

Regarding the diagnostic properties of ultrasonography in detecting SS, pooled sensitivity (Figure 3) was 0.69 (95% CI: 0.67 to 0.71) and pooled specificity (Figure 4) was 0.92 (95% CI: 0.91 to 0.93). We used a random effects model to determine the pooled diagnostic odds ratio (Figure 5): 33.89 (95% CI: 20.75 to 55.35). Considerable inconsistency was detected between studies when assessing sensitivity, specificity and diagnostic odds ratio (I^2= 89.1%, 81.7% and 72.4%).

Figure 4: Forest plot showing study-specific and pooled specificity of ultrasonography in diagnosing Sjögren's syndrome. The point estimates of specificity for each study are shown as solid circles, and the size of each circle indicates the weight that it is given based on the sample size of each study. Error bars are 95% confidence intervals.



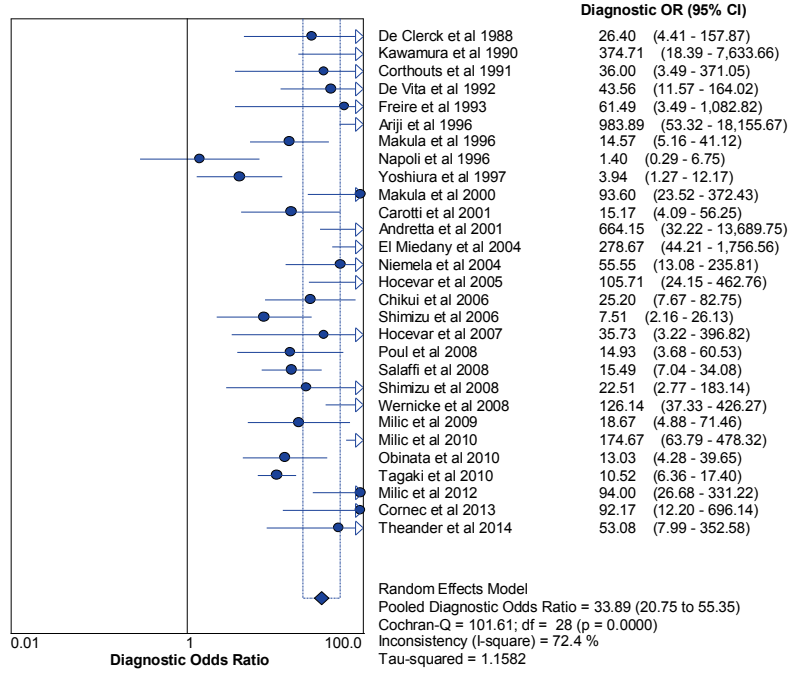
Meta-regression analysis

In the meta-regression analysis, it was found that studies with a high risk of bias in 'the conduct and interpretation of the index test' and studies using an ultrasonographic scoring system evaluating only homogeneity had a significantly higher natural logarithm of the diagnostic odds ratio (1.09 and 2.49, respectively; supplementary Table 4) in comparison to studies with a low risk of bias in the index test that used an ultrasonographic scoring system evaluating both ultrasonographic characteristics (echogenicity and homogeneity) for diagnosing SS.

Discussion

In the diagnosis of SS, involvement of salivary glands is currently assessed by sialography, scintigraphy, sialometry and histopathology. Recent discussion has focused on the accuracy of ultrasonography to evaluate the involvement of the major

Figure 5: Forest plot showing study-specific and pooled diagnostic odds ratio of ultrasonography in diagnosing Sjögren's syndrome. The point estimates of diagnostic odds ratio for each study are shown as solid circles, and the size of each circle indicates the weight that it is given based on the sample size of each study. Error bars are 95% confidence intervals. Calculations were performed using a random effects model. Dotted lines indicate whether the point estimate of each study falls within the confidence intervals of the pooled diagnostic odds ratio.



salivary glands and eventually to diagnose the disease [55]. Our study demonstrated that ultrasonography has a sensitivity, specificity and diagnostic odds ratio of 69%, 92% and 33.89, respectively, to diagnose SS in the major salivary glands. However, a high risk of bias in 'patient selection', 'index test' and 'flow and timing' was found in the included studies. Additionally, different ultrasonography scoring systems and study populations (study and control groups) were used in the studies, and publication bias was common. These methodological shortcomings probably inflated the diagnostic properties of ultrasonography in this meta-analysis. Additionally, studies with a poorer result (left lower part of the funnel plot) are missing (supplementary Figure 1). Several types of diagnostic criteria were used to identify SS patients in the included studies (Table 1), viz., AECG [47], ECSG [48], FC [49], RJDC [50], as well as less common ones like the Copenhagen Criteria [51], Takashima Criteria [43], and The Sjögren's Disease research Committee of the Japanese Ministry of Health and Welfare 1977 Diagnostic Criteria for definite Sjögren's Syndrome [52]. According to the meta-regression, the type of the cri-

teria used to diagnose SS did not seem to influence study outcome significantly. However, lack of power could explain this result.

The majority of included studies used a case control design. This means that the contrast in disease characteristics is large and that ultrasonography can 'easily' distinguish healthy controls from SS patients. The pooled specificity was high (92%), suggesting that ultrasonography may successfully identify patients who do not have SS. On the other hand, pooled sensitivity was considerably lower (69%), indicating that having a negative test is not adequate to exclude the presence of the disease.

Recently, a meta-analysis reported on the diagnostic properties of ultrasonography and sialography in SS [5]. In that study, 57 papers were initially identified and only 6 studies were finally included. The pooled sensitivity, specificity and diagnostic odds ratio was 77%, 81% and 17.48. In contrast to our study, the authors of that meta-analysis were less rigorous in their assessment of research methodology, since the number of studies with high risk of bias was limited in all QUADAS-2 domains. Concerns have been expressed regarding the outcome of their study [12], particularly the discrepancy between the data as included in that meta-analysis and the data provided by the source studies [6-11]. Additionally, the authors did not provide any data regarding inter-observer agreement.

Strengths and limitations

Strength of the current meta-analysis was the detailed literature search on eight databases, no language restriction, assessment of study eligibility by two reviewers, good inter-observer agreement, and application of the QUADAS-2 tool. The major limitation in the interpretation of the pooled outcomes (sensitivity, specificity and diagnostic odds ratio) is the low quality of the included studies and their clinical as well as their methodological heterogeneity. The likely sources of this heterogeneity are the variation in study populations, the ultrasonography scoring systems and the study designs. Another limitation in the interpretation of the pooled outcomes is publication bias, since small or large studies with positive results were overpresented in the funnel plot.

Implications and future research

We suggest that future studies should comply with the QUADAS-2 guidelines in order to ensure high diagnostic quality. Particular interest should be paid in the QUADAS-2 domains, where high risk of bias was observed: 1) a consecutive or random sample of patients should be used; a case control design and inappropriate exclusion of patients should be avoided, 2) ultrasonography results should be interpreted by observers blinded for each other as well as for the results of the reference test (diagnostic criteria, histology, sialography, scintigraphy, etc.); ideally, the applied threshold scoring should be pre-specified, 3) an appropriate and rather short interval should elapse between the application of ultrasonography and the reference test, the whole study population should receive the reference test (which should be always the same) and the whole study population should be included in the analysis.

The aforementioned features should be stated clearly by authors of the various papers to avoid potential misunderstanding and undervaluation of the study design. We acknowledge the need for a universally accepted ultrasonography scoring system to ensure uniform and standardized evaluation of the major salivary glands.

Conclusions

From the results of this meta-analysis we conclude that ultrasonography has the potential to evolve into a viable alternative in the evaluation of the major salivary glands in patients with SS, and therefore may be used as a non-invasive tool in the diagnosis of the disease. However, due to the low quality of the included studies, further research is required to elucidate the properties of ultrasonography in diagnosing SS.

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Disclosure of conflict of interests

The authors state that they have no conflict of interests.

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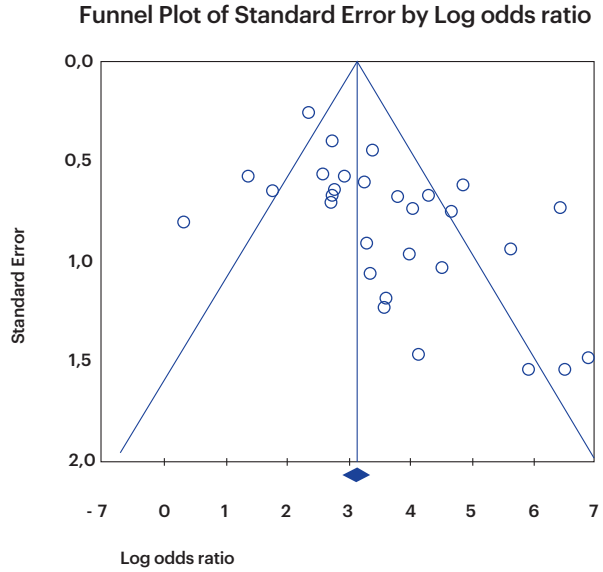
Supplementary files

Supplementary Figure: Funnel plot of standard error by log odds ratio. No studies were detected in the lower left part, indicating that publication bias is very likely.



Supplementary Table 1: Electronic databases and search details (according to the syntax rules of each database).

Database	Search Details
PubMed (http://www.ncbi.nlm.nih.gov/pubmed/)	("Sjogren's Syndrome"[Mesh] OR sjogren*[tw] OR sicca syndrome[tw])AND("Ultrasonography"[Mesh] OR "ultrasonography" [Subheading] OR ultraso*[tw] OR echograph*[tw] OR echotomograph*[tw] OR sonograph*[tw])
EMBASE (http://www.embase.com/home)	(Sjogren* OR 'sicca syndrome') AND (ultraso* OR echograph* OR echotomograph* OR sonograph*)
LILACS (bases.bvs.br)	(tw:((Sjogren* OR "sicca syndrome"))) AND (tw:((ultraso* OR echograph* OR echotomograph* OR sonograph*)))
Web of Science (scientific.thomson.com/products/wos/)	(Sjogren* OR "sicca syndrome") AND (ultraso* OR echograph* OR echotomograph* OR sonograph*)
OpenGrey (http://www.opengrey.eu/)	sjogren* AND ultrasound
African Journals online (www.ajol.info)	sjogren*
Cochrane Central Register of Controlled Trials (www.thecochranelibrary.com)	(Sjogren* OR "sicca syndrome") AND (ultraso* OR echograph* OR echotomograph* OR sonograph*)
National Institutes of Health Clinical Trials Registry (clinicaltrials.gov)	Sjogren AND ultrasound [Recognized Terms and Synonyms: ultrasound: 3947 studies, (echography, echotomography, gen.us, sonogram, sonography, ultrasonic, ultrasonic imaging, ultrasonic shockwave, ultrasonogram, ultrasonography), sjogren: 85 studies (gougerot-nulock-houwer syndrome, sjoegren syndrome, syndrome sjogren's)]

Supplementary Table 2: Outcomes of the risk of bias assessment and concerns regarding applicability of studies included in the quality assessment.

		Risk of Bias				Concern regarding applicability		
Publication Year	Author	f/t	rs	it	ps	rs	It	ps
1987	Diederich et al	high	unclear	high	high	unclear	low	low
1988	De Clerck et al	high	unclear	high	high	high	high	high
1990	Kawamura et al	high	unclear	high	high	low	high	low
1991	Akin et al	high	unclear	high	high	low	low	low
1991	Corthouts et al	high	unclear	high	low	low	low	low
1992	De Vita et al	unclear	unclear	high	high	low	low	low
1992	Takashima et al	high	unclear	high	high	unclear	low	unclear
1993	Freire et al	low	high	low	high	high	low	low
1996	Ariji et al	high	low	unclear	high	low	low	low
1996	Makula et al	high	unclear	high	high	low	low	low
1996	Napoli et al	unclear	unclear	high	high	low	low	low
1996	Nitsche et al	low	high	low	unclear	unclear	low	unclear
1997	Yoshiura et al	high	unclear	high	high	low	low	low
1999	Varshavsky	unclear	unclear	high	high	unclear	unclear	unclear
2000	Makula et al	high	high	high	high	low	low	low
2000	Salaffi et al	unclear	unclear	unclear	high	low	low	low
2001	Andretta et al	unclear	unclear	high	high	low	low	low
2001	Carotti et al	low	low	low	high	low	low	low
2004	El Miedany et al	high	low	high	high	low	low	low
2004	Niemela et al	high	unclear	high	high	low	low	low
2005	Hocevar et al	high	unclear	high	low	high	low	low
2006	Chikui et al	low	low	high	low	low	low	low
2006	Decuzzi et al	unclear	unclear	high	high	high	low	high
2006	Shimizu et al	unclear	unclear	high	high	low	low	low
2007	Hocevar et al	high	low	low	high	low	low	low
2008	Poul et al	high	unclear	high	high	low	low	low
2008	Salaffi et al	low	low	low	high	low	low	low
2008	Shimizu et al	high	unclear	low	high	low	low	low
2008	Wernicke et al	high	unclear	high	high	low	low	low
2009	Chikui et al	low	high	low	low	low	low	low
2010	Milic et al	high	unclear	high	high	low	low	low
2010	Obinata et al	high	unclear	low	high	low	low	low
2010	Tagaki et al	unclear	unclear	low	high	low	low	low
2012	Milic et al	low	unclear	high	low	low	low	low
2013	Cornec et al	unclear	unclear	high	low	low	high	low
2013	Milic et al	low	unclear	high	low	low	low	low
2014	Theander et al	unclear	unclear	high	high	low	low	low

f/t: flow of patients and timing of performance of index test and reference standard, it: conduct and interpretation of index test, ps: patient selection, rs: conduct and interpretation of reference standard.

Supplementary Table 3: Summary of ultrasonographic scoring systems for Sjögren's syndrome applied in the different studies.

Author	Year	US scoring system
Diederich et al	1987	1) Homogenicity 2) Echogenicity (compared to thyroid gland) 3) Size (normal: parotid gland 40x15mm and SM 30x15mm)
De Clerck et al	1988	2-grade system: decreased echogenicity and normal echogenicity (compared to the masseter muscle and mylohyoid muscle)
Kawamura et al	1990	2-grade system: Homogenous parenchyma and heterogeneous parenchyma
Akin et al	1991	Evaluation based on reports of: Wittich et al 1985 [53] and Da-Xi et al 1987 [54]
Corthouts et al	1991	2-grade system: decreased reflectivity and normal reflectivity (compared to the masseter muscle and mylohyoid muscle)
De Vita et al	1992	1) Parenchymal inhomogenicity (mild/evident/gross) 2) Parenchymal echogenicity (increased/decreased) 3) Glandular volume (arbitrary evaluated; increased/decreased) 4) Posterior glandular border (definite/ill defined/not visible) 5) Lymph nodes (evidence of peri-glandular/evidence of intra-glandular)
Takashima et al	1992	US was positive for SS if multiple hypoechoic areas were found and intermediate on the presence of non-homogenous parotid glands
Freire et al	1993	Heterogeneity of the parenchyma and decrease of the size of the glands (parotid and submandibular) was considered as positive for SS.
Ariji et al	1996	A) 5 grade-system: 0= regular contour, no hypoechoic spots/areas, no echogenic bands 1= regular contour, small hypoechoic spots/areas, no echogenic bands 2= regular contour, round multiple hypoechoic spots/areas, no echogenic bands 3= irregular contour, round multiple hypoechoic spots/areas, presence of echogenic bands 4= irregular contour, irregular multiple hypoechoic spots/areas, presence of echogenic bands B) Quantitative characteristics: S value and SD value
Makula	1996	1) Parenchymal inhomogenicity (mild/evident/gross) 2) Parenchymal echogenicity compared to masseter (increased/decreased) 3) Glandular size (Parotid gland was normal if width= 27±7mm)
Napoli et al	1996	1) Ultrasonography of parotid gland: negative=0, positive=1 2) Volume: normal=0, increased=1 3) Border: normal=0, abnormal=1 4) Structure: homogeneous=0, non-homogeneous=1 5) Hypoechogenic areas: absence=0, <1cm=1, >1cm=2 6) Ductal ecstasia: absent=0, 2mm=1, 2-4mm=2, 4mm=3 7) Lymph nodes: absence=1, presence=1
Nitsche et al	1996	1) parenchyma echogenicity (homogenous or non-homogenous) 2) size of gland (longitudinal and transverse diameter) 3) presence/absence of lymph nodes
Yoshiura et al	1997	Five-point-rating scale: 1: definitely normal/ 2: probably normal/ 3: not sure/ 4: probably abnormal/ 5: definitely abnormal
Makula et al	2000	1) parenchymal inhomogeneity (PIH), three grades of PIH were distinguished. In mild PIH (grade 1), a diffuse microareolar structure can be seen, the borders of the hypoechogenic areolae are blurred, and the areolae are <2 mm in diameter. In evident (moderately severe) PIH (grade 2), the hypoechogenic areas are larger (2–6 mm in diameter), with a sharper border. In gross (severe) PIH (grade 3), large (>6 mm in diameter) circumscribed hypoechogenic areas are also present 2) The parenchymal echogenicity was determined in comparison with that of the thyroid gland 3) The size of the parotid was considered to be normal if its width was 27±7 mm Two groups of SS patients were differentiated on the basis of the US findings: patients with a homogeneous parotid gland parenchyma and mild PIH (grade 1), and patients with more advanced abnormalities (grade 2 or 3) which are of true diagnostic value
Salaffi et al	2000	-Grade 0: normal gland -Grade 1: regular contour, small hypoechoi spots/areas without echogenic bands, regular or increased glandular volume (mean values 20±3mm for the parotids and 13±2mm for the submandibular glands), and ill defined posterior glandular border -Grade2: regular contour, evident multiple scattered hypoechogenic areas usually of variable size, (<2mm) and not uniformly distributed, without echogenic bands, regular or increased glandular volume and ill defined posterior glandular border -Grade 3: irregular contour, multiple large circumscribed or confluent hypoechogenic areas (2-6mm) and/or multiple cysts, with echogenic bands, regular or decreased glandular volume and posterior glandular border not visible -Grade 4: irregular contour, multiple large circumscribed or confluent hypoechogenic areas (>6mm) and/or multiple cysts or multiple calcifications, with echogenic bands, resulting in severe damage to the glandular architecture, decreased glandular volume, and posterior glandular border not visible Score ranges from 0-16 (comprising of the sums of the single scores 0-4 for each parotid and submandibular gland Optimal grade cutoff=8
Andretta et al	2001	Grade 0: no alterations Grade 1: gland increased in size or with normal echogenicity of the most reflective background Grade 2: gland increased in size or normal with sinuous profiles and inhomogeneous echogenicity with aspects of microareolara Grade 3: gland increased in size with sinuous profiles, inhomogeneous echogenicuty and coarse areola Grade 4: as above with ecstatic ducts and microcalculi in gland or small, sinuous profiles and grossly inhomogeneous echogenicity (type atrophic gland)

Supplementary Table 3 (continued)

Author	Year	US scoring system
El Miedany et al	2004	Grade 0: Normal homogenous parenchyma Grade 1: Mild PIH seen as diffuse hypoechoic areolae less than 2 mm with blurred borders Grade 2: Moderate PIH seen as large hypoechoic areas, 2–6 mm diameter, with sharp borders Grade 3: Severe PIH with large, more than 6 mm circumscribed hypoechoic areas
Niemela et al	2004	-stage 0=normal parenchymal structure; -stage 1=mild parenchymal inhomogeneity (PIH) (hypoechoic areas <2 mm) -stage 2=evident PIH (hypoechoic areas of 2–6 mm) -stage 3=gross PIH (hypoechoic areas >6 mm); and -stage 4=adipose degeneration of the gland (adipose tissue echogenicity and parenchymal atrophy)
Hocevar et al	2005	1) Parenchymal echogeneity was evaluated in comparison with the thyroid gland or when there was coincident thyroid gland disease by surrounding anatomical structures (muscular structures, subcutaneous fat). If the echogeneity was comparable to the thyroid, the grade was 0; if it was decreased, we graded it 1 2) Homogeneity was graded from 0 to 3. Grading 0 was for a homogeneous gland, 1 for mild inhomogeneity, 2 for evident inhomogeneity, and 3 for a grossly inhomogeneous gland 3) The presence of hypoechogenic areals was graded from 0 to 3 (grade 0, absent; grade 1, a few, scattered; grade 2, several; grade 3, numerous hypoechogenic areas) 4) Hyperechogenic reflections were graded from 0 to 3 in the parotid glands (grade 0, absent; grade 1, a few, scattered; grade 2, several; grade 3, numerous hyperechogenic reflections) and from 0 (absent) to 1 (present) in the submandibular glands. 5) Clearness of salivary gland borders was graded from 0 to 3 (grade 0, clear, regular defined borders; grade 1, partly defined borders; grade 2, ill-defined borders; grade 3, borders not visible) Final score: summation of the grades for the five parameters described above for all four glands.US score ranged from 0 to 48 Optimal grade cutoff=17
Chikui et al	2006	A) 3-grade system +: definitely present, ±: probably present,-: definitely absent B) Quantitative characteristics: Standard deviation of regions of interest (SD), Angular second moment (ASM), Correlation (COR), Inverse difference moment (IDM), Entropy (ENT), Hurst co-efficient (H)
Decuzzi et al	2006	0= normal I= early destructive changes (regular border, normal or enlarged dimensions and puntate hypoechoic lesions) II= late destructive changes (irregular border, reduced dimensions, multicystic or reticular pattern, calcifications and hyperechoic stripes)
Shimizu et al	2006	- multiple hypoechoic areas - multiple hyperechoic lines and/or spots - multiple hypoechoic areas surrounded with hyperechoic lines and/or spots On the sonographic images of the submandibular glands obscuration of the gland configuration was also evaluated additionally. Findings in the parotid gland are designated "P" and in the submandibular gland "S". A total of 7 findings were evaluated: P1, P2, P3, S1, S2, S3, and S4 combining both types of gland. Evaluations were recorded using 3 grades: positive, probable, and negative.
Poul et al	2008	US was considered positive for SS if the following features were detected: bilateral decreased parotid gland reflectivity, and heterogeneous or nodular parenchyma with a honeycomb appearance.
Salaffi et al	2008	Salaffi et al 2000, BUT: Optimal grade cutoff=6

Supplementary Table 4: Results of the meta-regression (method of moments) to analyze effects of study methodology on the natural logarithm of the diagnostic odds ratio using a random effects model.

Study characteristics	Regression Coefficient	95% CI	P
Intercept (reference study)	2.46	1.65 - 3.27	< 0.001
High risk of bias in index test	1.09	0.17 - 2.02	0.021
US characteristic: echogenicity assessed only	0.10	-0.94 - 1.15	0.845
US characteristic: homogeneity assessed only	2.49	0.82 - 4.15	0.003

95% CI: 95% confidence interval, reference study is a study with: a low risk of bias in the index test using both ultrasonographic characteristics (echogenicity and homogeneity) for diagnosing SS. High risk of bias in index test: the conduct and interpretation of the index test is highly likely to be biased. (Tau^2 = 0.6661, I^2 = 58.98%, Q = 60.95, df = 25, p = 0.0001). Other potential predictors were not significantly related to diagnostic properties of ultrasound in SS found in the studies.

Chapter 3B

Comment on 'Diagnostic accuracies of sialography and salivary ultrasonography in Sjögren's syndrome patients: a meta-analysis' by Song and Lee (2014)

Konstantina Delli[1], Pieter U Dijkstra[1,2], Frederik KL Spijkervet[1], Hendrika Bootsma[3], Arjan Vissink[1]

Affiliations:

1. Department of Oral and Maxillofacial Surgery

2. Department of Rehabilitation, Center for Rehabilitation

3. Department of Rheumatology and Clinical Immunology

University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

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With great interest we have read the recently published meta-analysis by Song and Lee [1] in your journal regarding the diagnostic properties of sialography and salivary ultrasonography in Sjögren's Syndrome (SS) patients. A systematic review and meta-analysis on this topic has been lacking so far from the literature and, thus, eagerly expected. We would like to express some concerns regarding Table 1 of their study in relation to the study outcomes. There seems to be a discrepancy between the data shown in the meta-analysis and the data presented by the source studies [2-7], viz.:

1. In the study of Tagaki et al. 2010 [2], the number of cases with SS is 188 as opposed to 177 reported by Song and Lee [1].
2. In the study of Obinata et al. 2010 [3], the number of cases with SS is 36 as opposed to 32 reported by Song and Lee [1].
3. In the study of Poul et al. 2008 [4], which is erroneously cited as Poul et al. 2009, the number of cases with SS is 45 as opposed to 32 reported by Song and Lee [1].
4. In the study of Salaffi et al. 2008 [5], the number of cases with SS is 77 as opposed to 68 reported by Song and Lee [1].
5. In the study of Yonetsu et al. 2002 [6], the number of cases is 171 as opposed to 151 reported by Song and Lee [1].
6. In the study of Yoshiura et al. 1997 [7], the number of cases with SS is 24 as opposed to 23 and the number of controls is 40 and 41 depending on the diagnostic technique tested, as opposed to 21 reported by Song and Lee [1].

Additionally, summing the numbers of true positive, true negatives, false positives and false negatives in Table 1 of Song and Lee's paper does not add up to the same numbers [1]. It is possible that the data set was not complete for every participant in the source studies. See, e.g., the study of Yoshiura et al. 1997, in which data of 2 control groups were used with different numbers for sialography and ultrasonography [7]. Furthermore, some source studies do not report the number of true positives, true negatives, false positives and false negatives. If Song and Lee calculated the number of true positives, true negatives, false positives and false negatives on basis of the reported sensitivity and specificity of the source populations, it is essential that the correct number of participants with SS and the number of controls in the various studies is entered in the calculations [1]. Finally, Song and Lee report that discrepancies between reviewers were resolved by consensus or a third reviewer [1]. However, they fail to present who the third reviewer was (it might be that there were no discrepancies that could not be resolved by consensus, so there was no need for a third reviewer) and do not report inter-observer agreement measures.

We were wondering which numbers were entered in the statistical program to perform the meta-analyses, since these numbers influence the outcome of the study. We would appreciate if the authors could comment on the above raised issues.

Table 1: Overview of the data presented in the source publications and the data presented by Song and Lee.

Source publications	Data from source papers			Data reported by Song and Lee (2014)			Sialography					Ultrasonography				
	SS	CO	SUM SS and CO	SS	CO	SUM SS and CO	TP	FP	FN	TN	SUM sialography	TP	FP	FN	TN	SUM ultrasonography
Takagi et al., 2010	188	172	360	177	172	349	146	31	42	141	360	154	50	34	122	360
Obinata et al., 2010	36	37	73	32	37	69	30	2	6	35	73	28	8	8	29	73
Poul et al., 2008	45	15	60	37	15	52	35	2	10	13	60	38	4	7	11	60
Salaffi et al., 2008	77	79	156	68	79	147	56	12	21	67	156	58	13	19	66	156
Yonetsu et al., 2002	171	123	294	151	123	274	149	2	30	121	302	130	7	41	116	294
Yoshiura et al., 1997				23	21	44										
-Sialography	24	40*	64				23	0	1	21	45					
-Ultrasonography	24	41**	65									11	1	13	21	46

SS: Sjögren syndrome patients, CO: Controls, TP: true positive, FP: False positive, FN: False negative, TN: True negative,
*:19 with nonspecific parotitis and 21 healthy volunteers, **: 19 nonspecific parotitis and 20 healthy volunteers.

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Chapter 3C

Ultrasound of the major salivary glands is a reliable imaging technique in patients with clinically suspected primary Sjögren's syndrome

Konstantina Delli[1], Suzanne Arends[2], Jolien F van Nimwegen[2], Pieter U Dijkstra[1,3], Alja J Stel[2], Frederik KL Spijkervet[1], Hendrika Bootsma[2], Arjan Vissink[1], on behalf of the EULAR US-pSS Study Group

1. Department of Oral and Maxillofacial Surgery

2. Department of Rheumatology and Clinical Immunology

3. Department of Rehabilitation, Center for Rehabilitation

University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

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Abstract

Objective: To assess inter- and intra-observer reliability of ultrasound of major salivary glands in patients clinically suspected with primary Sjögren's syndrome (pSS) as well as to assess sources of variation in outcomes of the ultrasonographic evaluation.

Methods: Eighty consecutive outpatients with clinically suspected pSS were subjected to ultrasound evaluation. The following ultrasound variables of the parotid and submandibular salivary glands were assessed: echogenicity, parenchymal homogeneity, presence of hypoechogenic areas, presence of hyperechogenic reflections and clearness of posterior glandular border, according to the scoring system of Hocevar et al. (total score range 0-48). Images were scored independently by three blinded observers in two sessions.

Results: Intra-observer reliability of the ultrasound total score was excellent, with Intraclass Correlation (ICC) ranging from 0.89 to 0.96. Inter-observer reliability was good to excellent, with ICCs of 0.84 and 0.76 for the ultrasound total score in the two sessions. Kappa ranged from 0.60 to 0.83 depending on the cut-offs applied (cut-off score ≥15 and ≥17). Hypoechogenic areas and homogeneity of parotid glands showed the highest inter-observer reliability. Median kappa for echogenicity was low. Ultrasound total scores were more widespread between observers on patients with higher ultrasonographic scores (approximately scores ≥20).

Conclusion: Ultrasound of major salivary glands is reliable in diagnosing pSS. Discrepancies between observers in assessing the severity of ultrasound findings may interfere with detecting 'true' changes over time. When monitoring the progression of pSS or treatment efficacy, it is advised that a particular patient is scored by the same ultrasonographer at every time point.

Introduction

Primary Sjögren's syndrome (pSS) is a chronic systemic auto-immune disease, with an estimated prevalence of 61 cases per 100,000 inhabitants in the general population [1]. pSS affects the exocrine glands, the salivary and lacrimal glands in particular, resulting in a sensation of dry eyes (keratoconjunctivitis sicca) and dry mouth (xerostomia) [2].

In the assessment of salivary gland component in pSS, ultrasonography of the major salivary glands merits currently special attention as a non-invasive, inexpensive, widely available, easily accessible and non-irradiating imaging modality [3]. Ultrasound is generally considered to be an operator-dependent technology with mediocre repeatability [4]. To date, numerous studies have been published investigating the diagnostic properties of ultrasound in pSS. In a recently published meta-analysis, it was estimated that ultrasound has pooled sensitivity of 0.69 and specificity of 0.92 for the diagnosis of SS [5]. However, the reliability of ultrasound and individual ultrasonographic variables as well as sources of variation in outcomes have not been thoroughly analyzed with regard to the salivary gland involvement in pSS [6].

The aim of this study was to assess the intra- and inter-observer reliability of B-mode ultrasonography in the diagnostic work-up of pSS with regard to the involvement of the major salivary glands in a representative study population of patients with clinically suspected pSS and to analyze sources of variation in outcomes of the ultrasonographic evaluation.

Materials and methods

Patients

Eighty consecutive patients with clinically suspected pSS were included in the study. All patients visited the outpatient clinic of the department of Rheumatology and Clinical Immunology, Multidisciplinary Sjögren's Expertise Center, University Medical Center Groningen, University of Groningen, between January 2015 and September 2015 and were subjected to ultrasonographic evaluation and routine diagnostic work-up, according to American European Consensus Group (AECG) criteria [7].

Procedures

All patients were examined with the same ultrasonographic scanner (Esaote MyLabSeven, Genova, Italy), equipped with a high resolution linear scanner (4-

13MHz). Each patient was scanned in a supine position with the neck slightly extended and the head turned a little to the opposite side. The parotid glands were examined in both axial and coronal planes, while the submandibular glands only in the coronal plane.

The following images were stored from each patient and used for this reliability study: one showing the thyroid gland, one showing the right submandibular salivary gland, one showing the left submandibular salivary gland, two providing an overview of the right parotid gland and two providing an overview of the left parotid gland. Images were processed in such a way that no patient data were visible, i.e. name, age, gender and date of examination (Supplementary Figure 1). The images were allocated to a random number.

All images were scored independently by three observers (KD, JFN and AJS; for scoring system see below) on the same monitor (MultiSync E231, 23 inches, NEC, Illinois, USA). The observers were blinded for the diagnostic work up, i.e. salivary gland biopsy, circulating auto-antibodies, salivary function tests, tear gland function tests and subjective oral and ocular symptoms [7]. The observers scored all patients in random order in two sessions with a 2-week interval. Prior to the study, exact instructions were given to all observers regarding the scoring and all observers were trained. Additionally, five pSS patients, who were not included in the reliability assessment, were scored in a consensus meeting for calibration, i.e. to train the observers to score the ultrasonographic images consistently.

Ultrasonographic assessments of B-mode images

The following ultrasonographic variables were assessed in each major salivary gland: echogenicity, parenchymal homogeneity, the presence of hypoechogenic areas, the presence of hyperechogenic reflections, and the clearness of posterior glandular border, according to the Hocevar scoring system [3]:

i. Parenchymal echogenicity was evaluated in comparison with the thyroid gland or when there was coincident thyroid gland disease by surrounding anatomical structures (muscular structures, sub-cutaneous fat). Echogenicity was graded 0 if echogenicity was comparable to the thyroid, and 1 if it was decreased.
ii. Homogeneity was graded 0 for a homogeneous gland, 1 for mild inhomogeneity, 2 for evident inhomogeneity, and 3 for a grossly inhomogeneous gland.
iii. Presence of hypoechogenic areas was graded 0 for no hypoechogenic areas, 1 for a few scattered areas, 2 for several areas, and 3 for numerous hypoechogenic areas.
iv. Presence of hyperechogenic reflections in the parotid glands were graded 0 for no hyperechogenic reflections, 1 for a few, scattered, 2 for several, and 3 for numerous hyperechogenic reflections, and in submandibular glands 0 for absent and 1 for present.

v. Clearness of salivary gland borders was graded 0 for clear, regularly defined borders, 1 for partly defined borders, 2 for ill-defined borders, and 3 for borders not visible.

Finally, ultrasound total score was calculated as the sum of the grades for the five variables described above for all four glands (range 0-48). According to the literature, the cut-off point to define positive or negative ultrasound for pSS was set at 15 [8] and 17 [3].

Data analysis

Patient characteristics were presented as number of patients (%) or median (interquartile range; IQR: Q1-Q3). Intra-observer reliability was assessed by comparing the ultrasound scores from the first and second session for each rater. Inter-observer reliability was assessed by comparing the ultrasound scores from both sessions between the observers. Intra-observer and inter-observer reliability was calculated using Cohen's Kappa and Fleiss' Kappa, respectively, in combination with the percentage of absolute agreement (calculated as: $n_{agreement}/n_{total}$) for nominal variables, Intraclass Correlation Coefficient (ICC; two-way mixed effects model, single measures, absolute agreement) was assessed for continuous variables. Kappa agreement was calculated with online statistical tools available at: http://www.statstodo.com/CohenKappa_Pgm.php and http://dfreelon.org/recal/recal3.php. Kappa and ICC values were interpreted as follows: 0.00-0.20, poor; 0.20-0.40, fair; 0.40-0.60, moderate; 0.60-0.80, good; and 0.80-1.00, excellent [9].

Variance components (type III ANOVA) were computed to determine the impact of factors influencing variation in ultrasonographic scores. The factors 'patient', 'session' and 'observer', were considered as random factors, and their 2-way interactions were analyzed. Error variation was calculated as the sum of all sources of variation minus patient variation. The contribution of factors to the total variation and the error variation was expressed as percentage. For each patient, the mean of the 6 observations (3 observers, 2 sessions) and the difference of the 6 observations with the mean were calculated and plotted against each other. Statistical analysis was performed with IBM SPSS Statistics 22 (SPSS, Chicago, IL, USA).

Results

Of the 80 included clinically suspected patients with pSS, the median age was 51 years (IQR=52-62), 69 (86%) were female, and median ultrasound total score was 15 (IQR=14-18).

Reliability of B-mode ultrasound

Intra-observer reliability of the US total score was excellent, with ICCs ranging from 0.89 to 0.96 for the three observers (Table 1).

Inter-observer reliability was good to excellent, with ICCs of 0.84 and 0.76 for the ultrasound total score in session one and two, respectively. The kappa ranged from 0.60 to 0.83 and the percentage of absolute agreement from 80 to 92 depending on the cut-offs applied to define positive or negative ultrasound for pSS (Table 2). Inter-observer reliability was higher for cut-off point of ≥17 compared to ≥15.

Regarding the individual ultrasonographic variables of the Hocevar's scoring system, hypoechogenic areas and homogeneity of the parotid glands showed the highest inter-observer reliability with median ICCs of 0.74 and 0.71, respectively, whereas median kappa was for echogenicity of the parotid glands (0.22) was low (Table 3).

Systematic differences and sources of variation

Differences between the three observers were larger for higher ultrasound total scores (Figure 1). The contribution of error variance to the total variance was 21.3%. The interaction of patient and observer made the largest contribution to error variance followed by the main effect of observer. The effects of session, interaction of session and observer, and interaction of session and patient contributed marginally to the error variance (Figure 2 and Supplementary Table 1).

Discussion

In the classification of pSS, involvement of the salivary glands is currently assessed with sialography, scintigraphy, sialometry and histopathology. Recent discussion has focused on the diagnostic accuracy of B-mode ultrasound to evaluate the involvement of the major salivary glands [10]. However, along with assessing the validity of ultrasound, evaluating the reliability of this imaging technique, i.e. the consistency or repeatability of the measurements, is of equal importance.

Our study, including patients with clinically suspected pSS, showed that the intra- and inter-observer reliability of the ultrasound total score, when applying the Hocevar scoring system [3] was excellent. When different cut-off points reported in the literature for ultrasound positivity were applied [3,8], the agreement ranged from moderate to excellent. These results are in accordance with previously published studies using various scoring systems and different study populations [11-19].

Table 1: Intra-observer reliability of ultrasound of the major salivary glands in patients with clinically suspected pSS.

		ICC	95% CI lower limit	95% CI upper limit	
ultrasound total score	Observer 1	0.96	0.90	0.98	
	Observer 2	0.92	0.95	0.99	
	Observer 3	0.89	0.83	0.96	
Diagnosis if:		Kappa	95% CI lower limit	95% CI upper limit	Absolute agreement (%)
Cut-off≥15 [8]	Observer 1	0.74	0.58	0.89	87
	Observer 2	0.77	0.64	0.91	89
	Observer 3	0.73	0.57	0.88	86
Cut-off≥17 [3]	Observer 1	0.85	0.73	0.97	93
	Observer 2	0.89	0.80	0.99	95
	Observer 3	0.70	0.54	0.85	85

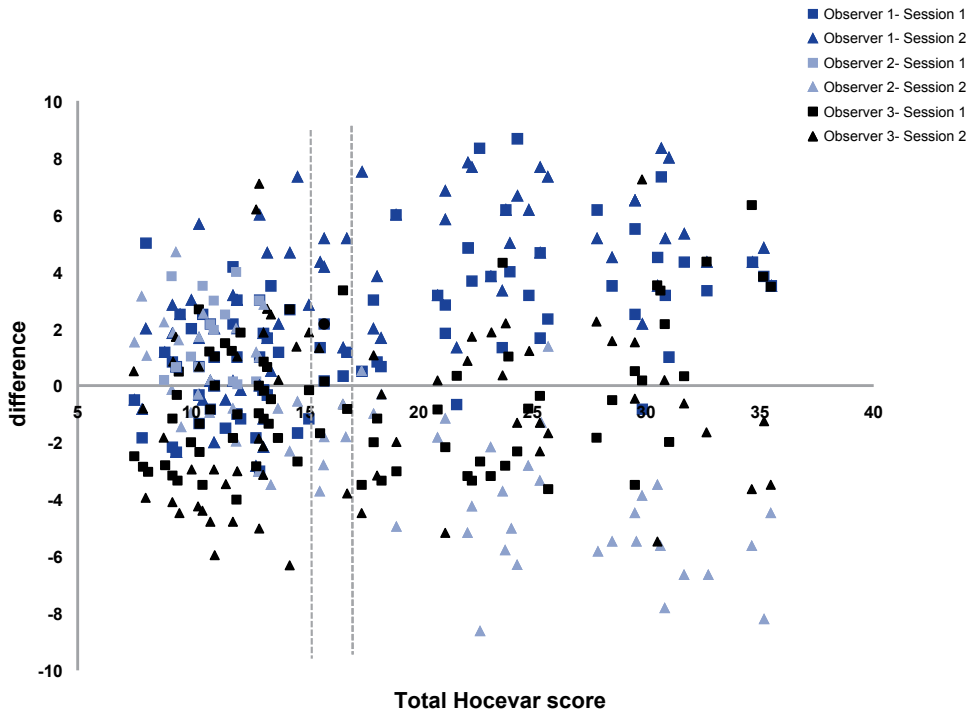
The cut-off point to define positive or negative ultrasound for pSS was set at 15 [8] and 17 [3].

Table 2: Inter-observer reliability of ultrasound of the major salivary glands in patients with clinically suspected pSS.

		ICC	95% CI lower limit	95% CI upper limit	
ultrasound total score	Session 1	0.84	0.71	0.91	-
	Session 2	0.76	0.52	0.87	-
Diagnosis if:		Kappa	95% CI lower limit	95% CI upper limit	Absolute agreement (%)
Cut-off≥15 [8]	Session 1	0.75	0.62	0.88	88
	Session 2	0.60	0.47	0.72	80
Cut-off≥17 [3]	Session 1	0.83	0.71	0.96	92
	Session 2	0.70	0.57	0.83	85

The cut-off point to define positive or negative ultrasound for pSS was set at 15 [8] and 17 [3].

Figure 1: Systematic differences in ultrasound total score using the Hocevar scoring system [3]. For each patient, the mean of the 6 observations (3 observers, 2 sessions) and the difference of these 6 observations with the mean were calculated and plotted against each other. The intermittent grey vertical lines indicate the different cut-off points applied.



When assessing the individual ultrasonographic parameters, the inter-observer reliability was good for homogeneity and the presence for hypoechogenic areas; moderate for hyperechogenic reflections and salivary gland border; and fair for echogenicity. The parotid glands scored lower compared to the submandibular glands for hyperechogenic reflections and salivary gland border. A possible explanation is that there is more room for interpretation when scoring the hyperechogenic reflections in the parotid glands, since these findings could be attributed to pSS as well as to normal ageing [20,21]. Furthermore, when it comes to echogenicity, it might be difficult for the observers to determine the overall echogenicity of a very inhomogenous gland. In general, in the absence of a well-defined consensual scoring system and standardized procedures, ultrasonography has an acceptable reliability for homogenicity of the parenchyma, but not for echogenicity [18]. The development of a validated automatic software may resolve these issues [22].

Additionally, we observed that results between observers were more widespread on patients with higher ultrasonographic scores (approximately scores≥20; Figure

Table 3: Median inter-observer reliability of different ultrasonographic variables scored by three observers in both sessions in the parotid and submandibular salivary glands.

	Parotid glands			Submandibular glands		
Variable	**Reliability***	**min value**	**max value**	**Reliability***	**min value**	**max value**
Echogenicity	0.22	0.18	0.32	0.25	0.07	0.42
Homogeneity	0.71	0.66	0.75	0.69	0.63	0.76
Hyperechogenic reflections	0.37	0.30	0.55	0.56	0.44	0.64
Hypoechogenic areas	0.74	0.73	0.75	0.71	0.44	0.64
Salivary gland border	0.39	0.34	0.43	0.59	0.55	0.60

*Fleiss kappa was calculated for echogenicity (nominal variable) and ICC for the rest of the ultrasonographic parameters (which were considered as continuous variables).

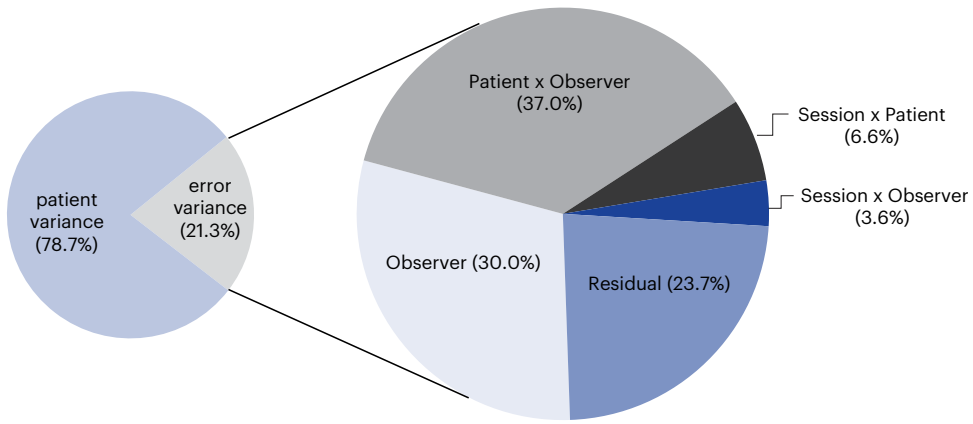
1). This suggests that different observers may rather consistently identify in which patients ultrasound of the major salivary glands supports the diagnosis of pSS, but scoring the severity of the ultrasonographic findings is more inconsistent between the observers. The reason for this variance could be mainly attributed to the observers and to the interaction between observer and patient (Figure 2). A possible consequence of this phenomenon is that when monitoring patients over time, the observed change might not be only attributed to the progression of the disease or to the effect of medication, but might be partly the result of the discrepancy in scoring between the different observers.

To the best of our knowledge, this is the first study where an analysis of the sources of variation in outcomes has been investigated in such detail. A next step is to identify the minimal clinically important difference, which is defined as the smallest difference in score in the domain of interest which patients perceive as important change and which would mandate a change in the patient's management [23].

Strengths and limitations of the study

Strength of the current study is that we included a representative study population reflecting the daily practice. Moreover, we focused on the Hocevar scoring system [3]. We have chosen to use this extensive scoring system as it is one of the most detailed ultrasound scoring systems used today [5]. The Hocevar scoring system can be easily 'transformed' to practically any of the existing ones by combining certain ultrasound variables. So, it is rather generally applicable. Additionally, the fact that we analyzed the reliability on the individual ultrasonographic characteristics allowed us a more detailed approach. The fact that we analyzed static images instead of live ones might be considered as a limitation of this study, although

Figure 2: Variance components of ultrasonographic examination of the major salivary glands. Total variance (left circle) comprised patient variance (main effect) and error variance. Several sources contributed to error variance. These sources (right circle) comprised main effects (session and observer), interaction effects (patient × observer and session × observer) and residual variance (all expressed as percentages of error variance).



this is common approach in similar studies. The development of a consensus and widely accepted ultrasonographic scoring system by the EULAR US-pSS Study Group for evaluating the major salivary glands of patients with pSS will allow better comparison between studies.

Conclusion

Our study showed that B-mode ultrasound of the major salivary glands is a reliable imaging technique for patients with clinically suspected pSS. The study also pointed that results between observers were more widespread on patients with higher ultrasonographic scores and that there are some discrepancies between observers in assessing the severity of ultrasonographic findings. Thus, when assessing 'true' changes over time, i.e. when monitoring the activity or progression of pSS, it is advised that each particular patient is scored by the same ultrasonographer at every time point.

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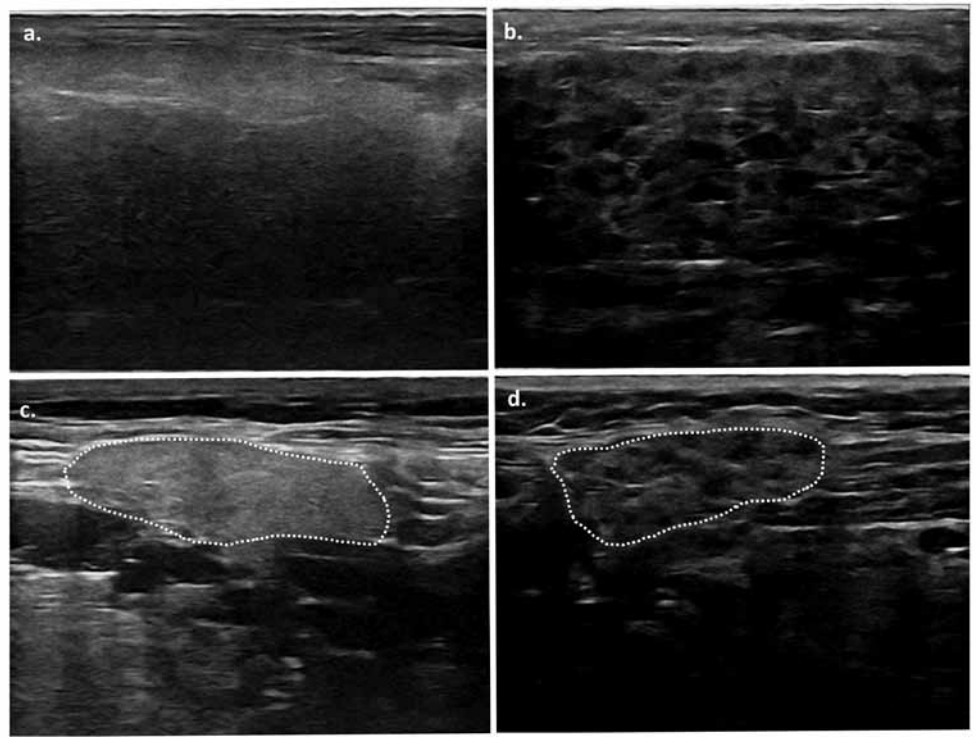
Supplementary Table 1: Impact of components influencing variation in ultrasonographic scores.

Variance Estimates		% of total variation	% of error variation
Sources of variation	Estimate		
Patients	59.05	78.7	-
Sessions	0.00	0.0	0.0
Observer	4.81	6.4	30.0
Patient x observer	5.90	7.9	37.0
Patients x session	1.06	1.4	6.6
Session x observer	0.43	0.6	2.8
Variance Error	3.78	5.0	23.7

Dependent Variable: ultrasound total score.
Method: ANOVA (Type III Sum of Squares).

Supplementary Figure 1: Representative ultrasonographic images of the major salivary glands: a. parotid gland with normal echostructure; b. parotid gland with echostructure corresponding to pSS; c. submandibular gland with normal echostructure; d. submandibular gland with echostructure corresponding to pSS. Images were processed in such a way that no patient data were visible.



Chapter 3D

Ultrasonography of major salivary glands compared to parotid and labial gland biopsy and classification criteria in patients with clinically suspected primary Sjögren's syndrome

Konstantina Delli[1*], Esther Mossel[2*], Jolien F van Nimwegen[2], Alja J Stel[2], Frans GM Kroese[2], Frederik KL Spijkervet[1], Arjan Vissink[1], Suzanne Arends[2] and Hendrika Bootsma[2], on behalf of the EULAR US-pSS Study Group

1. Department of Oral and Maxillofacial Surgery

2. Department of Rheumatology and Clinical Immunology

University of Groningen, University Medical Center Groningen, Groningen, the Netherlands

* Both authors contributed equally

Under submission

Abstract

Objective: To assess the validity of ultrasound of major salivary glands (sUS) compared to parotid and labial gland biopsies, sialometry, anti-SSA antibody status and classification criteria in patients clinically suspected with primary Sjögren's syndrome (pSS).

Methods: 103 consecutive outpatients clinically suspected with pSS underwent sUS. Parenchymal echogenicity, homogeneity, hypoechogenic areas, hyperechogenic reflections and clearness of salivary gland border were scored according to the Hocevar scoring system. Total ultrasound score was calculated as the sum of these domains (range 0-48).

Results: sUS outcome showed good agreement with parotid (kappa 0.635) and moderate agreement with labial (kappa 0.573) gland biopsy outcome. Negative sUS predicts negative parotid gland biopsy and positive sUS predicts positive labial gland biopsy. sUS outcome showed fair agreement with unstimulated whole saliva and good agreement with anti-SSA antibody status.

sUS outcome demonstrated good agreement with AECG (kappa 0.643, sensitivity 71%, specificity 92%), ACR (kappa 0.705, sensitivity 77%, specificity 92%) and ACR-EULAR (kappa 0.601, sensitivity 67%, specificity 94%) classification criteria. Positive sUS predicts classification, but negative sUS does not exclude classification. The combination of positive sUS with presence of anti-SSA antibodies or negative sUS with absence of anti-SSA antibodies showed a high predictive value for classification as pSS or non-pSS.

Conclusion: In our prospective inception cohort study derived from daily clinical practice, sUS outcome shows good agreement with parotid and moderate agreement with labial gland biopsy outcome. Additionally, the combination of positive sUS and presence of anti-SSA antibodies highly predicts classification according to the AECG, ACR and ACR-EULAR classification criteria.

Introduction

Primary Sjögren's syndrome (pSS) is a chronic, systemic auto-immune disease, which is characterized by inflammation of the exocrine glands, with an estimated prevalence of 0.05% in the general population [1]. Most patients with pSS suffer from xerostomia, keratoconjunctivitis sicca and extreme fatigue [2]. In addition, many different extraglandular manifestations may be present, of which arthralgia, arthritis and myalgia occur most frequently [2].

Currently, multiple criteria sets are available for the classification of pSS. In 2002, the American European Consensus Group (AECG) criteria were developed and, although not endorsed by the American College of Rheumatology (ACR) or European League Against Rheumatism (EULAR), these are till now the most commonly used in daily clinical practice [3,4]. In 2012, the ACR criteria were developed and provisionally approved by the ACR [5], but these criteria were not well accepted by many SS experts [6]. In order to develop international consensus classification criteria, the ACR-EULAR criteria were recently introduced and endorsed by both EULAR and ACR [4,7]. In all three sets, salivary gland biopsies and presence of anti-SSA antibodies play a significant role in classifying patients as pSS [3-5]. In the AECG and ACR-EULAR criteria, salivary gland involvement is also assessed by unstimulated whole salivary flow (UWS) [3,4].

Ultrasound of major salivary glands (sUS) is an upcoming diagnostic method for assessing involvement of major salivary glands in pSS [8,9]. sUS is well tolerated, non-invasive, inexpensive, non-irradiating and widely available in the rheumatologic outpatient clinics, but its reliability depends greatly on the operator. A recent meta-analysis assessing the diagnostic properties of sUS in pSS reported a pooled sensitivity of 69% and specificity of 92%. This meta-analysis also revealed a large clinical and methodological heterogeneity between studies, which not only hampered interpretation of pooled outcomes but also influenced the results reported in the various studies [8]. Thus, the possible role of sUS in the diagnosis of pSS remains unclear [8-10].

This study assesses the validity of sUS compared to parotid and labial gland biopsies, sialometry, anti-SSA antibody status and classification criteria in patients clinically suspected with pSS.

Materials and methods

Patients

The present cross-sectional study is based on prospectively collected data from the multidisciplinary Sjögren's expertise center in the University Medical Center Groningen (UMCG). One hundred and ten consecutive patients clinically suspect-

ed with pSS, who underwent sUS as part of the diagnostic work-up (in accordance with the Medical and Ethics Committee of the UMCG; waiver 016/120) and were over 18 years of age, were included. All patients underwent the diagnostic work-up. All domains of the AECG, ACR and ACR-EULAR criteria were assessed, including parotid or labial gland biopsy.

Primary assessment

Ultrasonography

All patients were examined with the same ultrasonographic scanner (Esaote MyLab-Seven, Genova, Italy), equipped with a high resolution linear scanner (4-13MHz). During the sUS examination, patients were examined in supine position with their neck slightly extended and turned away from the examined side [11,12]. The Hocevar scoring system was used. The following variables were investigated in the parotid and submandibular salivary glands: (I) parenchymal echogenicity compared to the thyroid gland, graded 0-1; (II) homogeneity, graded 0-3; (III) presence of hypoechogenic areas, graded 0-3; (IV) hyperechogenic reflections, graded 0-3 in parotid glands and 0-1 in submandibular glands; and (V) clearness of the salivary gland border, graded 0-3 [12]. Total ultrasound score was the sum of these five domains and can range from 0 to 48 [12].

Other assessments

Parotid and/or labial gland biopsies were considered positive if focus score (defined as the number of mononuclear infiltrates containing ≥50 lymphocytes per 4mm^2 of glandular tissue) was ≥1 [13-15]. UWS was evaluated by measuring the saliva production in 15 minutes [3]. UWS ≤1.5mL/15min was considered abnormal [16]. Serum levels of anti-SSA and anti-SSB antibodies were assessed with ELISA tests.

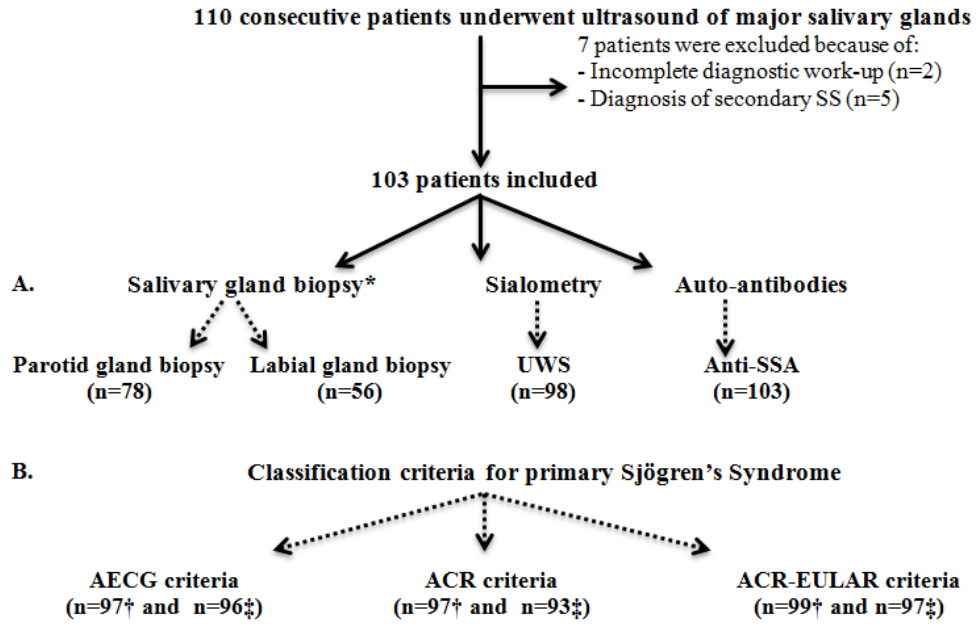
Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics 23 (SPSS, Chicago, IL, USA). Descriptive parameters were expressed as number of patients (%) and median (range) for categorical and non-normally distributed data, respectively.

Receiver operating characteristic (ROC) analysis was performed to determine the accuracy of sUS to predict parotid or labial gland biopsies, UWS, anti-SSA antibody status and classification as pSS. Area under the curve (AUC) was interpreted as no discrimination (0–0.5), poor accuracy (0.5–0.7), fair (0.7–0.8), good (0.8–0.9) or excellent (0.9–1.0) [17]. The optimal cut-off point for sUS positivity was determined according to the highest combination of sensitivity and specificity.

Agreement between sUS outcome and parotid or labial gland biopsies, UWS, anti-

Figure 1: Flowchart of patients included in the study with information about the availability of A. salivary gland biopsy, sialometry and auto-antibodies and B. classification according to the AECG, ACR and ACR-EULAR criteria. * 43 patients underwent parotid gland as well as labial gland biopsy. Separate analyses were done considering either † parotid gland biopsy or ‡ labial gland biopsy as an item, when applying these classification criteria. UWS = unstimulated whole saliva. n= total number of patients included in the analysis.



SSA antibody status and classification according to the classification criteria was determined by Cohen's kappa (κ) [18]. Furthermore, the association between ultrasound and UWS was analyzed using Spearman correlation coefficient (ρ) and was interpreted as poor (0.0–0.2), fair (0.2–0.4), moderate (0.4–0.6), good (0.6–0.8) or excellent (0.8–1.0). To further assess the diagnostic properties of sUS, the percentage of absolute agreement, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+) and negative likelihood ratio (LR-) were calculated.

Mann-Whitney U test was used to evaluate differences in total ultrasound score between patients with: (I) positive versus negative parotid or labial gland biopsies, (II) UWS ≤1.5ml/15min versus UWS >1.5ml/15min, (III) presence versus absence of anti-SSA and/or anti-SSB antibodies and (IV) pSS versus non-pSS according to the classification criteria. P-values <0.05 were considered statistically significant.

Table 1. Ultrasound of major salivary glands versus salivary gland biopsy, sialometry and anti-SSA antibodies status.

	Parotid gland biopsy (n=78)	Labial gland biopsy (n=56)	UWS (n=98)	Anti-SSA antibodies (n=103)
Optimal cut-off point	15	14	15	15
Cohen's kappa	0.635	0.573	0.345	0.633
% absolute agreement	83.3%	78.6%	66.3%	81.6%
Sensitivity	75.0%	72.4%	54.5%	69.8%
Specificity	88.0%	85.2%	81.4%	94.0%
PPV	77.8%	84.0%	78.9%	92.5%
NPV	86.3%	74.2%	58.3%	74.6%
LR+	6.25	4.89	2.93	11.63
LR-	0.28	0.32	0.56	0.32

UWS = unstimulated whole saliva; PPV = positive predictive value; NPV = negative predictive value; LR+ = positive likelihood ratio; LR- = negative likelihood ratio.

Results

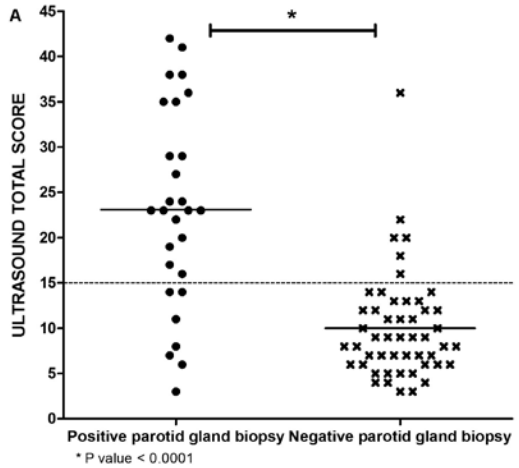
A flowchart of inclusion and exclusion of patients and information about the number of patients included in the analyses regarding salivary gland biopsies, sialometry, anti-SSA antibody status and classification criteria is presented in Figure 1. Of the 103 included patients, median age was 51 years (range 18-82), 90% were female and median total ultrasound score was 12 (range 3-43; Supplementary Table 1). For research purposes, 43 patients underwent parotid and labial gland biopsies. Of the remaining patients, 35 had only a parotid gland biopsy and 13 had only a labial gland biopsy.

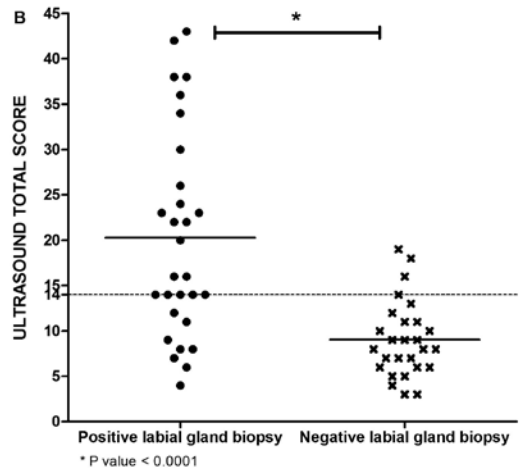
Ultrasound versus salivary gland biopsy

The accuracy of sUS to predict a parotid gland biopsy outcome was good, with an AUC of 0.849 (95% CI: 0.746-0.952) and optimal cut-off point of 15 (Supplementary Table 2). The agreement between sUS outcome and parotid gland biopsy was good (κ=0.635), with a sensitivity of 75%, specificity of 88%, PPV of 78% and NPV of 86% (Table 1).

The accuracy of sUS to predict a labial gland biopsy outcome was good, with an AUC of 0.824 (95% CI 0.714-0.934) and optimal cut-off point of 14 (Supplementary Table 2). The agreement between sUS outcome and labial gland biopsy was moderate (κ=0.573), with a sensitivity of 72%, specificity of 85%, PPV of 84% and NPV of 74% (Table 1).

Figure 2: Ultrasound total score compared to salivary gland biopsy. A. positive versus negative parotid gland biopsy; B. positive versus negative labial gland biopsy. Histopathology: positive parotid or labial gland biopsy was defined as focus score ≥1.





Total ultrasound score was significantly higher in patients with positive parotid or labial gland biopsies compared to patients with negative parotid or labial gland biopsies (Figure 2).

Ultrasound versus sialometry

The accuracy of sUS to predict UWS outcome was poor, with AUC of 0.696 (95% CI: 0.593–0.799). The agreement between sUS outcome and UWS was fair (κ=0.345; Table 1).

Table 2. Ultrasound of major salivary glands versus classification criteria.

	AECG	
	parotid glandbiopsy	labial gland biopsy
Optimal cut-off point	15/16	15/16
Cohen's kappa	0.643	0.585
% absolute agreement	82.4%	79.2%
Sensitivity	71.1%	67.4%
Specificity	92.3%	91.4%
PPV	88.9%	89.2%
NPV	78.7%	72.8%
LR+	9.23	7.84
LR-	0.31	0.36
	ACR	
	parotid gland biopsy	labial gland biopsy
Optimal cut-off point	15	15
Cohen's kappa	0.705	0.627
% absolute agreement	85.6%	81.7%
Sensitivity	77.3%	69.8%
Specificity	92.4%	92.0%
PPV	89.4%	88.2%
NPV	83.1%	78.0%
LR+	10.17	8.73
LR-	0.25	0.33
	ACR-EULAR	
	parotid gland biopsy	labial gland biopsy
Optimal cut-off point	15	15
Cohen's kappa	0.601	0.520
% absolute agreement	79.8%	75.3%
Sensitivity	67.3%	61.8%
Specificity	93.6%	92.9%
PPV	92.1%	91.9%
NPV	72.1%	65.0%
LR+	10.52	8.70
LR-	0.35	0.41

PPV = positive predictive value; NPV = negative predictive value; LR+ = positive likelihood ratio; LR- = negative likelihood ratio. In the left column parotid gland biopsies are considered an item of the AECG, ACR and ACR-EULAR classification criteria. In the right column, labial gland biopsies are considered an item of the AECG, ACR and ACR-EULAR criteria.

Total ultrasound score, however, was significantly higher in patients with UWS ≤1.5ml/15min compared to patients with UWS >1.5ml/15min (Supplementary Figure 1A). There was a fair reversed association between total ultrasound score and UWS total flow (ρ=-0.366) (Supplementary Figure 1B).

Ultrasound versus auto-antibodies

The accuracy of sUS to predict anti-SSA antibody status was good, with an AUC of 0.803 (95% CI: 0.711–0.894). The agreement between sUS outcome and anti-SSA antibody status was good (κ=0.633; Table 1).

Total ultrasound score was significantly higher in patients with anti-SSA antibodies compared to patients without anti-SSA antibodies (Supplementary Figure 2).

Ultrasound versus classification criteria

For the following analyses, the outcome of the parotid gland biopsy was an item of the classification criteria.

The accuracy of sUS to predict AECG classification was good, with an AUC of 0.826 (95% CI: 0.735–0.918) and optimal cut-off point of 15 (Supplementary Table 3). The agreement between sUS outcome and AECG classification was good (κ=0.643), with a sensitivity of 71%, specificity of 92%, PPV of 89% and NPV of 79% (Table 2).

The accuracy of sUS to predict ACR classification was good, with an AUC of 0.862 (95% CI: 0.777–0.947) and optimal cut-off point of 15 (Supplementary Table 3). The agreement between sUS outcome and ACR classification was good (κ=0.705), with a sensitivity of 77%, specificity of 92%, PPV of 89% and NPV of 83% (Table 2).

The accuracy of sUS to predict ACR-EULAR classification was good, with an AUC of 0.802 (95% CI: 0.710–0.894) and optimal cut-off point of 15 (Supplementary Table 3). The agreement between sUS outcome and ACR-EULAR classification was good (κ=0.601), with a sensitivity of 67%, specificity of 94%, PPV of 92% and NPV of 72% (Table 2).

Total ultrasound score was significantly higher in pSS versus non-pSS according to the classification criteria (Figure 3).

For analyses when the outcome of the labial gland biopsy was an item of the classification criteria, see Table 2 and Figure 3.

Figure 3: Ultrasound total score compared to AECG, ACR and ACR-EULAR classification criteria for primary Sjögren's syndrome. In A-C: parotid gland biopsy outcome is considered as an item of the criteria; in D-F: labial gland biopsy outcome is considered as an item of the criteria. POSITIVE or NEGATIVE indicate fulfilling or not fulfilling the classification criteria, respectively.

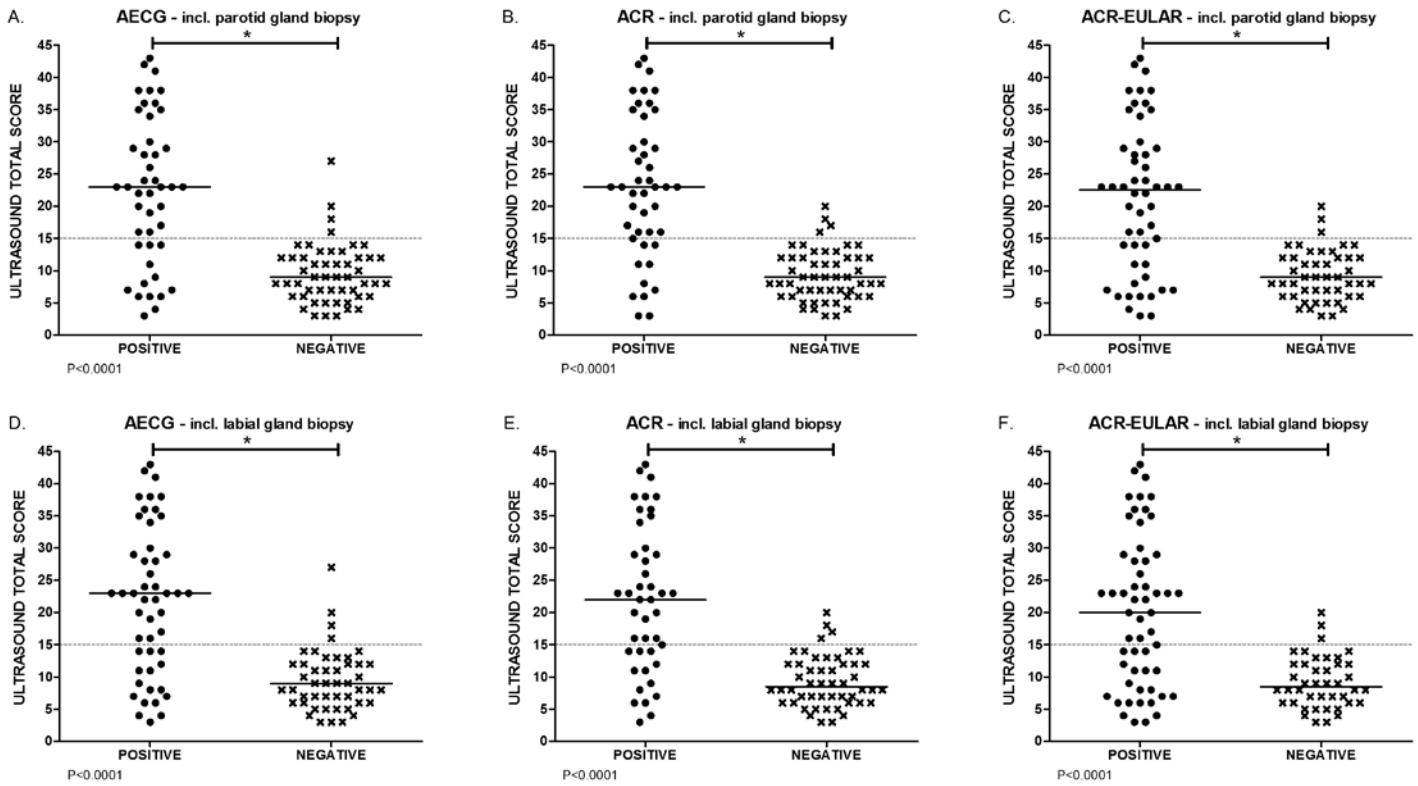
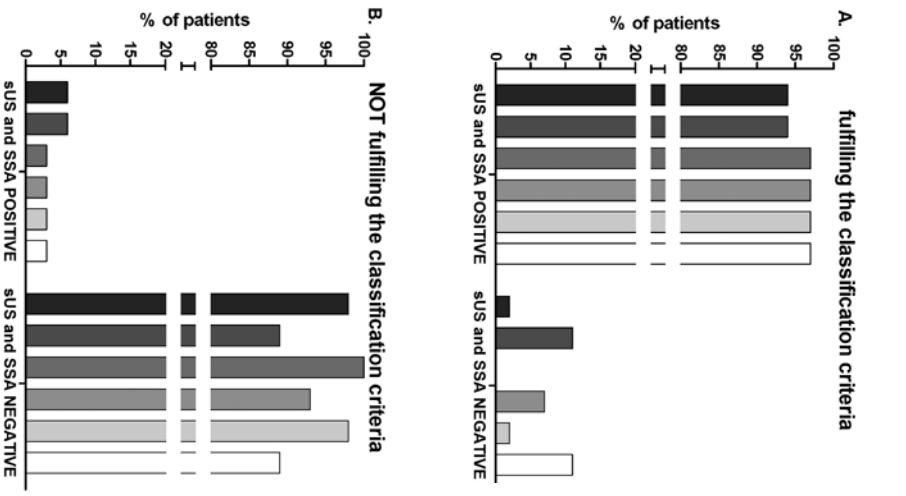


Figure 4: Percentage of patients fulfilling or not fulfilling the AECG, ACR or ACR-EULAR criteria. When 'parotid gland' is indicated, parotid gland biopsy outcome is considered an item of the AECG, ACR and ACR-EULAR criteria. When 'labial gland' is indicated, labial gland biopsy outcome is considered an item of the AECG, ACR and ACR-EULAR criteria.
A. fulfilling the AECG, ACR or ACR-EULAR classification criteria; on the left positive sUS in combination with presence of anti-SSA antibodies and on the right negative sUS in combination with absence of anti-SSA antibodies; and
B. NOT fulfilling the AECG, ACR or ACR-EULAR classification criteria; on the left positive sUS in combination with presence of anti-SSA antibodies and on the right negative sUS in combination with absence of anti-SSA antibodies.



Predictive value of the combination of sUS and antibody status

In patients with positive sUS combined with anti-SSA antibodies, 14 out of 18 (78%) had a positive parotid gland biopsy and 17 out of 18 patients (94%) had a positive labial gland biopsy.

In patients with negative sUS combined with absence of anti-SSA antibodies, 37 out of 40 (93%) had a negative parotid gland biopsy and 17 out of 22 patients (77%) had a negative labial gland biopsy.

In patients with positive sUS combined with anti-SSA antibodies, 32 out of 34 (94%) fulfilled the AECG, 34 out of 35 (97%) fulfilled the ACR and 35 out of 36 patients (97%) fulfilled the ACR-EULAR criteria, considering the outcome of parotid gland biopsies as an item for classification. Percentages are equal when the outcome of labial gland biopsies are considered an item for classification (Figure 4).

In patients with negative sUS combined with absence of anti-SSA antibodies, 45 out of 46 (98%) did not fulfill the AECG, 45 out of 45 (100%) did not fulfill the ACR and 44 out of 45 patients (98%) did not fulfill the ACR-EULAR criteria, considering the outcome of parotid gland biopsies an item for classification (Figure 4). Percentages are lower when the outcome of labial gland biopsies is considered an item for classification: 40 out of 45 patients (89%) did not fulfill the AECG, 43 out of 46 (93%) did not fulfill the ACR and 39 out of 44 (89%) did not fulfill the ACR-EULAR criteria (Figure 4).

Discussion

This study assessed the validity of sUS in a representative population of patients with clinically suspected pSS. It is the first study that directly compared the validity of sUS to parotid gland biopsy outcome and to the best of our knowledge the first that compared the validity of sUS to the ACR-EULAR criteria.

Previously, three studies evaluated the optimal cut-off of the Hocevar sUS scoring system [12]. These studies used different gold standards, viz. AECG criteria [12], labial gland biopsy [19] or AECG and ACR criteria [20]. The cut-off points ranged from 15 to 19. In the present study, the optimal cut-off point for the Hocevar score was found to be 15 in almost all analyses.

Applying a higher cut-off value, e.g. 17 as used by Hocevar et al. [12], would have led to increased specificity and PPV for parotid and labial gland biopsies as well as for the AECG, ACR and ACR-EULAR classification criteria, at the expense of sensitivity and NPV (Supplementary Table 1 and 2). At this moment, there is no consensus about the optimal cut-off point. A consensus scoring system and cut-off point should be developed, which would further elucidate the role of sUS in the diagnosis of pSS by enabling a direct comparison between the different studies.

Agreement was good between sUS outcome and parotid gland and moderate between sUS outcome and labial gland biopsy outcome. The specificity of sUS was slightly higher when parotid gland biopsies instead of labial biopsies were used as gold standard, which may be due to the fact that the parotid gland is included in the sUS evaluation, whereas the labial gland is not.

Interestingly, the parotid gland biopsies were negative in most patients with a negative sUS, but the labial gland biopsies were positive in 26% of patients with a negative sUS. On the other hand, positive sUS predicts positive labial gland biopsies, while 22% of patients with a positive sUS had a negative parotid gland biopsy.

There was a fair reversed association between total ultrasound score and UWS total flow. In other words, patients with more pronounced abnormalities on ultrasound tend to have a reduced UWS production. However, there was also a significant number of patients with few abnormalities on ultrasound who did have a reduced UWS production. This group may have consisted of early pSS patients, where UWS was reduced but no sUS abnormalities were yet seen or where another condition was causing the decrease in UWS.

Agreement between sUS outcome and anti-SSA antibody status was good, and sUS showed high specificity, confirming the findings of Luciano et al., who showed that higher total ultrasound scores correspond to presence of anti-SSA and/or anti-SSB antibodies [21].

Agreement between sUS outcome and the various sets of classification criteria for pSS was good. Our findings regarding the sensitivity and specificity of sUS outcome compared to the classification criteria for pSS are similar with the results described in a recent meta-analysis, where various sUS scoring systems showed an overall sensitivity of 69% and specificity of 92% [8]. Sensitivity of sUS was lower when compared to the recently published ACR-EULAR criteria, when either parotid or labial gland biopsies were considered as an item for classification. It is currently unknown if sUS is sensitive enough to detect changes in the major salivary glands early in the disease course. In our inception cohort, more patients with low sUS scores fulfilled the ACR-EULAR criteria compared to the AECG and ACR criteria. This may suggest either that patients are classified as pSS at an earlier stage of disease according to the ACR-EULAR criteria or that the ACR-EULAR criteria are more liberal [7]. Finally, the PPV of sUS compared to the classification criteria was higher than the NPV. Thus, positive sUS predicts fulfillment of the AECG, ACR and ACR-EULAR criteria, but negative sUS does not exclude classification.

In accordance with our findings, Astorri et al. [22] reported that positive sUS was highly predictive of positive labial gland biopsies. Therefore, one could consider not performing a labial gland biopsy in patients with a positive sUS. This previous study also showed that negative sUS was highly predictive of negative labial gland

biopsies in patients with sicca symptoms. However, we were unable to confirm this observation, as we found a moderate NPV of sUS for labial gland biopsies. Therefore, in patients with a negative sUS the result of labial gland biopsies could not fully be predicted. There are some possible explanations for this discrepancy. None of the "non-Sjögren's sicca patients" in the study of Astorri et al. [22] had a positive labial gland biopsy, while some of our "non-Sjögren sicca patients" did have a positive biopsy. Moreover, Astorri et al. [22] did not mention the time interval between sUS and labial gland biopsy, which might have been longer than in our study. Last but not least, a different sUS scoring system was used.

Astorri et al. [22] stated that labial gland biopsies should not be performed in ENA-negative patients with negative sUS, unless there are other strong clinical indications for SS. Based on our data, we cannot support this conclusion. It is well established that negative serology occurs in 10-50% of pSS patients and correlates with milder disease [23-25]. Interestingly, when labial gland biopsies are considered as an item of the classification criteria, 7-11% of our patients with the combination of negative sUS and absence of anti-SSA antibodies were classified as pSS according to the different criteria sets. In these anti-SSA negative patients, positive biopsies are decisive for classification [26]. Thus, ultrasound cannot fully replace labial gland biopsies, as there is a risk of underdiagnosing serologic negative patients. Consequently, we recommend that physicians should still consider performing biopsies in patients with absence of anti-SSA antibodies and negative sUS, especially when gland function (e.g., abnormal Schirmer or UWS) is impaired or when there are other signs and symptoms pointing to pSS.

Strikingly, almost all of our patients with both a positive sUS and presence of anti-SSA antibodies fulfilled the classification criteria for pSS. In this patient group, physicians could consider to skip the salivary gland biopsy as the combination of positive sUS and presence of anti-SSA antibodies is already highly suggestive of pSS. These rather interesting results are to be confirmed in other cohorts.

The main strength of our study is that consecutive patients clinically suspected with pSS were included. Thus, the study population clearly represents the clinical circumstances in daily clinical practice. Moreover, our Sjögren's expertise center is one of the few centers in which both parotid and labial gland biopsies can be performed [14]. Having access to parotid gland biopsies has several advantages, i.e. repeated biopsies of the same parotid gland can be performed (e.g. for monitoring treatment efficacy) and, MALT-lymphoma might be identified at an earlier stage [14].

In conclusion, in our prospective inception cohort study derived from daily clinical practice:

(i) sUS showed good agreement with parotid and moderate agreement with labial gland biopsy, fair agreement with sialometry, good agreement with anti-SSA antibody status and good agreement with classification criteria in patients suspected with pSS;

(ii) positive sUS predicts classification according to the AECG, ACR and ACR-EULAR classification criteria, but negative sUS does not exclude classification;

(iii) positive sUS in combination with presence of anti-SSA antibodies highly predicts classification according to the AECG, ACR and ACR-EULAR criteria. The combination of negative sUS and absence of anti-SSA antibodies highly excludes classification when parotid gland biopsy outcome is considered an item for classification, but when the outcome of labial gland biopsy is considered an item for classification, combining negative sUS with absence of anti-SSA antibodies does not exclude classification.

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Supplementary Table 1. Characteristics of the study population.

	Age (years)	Females	Ultrasound total score
Classification criteria including parotid gland biopsy			
Fulfilling AECG criteria (n=45)	49 (18-82)	42 (93)	23 (3-43)
Fulfilling ACR criteria (n=44)	46 (18-82)	40 (91)	23 (3-43)
Fulfilling ACR-EULAR criteria (n=52)	48 (18-82)	48 (92)	23 (3-43)
Classification criteria including labial gland biopsy			
Fulfilling AECG criteria (n=49)	51 (18-82)	47 (96)	23 (3-43)
Fulfilling ACR criteria (n=43)	47 (20-82)	39 (91)	22 (3-43)
Fulfilling ACR-EULAR criteria (n=55)	51 (18-82)	51 (93)	20 (3-43)
Not fulfilling AECG, ACR and ACR-EULAR criteria (n=44)	53 (20-71)	38 (86)	9 (3-20)
Total groups (n=103)	51 (18-82)	93 (90)	12 (3-43)

Values are presented as number of patients (%) or median (range). n=number of patients fulfilling the AECG, ACR and ACR-EULAR classification criteria. When 'including parotid gland biopsy' is indicated, parotid gland biopsy outcome is considered an item of the AECG, ACR and ACR-EULAR criteria. When 'including labial gland biopsy' is indicated, labial gland biopsy outcome is considered an item of the AECG, ACR and ACR-EULAR criteria.

Supplementary Table 2. Different cut-off points for ultrasound total score compared to salivary gland biopsy.

Cut-off point	17	16	15	14	13
Parotid gland biopsy (n=78)					
Cohen's kappa	0.629	**0.635**	**0.635**	0.622	0.551
% absolute agreement	83.3%	**83.3%**	**83.3%**	82.1%	78.2%
Sensitivity	71.4%	**75.0%**	**75.0%**	82.1%	82.1%
Specificity	90.0%	**88.0%**	**88.0%**	82.0%	76.0%
PPV	80.0%	**77.8%**	**77.8%**	71.9%	65.7%
NPV	84.9%	**86.3%**	**86.3%**	89.1%	88.4%
LR+	7.14	**6.25**	**6.25**	4.56	3.42
LR-	0.32	**0.28**	**0.28**	0.22	0.24
Labial gland biopsy (n=56)					
Cohen's kappa	0.402	0.435	0.435	**0.573**	0.537
% absolute agreement	69.6%	71.4%	71.4%	**78.6%**	76.8%
Sensitivity	48.3%	55.2%	55.2%	**72.4%**	72.4%
Specificity	92.6%	88.9%	88.9%	**85.2%**	81.4%
PPV	87.5%	84.2%	84.2%	**84.0%**	80.8%
NPV	62.5%	64.7%	64.7%	**74.2%**	73.3%
LR+	6.53	4.97	4.97	**4.89**	3.89
LR-	0.56	0.50	0.50	**0.32**	0.34

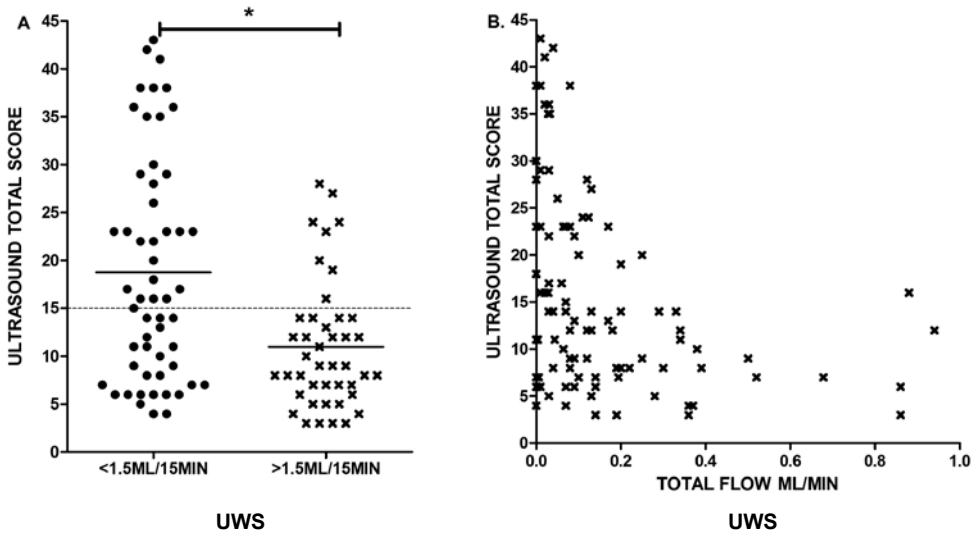
PPV = positive predictive value; NPV = negative predictive value; LR+ = positive likelihood ratio; LR- = negative likelihood ratio. The bold **columns** show the optimal cut-off point(s). Cut-off ≥15 and cut-off ≥16 were found to have identical diagnostic accuracy for parotid gland biopsy.

Supplementary Table 3. Different cut-off points of ultrasound total score compared to classification criteria.

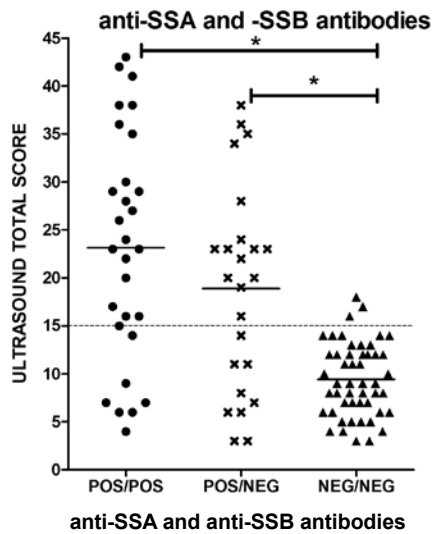
Cut-off point	17	16	15	14	13
AECG criteria (incl. parotid gland biopsy)					
Cohen's kappa	0.620	**0.643**	**0.643**	0.626	0.565
% absolute agreement	81.4%	**82.4%**	**82.4%**	81.4%	78.4%
Sensitivity	66.7%	**71.1%**	**71.1%**	77.8%	77.8%
Specificity	94.2%	**92.3%**	**92.3%**	84.6%	78.8%
PPV	90.9%	**88.9%**	**88.9%**	81.4%	76.1%
NPV	76.6%	**78.7%**	**78.7%**	81.4%	80.3%
LR+	11.5	**9.23**	**9.23**	5.05	3.67
LR-	0.35	**0.31**	**0.31**	0.26	0.28
AECG criteria (incl. labial gland biopsy)					
Cohen's kappa	0.565	**0.585**	**0.585**	0.584	0.521
% absolute agreement	78.1%	**79.2%**	**79.2%**	79.2%	76.0%
Sensitivity	63.3%	**67.4%**	**67.4%**	73.4%	73.4%
Specificity	93.6%	**91.4%**	**91.4%**	85.1%	74.0%
PPV	91.2%	**89.2%**	**89.2%**	83.7%	78.3%
NPV	71.0%	**72.8%**	**72.8%**	75.4%	74.0%
LR+	9.89	**7.84**	**7.84**	4.93	2.78
LR-	0.39	**0.36**	**0.36**	0.31	0.36
ACR criteria (incl. parotid gland biopsy)					
Cohen's kappa	0.639	0.684	**0.705**	0.667	0.607
% absolute agreement	82.4%	84.5%	**85.6%**	83.5%	78.0%
Sensitivity	68.2%	75.0%	**77.3%**	81.8%	81.8%
Specificity	94.3%	92.4%	**92.4%**	84.9%	79.2%
PPV	90.9%	89.2%	**89.4%**	81.8%	76.6%
NPV	78.1%	81.7%	**83.1%**	84.9%	84.0%
LR+	11.96	9.87	**10.17**	5.42	3.93
LR-	0.34	0.27	**0.25**	0.21	0.23
ACR criteria (incl. labial gland biopsy)					
Cohen's kappa	0.557	0.604	**0.627**	0.609	0.547
% absolute agreement	78.4%	80.6%	**81.7%**	80.6%	77.4%
Sensitivity	60.4%	67.4%	**69.8%**	76.7%	76.7%
Specificity	94.0%	92.0%	**92.0%**	84.0%	78.0%
PPV	89.7%	87.9%	**88.2%**	80.4%	75.0%
NPV	73.4%	76.7%	**78.0%**	80.8%	79.6%
LR+	10.07	8.43	**8.73**	4.79	3.49
LR-	0.42	0.35	**0.33**	0.28	0.30
ACR-EULAR (incl. parotid gland biopsy)					
Cohen's kappa	0.562	0.581	**0.601**	0.578	0.516
% absolute agreement	77.8%	78.8%	**79.8%**	78.8%	75.8%
Sensitivity	61.5%	65.4%	**67.3%**	73.1%	73.1%
Specificity	95.7%	93.6%	**93.6%**	85.1%	78.7%
PPV	94.1%	91.9%	**92.1%**	84.4%	79.2%
NPV	69.2%	71.0%	**72.1%**	74.1%	72.5%
LR+	14.30	10.21	**10.52**	4.91	3.43
LR-	0.40	0.37	**0.35**	0.32	0.34
ACR-EULAR (incl. labial gland biopsy)					
Cohen's kappa	0.486	0.502	**0.520**	0.513	0.447
% absolute agreement	73.2%	74.2%	**75.3%**	75.3%	72.2%
Sensitivity	56.4%	60.0%	**61.8%**	67.3%	67.3%
Specificity	95.2%	92.9%	**92.9%**	85.7%	78.6%
PPV	93.9%	91.7%	**91.9%**	86.0%	80.4%
NPV	62.5%	63.9%	**65.0%**	66.7%	64.7%
LR+	11.75	8.45	**8.70**	4.71	3.14
LR-	0.46	0.43	**0.41**	0.38	0.42

PPV = positive predictive value; NPV = negative predictive value; LR+ = positive likelihood ratio; LR- = negative likelihood ratio. The bold **columns** show the optimal cut-off point(s). Cut-off ≥15 and cut-off ≥16 were found to have identical diagnostic accuracy for AECG criteria including either parotid gland biopsy or labial gland biopsy. When 'including parotid gland biopsy' is indicated, parotid gland biopsy outcome is considered an item of the AECG, ACR and ACR-EULAR criteria. When 'including labial gland biopsy' is indicated, labial gland biopsy outcome is considered an item of the AECG, ACR and ACR-EULAR criteria.

Supplementary Figure 1: Ultrasound total score compared to sialometry. A. UWS flow ≤1.5mL/15min versus >1.5mL/min; B. association with UWS total flow ml/min; UWS = unstimulated whole saliva; * indicates P value=0.0009.



Supplementary Figure 2: Ultrasound total score compared to presence or absence of anti-SSA and anti-SSB antibodies. In patients with absence of anti-SSA antibodies, anti-SSB antibodies were also absent. In 29 patients anti-SSB antibodies were present.* indicates P value <0.001.



Chapter 3E

Can ultrasound of the major salivary glands differentiate primary Sjögren's syndrome from other systemic diseases with salivary gland involvement?

Konstantina Delli[1], Jolien F van Nimwegen[2], Suzanne Arends[2], Alja J Stel[2], Maarten Van den Berge[3], Hendrika Bootsma[2], Wouter F Bierman[4], Frederik KL Spijkervet[1], Arjan Vissink[1], on behalf of the EULAR US-pSS Study Group

Departments of:

1. Oral and Maxillofacial Surgery

2. Rheumatology and Clinical Immunology

3. Pulmonology

4. Internal Medicine, Infectious Diseases Service

University of Groningen, University Medical Center Groningen,
The Netherlands

Under submission

Abstract

Purpose: To assess the diagnostic accuracy of ultrasound of the major salivary glands (SGUS) to differentiate primary Sjögren's syndrome (pSS) from other diseases with salivary gland involvement.

Patients and methods: SGUS was performed in 20 consecutive patients with pSS and 20 consecutive patients with well-established systemic disease, i.e. 5 patients with either sarcoidosis, amyloidosis, HIV infection or HCV infection. Images were scored independently by two blinded observers according to the Hocevar scoring system. Diagnostic accuracy to discriminate between the patient (sub-)groups was explored.

Results: The accuracy of SGUS to differentiate pSS from other systemic diseases was excellent (area under ROC curve of 0.91). The optimal cut-off value to define positive or negative ultrasound for pSS was 15. Sensitivity, specificity, positive predictive value and negative predictive value were high, varying from 85-90%, and diagnostic odds ratio was 51. SGUS was positive in the vast majority of pSS patients (n=18), but also in 2 patients with HIV infection and one patient with sarcoidosis. UTS differed significantly between patients with pSS and other systemic diseases (median 27 vs. 10, $p<0.001$) as well as between pSS patients and patients with either sarcoidosis, amyloidosis, HIV or HCV infection (all $p<0.05$).

Conclusion: This pilot study indicates that SGUS has a potentially high diagnostic accuracy to discriminate pSS from associated systemic diseases with salivary gland involvement.

Introduction

The accuracy of B-mode ultrasound to evaluate the involvement of the major salivary glands in primary Sjögren's syndrome (pSS) and eventually to diagnose the disease continues to be a topic of interest [1,2]. It is generally agreed that salivary gland ultrasonography (SGUS) is a well-tolerated, non-invasive, inexpensive and non-irradiating imaging technique [3]. Thus, there are considerations that in the future, SGUS may be added to the classification criteria for pSS and may even replace more invasive diagnostic tests, like the salivary gland biopsy [2,4-6]. A recent meta-analysis demonstrated that SGUS has a sensitivity, specificity and diagnostic odds ratio of 69%, 92% and 33.9, respectively, to diagnose SS in the major salivary glands [7]. This meta-analysis also detected a considerable variety in the study populations (patient and control groups) used in the included studies. However, none of those studies included a control group of patients with sarcoidosis, amyloidosis, and human immunodeficiency virus (HIV) and hepatitis C virus (HCV) infection. These diseases are known to frequently affect the major salivary glands, cause dry mouth or they have similar histopathological features with pSS [8-11]. The aim of this pilot study was to assess the potential diagnostic accuracy of SGUS to differentiate patients with pSS and patients with sarcoidosis, amyloidosis, HIV or HCV infection.

Materials and methods

Patients

Twenty consecutive patients fulfilling the American European Consensus Group Criteria (AECG) criteria for pSS [12] and 20 consecutive patients with well-established systemic diseases mimicking pSS, i.e. 5 patients with sarcoidosis, 5 patients with amyloidosis, 5 patients with HIV infection and 5 patients with HCV infection, were included in this pilot study. The diagnosis of the systemic diseases was made by clinical presentation, histologic proof of granulomatous inflammation, and exclusion of malignancy and infection as alternative cause of granulomas for sarcoidosis, biopsy for amyloidosis and with detection of circulating antibodies and polymerase chain reaction (PCR) for HIV and HCV infection. All patients visited the outpatient clinic of the department of Rheumatology and Clinical Immunology and the department of Internal Medicine, Infectious Diseases Service of the University Medical Center Groningen. All patients with pSS were subjected to SGUS evaluation as part of the routine diagnostic work-up, and patients with sarcoidosis, amyloidosis, HIV infection and HCV infection provided written informed consent in accordance with the requirements of the ethics committee of the University Medical Center Groningen (METC waiver 016/120).

Ultrasonography

All patients were examined with the same ultrasonographic scanner (Esaote MyLab-Seven, Genova, Italy), equipped with a high resolution linear scanner (4-13MHz). Each patient was scanned in a supine position with the neck slightly extended and the head turned slightly to the opposite side. The parotid glands were examined in both axial and coronal planes, the submandibular glands only in the coronal plane.

The following images were stored from each patient and used: one showing the thyroid gland, one showing the right submandibular salivary gland, one showing the left submandibular salivary gland, two providing an overview of the right parotid gland and two providing an overview of the left parotid gland (Figure 1). Images were anonymized and allocated to a random number.

All images were scored independently by two observers (KD and JFN; for scoring system see below) on the same monitor (MultiSync E231, 23 inches, NEC, Illinois, USA). The observers were blinded for the diagnostic work-up, i.e., salivary gland biopsy, circulating auto-antibodies, salivary gland function tests, tear gland function tests and subjective oral and ocular symptoms. Both observers scored all patients in a random order.

Ultrasonographic assessments

The following ultrasonographic variables were assessed in the parotid and submandibular salivary glands: echogenicity, parenchymal homogeneity, presence of hypoechogenic areas, presence of hyperechogenic reflections, and clearness of posterior glandular border, according to the Hocevar scoring system [3]:

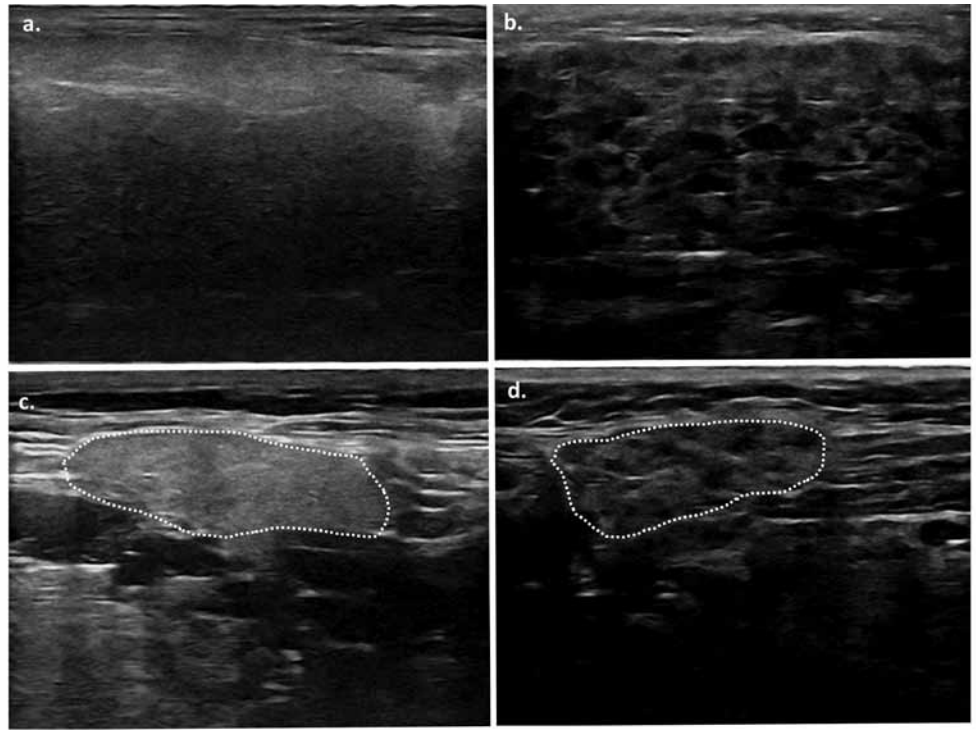
i. Parenchymal echogenicity was evaluated in comparison with the thyroid gland or when there was coincident thyroid gland disease by surrounding anatomical structures (muscular structures, sub-cutaneous fat). Echogenicity was graded 0 if echogenicity was comparable to the thyroid, and 1 if it was decreased.

ii. Homogenity was graded 0 for a homogeneous gland, 1 for mild inhomogeneity, 2 for evident inhomogeneity, and 3 for a grossly inhomogeneous gland.

iii. Presence of hypoechogenic areas was graded 0 for no hypoechogenic areas, 1 for a few scattered areas, 2 for several areas, and 3 for numerous hypoechogenic areas.

iv. Hyperechogenic reflections in the parotid glands were graded 0 for no hyperechogenic reflections, 1 for a few, scattered, 2 for several, and 3 for numerous hyperechogenic reflections, and in submandibular glands 0 for absent and 1 for present.

Figure 1. Representative ultrasonographic images of the major salivary glands: a. parotid gland with normal echostructure; b. parotid gland with echostructure corresponding to pSS; c. submandibular gland with normal echostructure; d. submandibular gland with echostructure corresponding to pSS.



v. Clearness of salivary gland borders was graded 0 for clear, regular defined borders, 1 for partly defined borders, 2 for ill-defined borders, and 3 for borders not visible).

Finally, ultrasound total score (UTS) was calculated as the sum of the grades for the five variables described above for all four glands (range 0-48). According to the literature, the cut-off value to define positive or negative ultrasound for pSS was set at 17 [3] and 15 [13]. Discrepancies between the two observers regarding the positivity or negativity of ultrasound for pSS were resolved in a consensus meeting.

Inter-observer reliability in scoring the ultrasonographic images was excellent, with ICC of 0.88 for the UTS. Cohen's kappa was 0.80 and 0.85 and the percentage of absolute agreement was 90% and 93%, respectively, when cut-off value ≥17 and ≥15 was applied to define positive or negative ultrasound for pSS.

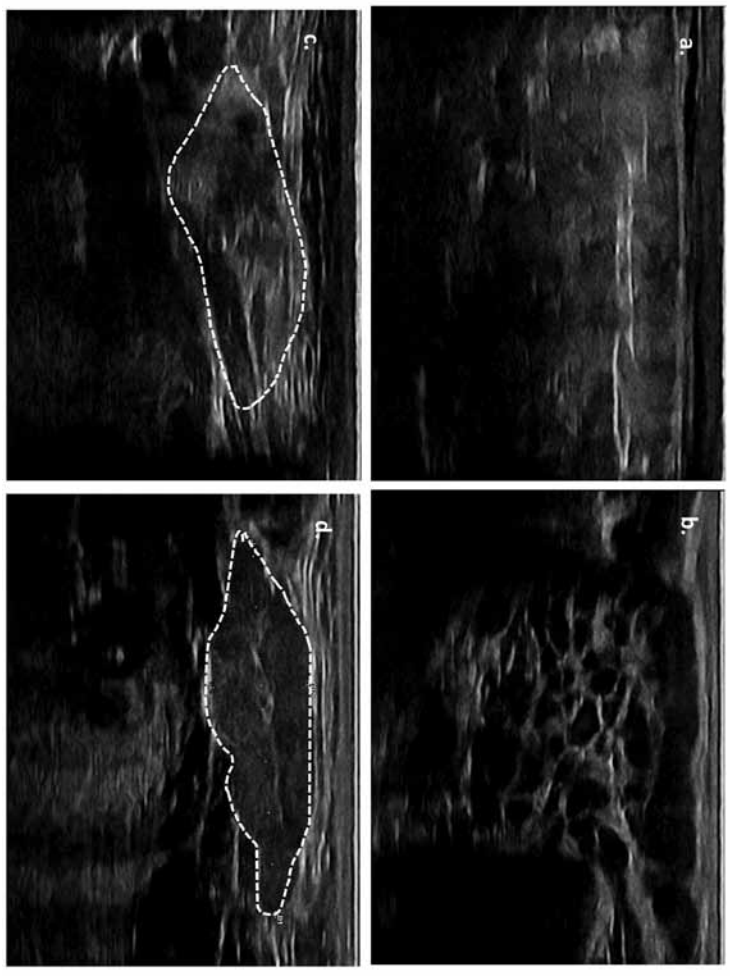


Figure 2. Ultrasonographic images of the major salivary glands of patients with systemic diseases who had positive ultrasound for pSS: a. parotid gland of patient with HIV infection; b. parotid gland of patient with sarcoidosis; c. submandibular gland of patient with HIV infection; d. submandibular gland of patient with sarcoidosis.

Data analysis

Statistical analyses were performed using IBM SPSS Statistics 23 (SPSS, Chicago, IL, USA).

Diagnostic accuracy of SGUS to discriminate between pSS and other systemic diseases was explored using area under the ROC curve (AUC), sensitivity, specificity, Youden's index, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+), negative likelihood ratio (LR-) and diagnostic odds ratio (DOR). AUC was interpreted as no discrimination (0-0.5), poor accuracy (0.5-0.7), fair (0.7-0.8), good (0.8-0.9) or excellent (0.9-1.0) [14]. Furthermore, differences in UTS between the patient (sub-)groups were analyzed using the Mann Whitney U test. P values <0.05 were considered statistically significant.

Table 1. Patients' characteristics.

Disease Group	Age median (range)	Gender male: female	Dry mouth >3 months n (%)	Recurrent/ swollen salivary glands n (%)	Need of liquid to swallow food n (%)	Ultrasound total score median (range)
pSS	50 (20-71)	1:19	19 (95)	14 (70)	17 (85)	27 (11-40)
Other systemic diseases	53 (25-80)	14:6	9 (45)	4 (20)	6 (30)	10 (6-29)
1. Sarcoidosis	44 (25-45)	3:2	3 (60)	4 (80)	1 (20)	10 (9-29)
2. Amyloidosis	74 (53-80)	2:3	3 (60)	0 (0)	2 (40)	11 (10-12)
3. HIV infection	58 (26-61)	5:0	3 (60)	0 (0)	3 (60)	10 (9-27)
4. HCV infection	53 (29-69)	4:1	0 (0)	0 (0)	0 (0)	10 (6-14)

Table 2. Ultrasound of major salivary glands versus classification diagnosis (pSS or other systemic disease). The cut-off point to define positive or negative ultrasound for pSS was set at 15 [13] and 17 [3].

Cut-off point	15	17
Sensitivity	90	85
Specificity	85	85
Youden's index	0.75	0.70
PPV	86	85
NPV	89	85
LR+	6.0	5.7
LR-	0.1	0.2
DOR	51.0	32.1

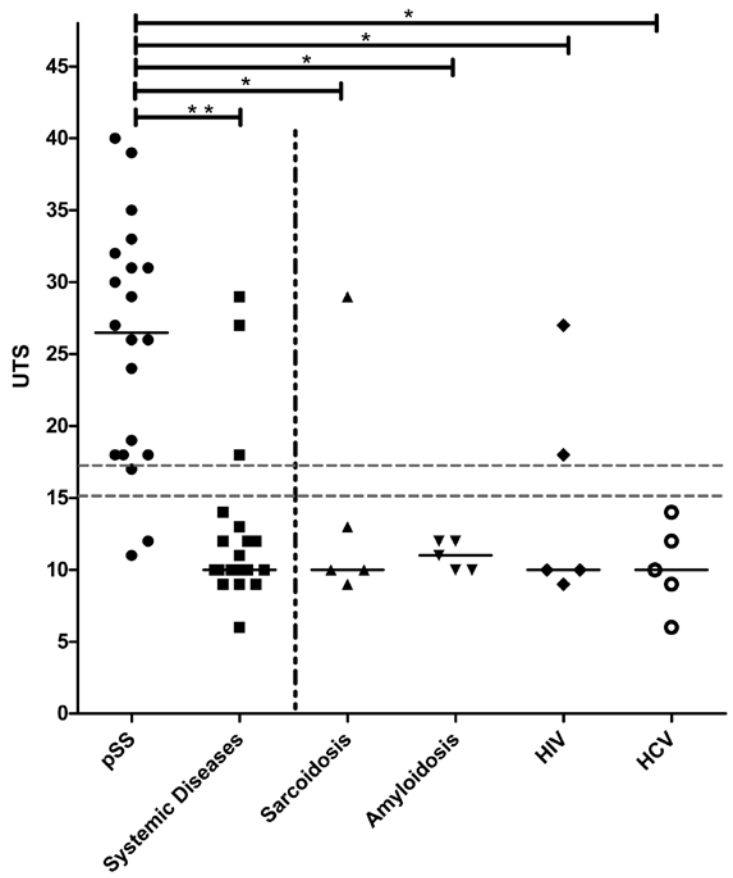
PPV = positive predictive value; NPV = negative predictive value; LR+ = positive likelihood ratio; LR- = negative likelihood ratio; DOR = diagnostic odds ratio.

Results

Of the 20 included patients with pSS, the median age was 50 years (range: 20-71), 19 were female, and the median UTS was 27 (range: 11-40). Of the 20 included patients with systemic diseases or infectious, the median age was 53 years (range: 25-80), 14 were male, and the median UTS was 10 (range: 6-29; Table 1). Regarding the oral symptoms, 95% of the patients with pSS reported to have daily complaints of dry mouth longer than 3 months, 85% needed liquid, e.g. water, to swallow food and 70% reported recurrent or persistent swelling of the major salivary glands. Interestingly, in the group of patients with systemic diseases, 45% of the patients reported to have daily complaints of dry mouth longer than 3 months, 20% reported recurrent or persistent swelling of the major salivary glands, and 30% needed liquid to swallow food. Table 1 summarizes the patient characteristics of all disease (sub-)groups.

The accuracy of SGUS to discriminate pSS from other systemic diseases was excellent, with area under ROC curve of 0.91 and the optimal cut off value was 15, in accordance with Zhang et al. [13]. The agreement between SGUS positivity and positive diagnosis for pSS was good (κ=0.75 and percentage of absolute agreement was 87.5), with sensitivity of 90%, specificity of 85%, PPV of 86% and NPV of 89% (Table 2). When the cut-off value was set at 17, the sensitivity, PPV, NPV, LR+, LR- and DOR slightly deteriorated (Table 2). UTS was positive in 2 patients with HIV infection and one patient with sarcoidosis (Figure 2), whereas UTS was negative in 2-3 patients with pSS (depending on the cut-off value).

Figure 3: Ultrasound total score (UTS) in patient (sub-)groups. ** indicate p<0.001 and * indicates p<0.05. Black horizontal lines indicate median values. The intermittent grey horizontal lines show the different cut-off values applied to define positive or negative ultrasound for pSS (cut-off values were set at 17 [3] and 15 [13]). The intermittent black vertical line separates the two major patient groups (pSS vs. other systemic diseases) from the subgroups of patients with a specific systemic disease (sarcoidosis, amyloidosis, HIV and HCV infection).



UTS differed significantly between patients with pSS and patients with systemic diseases mimicking pSS; (median 27 vs. 10, p<0.001) as well as between patients with pSS and the subgroup of patients with either sarcoidosis, amyloidosis, HIV or HCV infection (p<0.05; Figure 3).

Discussion

The present pilot study explored the use of SGUS in a representative population of consecutive patients diagnosed with pSS or sarcoidosis, amyloidosis, HIV infection and HCV infection. The latter are systemic diseases that could also affect the major salivary glands, cause dry mouth or have similar histopathological features with pSS. These diseases are considered exclusion criteria for the classification of patients according to the AECG [12], American College of Rheumatology (ACR) [15] and the newly published American College of Rheumatology – European League Against Rheumatism (ACR-EULAR) [16] classification criteria, because patients with these diseases can mimic pSS and thus lead to a false positive diagnosis. This pilot study indicates that SGUS has a potentially excellent diagnostic accuracy to discriminate pSS from associated systemic diseases with salivary gland involvement, viz. area under ROC curve of 0.91. The optimal cut off value was 15 and showed DOR of 51. Furthermore, the median UTS was significantly higher in patients diagnosed with pSS compared to patients with these systemic diseases or infectious diseases.

Interestingly, UTS was positive in 2 patients with HIV infection. The first one (UTS=27) reported having both dry mouth for longer than 3 months and recurrent/swelling of the major salivary glands. The second patient (UTS=18) did not report having any oral clinical symptoms that could point towards SS, i.e. neither dry mouth, nor need of liquid to swallow food nor recurrent/persistent swelling of the major salivary glands. Benign lymphoepithelial cysts (BLEC) are a common manifestation in the HIV-positive patient [10], and it is speculated that they might result in ultrasonographic characteristics resembling pSS. UTS was also positive in one patient with sarcoidosis (UTS=29), who presented at the time of the SGUS examination persistent swelling of the parotid glands. Possibly, the presence of non-caseating granulomas in the parotid glands [11] might have led to this ultrasonographic appearance.

In accordance with our study, Luciano et al. showed that SGUS is a highly specific tool for distinguishing pSS from undifferentiated connective tissue diseases [17], a set of unclassifiable systemic autoimmune diseases that shares clinical and serological manifestations with definite connective tissue diseases, which, however, do not fulfill over time, any of the foreseen classification criteria [18]. Similarly, Simizu et al. investigated SGUS in patients with IgG4-related sialadenitis and whether it can differentiate them from pSS [19]. They concluded that changes in the submandibular glands affected by IgG4-related disease could be easily detected using SGUS and that SGUS could also differentiate IgG4-related disease from pSS [19].

The most important strength of the current study is that we included consecutive patients diagnosed with pSS or another systemic disease, avoiding possible selection bias. Moreover, we focused on the Hocevar scoring system [3]. We chose to use this extensive scoring system as it is one of the most detailed ultrasound scoring systems used today and it can easily be transformed to almost any of the existing ones [7].

As with any pilot study, the number of included patients was limited and, thus, the interpretation of data should be done cautiously. Patients with a systemic disease were at different stages of the disease, i.e., some were just diagnosed while others were being in a long term follow up, and additionally patients were treated with different medications. However, it should be kept in mind that pilot studies are a necessary first step in exploring novel applications, and their principal role is to examine the feasibility of a research enterprise [20], and therefore such limitations are up to some extent expected.

Conclusion

This pilot study indicates that SGUS has a potentially high diagnostic accuracy to discriminate pSS from associated systemic diseases with salivary gland involvement, like sarcoidosis, amyloidosis, HIV infection and HCV infection. Further studies including more patients with different stages of systemic diseases are required to confirm and elucidate these preliminary findings.

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Chapter 3F

Salivary gland biopsy for Sjögren's syndrome

Konstantina Delli[1], Arjan Vissink[1], Frederik KL Spijkervet[1]

Department of Oral and Maxillofacial Surgery
University of Groningen, University Medical Center Groningen,
Groningen, the Netherlands

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Key Points

- Lymphocytic sialadenitis in labial salivary glands is a widely accepted criterion for histological confirmation of Sjögren's syndrome (SS).
- Sensitivity and specificity of parotid and labial biopsies for diagnosing SS are comparable.
- Parotid gland incision biopsy can overcome most of the disadvantages of labial gland excision biopsy.
- In contrast to labial salivary glands, lymphoepithelial lesions and early stage lymphomas can be often observed in parotid gland tissue of SS patients.
- Parotid tissue can be harvested easily, repeated biopsies from the same parotid gland are possible, and histopathological results can be compared with other diagnostic results derived from the same gland.
- Parotid biopsies, in contrast to labial salivary gland biopsies, allow the clinician to prospectively monitor disease progression and to assess effects of intervention treatment at a glandular level.

Introduction

Salivary gland biopsy is a technique broadly applied for the diagnosis of Sjögren's syndrome (SS), lymphoma accompanying SS, sarcoidosis, amyloidosis and other connective tissue disorders. SS has characteristic microscopic findings, involving lymphocytic infiltration surrounding the excretory ducts in combination with destruction of acinar tissue (Figure 1). In affected parotid glands, epimyoepithelial islands in a background of lymphoid stroma can be additionally seen and lymphoepithelial lesions (LELs) are a common phenomenon (Figure 2).

Biopsy of the labial salivary glands is considered as one of the four objective European- American Consensus Group classification criteria (AECG) and one of the three objective American College of Rheumatology (ACR) classification criteria for SS (Table 1). While the parotid biopsy has been shown as an alternative for labial salivary gland biopsy when applying AECG classification criteria, it has still to be validated in regard to the ACR classification criteria [1].

This chapter will focus on the main techniques used for taking labial and parotid salivary gland biopsies in the diagnostic work-up of SS with respect to their advantages, their post-operative complications, and their usefulness for diagnostic procedures, monitoring disease progression and treatment evaluation.

Figure 1: Lymphocytic infiltration (*) surrounding excretory ducts and destruction of acini.

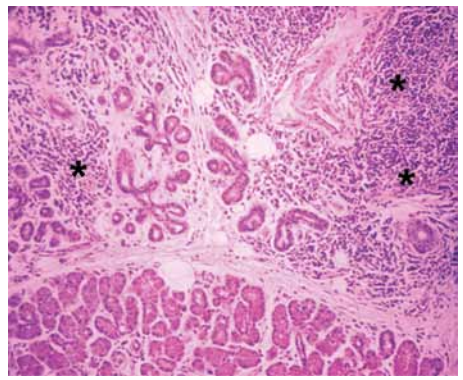


Figure 2: Lymphoepithelial lesions (*) form as the result of atrophy of the columnar ductal epithelium and proliferation of basal epithelial cells, associated with intraepithelial infiltration.

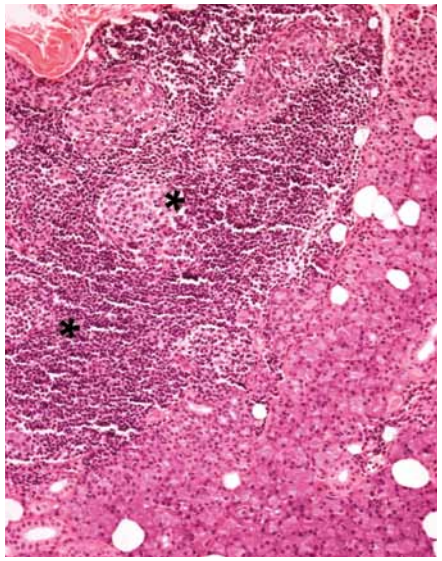


Table 1: Histological criteria for diagnosing SS on salivary gland biopsies [1,18,19].

Type of biopsy	Positivity
Labial gland	if minor salivary glands (obtained through normal appearing mucosa) demonstrate focal lymphocytic sialadenitis, evaluated by an expert histopathologist, with a focus score ≥1, defined as a number of lymphocytic foci (which are adjacent to normal appearing mucous acini and contain more than 50 lymphocytes) per 4 mm^2 of glandular tissue
Parotid gland	if one of the two following criteria is fulfilled: i. a focus score of ≥1, defined as the number of lymphocytic foci (which are adjacent to normal-appearing acini and contain >50 lymphocytes) per 4 mm^2 of glandular parotid tissue (including fat tissue), irrespective of the presence of benign LELs ii. small lymphocytic infiltrates, not fulfilling the criterion of a focus score of ≥1, in combination with the presence of benign LELs

Labial salivary gland biopsy

Minor salivary glands are widely distributed in the labial, buccal and palatal mucosa of the oral cavity [2]. Since pathognomonic changes are seen in minor salivary glands, the minor salivary gland biopsy is largely used for assisting the diagnosis of SS. Labial salivary glands in particular are easily accessible, lie above the muscle layer and are separated from the oral mucous membrane by a thin layer of fibrous connective tissue. Although the chance of excessive bleeding is minimal, since the arterial supply to the lip lies deep [3], there is a risk of sensible nerve injury, as the branches of the mental nerve in the lower lip are closely associated to the minor salivary glands (Figure 3) [4].

Labial salivary gland biopsies in the diagnosis of SS was introduced by Chisholm and Mason in 1968 and involved oral preparation of the patient with local anesthetic infiltration followed by excising an ellipse of oral mucous membrane down to the muscle layer [5]. The wound was closed with 4-0 gauge silk sutures, which were removed after 4-5 days. Ideally 6 to 8 minor glands must be harvested and sent for histopathologic examination.

Several clinicians have revised this technique (Table 2). Greenspan and colleagues described a 1.5-2 cm linear incision of mucosa, parallel to the vermillion border and lateral to the midline [6]. Marx et al. modified Greenspan's technique with a mucosal excision of 3x0.75 cm [7]. Delgado and Moscueda preferred a longitudinal incision of 1 cm in the labial mucosa in front of the mandibular cuspids [8]. Guevara-Gutierrez and coworkers proposed the punch biopsy technique, performed with a 4 mm punch just penetrating the epithelium of the lower lip [9]. Mahlsted et al. recommended a 1-1.5 cm wedge-shaped excision of mucosa between the midline and commissure [10]. Gorson and Ropper reported a 1 cm vertical incision just behind the wet line through the mucosa and submucosa [11]. An oblique incision, starting 1.5 cm from the midline and proceeding latero-inferiorly, avoiding the glandular free zone in the center of the lower lip was advocated by Berquin and colleagues [12]. Caporali et al. reported a small incision of 2-3 mm on the inner surface of the lower lip [13]. In view of the lack of sufficient evidence to support the superiority of one technique over the others, especially in respect to short and long term morbidity, the shape and the size of the incision can be considered a matter of preference. Incision shape has included elliptical, horizontal, vertical and wedge shapes, and incision length has varied from a few mm to 2 cm. The authors of the present article, based on their clinical experience, suggest a horizontal incision of approximately 2 cm in agreement with the technique proposed by Greenspan and colleagues [6], where the surgeon uses loupe operation glasses (magnification x2.5) to precisely excise the salivary glands without disturbing the direct underlying sensible nerves (Figure 4).

The first grading system for salivary gland biopsies was employed by Chisholm and Mason in an attempt to standardize the examined area and record the degree of histopathological change [5]. At present, according to the revised AECG classification criteria and the ACR classification criteria for SS, a labial salivary gland biopsy is considered positive if minor salivary glands (obtained through normal appearing mucosa) demonstrate focal lymphocytic sialadenitis, evaluated by an expert histopathologist, with a focus score ≥1, defined as a number of lymphocytic foci, containing more than 50 lymphocytes per 4 mm^2 of glandular tissue (Table 1).

Complications

The most commonly reported complications of labial gland biopsy are [1,6-8,10,12-17]:

1. Localized sensory alteration, which is frequently described with the terms anesthesia, reduced or partial loss of sensation, transitory numbness and hypoesthesia. The condition may last for a few months or can be even permanent;
2. External haematoma;
3. Local swelling;
4. Formation of granulomas;
5. Internal scarring and cheloid formation;
6. Failing sutures;
7. Local pain.

Suitability for diagnostic and treatment evaluation purposes

A widely accepted criterion for histological confirmation of SS is focal lymphocytic sialadenitis in labial salivary glands [18,19]. Labial biopsies are mainly well suited for the diagnostic work-up, but not for treatment and disease activity evaluation [20],

Figure 3: The branch of the mental nerve (*) that supplies the mucous membrane of the lower lip divides usually into two sub-branches: a horizontal and a vertical, which has an ascending course toward the vermillion border and is in close relation to the labial salivary glands (**).

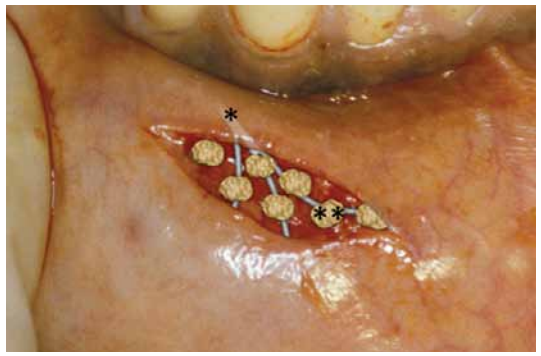
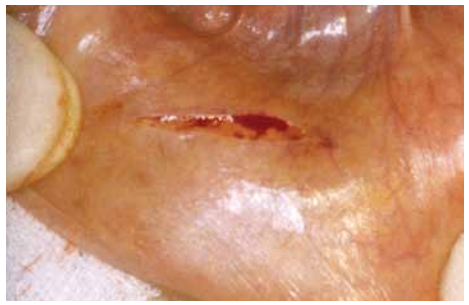
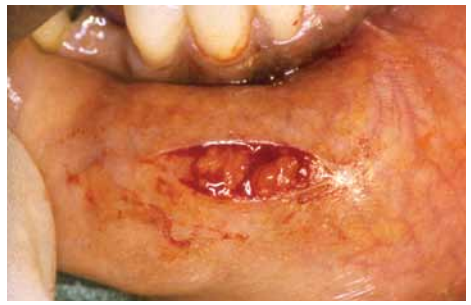


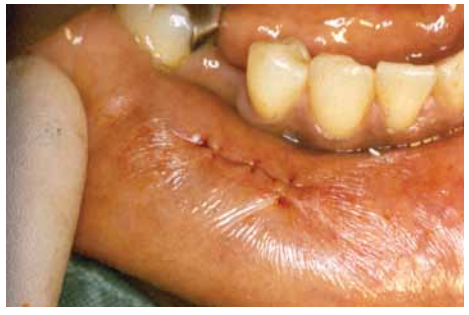
Figure 4: Harvesting labial salivary glands.



A. Horizontal incision of approximately 1.5 cm.



B. Harvest of 6-8 minor salivary glands.



C. Closure of the wound with 5-0 Vicryl rapide® (resorbable) inverted buried notch sutures.

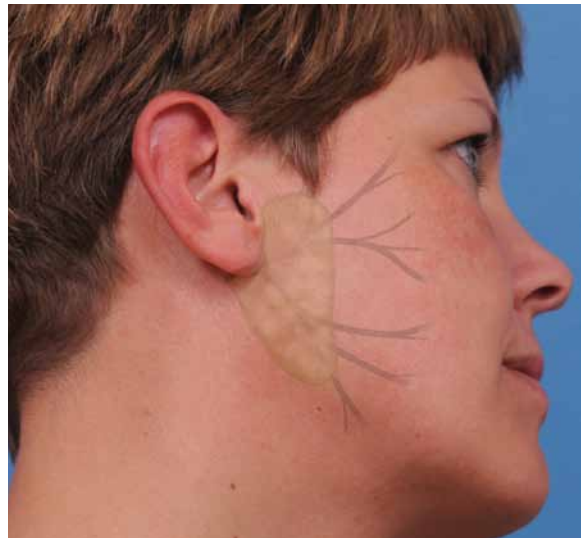
although very rare, B-cell MALT lymphomas can be found in labial biopsies of SS patients [21,22].

Parotid gland biopsy

The parotid gland is the largest salivary gland and is positioned on the lateral aspect of the face overlying the posterior surface of the mandible and antero-inferiorly to the auricle [23]. Traditionally, the gland is divided into a superficial and deep lobe based on the course of the facial nerve as it passes through. When the facial nerve enters the parotid gland, it forms a characteristic branching pattern that resembles a goose foot and is known as 'the pes anserinus', giving two main divisions of the facial nerve (Figure 5). Surgically, the facial nerve can be located in approximately 2-4 mm deep to the inferior end of the tympanomastoid suture line and 1 cm deep and slightly antero-inferior to the tragal pointer.

The technique of the parotid gland biopsy was initially described by Kraaijenhagen [24]: the area is anesthetized with local infiltration anesthesia after the standard preparation. With a No 15 blade, a small 1-2 cm incision is made just below the earlobe near the posterior angle of the mandible. The skin is incised and the pa-

Figure 5: The facial nerve enters the parotid gland forming a characteristic branching pattern that resembles a goose foot and is known as the pes anserinus, giving two main divisions of the facial nerve. The parotid gland is divided into a superficial and deep lobe based on the course of the facial nerve as it passes through. In the area of the incisional biopsy of the parotid gland, the distance between the gland surface and the facial nerve is approximately 1.5 cm.



rotid capsule is exposed by blunt dissection. The capsule of the gland is carefully opened and a small amount of superficial parotid tissue is removed. The procedure is completed with a 2 to 3-layered closure. The capsule must be cautiously closed to avoid future leakage or development of sialocele (Figure 6).

The technique was slightly modified by the present authors with an incision below and slightly behind the earlobe (Figure 7). The capsule of the parotid gland and subcutaneous tissue is closed with 4-0 Vicryl® sutures, whereas the skin is closed with 5-0 Ethilon® sutures. In this way, aesthetic results are excellent and future scar is invisible to the eye from anterior/lateral point of view.

Pijpe and coworkers established a new set of validated histopathological criteria for diagnosing SS according to the AECG classification criteria based on biopsy of the parotid gland (Table 1) [1]. A parotid biopsy was considered positive if one of the two following criteria was fulfilled:

i. a focus score of ≥1, defined as the number of lymphocytic foci (which are adjacent to normal-appearing acini and contain >50 lymphocytes) per 4 mm^2 of glandular parotid tissue (including fat tissue), irrespective of the presence of benign LELs.

ii. small lymphocytic infiltrates, not fulfilling the criterion of a focus score of ≥1, in combination with the presence of benign LELs.

Complications

Despite the potential risk of facial nerve damage, the development of sialoceles and salivary fistulae, temporary change in sensation in the skin area of the incision is the only well documented complication described to date [1,7].

Suitability for diagnostic and treatment evaluation purposes

Parotid biopsies allow the clinician to monitor disease progression and to assess the effect of an intervention treatment in SS. This is feasible due to the fact that parotid tissue can be harvested easily, repeated biopsies from the same parotid gland are possible, and the histopathological results can be compared with other diagnostic results derived from the same gland (e.g. secretory function, sialographic appearance, and ultrasound) [25]. Additionally, by performing parotid biopsies as a routine diagnostic procedure for SS, LELs and lymphomas located in the parotid gland can be identified [7,26].

Sublingual salivary gland biopsy

The sublingual salivary gland is the smallest of the major salivary glands. It lies in the floor of the mouth on both sides of the tongue and is covered only by oral mucosa. There are a few reports about taking a biopsy of the sublingual salivary gland for the diagnosis of SS [12,27,28]. The technique is performed with an 1 cm linear mucosal incision in the floor of the mouth, 1 cm anterolaterally from Wharton's duct to 1 cm antero-posteriorly [12,28,28].

Complications

The post-operative complications of sublingual salivary gland biopsy are [12,27]:

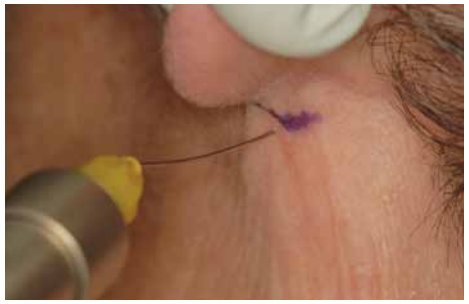
1. Ligaturing Wharton's duct, resulting from the placement of sutures;
2. Bleeding;
3. Swelling in the floor of the mouth.

Comparison of techniques (Table 2)

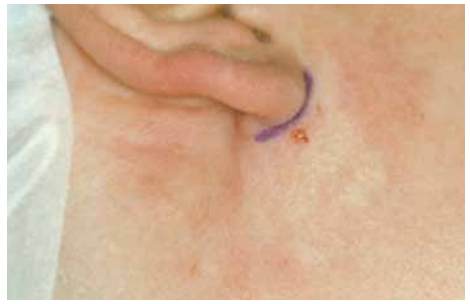
Although focal lymphocytic sialadenitis in the labial salivary gland is a widely accepted criterion for histological confirmation of SS, biopsies of the labial salivary glands may have several disadvantages. The sensitivity and specificity of labial salivary gland biopsies vary in the literature. Data from different studies are often difficult to compare because different sets of criteria for diagnosing SS have been used and the outcome of the labial biopsy is a strong determinant for the final diagnosis. In a normal population, the labial biopsy resulted in 6–9% false-positive diagnoses, and 18–40% of the patients with a clinical diagnosis of SS have a negative labial biopsy, resulting in a sensitivity of 60–82% and a specificity of 91–94% (Table 3) [14,29-33]. According to the ACR classification criteria, the labial biopsy has a sensitivity of 89.8 (95% CI: 87.2–92.0), but a lower specificity of 74.3 (95% CI: 71.0–77.5) [19]. Moreover, it may be difficult to harvest a sufficient number of labial salivary glands in atrophic submucosa of patients with longstanding SS [30]. In addition, permanent sensory loss of the mucosa of the lower lip, occurring in 1–10% of the patients, is a known complication of a labial biopsy [7,14,15]. Pijpe and coworkers report sensory loss in 6% of patients after labial biopsy, while no permanent sensory loss was observed after parotid biopsy [1].

Incisional biopsy of the parotid gland can overcome most of the disadvantages of the labial biopsy. When evaluating the parotid and the labial biopsy, sensitivity and specificity are comparable (Table 3), estimated in 78% and 86% respectively [1]. Parotid gland tissue can be harvested easily, repeated biopsies from the same parotid gland are possible (an important asset in studies assessing the efficacy of a treatment in SS patients or monitoring disease progression), and the histopathological results can be compared with other diagnostic results derived from the same gland (secretory function, sialographic appearance, ultrasound). In contrast to labial salivary glands, LELs are often observed in parotid gland tissue of SS patients. These LELs, a characteristic histological feature of the major salivary glands

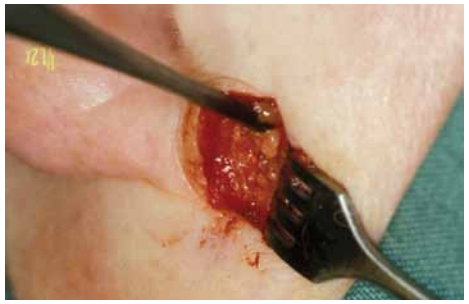
Figure 6: Incisional biopsy of the parotid gland.



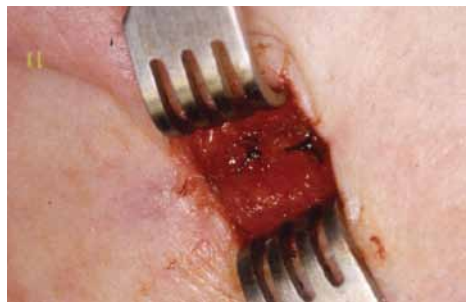
A. The area is anesthetized with local infiltration anesthesia.



B. With a No 15 blade a small 1-2 cm incision is made just below and behind the earlobe near the posterior angle of the mandible.



C. The skin is incised and the parotid capsule is exposed by blunt dissection. The capsule of the gland is carefully opened and a small amount of superficial parotid tissue is removed.



D. The procedure is completed with a 2 to 3-layered closure with 4-0 gauge absorbable sutures (polyglycolic acid), while the skin layer is closed with 5-0 nylon sutures.

in SS [33], develop as a result of hyperplasia of ductal basal cells within a lymphocytic infiltrate. In addition, well-formed lymphoid follicles or germinal centers, often adjacent to ductal epithelium, can be found in the major salivary glands [34]. Since both LELs and reactive lymphoid follicles are also indicative of malignant lymphoma, benign LELs must be discriminated from (pre)malignant lesions, using strict criteria [35,36].

Four to seven per cent of patients with SS develop malignant B cell lymphoma [37, 38], 48%–75% of which are of the MALT-type. These B cell lymphomas are most frequently located in the parotid gland [39,40,41]. Assessment of SS patients who may have developed a MALT lymphoma is not always easy, but an incisional biopsy of the parotid gland can safely be performed under local anesthesia [4] and can help towards this diagnosis. Pollard and coworkers have established an algorithm

Figure 7: The technique of the biopsy of the parotid gland was slightly modified by the present authors with an incision below and slightly behind the earlobe.

for the management of MALT-type lymphoma of parotid gland and associated SS (MALT-SS), showing the importance of a parotid gland biopsy for controlling the disease [26].

Additionally, in pediatric patients with clinical suspicion of SS and a negative minor salivary gland biopsy result, a parotid gland biopsy could be safe and effective in order to establish histopathologic evidence for the diagnosis of SS [43].

Finally, it is noteworthy that the pain following labial and parotid biopsies is comparable in severity and disappears within 1 month [1].

Notwithstanding these aforementioned advantages, biopsies of the parotid gland have not become commonplace because of the concern for damage to the facial nerve, development of sialoceles and salivary fistulae (Table 2). In addition, parotid gland biopsies are not part of the established criteria for diagnosing SS and demand higher surgical expertise. They are validated for the AECG classification criteria, but not yet for the ACR classification criteria.

Comparison of sublingual gland biopsy to labial gland biopsy has shown that the sensitivity of sublingual gland biopsy is better than the one of the labial gland biopsy, while the specificity of the latter is better than that of the former (Table 3) [27]. As far as the post-operative complications are concerned, researchers claim that sublingual gland biopsy is a relatively safe procedure (Table 2). Owing the fact that placing a suture might increase the risk of ligaturing Wharton's duct and lead to swelling of the floor of the mouth, no suture [28] or careful placement of one to two sutures could be an alternative [29]. A damage to the mental nerve is obviously not feasible, because of the operation site, while a damage to the lingual nerve related to this biopsy technique has never been reported in the literature. Advanced risk

Table 2: Comparison of techniques [5-13,24,27,28,42].

	Technique	Advantages	Complications
Labial gland			
Chisholm and Mason, 1968	Ellipse of oral mucous membrane down to the muscle layer. Harvest of 6-8 glands. Wound closure with 4-0 gauge silk sutures, which must be removed after four-five days.	1. Widely distributed glands 2. Easily accessible glands 3. Minimal chance of bleeding	1. Temporary or permanent alteration in sensation in the area of the incision 2. External haematoma 3. Local swelling 4. Granulomas formation 5. Internal scarring and cheloid formation 6. Suture failing 7. Local pain
Greenspan et al., 1975	1.5-2 cm linear incision of mucosa, parallel to the vermillion border and lateral to the midline		
Marx et al., 1988	Mucosal incision of 3x0.75 cm		
Delgado and Moscueda, 1989	Longitudinal incision of 1 cm in the labial mucosa in front of the mandibular cuspids		
Guevara-Gutierrez et al., 2001	Punch biopsy		
Mahlsted et al., 2002	1-1.5 cm wedge-shaped incision between the midline and commissure		
Gorson and Ropper, 2003	1 cm vertical incision just behind the wet line through the mucosa and submucosa		
Berquin et al., 2006	Oblique incision, starting 1.5 cm from the midline and proceeding latero-inferiorly, avoiding the glandular free zone in the center of the lower lip		
Caporali et al., 2008	Small incision of 2-3 mm on the inner surface of the lower lip		
Parotid gland			
Kraaijenhagen 1975 Marx et al., 1988 Mc Guirt et al., 2002 Baurmash et al., 2005 Pijpe et al., 2007	1-2 cm incision just below and behind the earlobe near the posterior angle of the mandible. The skin is incised and the parotid capsule is exposed by blunt dissection. The capsule of the gland is opened and adequate amount of superficial parotid tissue is removed. The procedure is completed with a 2 to 3-layered closure	1. Presence of LELs 2. Identification of MALT 3. Possibility of repeated biopsy from the same gland 4. Comparison with other diagnostic results derived from the same gland (e.g. secretory function, sialographic appearance)	1. Temporary alteration in sensation in the area of the incision 2. Facial nerve damage 3. Sialoceles 4. Salivary fistulae 5. Risk of harvesting only fat tissue 6. Demanding surgical expertise
Sublingual salivary gland			
Pennec et al., 1990	Incision between the first premolar and the lateral cutting tooth	Collection of sufficient amount of tissue	1. Uncomfortable scars 2. Bleeding 3. Swelling in the floor of the mouth 4. Risk of ligaturing Wharton's duct 5. Not established histopathologic criteria
Adam et al., 1992 Berquin et al., 2006	Mucosal incision 1 cm anterolarerally from the Whartonian duct to 1 cm anteroposteriorly. Blunt dissection and harvest of 0.5 ml of gland. The wound edges are joined with 1-2 resorbable stiches		

Table 3: Sensitivity and specificity of biopsy techniques [1,27].

Technique	Sensitivity	Specificity
Labial gland biopsy	60-82%	91-94%
Parotid gland biopsy	78%	86%
Sublingual gland biopsy	66%	52%

of bleeding is encountered in cases a more posterior incision is made, which, however, resolves spontaneously [12]. To date, specialized histopathological criteria have not been established for the diagnosis of SS after a sublingual gland biopsy and researchers merely employed the criteria for labial gland biopsies [12,27,29].

Conclusion

Early diagnosis and treatment are of high importance for preventing the complications associated with SS. Unfortunately, so far there is not a single test capable of confirming the diagnosis of SS. A positive salivary gland biopsy provides strong evidence, which in correlation with additional diagnostic tests can establish the classification of SS. Parotid gland biopsy is a relatively simple technique, has the potential to overcome most of the disadvantages of the labial biopsy and can additionally aid in monitoring disease progression and the effect of an intervention treatment in SS.

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Chapter 4

Treatment

Chapter 4A

Towards personalized treatment in primary Sjögren's syndrome: baseline parotid histopathology predicts responsiveness to rituximab treatment

Konstantina Delli[1]*, Erlin A Haacke[2,3]*, Frans GM Kroese[2], Rodney P Pollard[1], Stephan Ihrler[4], Bert van der Vegt[3], Arjan Vissink[1], Hendrika Bootsma[2], Frederik KL Spijkervet[1]

Affiliations

1. Department of Oral and Maxillofacial Surgery, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands

2. Department of Rheumatology and Clinical Immunology, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands

3. Department of Pathology and Medical Biology, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands

4. Laboratory for Dermatohistology & Oral Pathology, Munich, Germany

* Authors contributed equally to the paper

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Abstract

Objectives: The aims of this study were (1) to assess the effect of rituximab (RTX; anti-CD20) treatment in primary Sjögren's syndrome (pSS) patients based on sequential parotid biopsies obtained in a placebo-controlled, randomized clinical trial, and (2) to assess the prognostic value of the histological characteristics of parotid gland tissue with regard to responsiveness to RTX treatment.

Methods: In a double-blinded, placebo-controlled trial, sequential parotid gland biopsies were taken from 20 RTX-treated and 10 placebo-treated pSS patients, at baseline and 12 weeks after treatment. The relative amount of lymphocytic infiltrate (stained for CD45), absolute number of T-cells and B-cells per mm^2 parenchyma (stained for CD3 and CD20, respectively), focus score, number of germinal centers and of lymphoepithelial lesions per mm^2 of parotid gland parenchyma were assessed. Histopathological data were compared between clinical responders (decrease in European League Against Rheumatism EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) score of ≥3 at 12 weeks compared to baseline) and non-responders (change in ESSDAI <3) to RTX treatment.

Results: In RTX-treated patients, a significant reduction in the number of $CD20^+$ B-cells/mm^2 parenchyma was observed, while no such reduction was observed in placebo-treated patients. The number of $CD3^+$ T-cells/mm^2 in parenchyma did not change in either group. Furthermore, the number and the severity of lymphoepithelial lesions/mm^2 and number of germinal centers/mm^2 was significantly reduced in RTX-treated patients, but did not change in placebo-treated patients. When comparing the pre-treatment characteristics of clinical responders with non-responders, the median number of $CD20^+$ B-cells/mm^2 parenchyma at baseline was significantly higher in responders (1871 versus 353 cells/mm^2, $p<0.05$). Other histopathological baseline characteristics were not predictive for response to RTX treatment.

Conclusion: RTX treatment in pSS leads to a major reduction of lymphocytic infiltration and to fewer B-cells, germinal centers and lymphoepithelial lesions in parotid gland parenchyma. A high pre-treatment number of $CD20^+$ B-cells/mm^2 parotid gland parenchyma predicts better responsiveness of pSS patients to RTX treatment. Pre-treatment parotid gland histopathological characteristics could therefore contribute to a more personalized treatment approach to pSS.

Introduction

Primary Sjögren's syndrome (pSS) is a common rheumatic disease, with a prevalence of 60.8 (95% CI: 43.7 to 77.9) cases per 100,000 inhabitants in the total population [1]. pSS commonly affects salivary and lacrimal glands, resulting in a sensation of dry mouth (xerostomia) and dry eyes (keratoconjunctivitis sicca). Although the exact pathogenic mechanism has not been fully elucidated, in patients with pSS the minor and major salivary glands are characteristically infiltrated by mononuclear lymphoid cells, which form periductal foci. The classic glandular lesion is composed of a lymphoid infiltrate of T and B lymphocytes, whose distribution may vary according to lesion severity [2]. A central role is attributed to B-cells, which tend to be hyperactive [3]. pSS patients have an increased risk of developing lymhoproliferative diseases, which is about 4% during the first 5 years, 10% at 15 years and 18% after 20 years post-diagnosis [4]. Consequently, about 7.5% of patients with pSS develop malignant B-cell lymphoma. In 48-75% of these cases, this is the mucosa-associated lymphoid tissue (MALT) type of lymphoma [5-7]. Most commonly, these lymphomas arise in the parotid glands. The assumed role of hyperactive B-cells in the pathogenesis of pSS is supported by the observed beneficial objective and subjective clinical effects of B-cell depletion by rituximab (RTX), a chimeric monoclonal antibody that binds to the B-cell surface antigen CD20 [8-16]. Significant response was observed in most trials, except from one large randomized clinical trial, the TEARS study [17]. Posthoc application of the Sjögren's syndrome response index (SSRI) showed also significant response rate difference between RTX and placebo in TEARS [18]. Because there are some concerns about the efficacy of rituximab, the TRACTISS study is aiming to provide evidence whether rituximab improves the clinical outcomes [19]. The final results of the TRACTISS study, including a subanalysis on responders and non-responders, are eagerly awaited.

In a previous open-label phase II study, based on sequential parotid biopsies of 5 pSS patients, we showed that RTX treatment might result in restoration of secretory tissue at a glandular level in responding patients [20]. In that study we observed a reduction of the lymphocytic infiltration with partial to complete loss of germinal centers (GC) and redifferentiation of lymphoepithelial lesions (LEL) to regular striated ducts. However, major limitations of the study by Pijpe et al. were the small number of patients and lack of a placebo group [20]. Therefore, the aims of the current study were (1) to assess the effect of RTX treatment in pSS patients based on sequential parotid biopsies obtained in a placebo–controlled, randomized clinical trial, and (2) to assess the prognostic value of the histological characteristics of parotid gland tissue with regard to responsiveness to RTX treatment.

Materials and methods

Patients

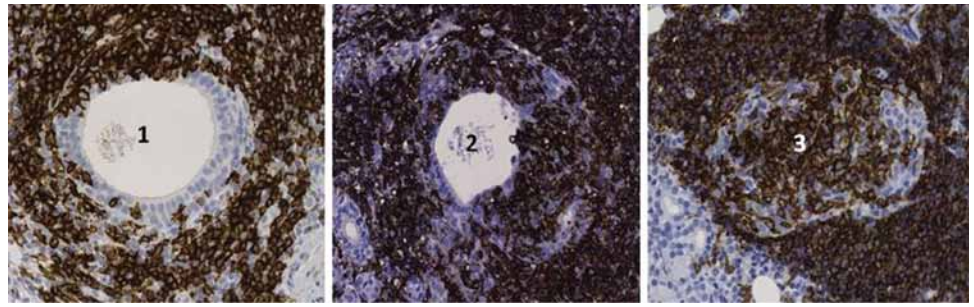
Thirty patients with pSS were treated in a randomized double-blinded placebo-controlled trial on days 1 and 15 with either 1000 mg RTX i.v. (Roche, Woerden, the Netherlands; n= 20) or placebo i.v. (n= 10) at the University Medical Center Groningen, the Netherlands, as described before [14]. All patients fulfilled the American European Consensus Group (AECG) criteria for pSS [21]. To minimise side effects (infusion reactions, serum sickness), all patients, including the placebo treated patients, were pre-medicated with methylprednisolone (100 mg i.v.), acetaminophen (1000 mg p.o.) and clemastine (2 mg i.v.), and received 60 mg oral prednisone on days 1 and 2, 30 mg on days 3 and 4, and 15 mg on day 5 after each infusion.

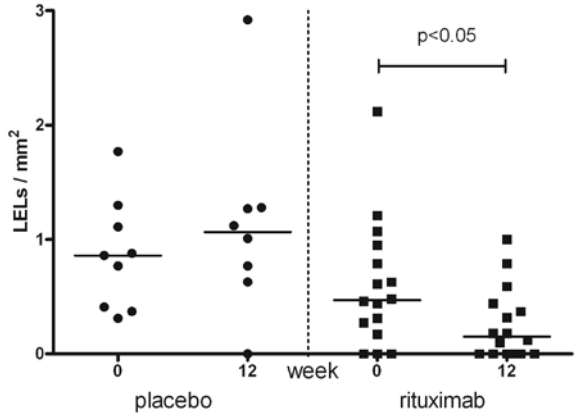
An incisional biopsy was taken under local anesthesia from the same parotid gland before and 12 weeks after therapy [22]. The European League Against Rheumatism (EULAR) Sjögren's Syndrome Disease Activity Index (ESSDAI) [23,24] was assessed at the same time points by two experienced rheumatologists, who were blinded to the treatment group, in order to evaluate possible systemic complications [16]. With regard to response to RTX treatment, patients were categorized into two groups: clinical responders, if the decrease in ESSDAI was 3 points or more at 12 weeks after treatment compared to baseline, and clinical non-responders, if the change of ESSDAI was less than 3 points. The cut-off of 3 points was chosen because this difference indicates a clinically relevant effect [25]. All patients provided informed consent in accordance with the ethics committee of the University Medical Center Groningen (METC approval: 05.229).

Histopathologic analysis

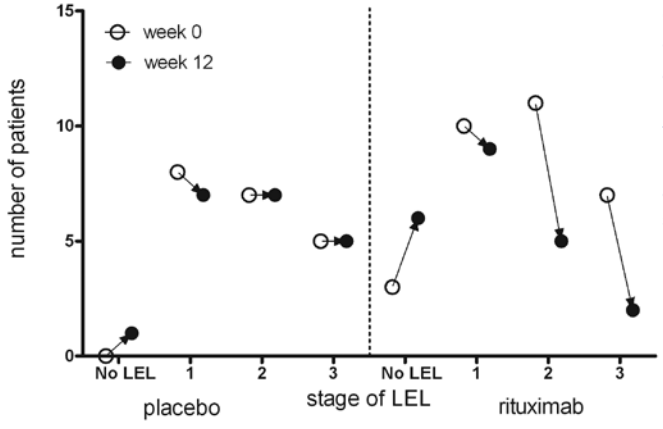
Parotid gland tissue biopsies were fixed in 4% formaldehyde, embedded in paraffin, cut at a thickness of 3 µm, and stained with hematoxylin and eosin. The focus score (defined as ≥50 lymphocytes per 4 mm^2 glandular tissue), and the number of GC/mm^2 parotid gland parenchyma were determined. GC were defined as a clearly visible lighter area in a lymphocytic infiltrate containing cells that are usually present in classical germinal centers, like follicular dendritic cells, centrocytes or centroblasts and macrophages. LEL are expressed in LEL/mm^2 of parotid gland parenchyma, excluding intraparenchymal connective and fat tissue. LEL were evaluated in immunohistochemically stained tissue sections, stained for CD20 as B-lymphocytes predominate in the LEL. A LEL was defined as a cross section of a striated duct with infiltration of $CD20^+$ B-cells within the basement membrane combined with hyperplasia of the epithelium. To evaluate regeneration of the ducts, LEL were subcategorized into three stages (Figure 1A [20]): stage 1) LEL affecting less than 50% of the epithelium of the striated duct (partial LEL); stage 2) LEL affecting be-

Figure 1: (A) Classification of lymphoepithelial lesions (LEL) in three stages as stained with CD20 [20]: (1) LEL affecting less than 50% of the epithelium of the striated duct (partial LEL); (2) LEL affecting between 50% and 100% of the epithelium of the striated duct (developed LEL); (3) LEL with fully circumferential affected epithelium without lumen (occluded LEL).





(B): Number of LEL/mm^2 of parenchyma in parotid glands of patients with primary Sjögren's syndrome (pSS). Horizontal lines indicate median values.



(C): Presence of three stages of LEL/mm^2 in placebo-treated and rituximab (RTX)-treated patients at week 0 and week 12; y axis indicates the total number of patients, while x axis the presence of different stages of LEL.

tween 50% and 100% of the epithelium of the striated duct (developed LEL); stage 3) LEL with fully circumferentially affected epithelium without lumen (occluded LEL). For histopathological evaluation, biopsies were independently scored by 2 investigators (R.P.P. and S.I.) in a blinded setting. In case of discrepancy, a definitive score was established by consensus.

Immunohistochemical analysis

Immunostaining was utilized for the analysis of lymphocytic infiltrate and was performed as follows. Parotid glands were fixed in 4% buffered formaldehyde, embedded in paraffin wax and sectioned into 4-µm-thick serial sections. Sections were stained after deparaffinisation, pre-treatment with Ultra CC1 (Ventana Medical Systems, Inc, USA), antigen retrieval and endogenous peroxidase blocking using the Benchmark machine. Sections were immunohistochemically stained with anti-CD45 (dilution 1:25, Dako, Heverlee, Belgium, clone 2B11+PD7/26), anti-CD79a (dilution 1: 100, Dako, Heverlee, Belgium, clone JCB117), anti-CD20 (dilution 1:200, Dako, Heverlee, Belgium, clone L-26) and anti-CD3 (dilution 1:20, Monosan, Uden, the Netherlands, clone PS-1) antibodies. The sections were then treated with peroxidase-labelled secondary antibody and visualized with the chromogen DAB (3,3′ Diaminobenzidine) solution.

The relative amount of CD45 positive lymphocytic infiltrates was assessed in relation to the total amount of tissue parenchyma by morphometry with use of ImageJ software (v1.46). Using HistoQuest software, version 3.5.3.0171, two markers were created, the DAB master marker (CD20) and the hematoxylin non-master marker (nucleus). For the master marker, multiple reference shade was set on 8 with a background threshold range of 5–255. Ring mask and identified cell mask were used. By using a color picker, the shade was chosen directly from a positively stained CD20 cell. One whole section was analyzed excluding intraparenchymal connective and fat tissue leaving multiple regions of interest (ROIs). For the assessment of $CD20^+$ cells, scattergrams were created for each ROI, allowing the visualization of corresponding positive cells in the source ROI, using the real-time back-gating feature. To correct false events, a specific gate according to cell size and intensity of CD20 staining was defined and applied to all analyzed samples. $CD20^+$ cells were quantified according to the selected marker and gate. By using the real-time back-gating feature, automatically counted $CD20^+$ cells were visualized and controlled. The $CD20^+$ cell count (number of cells/mm^2) for each analyzed ROI was obtained. The same procedure was followed for $CD3^+$ cell count.

Statistical analysis

Analysis was carried out with IBM SPSS Statistics 20 (SPSS, Chicago, Illinois, USA). Mann-Whitney U test was used to compare differences between the RTX and place-

Table 1: Histopathological and immunohistochemical data [median values and interquartile range (Q1-Q3)] before and after RTX or placebo therapy.

	Placebo (n=9)			RTX-treated (n=16)		
	baseline	**week 12**	**p**	**baseline**	**week 12**	**p**
Focus score	1.63 (0.84-3.27)	1.97 (1.47-2.88)	0.678	1.7 (0.87-2.5)	1.19 (0.59-1.23)	0.179
LELs/mm^2	0.77 (0.38-1.05)	1.11 (0.67-1.23)	0.310	0.48 (0.24-0.91)	0.18 (0-0.42)	**0.011**
GCs/mm^2	0.06 (0-0.23)	0.09 (0.03-0.15)	0.735	0.07 (0-0.28)	0 (0-0.1)	**0.004**
CD45 (%)	15.2 (5.86-16.92)	14.8 (5.2-28.06)	0.374	7.45 (1.85-22.35)	3.96 (0.48-7.71)	**0.011**
$CD20^+$cells/mm^2	2709 (1469-4395)	3664 (2256-7979)	0.173	1172 (389-5278)	355 (51-743)	**0.001**
$CD3^+$cells/mm^2	863 (359-1483)	1712 (725-2878)	0.953	315 (124-2157)	180 (91-570)	0.535

bo groups or between clinical responders and non-responders. Wilcoxon signed-rank test was used to compare differences over time within groups. Spearman's correlation coefficient was used to analyze the relationship between histopathology and ESSDAI. Correlations (ρ) <0.3 were interpreted as a poor association, 0.3–0.6 as moderate, 0.6–0.8 as good and >0.8 as excellent [15]. P-values <0.05 were considered as statistically significant. Power analysis was performed with Statistical Power Calculator (DDS Research, Washington DC, USA).

Results

From the total group of 30 patients, five patients had to be excluded from histopathological analysis, due to serum sickness (n=1, RTX-group) or insufficient biopsy material (n=3, RTX-group); one patient dropped out of the study (placebo group). Thus, complete evaluation could be performed of parotid gland biopsies taken from 16 RTX treated patients and 9 placebo-treated patients.

i. Lymphocytic infiltrate in parotid glands

No differences at baseline between the RTX-treated group and the placebo-treated group were found regarding the focus score, relative area of CD45 staining, numbers of $CD20^+$ B-cells and $CD3^+$ T-cells and proportion of biopsies containing GC (data not shown).

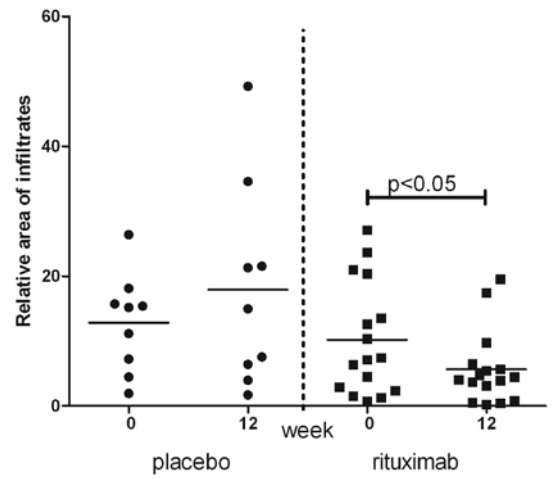
After treatment, the focus score did not change significantly in either the RTX-treated group or the placebo-treated group (Table 1). However, CD45 staining demonstrated a significant decrease of the relative area of infiltrates at 12 weeks after RTX treatment. In the placebo group no change was observed between baseline values and 12-week post-treatment values (Figure 2, Table 1).

By counting the number of $CD20^+$cells/mm^2 of parenchyma, a significant decrease was observed in the number of B-cells (1172 versus 355 cells/mm^2, p=0.001) in the glandular tissue at 12 weeks after RTX treatment compared to baseline (Figure 3, Table 1). In the placebo-treated group, the number of $CD20^+$/mm^2 of parenchyma of the parotid glands at week 12 was not statistically different from the number of $CD20^+$ cells at baseline (Table 1).

The number of $CD3^+$cells/mm^2 of parenchyma remained unaffected after 12 weeks both in the placebo and RTX-treated group (Table 1).

GC were present at baseline in 67% and 68% of the parotid glands of the placebo and RTX-treated patients, respectively. RTX treatment resulted in a significant decrease in the total number of GC/mm^2 (Figure 4, Table 1). Twelve out of 16 parotid glands (75%) were completely devoid of GC 12 weeks after treatment with RTX. In

Figure 2: Effect of placebo (n=9) and rituximab (RTX) (n=16) treatment on relative areas of infiltrates (stained with CD45; %), in parotid gland parenchyma of patients with primary Sjögren's syndrome (pSS). Horizontal lines indicate median values.



the placebo group, no significant difference was observed in the number of GC/mm^2 between baseline levels and 12 weeks after treatment.

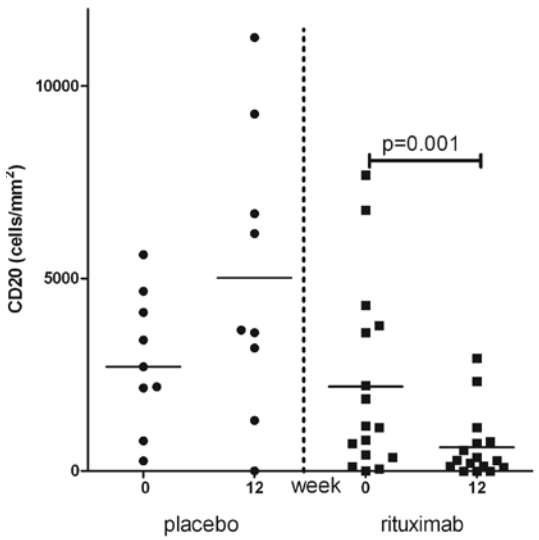
ii. Lymphoepithelial lesions

At baseline, no differences were observed in the presence of LEL in the parotid gland parenchyma between the group of RTX-treated patients and the placebo-treated patients (data not shown). In the RTX-treated group, a significant decrease in the total number of LEL/mm^2 was observed after 12 weeks of treatment (Figure 1B, Table 1). In 6 out of 16 patients (38%), LEL were completely absent after RTX treatment. In the placebo group, no significant change was observed in the amount of LEL/mm^2 after 12 weeks (Figure 1B, Table 1). Besides the number of LEL/mm^2, the severity of the lesions also appeared to decrease; all stages of LEL seemed to transform to a less severe stage. Detailed data regarding the presence of all stages of LEL/mm^2 in placebo and RTX-treated patients at week 0 and week 12 is presented in Figure 1C.

Histopathology and ESSDAI

Of the 16 patients that were treated with RTX, 11 patients (69%) improved by 3 or more ESSDAI points and were therefore considered to be clinical responders

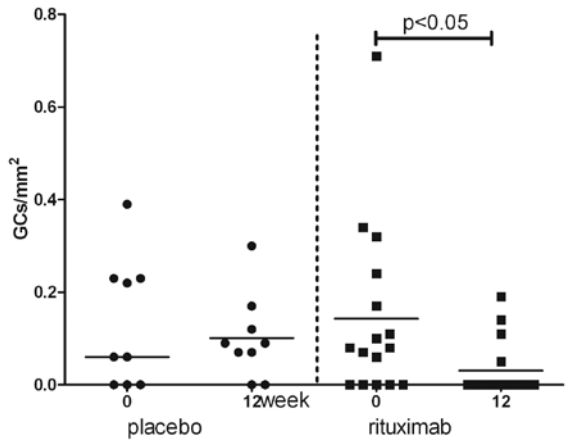
Figure 3: Effect of placebo (n=9) and rituximab (RTX) (n=16) treatment on number of CD20⁺ cells/mm² of parenchyma in parotid glands of patients with primary Sjögren's syndrome (pSS). Horizontal lines indicate median values.



[25]. The other 5 were considered to be non-responders. The supplementary table shows the number (%) of patients having any degree of activity per ESSDAI domain (score at least 1) before and after RTX therapy, stratified for responders and non-responders.

The baseline (pre-treatment) histopathological parameters (CD20⁺ cells/mm², CD3⁺ cells/mm², CD45⁺ relative infiltrate, GC/mm², LEL/mm² and focus score) as well as CD19-positive B-cell subsets determined by flow-cytometry in the peripheral blood (i.e., $CD38^{Low}CD27^{-}$, $CD38^{High}CD27^{-}$, $CD27^{Low}CD38^{-}$, $CD27^{High}CD38^{-}$, $CD38^{Low}CD27^{Low}$, $CD38^{High}CD27^{High}$, $CD27^{Low}CD38^{High}$, $CD27^{High}CD38^{Low}$,$CD38^{-}CD27^{-}$) of responders and non-responders to RTX treatment were subjected to additional statistical analysis. In responders, the baseline number of CD20⁺ cells/mm² was significantly higher in comparison to non-responders [1871 (Q1-Q3=801-4310) cells/mm² versus 353 (Q1-Q3=35-2102) cells/mm²; Figure 5]. At an alpha level of 5%, it was calculated that the number of responders and non-responders would give us a power of 94.2% to assume that their baseline number of CD20⁺ cells/mm² could serve as potentially prognostic factor with regard to responsiveness to RTX treatment. The other baseline histological characteristics, as well as baseline B-cell subsets determined by flow-cytometry in the peripheral blood, did not differ significantly between responders and non-responders. Of note, there was no correlation between the absolute numbers of CD20⁺ B-cells/mm² of parenchyma and ESSDAI or between B-cell subsets in peripheral blood and ESSDAI.

Figure 4: Number of germinal centres (GCs)/mm² of parenchyma in parotid glands of patients with primary Sjögren's syndrome (pSS). Horizontal lines indicate median values.



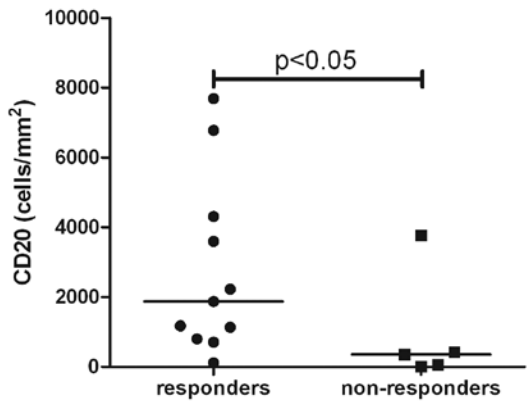
Furthermore, in RTX treated patients the change in ESSDAI correlated with the change in the number of CD20⁺ cells/mm² of parenchyma (ρ=0.706 and p<0.05). No other statistically and clinically significant correlations were found for the changes between baseline and 12 weeks.

Discussion

We demonstrated that RTX treatment significantly reduced the overall lymphocytic infiltrate with a major loss of the B-cell component and number of GC/mm² of parotid gland parenchyma in pSS patients. In addition, a major reduction of the quantity and severity of LEL was apparent, reflecting significant restoration of the striated ducts.

RTX treatment results in a considerable decrease in the number of B-cells in the parotid gland tissue. Although this is reflected by a decrease in the amount of infiltrate, as measured by staining for CD45, this is not manifested by a decrease in the focus score. This apparent discrepancy can be explained by the fact that the foci also contain high numbers of T-cells, which may outnumber the number of B-cells [2], and which are not affected in significant numbers by RTX treatment. The focus score is therefore not an appropriate criterion to measure the local effect of RTX on the periductal lymphocytic infiltration. Although RTX treatment results in the almost complete absence of B-cells in the peripheral blood of patients with pSS [26], this is thus not accompanied by a complete loss of B-cells in parotid salivary gland tissue. These results are in line with other studies in pSS [20,28] and rheuma-

Figure 5: Number of baseline CD20⁺ cells/mm² in clinical responders (n=11) and non-responders (n=5), p<0.05. Horizontal lines indicate median values.



toid arthritis [29-31] showing a certain degree of persistence of B-cells in the local tissue after RTX treatment. In contrast, Devauchelle-Pensec et al. reported a total depletion in B-cells in labial salivary glands of pSS patients after RTX treatment [10]. However, in that study only a very low number of the pSS patients (6 out of 15) showed significant numbers of B-cells in the periductal infiltrates at baseline. This is remarkable, since B-cells usually make up to 20-60% of the lymphocytes in the infiltrates of the labial glands of pSS patients, depending upon the grade of the lesion [2].

In this study, at baseline the included patients had high systemic activity, as indicated by the relatively high ESSDAI scores, and the high numbers of GC/mm² [32]. We observed a strong reduction of GCs in the parotid tissue after RTX treatment; in several patients we even observed a complete absence of GCs. This is striking, since not all B-cells are depleted in the parotid glands, and GC B-cells may be relatively more resistant for anti-CD20 therapy compared to other B-cells, as shown by Gong et al. in a murine model for human CD20 expression [33]. A possible explanation for the strong depletion of GCs in the parotid tissue might be that RTX treatment also results in a significant reduction of follicular helper T cells (T_{FH}), as indicated by analysis of peripheral blood samples (Verstappen et al. 2015, manuscript in preparation). T_{FH} cells are essential for the development of GCs at local sites. These cells are present in the salivary gland tissue of pSS patients [34], where they may drive GC formation and generation of plasma cells. It is therefore possible that the relative absence of T_{FH} in the salivary gland tissue after RTX treatment contributes to the loss of GC activity in these pSS patients.

LEL develop in striated ducts in pSS patients, particularly in the parotid glands. The epitheliotropic autoimmune inflammation of the intraepithelial lymphocytes results in the reaction of the epithelium and induction of these lesions [35]. RTX

treatment not only results in a significant reduction of the number CD20⁺ B-cells in the periductal infiltrates, but also in a recovery of the LEL, as revealed by a considerable reduction of the severity of the lesions at all stages (Figure 1C). Such a restoration/redifferentiation of LEL was also observed in the small RTX treatment study (5 patients) described by Pijpe et al. [20]. Apparently, RTX treatment also results in depletion of B-cells within the basement membrane of striated ducts. To explain this, we have hypothesized that the trigger for LEL formation is diminished, and as a result less epithelial reaction takes place leading to reduced proliferation and finally anatomical restoration of the striated ducts. The trigger for LEL formation is unknown, but B-cell derived cytokines may possibly be responsible for this. This notion is in line with the finding of Pollard et al., who showed in the same cohort of RTX-treated patients that the serum levels of pro-inflammatory cytokines (e.g. IL-6) decreased significantly [36].

Patients with pSS have different genetic backgrounds, demographic features and prognosis and exhibit a wide variety of clinical manifestations, involving a number of pathophysiological pathways [37]. Personalized treatment, i.e. providing 'the right patient with the right drug at the right dose at the right time' [38], will therefore be the key to treating pSS. An important finding in our study was that clinical responders to RTX treatment had a higher number of CD20⁺ B-cells/mm² of parenchyma of parotid gland tissue at pre-treatment (baseline) compared to non-responders. Furthermore, we also observed a correlation between the change in the number of CD20⁺ cells/mm² of parenchyma and the change in ESSDAI. When higher numbers of B-cells are present in the parotid gland parenchyma, it is therefore possible that RTX treatment may result in depletion of more absolute numbers of B-cells responsible for the disease activity (measured by ESSDAI) than when lower numbers of B-cells are present in the tissue. The baseline number of B-cells/mm² of parenchyma of parotid gland may therefore determine patients' response to treatment with RTX and may be considered as a biomarker for a more personalized treatment approach to pSS patients. The nature of these disease-associated B-cells that are reduced after RTX treatment needs to be elucidated. These cells are probably not antibody-producing cells, since antibody producing cells persist in the parotid salivary glands after RTX treatment [28]. Alternatively, these cells may represent cytokine-producing B-cells [36].

Although the change in number of B-cells in the infiltrates of the salivary glands correlated to the change in ESSDAI after RTX treatment, the absolute number of B-cells at baseline did not correlate to the ESSDAI. Furthermore, the focus score of the salivary glands did not correlate to the ESSDAI. However, Risselada et al. showed a significant correlation at baseline between the focus score and the cumulative ESSDAI in labial salivary glands of 174 pSS patients (ρ=0.166-0.284, p≤0.04) [39]. This discrepancy could be ascribed to the fact that the study by Riselada et al. was retrospective, where ESSDAI was assessed at any time point during disease (not necessarily at diagnosis and biopsy), that 21% of patients used immunomodulating medication at the time of the biopsy and correlations were considered to be sig-

nificant even if ρ was as low as 0.166-0.284. Moreover, the size of the focus (as we assessed by CD45 staining) is probably more relevant than the absolute number of present foci in the salivary gland tissue.

In conclusion, we demonstrated that in parotid gland tissue of pSS patients:

1. RTX treatment leads to major reduction of B-cells and a significant reduction in the number of GCs and LEL. This reduction in the LEL may be the consequence of a major decrease of local B-cell infiltration and may result in structural regeneration of the glands, especially the striated ducts.

2. The baseline number of $CD20^{+}$B-cells/mm^2 of parenchyma may serve as a prognostic biomarker to predict response to RTX treatment. As a result, baseline histopathological characteristics of a parotid biopsy may strongly contribute to a more personalized treatment approach to pSS patients.

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Supplementary table: Number (%) of patients having any degree of activity per ESSDAI domain (score at least 1) before and after RTX therapy, stratified for clinical responders and non-responders.

	responders		non-responders	
	before	after	before	after
cutaneous	1 (9)	0 (0)	0 (0)	0 (0)
pulmonary	5 (45)	0 (0)	0 (0)	0 (0)
renal	0 (0)	0 (0)	0 (0)	0 (0)
articular	8 (73)	2 (18)	5 (100)	5 (100)
muscular	0 (0)	0 (0)	0 (0)	0 (0)
peripheral nervous system	0 (0)	0 (0)	0 (0)	0 (0)
central nervous system	0 (0)	0 (0)	0 (0)	0 (0)
hematological	6 (55)	5 (45)	2 (40)	2 (40)
glandular	9 (82)	4 (36)	4 (80)	4 (80)
constitutional	1 (9)	0 (0)	0 (0)	0 (0)
lymphadenopathy	0 (0)	0 (0)	0 (0)	0 (0)
biological	11 (100)	8 (73)	2 (40)	2 (40)

Chapter 4B

In primary Sjögren's syndrome high absolute numbers and proportions of B cells in parotid glands predict responsiveness to rituximab as defined by ESSDAI, but not by SSRI

Konstantina Delli[1], Erlin A Haacke[2,3], Frans GM Kroese[2], Rodney P Pollard[1], Stephan Ihrler[4], Bert van der Vegt[3], Arjan Vissink[1], Hendrika Bootsma[2], Frederik KL Spijkervet[1]

Affiliations

1. Department of Oral and Maxillofacial Surgery, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands

2. Department of Rheumatology and Clinical Immunology, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands

3. Department of Pathology and Medical Biology, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands

4. Laboratory for Dermatohistology & Oral Pathology, Munich, Germany

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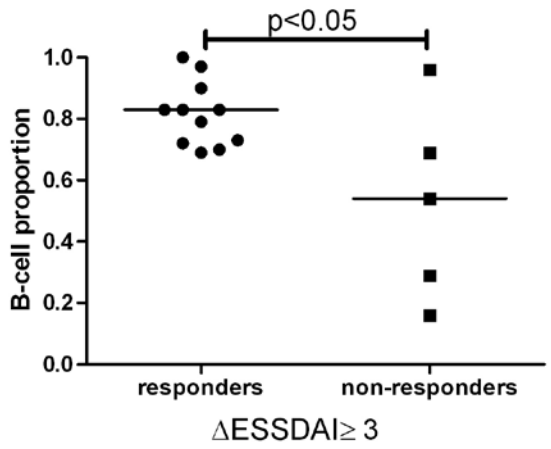
With great interest, we have read the article by Cornec et al. [1] regarding our paper 'Towards personalised treatment in primary Sjögren's syndrome (pSS): baseline parotid histopathology predicts responsiveness to rituximab treatment' [2]. In essence, we showed in our paper that absolute numbers of $CD20^+$ cells/mm^2 of parenchyma of parotid gland tissue are predictive for the responsiveness of patients with primary Sjögren's syndrome (pSS) to rituximab (RTX) treatment. Cornec et al. argue that there is a discrepancy in outcomes presented in their study and our study [1], as they observed that a high proportion of minor salivary gland B cells predict absence of a clinical response to RTX [3]. As we will show and explain here, there is no inconsistency between the two studies and most of the apparent discrepancy is likely the result of differences in how the tissues are analyzed and how the disease activity is established.

1. Absolute numbers versus proportions of B cells and technique applied

A major difference in the two studies is how B cells are assessed in tissue sections of salivary gland biopsies of pSS patients before (and after) RTX treatment. We measured absolute numbers of $CD20^+$ B cells/mm^2 of parenchyma, while Cornec et al. assessed the proportion of B cells [1,3]. Obviously, even when there is a change in absolute numbers of B cells in the tissue, the B/T cell ratio still can remain the same. Thus, although higher numbers of B cells, do not need to be reflected per se in higher proportions of B cells, we also found in our study that patients with higher absolute numbers of B cells in the glandular tissue, had a higher B/B+T cell ratio. Furthermore, responders to RTX, as defined by a decrease in European League Against Rheumatism EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) score of ≥3 (ΔESSDAI≥3) at 12 weeks after treatment compared to baseline [4], had a higher B/B+T cell ratio compared to non-responders (Figure 1).

Nevertheless, there are some technical differences between the two studies that warrant some attention. We counted the absolute number of cells with HistoQuest software (version 3.5.3.0171, Tissuegnostics, Vienna, Austria), a well-known and widely used image analysis software package within pathology. Since parotid gland biopsies include areas of fat and fibrous tissue and intra parenchymal lymph nodes, we excluded these areas manually from the analysis, in order to increase the accuracy of the data. We would like to emphasize that, as stated in the section on 'Immunohistochemical analysis' in the materials and methods of our article [2], the whole slide (except from fat and fibrous tissue) was evaluated and areas of interest were not electively chosen, as implied by Cornec et al. [1]. The methodology used by Cornec et al. is based on digital pixel counting procedure developed by Costa et al. [1,3,5]. We have some concerns about this method. First, with the method of Costa et al. extra-glandular areas are not excluded from the tissue specimen studied [5], which has the risk to include in the counting infiltrating cells located in areas of non-interest, e.g., intraparenchymal fat tissue, fibrous tissue, perineural tissue, etc. Second, although the number of pixels is reported to correlate to the

Figure 1: Proportions of baseline $CD20^+$ cells in clinical responders (n=11) and non-responders (n=5) as defined by ESSDAI, p<0.05. Horizontal lines indicate median values.

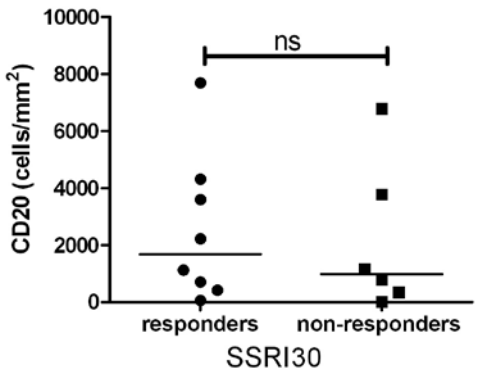


manually counted cells [5], the exact number of pixels corresponding to one cell remains unknown. Moreover, although the B-cell proportions assessed with the method of Costa et al. correlate moderately to focus scores [5], the focus score does not give an indication about the severity of inflammation, as discussed in our paper [2]. As a consequence, when the area covered by a single focus at baseline is rather large, a clinically relevant decrease in the inflamed area is not necessary reflected by a decrease in focus score. Apparently the HistoQuest method of counting the absolute number of cells is more precise in these aspects. Thus, it would be interesting to know whether the results reported by Cornec et al. change if biopsies would have been analyzed with the HistoQuest software approach [3].

2. ESSDAI versus SSRI

Another factor that contributes significantly to the apparent difference between the two studies concerns the way disease activity has been defined. Cornec et al. pose that the discrepancy in outcomes can partially be attributed to ineffectiveness of RTX in improving systemic involvement as measured by ESSDAI [1]. This prompted them to develop and use the Sjögren's Syndrome Response Index (SSRI), an index reflecting mainly the objective and subjective sicca symptoms [6]. When we applied the SSRI to classify patients as responder or non-responder, we were unable to detect any difference in our data in baseline $CD20^+$ B-cells/mm^2 parenchyma between responders and non-responders (Figure 2). Importantly, the agreement between ESSDAI and SSRI in defining responders in our study was rather poor (κ=0.25, percentage of agreement 64%; data not shown). Based on these

Figure 2: Numbers of baseline CD20+ cells/mm^2 in clinical responders (n=8) and non-responders (n=6) as defined by SSRI. Horizontal lines indicate median values, ns: non-significant.



findings, we conclude that it is evident that ESSDAI and SSRI measure different outcomes; the ESSDAI focuses on systemic disease activity and the SSRI mainly on sicca related complaints. In this respect, it is also worth mentioning that in our placebo treated patients, SSRI characterized 40% of the patients as responders, while ESSDAI only 11%. ESSDAI has been proven to be sensitive to measure the change in disease activity after therapeutic interventions and also showed that RTX was effective in our double-blind placebo-controlled RTX trial [4,7-10]. Thus, further validation is necessary for the SSRI.

3. Differences in general features

In addition to these two main aspects that result in the apparent discrepancies between the two studies, there are also some other differences that may influence differences in outcomes.

Baseline ESSDAI: Although the baseline ESSDAI scores were rather similar between the two studies (8 in our study and 10 in the TEARS study) only in our study the ESSDAI was prospectively evaluated [11,12]. In the TEARS study, the ESSDAI was retrospectively evaluated.

Baseline salivary gland positivity: Another major difference between the two studies is the positivity of salivary gland biopsy of the included patients; all patients in our study had a positive parotid gland biopsy at baseline, while only 64% of the patients included in the study by Cornec et al. had a positive minor salivary gland biopsy [1]. When excluding patients with a negative minor salivary gland biopsy from the study by Cornec et al., the median proportion of B-cells in responders would have been probably higher than in non-responders, which is in agreement with the conclusion of our study.

Table 1: Comparison of two studies.

STUDY	Cornec et al., 2015	Delli et al., 2015
Outcome	Proportion of B cells	Absolute number of CD20$^+$ B-cells/mm^2 parenchyma
Software	Digital pixel counting software, developed by the same team [5]	HistoQuest software, version 3.5.3.0171, Tissuegnostics, Vienna, Austria
Tool for measuring response to RTX	SSRI [6]	ESSDAI [4]
Salivary gland	Minor salivary glands	Parotid gland
General features	• Baseline ESSDAI: 10 • Median age: 50.4 and 54.8 (± 9.5 & ± 13.8) • Baseline salivary gland biopsy positivity: 64% • Baseline anti-SSA positivity: 80%	• Baseline ESSDAI: 8 • Median age: 43 (± 11 years) • Baseline salivary gland biopsy positivity: 100% • Baseline anti-SSA positivity: 100%

Parotid versus minor salivary gland biopsy: The different histopathological characteristics observed in parotid and minor salivary gland biopsies complicate the comparison as, e.g., the B/T-cell ratio differs greatly. Minor salivary glands of healthy controls may have a physiological infiltrate that consists mainly of T-lymphocytes (and plasma cells), while parotid salivary gland tissue of healthy controls shows rarely a lymphocytic infiltrate. Although those differences have been shown by Pijpe et al. [13], there is still a need for larger studies focusing on the inherent differences in the histopathological characteristics of parotid and minor salivary gland tissue in both pSS patients and healthy controls.

4. Salivary gland ultrasound

Like Cornec et al., we also feel that ultrasound has merit in the diagnosis and assessment of the disease activity of pSS [1,14,15]. However, before making salivary gland ultrasound a standard in pSS diagnostics, disease monitoring and treatment evaluation, there are several questions that need to be answered first, i.e. the reliability of ultrasound in the evaluation of changes that occur in the major salivary glands of pSS patients, and the validity of ultrasound to detect the histopathologic changes occurring in the parotid tissue of patients (suspected) with pSS, in particular (direct comparison of ultrasonographic and histhopatologic features).

From the abovementioned, it may be concluded that our study and the study of Cornec et al. differ in some respect [2,3], but do not present contradicting results.

Most likely, differences in assessment of patients' responsiveness to RTX treatment by using different methods and techniques lead to different results and apparent differences (Table). Probably, by combining theirs and our analyses, we might even be able to more efficiently select patients at baseline, who probably will benefit from RTX treatment.

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Chapter 4C

Need for consensus guidelines to standardize the assessment of germinal centers and other histopathological parameters in salivary gland tissue of patients with primary Sjögren's syndrome

Konstantina Delli[1], Erlin A Haacke[2,3], Stephan Ihrler[4], Bert van der Vegt[3], Arjan Vissink[1], Hendrika Bootsma[2], Frederik KL Spijkervet[1], Frans GM Kroese[2]

Affiliations

1. Department of Oral and Maxillofacial Surgery, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands

2. Department of Rheumatology and Clinical Immunology, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands

3. Department of Pathology and Medical Biology, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands

4. Laboratory for Dermatohistology & Oral Pathology, Munich, Germany

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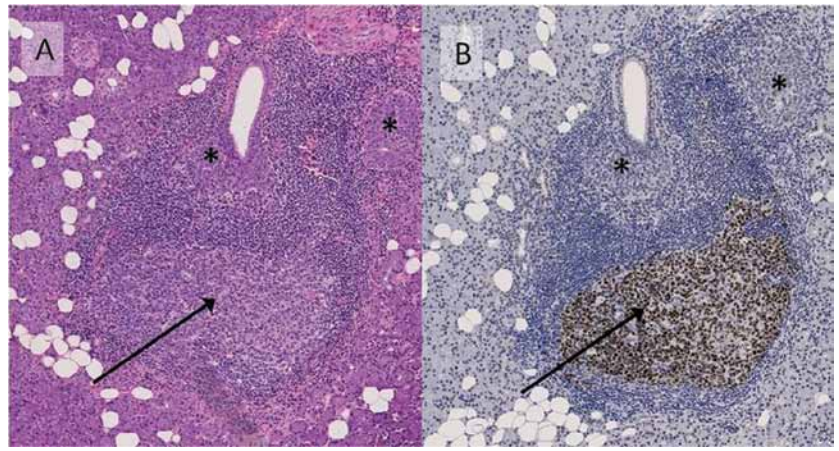
We have read with great interest the article by van Roon et al. [1] commenting to our paper 'Towards personalised treatment in primary Sjögren's syndrome: baseline parotid histopathology predicts responsiveness to rituximab treatment' [2]. The authors argue that there is a need of standardization of the histopathological characteristics of salivary gland tissue of patients with primary Sjögren's syndrome (pSS), in general, and of the presence of germinal centers (GCs), in particular.

We fully agree with van Roon et al. and other authors about the need for consensus guidelines to standardize the histopathological evaluation of salivary gland biopsies in pSS patients [1,3]. A standardized scoring system may facilitate prognostication and stratification of pSS patients and is needed for a valid evaluation of various clinical trials [3]. In particular, histological definition of GCs in salivary gland tissue is warranted, since these structures can be difficult to detect in diagnostic hematoxylin & eosin (H&E)-stained tissue sections. Detection of GCs in the periductal lymphoid infiltrates of the salivary glands is clinically relevant, because the presence of these structures is associated with more severe disease [4]. Importantly, the presence of GCs in minor salivary gland biopsies has been postulated to be a predictor of patients who are at risk for lymphoma development [5,6]. It has to be mentioned, however, that recently, we were not able to confirm these findings for a larger number of mucosa associated lymphoid tissue (MALT) lymphomas in parotid glands of pSS patients (Haacke et al., unpublished data).

In our study, we defined GCs in H&E stained sections as lighter areas within the lymphoid infiltrate composed of both lymphoid cells (centrocytes, centroblasts) and of cells with a non-lymphoid nature (macrophages and follicular dendritic cells (FDCs)) (Figure 1A) [1]. Furthermore, the GCs were scored independently by two experienced pathologists. For the inexperienced eye, GCs may be overlooked, because of their small size, or lighter areas within the infiltrate may erroneously be scored as GCs, while in fact they represent lymphoepithelial lesions. For proper and easy detection of GCs, also by less trained persons, additional immunohistochemical staining might be helpful. Therefore, we propose to stain for B-cell lymphoma 6 (Bcl-6) to define and identify GCs. Bcl-6 is a transcription factor expressed at high levels by GC B-cells. Like GCs in peripheral lymphoid organs, GCs in salivary glands of patients with pSS are also consistently positive for Bcl-6 [5]. As shown in Figure 1B, staining for Bcl-6 allows easy and unequivocal detection and scoring of GCs in salivary gland biopsies, both in minor and major (parotid) salivary glands. Implementation of Bcl-6 staining is relatively easy, since it is routinely used in pathology laboratories worldwide for the diagnosis of lymphomas [7]. Other markers, as proposed by Fisher et al. and van Roon et al. [1,3], are less specific and less suitable to detect GCs in routine diagnostics. For example, activation induced deaminase, an enzyme essential for the function of GCs B-cells, is expressed only by a minority of GCs B-cells in minor salivary glands of pSS patients [5], which may make GCs harder to detect. The long isoform of CD21 (CD21L) has also been suggested for detection of GCs. CD21L is expressed by follicular dendritic cells (FDCs). However, although FDCs are a prerequisite for GC function and development, the

Figure 1: Images showing serial paraffin sections of a parotid gland biopsy of a patient with primary Sjögren's syndrome (pSS) stained with H&E (A) and for Bcl-6 (B). A clearly visible germinal centre (GC) (indicated with an arrow) is seen in the periductal infiltrate. Asterisks indicate lymphoepithelial lesions.



presence of these cells does not necessarily imply that GCs are present. Indeed, ectopic lymphoid infiltrates in salivary gland tissue of pSS patients can contain FDC-networks in the absence of GCs [8,9]. Staining for the long isoform of CD21 may therefore result in an overestimation of the number of GCs present in salivary gland tissue.

In our study, we observed that a relative high proportion of the parotid salivary gland biopsies presented with GCs at baseline; 67% and 68% of patients in the placebo and rituximab treated group, respectively [2]. These are relatively high percentages compared to the general pSS population, in which approximately one-quarter of the minor salivary gland biopsies exhibit GCs [4]. The reason of this high baseline characteristic can be attributed to the inclusion criteria of our study. In our study the pSS patients were all positive for anti-SSA antibodies and had high systemic activity, as indicated by the relatively high ESSDAI scores [10]. Indeed, presence of GCs in minor salivary glands has been associated with more severe disease, including systemic pro-inflammatory mediators and anti-SSA antibodies [4]. A second explanation for the high number of GCs at baseline might be related to histopathological differences between minor and parotid salivary gland biopsies. Although a previous study in a small cohort of pSS patients (n=30) did not report a difference in numbers of GCs [11], it remains possible that there are more and/or larger GCs in parotid gland biopsies compared to minor salivary glands. Apparently, there is a petition for larger studies focusing on the inherent differences in the histopathological characteristics of parotid and minor salivary gland tissue in both pSS patients and healthy controls.

In summary, in agreement with van Roon et al. [1], we would also like to emphasize that there is a need for consensus guidelines to standardize the evaluation of ectopic lymphoid infiltrates and GCs in salivary gland tissue of pSS patients. The various methods used for automated analysis of several parameters should also be taken into account [12]. Consensus guidelines will assist the pathologist to correctly identify and quantify histopathological parameters in pSS and contribute to a more accurate prediction of disease progression and personalized treatment, as well as allowing the comparison between study cohorts and different clinical trials.

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Chapter 5

e-Patient education

Chapter 5A

Internet information on xerostomia. What should patients expect?

Konstantina Delli[1], Christos Livas[2], Frederik KL Spijkervet[1], Arjan Vissink[1]

1. Department of Oral and Maxillofacial Surgery

2. Department of Orthodontics

University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

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Abstract

Objective: To assess the qualitative standards of the information distributed via the Internet regarding xerostomia.

Materials and Methods: A computerized electronic search was performed for 'xerostomia' and 'dry mouth' separately using four search engines. The first 30 results from each search term-engine combination were pooled for analysis. After excluding promotional product sites, discussion groups, video feeds, scientific articles, non-operative sources, sites with denied direct access through password requirement, non-English language domains, and online medical dictionaries, 50 Web pages were evaluated in terms of readability, accessibility, usability, and reliability using recommended research methodology the Flesch Reading Ease Score and the LIDA instrument. Author and information details were also recorded.

Results: The results revealed a variable quality of the available Internet information on xerostomia. The Web sites required advanced reading skills, while LIDA scores for accessibility, usability and reliability ranged from medium to low with average scores extending from 29.1% to 81.3%.

Conclusions: The quality of information about xerostomia among Web sources presents high variability. The existing discrepancy should be alleviated by referring patients to evidence-based education materials on the Internet. Improvement of xerostomia information e-resources will contribute to a more advanced quality in oral health care.

Introduction

Xerostomia is the subjective feeling of oral dryness. The term derives from the Greek words *"xeros"* *(ξηρός)*, meaning dry and *"stoma"* *(στόμα)*, meaning mouth. Whereas the prevalence of xerostomia remains difficult to be determined, overall estimates have been summarized in 13-63% [1]. Dry mouth complaints are generally more prominent in women, elderly and individuals housed in long-term care facilities. The plethora of medical conditions, which has been associated with transient or persistent xerostomia, may complicate therapeutic procedures for the parties concerned. As a consequence, symptomatic individuals or caregivers may refer to available health services for relevant patient education materials.

Internet is becoming an increasingly popular source of health information as well as a potential communication channel among e-health seekers [2,3]. A nationwide survey in the United States (U.S.) revealed that 72% of the adult Internet users searched online for health information in 2012 [4]. Likewise, interview-based research in seven European countries, deduced that 71% of the e-consumers went online for health purposes within a year [3]. Albeit the Web enables access to vast amounts of miscellaneous health related data, the anarchical nature of the medium along with the limited navigation skills of the average user may entail plausible risks [5]. Hypothetically, persons experiencing xerostomia, like any other online health seeker, may be prone to track inaccurate citations while searching the Internet for background knowledge. Therefore, this study was aimed to assess the qualitative standards of the disseminated via the Internet information on xerostomia.

Materials and methods

Search method

An e-search was undertaken in August 2013 using four popular search engines: Google™ (www.google.com), bing™ (www.bing.com), YAHOO!® (www.yahoo.com) and Ask® (www.ask.com). The terms 'dry mouth' and 'xerostomia' were entered individually to mimic a common, performed by a layperson, online search. The top 30 results for each search combination were collected for content analysis. Exclusion criteria were promotional product sites, discussion groups, video feeds, scientific articles, non-operative sources, sites with denied direct access through password requirement, non-English language domains, and online medical dictionaries (Figure 1). Author's name, profession, and type of information of all eligible Web portals were documented.

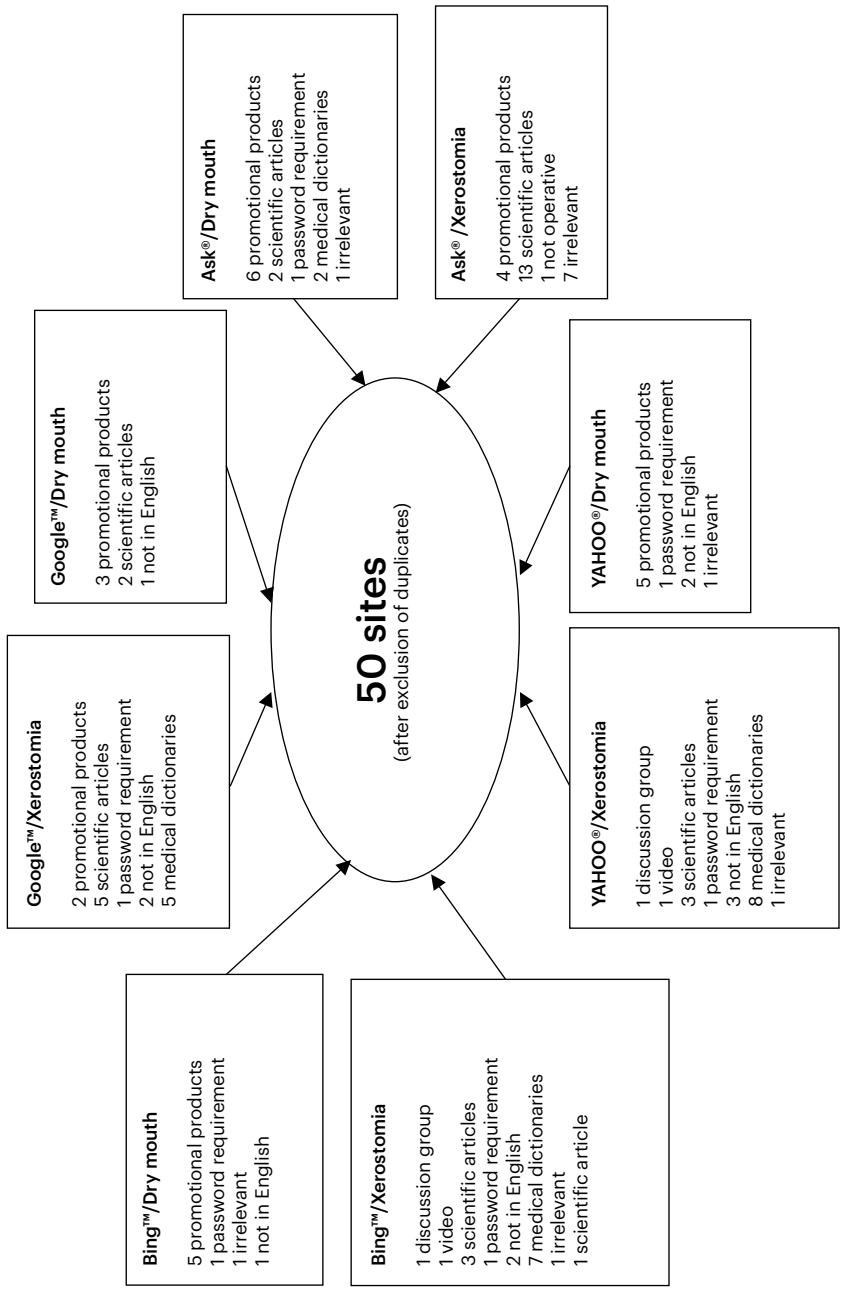


Figure 1: Flowchart diagram of the selection process.

Quality evaluation

Readability

Readability is defined as the determination, by systematic formulae, of the reading comprehension level a person must possess to understand written texts [6]. The Flesch Reading Ease (FRE) Score, one of the oldest textual difficulty measures, was applied to calculate readability of the analyzed sites according to the equation: FRE= 206.835 – (1.015 x ASL) – (84.6 x ASW), where ASL stands for average sentence length, and ASW for average number of syllables per word [7]. The outcome is a number ranging from 0 to 100 with higher score indicating easier text reading. Scores between 90 and 100 are considered easily understandable by an average fifth grade student. Eighth and ninth grade students are supposed to easily comprehend numbers between 60 and 70. As a final point, understanding of up to 30 score texts is considered representative of higher education level, i.e. college or university graduate level. In the present study, a piece of text from each site consisting of 200-500 words was copied and pasted into an online FRES calculator program (http://www.readabilityformulas.com/free-readability-formula-tests.php). The accuracy of the online method has been previously confirmed by comparison of automate and manual calculation modes [8].

Detailed qualitative analysis was performed by means of the LIDA instrument (The LIDA Instrument, Version 1.2, Minervation Ltd, Oxford, UK). This validation tool was invented for the assessment of the format and content of health care Web sites. There are three distinctive categories rated: accessibility, usability, and reliability. The accessibility score is computed by completing the Web address of the site on a customised Web platform (LIDA tool, accessed on August 2013) [9]. A list of nine questions is utilized to determine usability and reliability (Table 1). The response options are graded from 0 to 3 (0: never; 1: sometimes; 2: mostly; 3: always). De-

Table 1: LIDA instrument questions intended to evaluate usability (1-4) and reliability (5-9) of Web sites.

Question No	Formulation
1	Is the site design clear and transparent?
2	Is the site design consistent from one page to another?
3	Can users find what they need on the site?
4	Is the format of information clear and appropriate for the audience?
5	Is it clear who has developed the website and what their objectives are?
6	Does the site report a robust quality control procedure?
7	Is the page content checked by an expert?
8	Is the page updated regularly?
9	Does the page cite relevant sources where appropriate?

Table 2: Author details of the Web sites analyzed in the study.

50 articles			
Author nm		**Author m**	
33		**17**	
	Profession nm		Profession m
	5		**12**
		Dentist	Other
		5	**7**

m indicates mentioned; nm not mentioned

pending on the data entry, this software generates final percentile scores that correspond to high, medium or low quality. The LIDA outcomes are regarded 'high', 'moderate', and 'low' in case of percentages larger than 90%, between 50%, and 90%, and smaller than 50%, respectively. Conclusively, the total ranking of the design and structure of the sites is estimated by averaging the three LIDA subscores.

Results

Search results

The search strategy initially produced 240 sites for relevance analysis. Following data filtering, a sample of 50 sites was finally selected based on the selection criteria and after excluding duplicates (Figure 1).

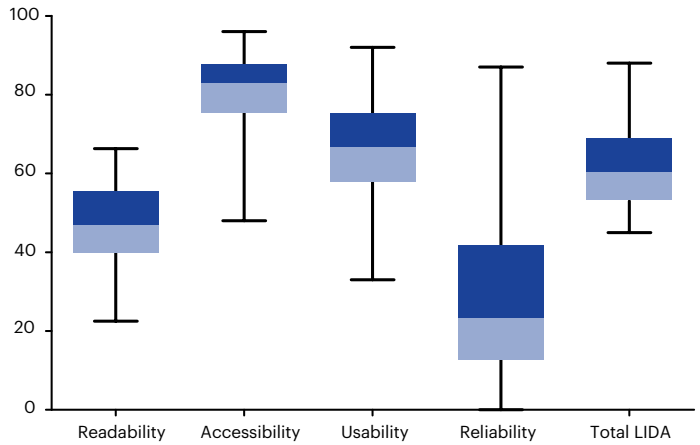
Authorship and type of information

In 66% of the sites, the author was anonymous (Table 2). A high proportion of Web platforms, 38 out of 50, did not enclose professional details of the contributor. The rest of the articles were written by 9 health professionals, 2 journalists and 1 search engine optimization (SEO) expert. Regarding information specificity, 4 pages claimed to provide exclusive information about saliva and/or xerostomia. Fourteen Web pages were found to cover topics of oral medicine and general dentistry, while general information for health conditions and symptoms was found in 17 sites. Of note, the rest of the reviewed pages had a broader scope of interest, posting information on fields such as nutrition, fitness, entertainment and lifestyle.

Quality assessment

The evaluation outcomes and the description statistics of percentile scores are summarized in Table 3. A box and whisker diagram was constructed to enable schematic representation of the distribution of the dataset (Figure 2). It appears that accessibility, usability and reliability were scattered over a wide range, implying therefore, the variable quality of these characteristics in the available Internet sources.

Figure 2: Box and whisker diagram illustrating the distribution of evaluation percentile scores.



Readability

FRE ratings ranged between 22.5 and 66.3. The total readability level (mean: 45.56, SD: 7.67) was considered appropriate for college and university graduates. The highest scoring site was designed by the U.S. Department of Health and Human Services, intending to protect and promote public health. Fairly difficult to very difficult reading style (FRES: 30-69) was identified for the 90% of the reviewed information sources (Figure 3).

Accessibility

The general accessibility was considered medium (mean: 81.3, SD: 9.83). The maximum achievable score (96%) was recorded for a Web platform addressing information for oral, dental and craniofacial health. High accessibility scores (>90%) were identified for eight Web pages. Surprisingly, the lowest score (48%) was ascribed to an Internet address designed to provide information about dry mouth exclusively.

Usability

The mean score of usability was 64 (SD: 14), which corresponded to medium quality. The highest score of 92% was achieved by two sites, in which information was provided for oral/dental health related issues and Sjögren's Syndrome, respectively. The vast majority of Internet addresses (84%) obtained moderate scores. One Web site of unknown authorship scored as low as 33%.

Reliability

The overall reliability of the Internet portals examined by this study was low (mean: 29.1, SD: 23.65). A Web page edited by a dentist, was graded with the highest score

Table 3: Evaluation scores of the Web sites in terms of Flesch Reading Ease Score (FRES), accessibility, usability, readability and LIDA (in brackets: the maximum achievable scores; in parentheses: the corresponding percentile score).

	Readability (FRES) [100]	Accessibility (LIDA) [54]	Usability (LIDA) [12]	Reliability (LIDA) [30]	LIDA total [96]
1. arthritis.about.com	55.6	50(93)	9(75)	6(20)	65(68)
2. betterhealth.vic.gov.au	62.9	35(65)	10(83)	29(70	47(49)
3. brighamandwomens.org	40.8	39(72)	5(42)	10(33)	54(56)
4. buzzle.com	57	45(83)	6(50)	4(13	55(56)
5. calmclinic.com	63.8	44(81)	7(58)	0(0)	51(53)
6. cancer.net	44.3	46(85)	7(58)	10(30)	63(66)
7. health.canoe.ca	49.5	42(78)	8(67)	0(0)	50(52)
8. chemocare.com	48.6	40(74)	7(58)	4(13)	51(53)
9. cosmetic-dentistry-center.com	40.8	45(83)	9(75)	10(33)	64(67)
10. dental.tufts.edu	51.7	47(87)	8(67)	12(40)	67(70)
11. dentanet.org.uk	37.7	51(94)	7(58)	4(13)	62(65)
12. dentistryiq.com	43.3	41(76)	5(42)	22(73)	68(71)
13. drdavidfox.com	59.4	36(67)	10(83)	16(53)	62(65)
14. drssmithandrobinson.com	61.7	39(72)	8(67)	12(40)	59(61)
15. drugs.com	42	47(87)	4(33)	0(0)	51(53)
16. drymouth.info	56	26(48)	10(83)	12(40)	48(50)
17. drymouthfoundation.org	52.3	50(93)	8(67)	12(40)	59(61)
18. earthclinic.com	50	49(91)	5(42)	2(7)	56(58)
19. ehow.com	46.9	46(85)	7(58)	6(20)	59(61)
20. en.wikipedia.org	31.3	45(83)	9(75)	22(73)	76(79)
21. fda.gov	66.3	42(78)	9(75)	10(33)	61(64)
22. health.howstuffworks.com	55.2	43(80)	8(67)	8(27)	59(61)
23. irishhealth.com	57.8	33(61)	8(67)	2(7)	43(45)
24. knowyourteeth.com	46.9	48(89)	8(67)	2(7)	58(60)
25. livestrong.com	52.8	45(83)	7(58)	14(47)	66(69)
26. localhealth.com	46	46(85)	8(67)	2(7)	56(58)
27. mayoclinic.com	48.3	42(83)	7(58)	14(47)	66(69)
28. medactive.com	25.8	41(76)	6(50)	2(7)	49(51)
29. medicalnewstoday.com	46	46(85)	10(83)	8(27)	64(67)
30. medicinenet.com	26.6	47(87)	11(92)	26(87)	84(88)
31. mouthhealthy.org	39	45(83)	6(50)	2(7)	53(55)
32. my.cleavelandclinic.org	42.8	47(87)	6(50)	2(7)	53(55)
33. nidcr.nih.gov	55.7	52(96)	8(67)	16(53)	76(79)
34. nihseniorhealth.gov	55.1	46(85)	10(83)	10(33)	66(69)
35. oncolink.org	40.1	48(89)	7(58)	6(20)	61(64)
36. oralcancerfoundation.org	26.8	47(87)	9(75)	22(73)	78(81)
37. patient.co.uk	50.6	48(89)	8(67)	24(80)	80(83)
38. pazienti.net	54.4	46(85)	7(58)	0(0)	46(53)
39. salivalis.com	27.8	34(63)	7(58)	6(20)	47(49)
40. simplestepsdental.com	55.4	37(69)	8(67)	6(20)	51(53)
41. sjogrens.org	40.1	36(67)	11(92)	20(67)	67(70)
42. smileforlife.com	22.5	48(89)	8(67)	4(13)	57(59)
43. studiodentaire.com	46.9	45(83)	8(67)	4(13)	57(59)
44. symptoms.rightdiagnosis.com	39	49(91)	10(83)	6(20)	65(68)
45. uic.edu	32.9	40(74)	5(42)	4(13)	49(51)
46. voices.yahoo.com	29.5	50(93)	7(58)	4(13)	49(51)
47. webmd.com	35	51(94)	10(83)	22(73)	83(86)
48. wellsphere.com	61.9	40(74)	5(42)	10(33)	55(57)
49. wikihow.com	46.7	47(87)	7(58)	12(40)	66(69)
50. wisegeek.com	57.6	41(76)	6(50)	4(13)	51(53)
Mean	**45.56**	**43.9(81.3)**	**7.68(64)**	**8.73(29.1)**	**59.73(62.22)**
SD	**7.67**	**5.3(9.83)**	**1.68(14)**	**7.1(23.65)**	**9.85(10.26)**

of 87%. In total, three out of the top-five ranking sites were managed by dentists. The other two were written by a general practitioner and a pharmacist, respectively. Disappointingly, no site was identified as highly reliable.

LIDA scores

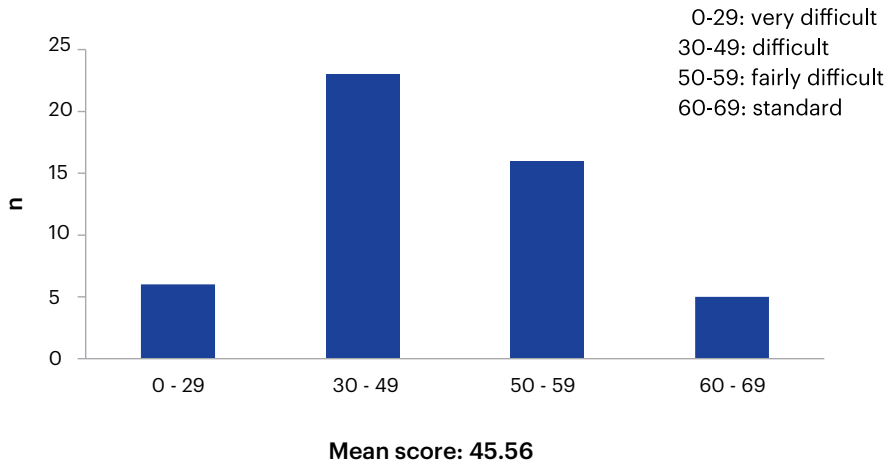
Similarly, no high rates were identified in any of the examined sites. The mean validation score for the 50 Web sites was 59.73 (SD: 9.85), suggestive of medium quality. LIDA values varied from 45% to 88%. The highest grade referred to a Web page run by a dentist. The lowest score was obtained from an especially developed domain for general health information.

Discussion

Internet is believed to have the power to modify patient-doctor relationship, by encouraging patients in the management of their health through a more shared decision making approach [10]. While speed and ease on obtaining the desired information are indisputable, validity and reliability might often be dubious.

To the best of our knowledge, studies investigating Web based information on oral medicine and dentistry have been so far scarce and dealt with either head and neck cancer [11,12], leukoplakia [13], orthodontic treatment [5,8,14-16], temporomandibular disorders [17,18], mouth guards [9] or periodontal diseases [20]. A wide variety of validation tools has been used in the aforementioned studies, e.g. JAMA benchmarks, DISCERN, HON seal, LIDA, FRES, Flesch-Kinkaid Grade Level, Fog Scale Level as well as personal scoring systems, making it difficult to compare the results and thus draw specific conclusions. As far as the quality of the investigated sites is concerned, it can be generally argued that sites displayed either low [11, 13], or low to moderate [12,17] or greatly variable quality [5,8,15,16,19,20], with the vast majority of studies underlining the lack of evidenced-based high quality sites. The level of readability was overall judged fairly difficult and appropriate for 8^{th}-9^{th} grade level students [5,8,15].

Figure 3: Distribution of FRES scores among included sites. n indicates number of articles.

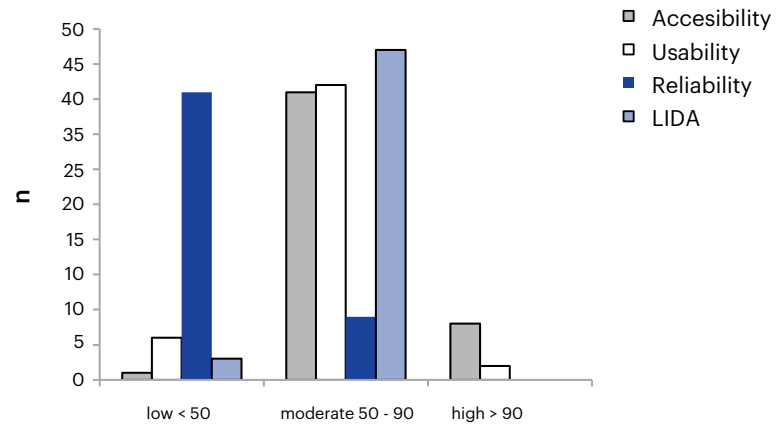


Our objective was to conduct a comprehensive examination of e-sources potentially visited by patients experiencing dry mouth as well as their caregivers. According to the updated Health Online report, 77% of online health inquiries initiate at a search engine such as Google™, bing™, YAHOO!® [4]. The search methodology was supplemented by Ask®, which follows the aforementioned Web browsers in popularity in the U.S. as indicated by the latest Internet traffic statistics (Google™: 66.7%, bing™: 17.9%, YAHOO!®: 11.4%, Ask®: 2.9%) [21]. Individuals tend to limit their search within the first eight to ten results of a search engine [22,23]. The broad inclusion of links that exceeded the first page may be also considered methodologically advantageous.

Readability

It has been previously suggested that patient education material should be written at an 'easy' to 'very easy' reading style [6]. By applying FRES, we found that the bulk of the Web information regarding xerostomia was written in a fairly difficult to very difficult understandable mode. As a result, readers may fail to broaden their understanding of this symptom or may even misinterpret crucial information on health care decisions. Misperception may be further aggravated in stressful conditions. Being a purely personal feature, stress may affect reading capacity and be related to lower comprehension of medical terminology [24,25]. Certain factors such as educational status, employment, income and origin have been regarded strong predictors of Internet behaviour [3]. Nevertheless, the demographic profile of the Internet users tends to transform and resemble more closely to the general population [2]. In the meantime, the writing style of the material intended for patients should be revised to a more favorable comprehensive level. Sentences should be kept short, while uniform and plain language should be maintained throughout the

Figure 4: Number of sites achieving low, moderate and high scores, when applying LIDA validation tool. n indicates number of articles.



text. Long lists should preferably be avoided and bullet points as well as graphics are advised to be used with caution [2].

LIDA

Various quality validating tools have been developed over the last years to cope with the absence of universally accepted criteria. In our study, systematic assessment of the health informative Web sites was carried using the LIDA instrument, a compact set of criteria earlier picked up by several research groups to measure quality of Web information across cardiology, endocrinology, gastroenterology and orthodontics [5,8,26-29]. With respect to the LIDA outcomes (Figure 4), the Web pages were rated with medium accessibility, even though higher than all the rest LIDA categories. Administrators of Web sites should by advised by search engine optimization (SEO) experts to ensure that their sites are easily accessible. Usability results exhibited a higher variability than accessibility results. Efficient presentation of the information data is as essential as the information per se, and this should be taken into account by health care providers when designing a Web page. Finally, we detected a considerably low reliability of the sites under investigation, with implicating for site transparency, expertised authorship, review procedures and update frequency. Since three out of the top-five ranking sites were managed by a dentist, indicating therefore their prominent expertise, patients and their intimates should be encouraged to use verified Web sites, generated by specialists, with structured interactive educational programs. Most recently, subjects provided computer-assisted instruction about major hypertension topics presented significantly improved knowledge scores and more positive outlook on the learning experience compared to counterparts ascribed to random Internet search [30].

Study limitations

The study exhibits particular limitations. The search, for example was carried out on one date. In view of the fact that sites have a dynamic character, their profile might change drastically in a very short period of time; content and design might be modified leading to different FRES and/or LIDA results. Additionally, the single language of choice, does not allow generalization of our findings to Web sites displayed in other languages, despite the fact that English is acknowledged by Internet usage and population standings as the prevailing language [31]. The major limitation of FRES is that it is unable to distinguish common words from unusual ones, since the difficulty of a word/sentence is only determined by the length of characters. Furthermore, Web site features, such as layout, font size and colour, are not taken into consideration, although they significantly influence readability [2].

Conclusion

The results of our study indicate a variable quality of the available Internet information on xerostomia. The Web sites required advanced reading skills, while LIDA scores for accessibility, usability and reliability ranged from medium to low. Electronic disposal of valid information in plain language may prove beneficial for xerostomic patients and caretakers. By gaining knowledge over aetiology and underlying conditions, impact on daily activities, and treatment strategies, patients can become more compliant, and at the same time, more active, keeping pace with the international trend in heath care field. However, Internet users should be aware of the current shortcomings while searching for online health advice for dry mouth. To conclude, oral medicine specialists should be actively involved in the development of information resources and refer patients to evidence based materials on the Internet.

Conflict of interest

None declared

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Chapter 5B

Is YouTube useful as a source of information for Sjögren's Syndrome?

Konstantina Delli[1], Christos Livas[2], Arjan Vissink[1], Frederik KL Spijkervet[1]

Affiliations

1. Department of Oral and Maxillofacial Surgery

2. Department of Orthodontics

University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

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Abstract

Objectives: To quantitatively and qualitatively assess the characteristics of YouTube videos dealing with Sjögren's syndrome.

Materials & Methods: A comprehensive electronic search was performed for ''Sjögren's syndrome'' in YouTube. After excluding duplicates, irrelevant videos and non-English language domains, 70 videos were included for analysis. Videos were classified as useful, misleading or personal experience. The overall quality of videos was scored according to the Global Quality Scale (GQS). Useful videos were assessed for reliability and comprehensiveness based on two 5-point scales. Key points of the misleading videos were explored and patients' personal experiences were further investigated.

Results: Thirty six videos (51.4%) were classified as useful, 6 (8.6%) as misleading and 28 (40%) as patients' personal experience. Independent users tend to upload videos with personal experience while university channels/professional organizations share useful videos with evidence-based information. Significant difference was observed in GQS among useful, misleading videos and patients' experiences. The mean reliability, comprehensiveness and GQS scores of useful videos were 2.5 (SD:1.2), 2.6 (SD:1.4) and 3.4 (SD:1.0) respectively, whereas only 6 videos (16.7%) were rated as complete vis-à-vis content. The most frequently misleading topics were etiology and treatment.

Conclusions: Specialists should refer their patients to validated e-information resources and actively participate in the development of video-sharing platforms.

Introduction

Sjögren's syndrome (SS) is the second most common rheumatic disease after rheumatoid arthritis, with prevalence of 60.8 (95% CI: 43.7 to 77.9) cases per 100,000 inhabitants in the total population [1]. SS is a chronic inflammatory and lymphoproliferative disorder that is principally characterized by chronic infiltration of the exocrine glands and is regarded as the most concerning autoimmune disorder for oral health care professionals [2]. The most prominent symptom of SS is xerostomia (sensation of dry mouth), due to reduced saliva production. This symptom is often accompanied by dysgeusia, difficulty in eating dry food (e.g., crackers), problems in speaking for long period of time, burning sensation of the mouth, discomfort while wearing dentures and increased risk of dental caries, especially cervically, as well as candidiasis and periodontal disease [2]. Nearly a third of SS patients present systemic manifestations, and due to the multiple organ involvement, general symptoms like fatigue, polyathralgia and myalgia, sleep disturbances, anxiety and depression are often evident, leading to diminished quality of life [3].

The Internet has grown through the years into a popular source of health information both for patients as well as for healthcare providers [4,5]. More than 70% of the adult Internet users in the United States searched online for health topics in 2012 [6]. YouTube is the most popular free video-sharing platform with more than 1 billion users and 300 hours of uploaded new video material per minute [7], increasingly being used for disseminating health information. According to Alexa's Internet traffic estimates, YouTube ranked third in 2014 in terms of page views and visitors, following Google and Facebook [8]. A recent systematic review on YouTube healthcare information concluded that YouTube portrays misleading information, primarily anecdotal that contradicts the reference standards [9]. Under this spectrum, lay YouTube users being suspected for or diagnosed with SS and their caregivers are highly likely to access patient education materials of such quality. Therefore, the aim of this study was to assess the potential of YouTube videos as a valid source of information on SS.

Materials and methods

Search strategy

YouTube (http://www.youtube.com) was searched using the keyword phrase 'Sjögren's syndrome', on May 21, 2015 for videos uploaded anytime since the advent of YouTube. These videos should contain information about the epidemiology, pathogenesis, clinical features, diagnosis and treatment of the disease.

'Sjögren's syndrome' without Umlaut (¨) was identified by the 'Google Trends' application as the most commonly used search term for SS [10]. Google Trends measures search interest in topics by calculating the frequency a search term is en-

tered in relation to the total search-volume across various regions of the world. The 'Incognito'/'Worldwide' settings were selected to limit filtering to previous user history and expand the search results [11].

The search generated a total of 3940 videos. The first 100 videos (first 5 pages) ranked by relevance were analyzed for information about SS. Non-English language videos, duplicated in part or whole or containing information irrelevant to SS were excluded [12]. Multipart videos were counted as one and the viewer interaction parameters were averaged for the purposes of the analysis.

Video classification

All videos were scrutinized by 2 reviewers independently (KD, CL) and interexaminer discrepancies were resolved in a consensus meeting. The content of included videos was classified according to the following system [12-15]:

i. useful, if they contained scientifically sound information about any aspect of SS.
ii. misleading, if they contained scientifically erroneous or unproven information about any aspect of SS.
iii. personal experience, if the videos described a user's personal experience while being diagnosed with or treated for SS.

All videos were also categorized by source into 5 groups [12]:

i. independent users
ii. government/news agencies
iii. university channels/professional organizations
iv. health information Web sites
v. medical advertisements/profit companies

Videos rated as useful were further examined for reliability using a 5-item questionnaire modified from the DISCERN validation tool for assessment of written consumer health information [16]. In this questionnaire, items 1, 2, 3, 6, 7 and 8 of DISCERN/reliability section have been implemented, while items 4 and 5 were not applicable, because of the different nature of videos, compared to written information. Positive responses scored 1 point, whereas negative responses scored 0 points (Table 1). Comprehensiveness of video information regarding 5 different areas of the disease (epidemiology, pathogenesis, clinical features, diagnostic tests, treatment) was also analyzed; videos were awarded with 1 point for each aspect covered, leading to a possible score range of 0-5 points [12]. The key points of the misleading videos were as well explored. Patient personal experiences were labeled positive (when providing either emotional support to the audience or useful information on SS

Table 1: Questions adapted from DISCERN tool intending to evaluate the reliability of videos (1 point is given for every Yes and 0 points for No) [16].

Item	Questions
1.	Are the aims clear and achieved?
2.	Are reliable sources of information used? (i.e., publication cited, speaker is specialist in SS)?
3.	Is the information presented balanced and unbiased?
4.	Are additional sources of information listed for patient reference?
5.	Are areas of uncertainty mentioned?

and its treatment) or negative (negatively depicting evidence-based remedies or promoting therapeutic alternatives with unproven scientific benefits).

Furthermore, the overall quality of the videos was graded using a 5-point scale, namely the global quality score (GQS), based on the quality of the information and how useful the reviewer assumed the particular video would be to a patient (Table 2) [17].

Data collection

Video features such as length and time since upload were recorded. Additionally, video popularity defined as the ratio of total views for video per number of days on YouTube since upload, number of 'likes', 'dislikes' and comments were noted.

Statistical analysis

Statistical analysis of the collected data was carried out with IBM SPSS Statistics 20 (SPSS, Chicago, Illinois, USA). One way ANOVA was performed to compare the means of variables. A p value <0.05 was considered significant.

Results

The first 100 videos were screened for relevance based on our selection criteria. A sample of 70 videos was finally included. In particular, 7 non-English, 15 duplicated in whole or in part, and 8 irrelevant videos were excluded (Figure 1). The mean length of the included videos was 5:27 (SD: 4:04) minutes and the mean video popularity was 10.37 (SD: 42). The videos were posted on YouTube on average 1,063 (SD: 2,018) days ago. Among the selected videos, 36 (51.4%) were classified

Table 2: Global quality scale (GQS) criteria used to score videos with information about SS on YouTube [17].

Item	Characteristics
1.	Poor quality, poor flow of the video, most information missing, not at all useful for patients
2.	Generally poor quality and poor flow, some information listed but many important topics missing, of very limited use to patients
3.	Moderate quality, suboptimal flow, some important information is adequately discussed but others poorly discussed, somewhat useful for patients
4.	Good quality and generally good flow. Most of the relevant information is listed, but some topics not covered, useful for patients
5.	Excellent quality and flow, very useful for patients

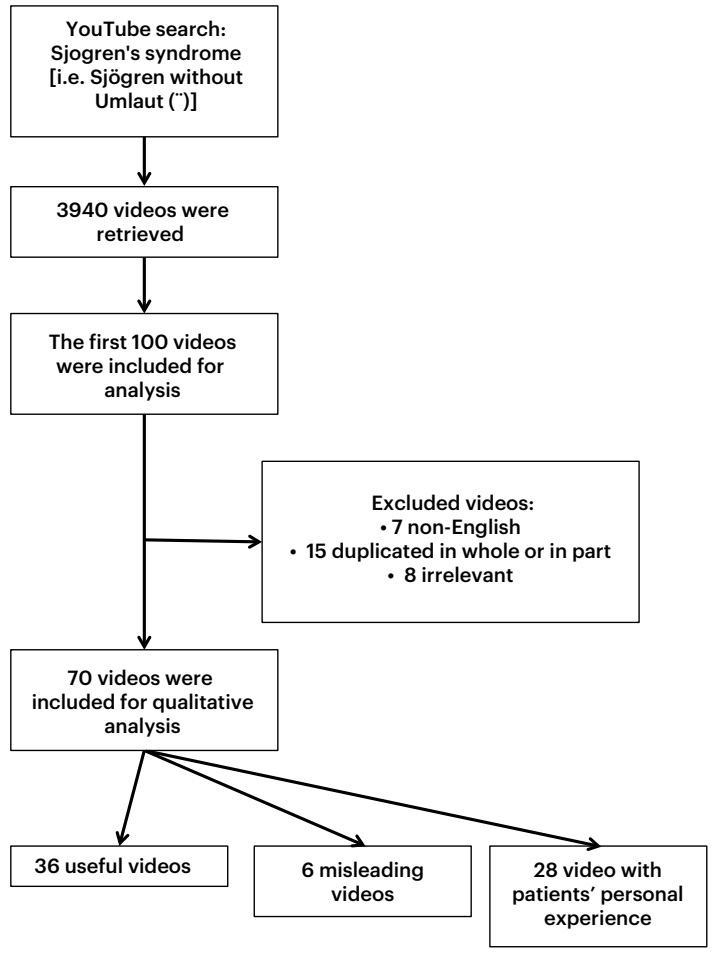
as useful, 6 (8.6%) as misleading and 28 (40%) as patient's personal experience (Table 3). Significant difference was observed in global quality among useful, misleading videos and patient's personal experiences.

The overall content of videos based on source of information is shown in Table 4. It appears that independent users usually upload videos with their personal experience. University channels/professional organizations usually share useful videos with evidence-based information, while no misleading videos have been accredited to them. Interestingly, there were no videos distributed by health information Web sites.

Useful videos

The mean reliability, comprehensiveness and GQS scores were 2.5 (SD: 1.2), 2.6 (SD: 1.4) and 3.4 (SD: 1.0), respectively. We observed that 21 videos (58.3%) used reliable source of information, while 8 videos (22.2%) listed additional sources of information for patient reference and 9 videos (25.0%) mentioned areas of uncertainty. From a content perspective, 17 videos (47.2%) discussed the epidemiology of SS, 15 videos (41.7%) explained the basic pathogenetic principals, 31 videos (86.1%) presented the most common clinical features, 12 videos (33.3%) analyzed the importance and scope of diagnostic tests, and 17 videos (47.2%) provided information regarding treatment options. Only 6 videos (16.7%) were rated as complete vis-à-vis content (score 5/5). The overall quality of useful videos based on source of information is shown in Table 5. Government/news agencies and university channels/professional organizations scored significantly higher ($p<0.05$) with regard to GQS, 4 (SD: 0.89) and 4 (SD: 0.92), respectively.

Figure 1: Flowchart diagram of the selection process.



Misleading videos

The most frequently misleading areas to be discussed appeared to be etiology and treatment. Dysbiosis, i.e. microbial imbalance in the body, low levels of vitamin D and Helicobacter pylori were presented as the sole cause of SS and not discussed with a broader view/perspective. Regarding treatment, essential oils against dryness, herbal natural remedies and pills with unknown ingredients were offered as the panacea against SS. With regard to source of the misleading videos, 2 were uploaded by independent users, 2 by news agencies and 2 by profit companies. No misleading videos were uploaded by university channels or professional organizations.

Table 3: Quantitative characteristics of videos characterized as useful, misleading and personal experience in mean values.

	Useful (n=36)	Misleading (n=6)	Personal experience (n=28)	p-value
uploaded since (days)	751.5	791	1522	0.34
duration (min)	4:27	6:16	6:35	0.05
views	8754	1780	4031.3	0.42
popularity	11.65	2.25	2.6	0.48
likes	29	8.3	18.5	0.56
dislikes	1.7	0.5	2.1	0.81
comments	10	1.3	23.5	0.45
GQS	3.42	1.83	2.14	*<0.01*

Patients' personal experience videos

Twenty eight videos were classified as patients' personal experience. Out of these, 20 videos provided emotional support to patients, shared useful information about SS and communicated treatment experience positively, and therefore deemed positive. By endorsing treatment alternatives lacking of scientific evidence or presenting the course of the disease or treatment in a negative way, the rest 8 videos were considered as negative.

Discussion

Nowadays, patients are increasingly turning to Internet and video-sharing Web sites like YouTube to make informed healthcare decisions. However, the diversity of authorship and the lack of peer-review process on this platform have led to dissemination of inaccurate and misleading information [18]. Practically speaking, any YouTube user without exception regarding his/her background, medical qualifications, professionalism and intentions is authorized to upload video clips. To the authors' knowledge, few studies have been published so far on the available Web information on oral medicine topics [19-22], but none of them has dealt with YouTube videos.

Our study showed that more than half of YouTube videos relevant to SS were deemed useful, a finding lying close to the range of 54.9-63.0% reported by studies with similar methodology [12-15,23]. Unlike misleading videos, useful videos seemed more recently uploaded, of shorter duration, with more views, likes, and higher popularity and GQS. However, the latter was the only outcome that reached statistical significance.

Table 4: Distribution of useful, misleading and personal experience videos by source.

	content		
	useful	misleading	personal experience
independent users	13	2	21[a]
government/news agencies	6	2	2
university channels/professional organizations	8[b]	0	1
health information Web sites	0	0	0
medical advertisements/profit companies	9	2	4

[a] Independent users predominantly uploaded a personal experience video ($p<0.05$).
[b] University channels/professional organization predominantly uploaded useful videos ($p<0.05$).
In the other categories of video source, no statistical significance was detected regarding the content of videos.

Popularity is the second most frequently cited quality measure on YouTube videos, often defined in relation to view counts [24]. Caution has to be taken with regard to popularity as a quality measure, since the number of views can easily be manipulated by, e.g., marketing strategies investing in pseudopopularity of products as well as by the YouTube viral effect attributed to longer availability or spreading across multiple Web pages of a YouTube video, which may account for higher view counts [24]. Moreover, negative popularity in the form of user comments and posts on YouTube has been claimed to harm the effectiveness of public health campaigns and reverse the initial positive attitude of laypeople towards a particular recommendation; the human papillomavirus vaccination is a well-known example [25]. In our study, we could not detect significant differences in numbers of 'dislikes' and comments among useful, misleading videos and personal experiences.

Incomplete information on the etiology of SS and drugs of unknown ingredients were posted by the misleading videos of the study. This observation confirms previously expressed safety concerns in retrieving YouTube information for healthcare decision making; promotion of unscientific therapies without authority approval, and dissemination of contradicting information to reference guidelines [9]. Useful videos were found, per definition, to discuss the abovementioned topics in a reliable way, sometimes incomplete or simplified, but never misleading.

The substantial proportion of YouTube video material related to personal experiences is also calling attention. Patient testimonials may be driven by financial motives. For example, plastic surgery clinics have rewarded patients with favorable opinion in testimonials with treatment discounts [26]. When merging misleading and patient experience video rates, our results related to SS are in line with previous YouTube reviews on other disease related videos [12-15,23].

Table 5: Quality of useful videos on SS (n = 36) based on source of information.

	independent users	government/ news agencies	university channels/ professional organizations	medical advertisements/ profit companies
	n=13	**n=6**	**n=8**	**n=9**
GQS (SD)	3.15 (0.7)	4 (0.8)	4 (0.7)	2.9 (1.2)
Reliability (SD)	2.2 (0.9)	3.2 (0.4)	2.75 (1.3)	2.1 (1.5)
1. The aims clear and achieved (%)	7 (53.8)	6 (100)	6 (75)	3 (33.3)
2. Reliable sources of information are used (%)	7 (53.8)	4 (66.7)	6 (75)	4 (4.4.)
3. The information is presented balanced and unbiased (%)	10 (71.4)	5 (83.3)	7 (87.5)	7 (77.8)
4. Additional sources of information are listed for patient reference (%)	3 (23.1)	5 (83.3)	1 (12.5)	2 (22.2)
5. Areas of uncertainty are mentioned (%)	2 (15.4)	2 (33.3)	2 (25)	3 (33.3)
Comprehensiveness (SD)	2.8 (1.4)	3.3 (1.1)	2.75 (1.5)	1.75 (1.5)
1. Epidemiology (%)	7 (53.8)	4 (66.7)	5 (62.5)	1 (11.1)
2. Pathogenesis (%)	6 (45.3)	2 (33.3)	4 (50)	3 (33.3)
3. Clinical features (%)	12 (92.3)	6 (100)	8 (100)	5 (55.6)
4. Diagnostic tests (%)	5 (38.5)	3 (50)	2 (25)	2 (22.2)
5. Treatment (%)	6 (45.3)	5 (83.3)	3 (37.5)	3 (33.3)

In terms of global quality, reliability and comprehensiveness of information, government/news agencies appeared to be the most creditable contributors. On the other hand, university channels/professional organizations presented as high GQS as government/news agencies, while none video was classified as misleading. Therefore, to increase the chances of accessing high quality information on SS, YouTube users should seek for videos of reliable origin. Nevertheless, this contradicts the search habits of Internet users, in which 75% of the e-health seekers occasionally or never trace the source of information [27].

By gaining knowledge over SS, patients can become more compliant, and at the same time, more active, keeping pace with the international trend in healthcare field. However, they are usually not able to identify an incomplete or misleading video, thus becoming prone to be deceived. YouTube users should be aware of the current shortcomings while searching for online health information for SS and seek advice from specialists regarding evidence-based videos.

Individuals searching at the Web tend to limit their search within the first eight to ten results of a search engine [28,29]. The broad inclusion of links that exceeded the first page in YouTube may be considered methodologically advantageous.

The results of our study underline the need for quality filtering of YouTube videos displaying health information on SS. YouTube encourages it's users to 'flag' videos of inappropriate content, however, such an option may be intentionally misused [30]. The social networking approach could offer the benefits of collective intelligence in assessing the trustworthiness of YouTube videos. Peer reviews by the crowd, like patient support groups, have been found capable of identifying and fixing incorrect information [31]. As indicated by our study, university and governmental institutions should be represented in these examination bodies. Interfaces that enable coupling of YouTube with evidence-based references could enhance the dissemination of accurate information [9]. Other researchers suggested modification of YouTube's ranking search algorithm to extract first the health related videos of trustworthy origin when a medical term is entered in YouTube's video search engine [32].

Limitations

As with any YouTube investigation, a number of limitations applied to our study. First, we focused on the analysis of English-language videos directly available on YouTube and not linked to other Web sites, at a single time-point. Although this approach might limit generalization of our findings, it has to be mentioned that English is acknowledged by Internet usage and population standings as the prevailing language [33]. Additionally, given the current lack of standardized tools to assess quality of patient health information videos on YouTube [24], we ran and further developed a multi-level but rather flawed by subjectivity evaluation system [12-15,23]. Lastly, the participation of a second examiner in applying the criteria optimized to some extent the evaluation process.

Conclusions

This study classified more than half of the included YouTube videos posting information on SS as useful. There was significant difference in global quality among useful, misleading videos and personal experiences. Government/news agencies and university channels/professional organizations appeared to be the most trustworthy sources of information. The vast majority of videos was found to be incomplete with regard to completeness of content. Specialists should be actively involved in the development of e-information resources and video-sharing platforms and should also refer their patients to evidence-based videos.

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Chapter 6

General discussion & future perspectives

The research described in this thesis concerns three different aspects of primary Sjögren's syndrome (pSS), viz. diagnosis, treatment and patient education. The focus was on the accuracy of ultrasound in diagnosing pSS, the development of markers to personalize treatment in pSS and the value of Internet as a source of information for patients with pSS. Although these three aspects at a first glance are not directly related to each other, they comprise the main interests of both clinicians and patients.

What is the accuracy of ultrasound in diagnosing pSS?

In the diagnosis of pSS, the involvement of salivary glands is currently usually assessed by sialometry and histopathology or less frequently by sialography and scintigraphy. Ultrasonography is a non-invasive, inexpensive, widely available, easily accessible and non-irradiating imaging modality [1], which has drawn a lot of attention in the diagnosis of pSS. The meta-analysis described in **Chapter 3A** demonstrated that major salivary gland ultrasonography has a sensitivity, specificity and diagnostic odds ratio of 69%, 92% and 33.89, respectively, to diagnose SS in the major salivary glands. However, the included studies were characterized by a high risk of bias in 'patient selection', 'index test' and 'flow and timing'. Additionally, different ultrasonographic scoring systems and study populations (study and control groups) were used, and publication bias was common.

Furthermore, the majority of included articles analyzed in the study described in **Chapter 3A** used a case-control design. This means that the difference in ultrasonographic characteristics is large and that ultrasonography can 'easily' distinguish healthy controls from pSS patients (**Chapter 3A**). The patient populations selected so far do not represent the daily practice, as the patients suspected for pSS exhibit a wide range of ultrasonographic characteristics from normal to extremely altered ultrasonographic structure. Consequently, the need of well-designed studies became evident in order to define the reliability and validity of ultrasound.

Moreover, none of the studies included in the meta-analysis (**Chapter 3A**) recruited as a control group patients with sarcoidosis, amyloidosis, human immunodeficiency virus (HIV) infection and hepatitis C virus (HCV) infection, all being diseases that could mimic a critical aspect of pSS, i.e. affect the major salivary glands, cause dry mouth or have similar histopathological features with pSS. The study described in **Chapter 3E** assessed these important disease-control groups. This pilot study showed that ultrasound is a potentially accurate imaging technique in diagnosing pSS and in differentiating it from sarcoidosis, amyloidosis, HIV and HCV infection. However, further studies with larger population series in patients with pSS and systemic diseases at different disease stages are required to confirm and elucidate these preliminary findings.

As far as the reproducibility of salivary gland ultrasonography for diagnosing pSS is concerned, the study described in **Chapter 3C** showed that the intra- and inter-observer reliability ranged from good to excellent. When assessing the individual ultrasonographic parameters, the inter-observer reliability was good for homogeneity and the presence for hypoechogenic areas, moderate for hyperechogenic reflections and salivary gland border and fair for echogenicity. Additionally, we observed that different observers may rather consistently identify in which patients ultrasound of the major salivary glands supports the diagnosis of pSS, but scoring the severity of the ultrasonographic findings is more inconsistent between observers (**Chapter 3C**). A possible consequence of this phenomenon is that when monitoring patients over time, the observed change by different observers might not be only attributed to the progression of the disease or to the effect of medication used to treat pSS, but might be partly the result of the discrepancy in scoring between different observers. Special attention is needed while following longitudinally an individual patient, i.e. when monitoring the activity or progression of pSS; it is advised that each particular patient is scored by the same ultrasonographer at every time-point.

Regarding the validity of ultrasound, we learned from the study described in **Chapter 3D** that the agreement between ultrasound and parotid or labial gland biopsy outcome was good and moderate, respectively. Specificity of ultrasound was higher when a parotid gland biopsy instead of labial biopsy was used as 'gold standard', which may be explained by the fact that the parotid gland is included in the ultrasonographic evaluation, whereas the labial gland is not. One of the most important findings is that negative ultrasound was a strong predictor of having also a parotid gland biopsy with focus score <1, while positive ultrasound was a strong predictor of having positive labial biopsy. Additionally, when ultrasound and serology were both negative, 94% of the patients had a negative parotid gland biopsy and 78% had a negative labial gland biopsy. Overall, ultrasound had high negative predictive value (NPV) for parotid gland biopsy and high positive predictive value (PPV) for labial gland biopsy (**Chapter 3D**), possibly, because patients with a positive parotid gland biopsy might have a distinct clinical profile from patients with a positive labial biopsy. There is still a need for larger studies focusing on the inherent differences in the histopathological characteristics of parotid and minor salivary gland tissue in both pSS patients and healthy controls. The 'NIH Salivary biomarkers for SS detection' study, currently running in the UMCG, will hopefully soon address this issue.

When combining positive ultrasound with presence of anti-SSA antibodies 94, 97 and 97% of the patients fulfilled the AECG, ACR and ACR-EULAR criteria, respectively. When combining negative ultrasound with absence of anti-SSA antibodies 98, 100 and 98% of the patients did not fulfill the AECG, ACR and ACR-EULAR criteria, respectively, if a parotid gland biopsy is amongst the items of the classification criteria. In case that a labial gland biopsy was considered as an item of the classification criteria, the combination of negative ultrasound with absence of anti-SSA

antibodies could not sufficiently exclude the classification of patients according to the AECG, ACR and ACR-EULAR criteria (**Chapter 3D**). Further research is needed to elucidate the accuracy of the classification criteria if ultrasound is added or if ultrasound replaces one of the existing items.

When interpreting the results, it should be kept in mind that the classification criteria are developed for research purposes to define homogenous study groups instead of diagnostic purposes, because of lack of sensitivity [2]. However, in clinical practice, classification criteria are often used for diagnostic purposes. This study showed that ultrasound is not able to replace salivary gland biopsy at group level. However, at individual patient level, and for diagnostic purposes, positive ultrasound may predict classification according to the AECG, ACR and ACR-EULAR classification criteria. Furthermore, ultrasound in combination with anti-SSA antibodies highly predicts classification according to the AECG, ACR and ACR-EULAR criteria, if parotid gland biopsy is performed as part of the diagnostic work-up.

Implications and future perspectives in ultrasound of the major salivary glands for diagnosing SS

We recommend that future diagnostic studies on ultrasound of the major salivary glands in pSS should comply with the QUADAS-2 guidelines in order to ensure high diagnostic quality [3]. Specifically, the following points from the guidelines should be followed:

1) a consecutive or random sample of patients should be used; a case control design and inappropriate exclusion of patients should be avoided;

2) ultrasonography results should be interpreted by observers blinded to each other as well as for the results of the reference test (diagnostic criteria, histology, sialography, scintigraphy, etc.). Ideally, the applied threshold scoring should be pre-specified;

3) an appropriate and rather short interval should elapse between the application of ultrasonography and the reference test, the whole study population should receive the reference test (which should be always the same) and the whole study population should be included in the analysis.

The aforementioned features should be clearly stated by authors of future studies to avoid potential misunderstanding and underestimation of the study design.

The development of validated automatic software has the potential to improve the reliability of salivary gland ultrasonography applied for pSS diagnostics [4]. Furthermore, so far static images have been analyzed instead of live ones for study purposes. Therefore, it is worth comparing the scoring of live images to static ones, since in daily practice live images are scored. Whether ultrasound can assess 'true' changes over time, e.g., when monitoring the activity or progression of pSS, remains also a burning question. That is why the next logical step should be to identify the minimal clinically relevant change, in other words, the smallest difference in score in the domain of interest, perceived as important change by the patient or the clinician, which could potentially mandate a change in patient management [5]. Last but not least, the development of a consensus and widely accepted ultrasonographic scoring system, by e.g., the EULAR US-pSS Study Group, for evaluating the major salivary glands of patients with pSS will allow better comparison between studies.

Is personalized treatment realistic in SS?

Patients with pSS have different genetic backgrounds, demographic features and prognosis and exhibit a broad variety of clinical manifestations, involving a number of pathophysiological pathways [6]. Personalized treatment, i.e. providing 'the right patient with the right drug at the right dose at the right time' [7] will therefore contribute to treating pSS. In previous studies it has been shown that rituximab (B cell depletion therapy) has beneficial objective and subjective clinical effects on patients with pSS [8-16]. However, not all patients seem to benefit from this treatment. An important finding of the study described in **Chapter 4A** was, therefore, that clinical responders to treatment with rituximab (RTX) had a higher number of $CD20^+$ B-cells/mm^2 of parenchyma of parotid gland tissue at pre-treatment (baseline) compared to non-responders. Moreover, this study also observed a correlation between the change in the number of $CD20^+$ cells/mm^2 of parenchyma and the change in ESSDAI. When higher numbers of B-cells are present in parotid gland parenchyma, it is presumed that RTX may result in depletion of more absolute numbers of B-cells responsible for the disease activity (measured by ESSDAI) than when lower numbers of B-cells are present in the tissue. The baseline number of B-cells/mm^2 of parenchyma of parotid gland may thus predict the patients' response to RTX and may be considered as a biomarker for a more personalized treatment approach to pSS patients. We arrived at the same conclusion when we tested the baseline proportions (and not only absolute numbers) of B-cells in the parotid gland tissue (**Chapter 4B**) of responders compared to non-responders. The nature of these disease-associated B-cells, which are reduced after RTX, needs to be elucidated.

The study described in **Chapter 4A** revealed also that RTX significantly reduced the overall lymphocytic infiltrate with a major loss of the B-cell component and number of germinal centers (GC)/mm^2 of parotid gland parenchyma in pSS patients. In addition, a major reduction of the quantity and severity of lymphoepithelial lesions (LEL) was apparent, reflecting significant restoration of the striated ducts. To explain this, we have hypothesized that the trigger for LEL formation is diminished, and as a result less epithelial reaction takes place leading to reduced proliferation and finally anatomical restoration of the striated ducts. The trigger for LEL forma-

tion is unknown, but B-cell derived cytokines may possibly be responsible for this. This notion is in line with the finding of Pollard et al. [17], who showed in the same cohort of RTX-treated patients that the serum levels of pro-inflammatory cytokines (e.g. IL-6) decreased significantly.

Implications and future perspectives in the treatment of pSS

A major aim of the treatment of pSS should be the restoration of the glandular tissue. To the best of our knowledge, RTX is the only medication that leads to restoration of the salivary gland ductal lesions [18]. The study described in **Chapter 4A** showed that pre-treatment histopathological evaluation of parotid gland biopsy in pSS patients may provide biomarkers to predict responsiveness to RTX treatment. However, there is lack of consensus guidelines to standardize the histopathological evaluation of salivary gland biopsies. Consensus guidelines will assist the pathologist to correctly identify and quantify histopathological parameters in pSS and contribute to a more accurate prediction of disease progression and personalized treatment, as well as to allow the comparison between study cohorts and different clinical trials. **Chapter 4C** points towards the urgent need for consensus guidelines. In particular, histological definition of germinal centers (GCs) in salivary gland tissue is warranted, since these structures can be difficult to detect in diagnostic hematoxylin & eosin (H&E)-stained tissue sections. Detection of GCs in the periductal lymphoid infiltrates of the salivary glands is clinically relevant, not only for assessing the responsiveness to treatment but also because the presence of these structures is associated with more severe disease [19]. Furthermore, the presence of GCs in minor salivary gland biopsies has been postulated to be a predictor of patients at risk for lymphoma development [20,21]. To confirm and elucidate the latter and to facilitate proper and easy detection of GCs, also by less trained persons, supplementary immunohistochemical staining might be helpful. Therefore, we recommend staining for B-cell lymphoma 6 (Bcl-6), a transcription factor expressed at high levels by GC B-cells (**Chapter 4C**).

Are Internet sites and YouTube reliable sources of information for patients with pSS?

Nowadays, patients are increasingly turning to Internet and video-sharing Web platforms, like YouTube, to make informed healthcare decisions for themselves and to rate the information provided by professionals or others. Internet is believed to have the power to modify patient-doctor relationship, by encouraging patients in the management of their health through a more shared decision making approach [22]. However, the diversity of authorship and the lack of peer-review process have led to dissemination of inaccurate and misleading information [23]. Practically speaking, any Internet user without exception regarding his/her background, medical qualifications, professionalism and intentions is authorized to create a site and upload video clips.

It was found that the bulk of the Web information regarding xerostomia was written in a fairly difficult to very difficult understandable mode and language (**Chapter 5A**). As a result, readers may fail to broaden their understanding of this symptom or may even misinterpret crucial information on healthcare decisions. Misperception may be further aggravated in stressful conditions [24,25]. Additionally, the Web pages were rated with medium accessibility and a considerably low reliability was detected for the sites under investigation, with implications for site transparency, expertise authorship, review procedures and update frequency (**Chapter 5A**). These findings have raised concerns about the susceptibility of patients to misinformation.

We also showed that approximately 50% of YouTube videos relevant to pSS were deemed useful (**Chapter 5B**), a finding lying close to the range of 54.9-63.0% reported by studies with similar methodology, which investigated the role of YouTube in the e-education of patients regarding H1N1 influenza pandemic, cardiopulmonary resuscitation, nephrolithiasis, rheumatoid arthritis and hypertension [26-30]. Incomplete information on the etiology of pSS and drugs of unknown ingredients were posted by the misleading videos of the study (**Chapter 5B**). This observation confirms previously expressed safety concerns in retrieving YouTube information for healthcare decision making; promotion of unscientific therapies without authority approval, and dissemination of contradicting information to reference guidelines [31]. The substantial proportion of YouTube video material related to personal experiences is also calling for attention. It has been previously demonstrated that patient testimonials may be driven by financial motives [32]. In terms of global quality score (GQS), reliability and comprehensiveness of information, government/news agencies appeared to be the most creditable contributors. On the other hand, university channels/professional organizations presented as high GQS as government/news agencies, while none video was classified as misleading. Therefore, to increase the chances of accessing high quality information on pSS, YouTube users should seek for videos of reliable origin.

Implications and future perspectives in e-education of pSS patients

The writing style of the e-material intended for patients preferably should be revised to a more favorable comprehensive level. Sentences should be kept short, while uniform and plain language should be maintained throughout the text. Long lists should preferably be avoided and bullet points as well as graphics are advised to be used with caution [33]. Administrators of Web sites should be advised by search engine optimization (SEO) experts to ensure that their sites are easily accessible. Patients and their intimates as well as patient organizations should be encouraged to use verified Web sites, generated by specialists, with structured interactive educational programs.

The results of our study underline also the need for quality filtering of YouTube videos displaying health information on pSS (**Chapter 5B**). YouTube encourages its

users to 'flag' videos of inappropriate content. Such an option may be intentionally misused by users with conflict of interest, however [34]. The social networking approach could offer the benefits of collective intelligence in assessing the trustworthiness of YouTube videos. Peer reviews by the crowd, like patient support groups, have been found capable of identifying and fixing incorrect information [35]. University and governmental institutions should be represented in these examination bodies. Interfaces that enable coupling of YouTube with evidence-based references could enhance the dissemination of accurate information [31]. In agreement with other researchers, we also suggest modification of YouTube's ranking search algorithm to extract first the health related videos of trustworthy origin when a medical term is entered in YouTube's video search engine [36].

Epilogue

The research described in this thesis has shown that:

i. ultrasound of the major salivary glands is a reliable imaging technique in the diagnostic process of patients suspected with pSS and has good and moderate agreement with parotid and labial gland biopsy, respectively. The combination of ultrasound with anti-SSA antibodies is highly predictive for fulfilling the classification criteria, when the outcome of the parotid gland biopsy is considered as an item of the classification criteria.

ii. baseline histopathological characteristics of parotid gland biopsy may strongly contribute to a more personalized treatment approach to pSS patients with RTX.

iii. online information on xerostomia and pSS exhibits currently variable quality and therefore should be approached with caution.

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Chapter 7

Summary

Sjögren's syndrome (SS) is a systemic autoimmune disease, second to rheumatoid arthritis, with an estimated prevalence of 0.05% in the general population. SS commonly affects the exocrine glands, in particular the salivary and lacrimal glands, resulting in a sensation of dry eyes (keratoconjunctivitis sicca) and dry mouth (xerostomia). SS can be distinguished in primary Sjögren's syndrome (pSS), in case no other autoimmune disease is present, and secondary Sjögren's syndrome (sSS), in case additional connective tissue diseases are present.

Recently, the accuracy of ultrasonography to evaluate the involvement of the major salivary glands as well as its suitability to be used as an alternative to or replacement of other diagnostic tests used to classify pSS gained a lot of interest. The true diagnostic properties of salivary gland ultrasonography remain, however, vastly unknown.

With regard to treatment of pSS, for many decades this was mainly symptomatic and aimed to, e.g., reduce the feeling of dryness and provide comfort to the patients. With the advent of 'disease-modifying antirheumatic drugs' (DMARDS) the possibilities for treating pSS have considerably expanded. DMARDS not only aim to reduce the symptoms, but are also aimed to have a systemic effect. There is, however, so far no agreement on which pSS patients are susceptible to a treatment with DMARDS.

Finally, as far as patient education is concerned, persons experiencing xerostomia or being diagnosed with pSS and undergoing treatment, like any other online health information seeker, may search the Internet for background knowledge. However, the diversity of authorship and the lack of peer-review process have led to dissemination of inaccurate and misleading information on the Internet.

Taking into account the abovementioned open issues regarding the diagnosis, treatment and patient education of patients with pSS, the overall aim of the research described in this PhD-thesis was to assess new challenges and trends in the diagnosis, treatment and e-education of pSS patients (**Chapter 1**).

The study described in **Chapter 2** reviews the three major causes of xerostomia, viz. side-effects of medication, radiotherapy in the head and neck area and SS. With regard to medication, certain classes of drugs are known to induce hyposalivation and/or xerostomia by, e.g., targeting neurotransmitters and receptors. As far as head and neck radiotherapy is concerned, the administration of high radiation doses to the major salivary glands leads to progressive loss of glandular function and a diminished salivary output. Reduction of the dose and the volume of the radiated salivary gland tissue by advanced radiotherapy techniques was shown to be highly beneficial for patients. As mentioned before, SS affects the exocrine glands, the salivary and lacrimal glands in particular. The pathogenesis underlying SS involves systemic B-cell hyperactivity and T-cell lymphocytes targeting glandular epithelial cells.

Diagnosis

Chapter 3A presents a meta-analysis on studies examining the properties of ultrasonography of major salivary glands (SGUS) for diagnosing pSS, a tool that is more frequently used in the diagnostic work-up of SS. This meta-analysis demonstrated that SGUS has a sensitivity, specificity and diagnostic odds ratio of 69%, 92% and 33.89, respectively, to diagnose pSS according to the ultrasonographic characteristics of the major salivary glands. Based on the results of this meta-analysis, it was concluded that SGUS has the potential to evolve into a viable alternative in the diagnostic work-up of the major salivary glands in patients with pSS. In the study described in chapter **Chapter 3B**, concerns were expressed regarding a similar meta-analysis performed by other authors, as discrepancies were detected between the data shown in that study and the data presented by the source studies.

With respect to the reproducibility of SGUS, the study presented in **Chapter 3C** showed that the intra- and inter-observer reliability of SGUS ranged from good to excellent. The results of this study also showed that scoring the severity of the ultrasonographic findings is less consistent between observers. Thus, when monitoring patients over time, the observed change might not be only attributed to the progression of the disease or to the effect of medication, but might also be partly due to the discrepancy in scoring between the different observers.

In order to investigate the validity, i.e. the ability of SGUS to indicate which individuals have pSS and which do not, SGUS results were compared to the results of the parotid and labial gland biopsy, as well as to the performance of the classification criteria in patients who were clinically suspected with pSS (**Chapter 3D)**. We learned from this study that the agreement between SGUS and parotid or labial gland biopsy outcomes was good and moderate, respectively, but also that SGUS cannot replace salivary gland biopsy. One of the most important findings of this study is that when combining positive SGUS with presence of anti-SSA antibodies, 94, 97 and 97% of the patients fulfilled the AECG, ACR and ACR-EULAR criteria, respectively.

The pilot study described in **Chapter 3E** addressed a major issue detected in the meta-analysis presented in **Chapter 3A**, viz. none of the studies included in the meta-analysis had recruited as controls patients with sarcoidosis, amyloidosis, human immunodeficiency virus infection or hepatitis C virus infection. These are all diseases that can mimic pSS. Therefore, a pilot study was performed to assess the performance of SGUS in these patient groups. It was shown that SGUS is a potentially accurate imaging technique in diagnosing pSS and in differentiating pSS from these systemic diseases.

Treatment

The study described in **Chapter 4A** revealed that treatment with rituximab (RTX) significantly reduced the overall lymphocytic infiltrate with a major loss of the B-cell component and number of germinal centers/mm^2 of parotid gland parenchyma in pSS patients. In addition, a major reduction of the quantity and severity of lymphoepithelial lesions was apparent. These findings clearly show that RTX treatment results in significant restoration of the salivary gland parenchyma of patients with pSS. The study described in this chapter showed also that clinical responders to treatment with RTX had a higher number of CD20$^+$ B-cells/mm^2 of parenchyma of parotid gland tissue at pre-treatment (baseline) compared to non-responders. The baseline number of B-cells/mm^2 of parenchyma of parotid gland may thus predict the patients' response to RTX and may be considered as a biomarker for a more personalized treatment approach.

The same conclusion as mentioned above could be drawn when baseline proportions of B-cells in the parotid gland tissue were used and thus not absolute numbers of these cells (**Chapter 4B**). The observations described in **Chapter 4C** further stressed the need for standardized guidelines to assess the histopathological characteristics of the salivary gland tissue of patients with pSS. Consensus guidelines will assist researchers to better identify and quantify histopathological parameters in pSS and thus contribute to a more accurate prediction of disease progression and targeted personalized treatment.

e-Patient education

In **Chapter 5A** an e-search was performed using four popular search engines: Google™, Bing™, YAHOO!® and Ask®. The terms 'dry mouth' and 'xerostomia' were entered individually to mimic a common, i.e., a search performed by a layperson, online search. It was found that the bulk of the Web information related to xerostomia was written in a fairly difficult to very difficult understandable mode and language. Furthermore, medium accessibility and a considerably low reliability were detected for the sites under investigation, with implications for site transparency, expertise authorship, review procedures and update frequency. These findings have raised concerns about the susceptibility of patients to misinformation.

In addition to the information available on Web sites, we also showed that approximately 50% of YouTube videos relevant to pSS were deemed useful (**Chapter 5B**) and thus may have the power to positively modify patient-doctor relationship, by encouraging patients in the management of their health through a more shared decision making approach. At the same time, incomplete information on the etiology of pSS and drugs of unknown ingredients were posted by the misleading videos. Videos were evaluated with the global quality score (GQS), a 5-point scale, which assesses the quality of the information and how useful the reviewer assumed the particular video would be to a patient. In terms of GQS, reliability and comprehensiveness of information, government/news agencies appeared to be the most creditable contributors. On the other hand, university channels/professional organizations presented as high GQS as government/news agencies, while none video was classified as misleading. Therefore, to increase the chances of accessing high quality information on pSS, YouTube users should seek for videos of reliable origin.

Conclusion

The research described in this thesis has shown that SGUS is a reliable imaging technique in the diagnostic process of patients suspected with pSS, but should be used with caution when following up patients longitudinally for assessing disease progression or treatment evaluation. Combining a positive SGUS with the presence of anti-SSA antibodies is highly predictive whether a patient might fulfill the classification criteria. As far as treatment is concerned, baseline histopathological characteristics of a parotid gland biopsy may strongly contribute to a more personalized treatment approach to pSS patients with RTX. Last but not least, the currently available online patient information on xerostomia and pSS exhibits a variable quality and therefore should be approached with caution.

Chapter 8

Samenvatting

Het syndroom van Sjögren (SS) is de vaakst voorkomende systemische auto-immuunziekte op reumatoïde artritis na, met een geschatte prevalentie van 0.05% in de populatie. SS tast in het bijzonder vocht producerende (exocriene) klieren aan, met name de speekselklieren en traanklieren. De beschadiging van de exocriene klieren leidt o.a. tot een droog gevoel van de ogen (keratoconjunctivitis sicca) en de mond (xerostomie). SS kent twee vormen, namelijk het primaire (pSS) en secundaire (sSS) syndroom van Sjögren. Men spreekt van sSS wanneer het syndroom van Sjögren gepaard gaat met een andere systemische auto-immuunziekte, bijvoorbeeld reumatoïde artritis of lupus erythematosus.

Bij de diagnostiek naar het bestaan van SS worden veelal invasieve onderzoekstechnieken gebruikt zoals een chirurgische biopsie van speekselklierweefsel. Het beschikbaar komen van betrouwbare maar minder invasieve onderzoekstechnieken zou een grote verworvenheid zijn. Momenteel wordt veel aandacht besteed aan de waarde van echografie in de diagnostiek van SS. Er wordt hierbij onderzocht wat de betrouwbaarheid van echografie van de grote speekselklieren voor het stellen van de diagnose SS is. Als het stellen van de diagnose met deze methodiek betrouwbaar blijkt, vormt echografisch onderzoek van de speekselklieren mogelijk een alternatief voor andere diagnostische onderzoeken die worden gebruikt om de diagnose SS te stellen. De eerste resultaten lijken veel belovend, maar over de werkelijke diagnostische waarde echografie van de speekselklieren is nog onvoldoende bekend. In het eerste deel van dit proefschrift (**DIAGNOSE**) wordt aandacht geschonken aan de waarde van echografie van de speekselklieren in het kader van diagnostiek naar pSS.

Tot voor kort was de behandeling van pSS voornamelijk gericht op het verlichten van symptomen, onder andere door klachten van droogheid te verminderen. Met de komst van 'Disease-Modifying Antirheumatic Drugs (DMARDs) zijn de mogelijkheden voor de behandeling van pSS toegenomen. Een behandeling met DMARDs is zowel gericht op symptoomverlichting als op het verminderen van de systemische ziekteactiviteit. Een behandeling met DMARDs is echter kostbaar. Daarom is het van belang om vooraf in te kunnen schatten of een bepaalde patiënt met pSS goed zal reageren op een behandeling met DMARDs. Momenteel kan deze inschatting nog niet worden gemaakt. In het tweede deel van dit proefschrift (**BEHANDELING**) wordt onderzoek beschreven op basis waarvan mogelijk deze inschatting wel kan worden gemaakt.

In het derde deel van dit proefschrift (**ONLINE PATIËNTEN EDUCATIE**) wordt ingaan op de betrouwbaarheid van beschikbare informatie op het internet t.a.v. het SS. Over de betrouwbaarheid van deze informatie op internet is onvoldoende bekend, terwijl zowel patiënten met xerostomie als patiënten met pSS vaak aanvullende informatie zullen zoeken op internet.

In **hoofdstuk 2** worden de drie belangrijkste oorzaken van xerostomie beschreven, namelijk bijwerkingen van medicatie, radiotherapie in het hoofd-hals gebied en SS. Medicatie-gerelateerde xerostomie kan worden veroorzaakt door bepaalde typen medicatie, bijvoorbeeld door medicamenten die de neurotransmissie beïnvloeden. Het blootstellen van de grote speekselklieren aan hoge dosis ioniserende straling leidt tot een progressief verlies van de functie van deze klieren. Aangezien de tumoren gewoonlijk niet in de speekselklieren zelf zijn gelegen, maar de straling deze klieren moet passeren om de tumor te bereiken, kunnen de patiënten veel voordeel hebben bij een vermindering van de cumulatieve stralingsdosis die de speekselklieren ontvangen en het volume van het speekselklierweefsel dat is bestraald. Door toepassing van geavanceerde radiotherapeutische technieken kan de dosis op het speekselklierweefsel worden verminderd, terwijl de tumor toch de gewenste cumulatieve dosis ontvangt. Zoals eerder genoemd, heeft SS invloed op de exocriene klieren, in het bijzonder de speeksel- en traanklieren. In dit proefschrift wordt ingaan op verschillende aspecten van SS.

Diagnose

In hoofdstuk 3A wordt door middel van een meta-analyse van de in de literatuur beschikbare data onderzocht wat de waarde van echografie van de grote speekselklieren is voor het stellen van de diagnose pSS. Uit deze meta-analyse kwam naar voren dat echografie van de grote speekselklieren een redelijke sensitiviteit (69%), een hoge specificiteit (92%) en een goede diagnostische odds ratio (33.89) heeft om de diagnose pSS te stellen. Op basis van deze resultaten werd de conclusie getrokken dat echografisch onderzoek van de grote speekselklieren een belangrijke rol kan spelen in de diagnostische work-up van patiënten die worden verdacht voor het pSS. In **hoofdstuk 3B** wordt commentaar gegeven op een vergelijkbare meta-analyse die door andere auteurs was uitgevoerd. De in dat onderzoek gebruikte gegevens kwamen echter niet overeen met de gegevens zoals deze waren vermeld in de oorspronkelijke artikelen waarop die meta-analyse gebaseerd was.

In **hoofdstuk 3C** wordt een studie gepresenteerd waarin de reproduceerbaarheid van echografisch onderzoek van de grote speekselklieren is onderzocht. Uit deze studie kwam naar voren dat de intra- en interbeoordelaarsbetrouwbaarheid van deze techniek goed tot uitstekend was. Met betrekking tot het scoren van de ernst van de aantasting van de speekselklieren stemden de echografische bevindingen tussen de verschillende beoordelaars minder goed overeen. Met andere woorden, wanneer patiënten in de tijd worden gevolgd, moet dezelfde echografist steeds de metingen doen zodat de waargenomen veranderingen in de ziekteprogressie en/of het waargenomen effect van een behandeling daadwerkelijk aan de ziekteprogressie of behandeling zijn toe te wijzen en niet aan een verschil in interpretatie van de echografische beelden door verschillende echografisten.

In **hoofdstuk 3D** wordt een studie beschreven waarin is onderzocht of op basis van echografisch onderzoek van de grote speekselklieren onderscheid kan worden gemaakt tussen patiënten met pSS en patiënten zonder pSS. Met andere woorden

hoe valide is echografie van de grote speekselklieren om vast te stellen of een patiënt daadwerkelijk pSS heeft. Daarvoor werden de uitkomsten van echografisch onderzoek van de grote speekselklieren vergeleken met die van het parotisbiopt, lipbiopt en verschillende classificatie criteria. Deze vergelijking werd gedaan in een groep patiënten met verdenking op pSS. De resultaten lieten zien dat de uitkomst van echografisch onderzoek van de grote speekselklieren goed overeenkwam met de histopathologische karakteristieken van het parotis biopt, maar minder goed met die van het lipbiopt. De mate van overeenkomst was niet voldoende het speekselklierbiopt te vervangen door de bevindingen van het echografisch onderzoek van de grote speekselklieren. Een belangrijke nevenbevinding van deze studie was dat bijna alle patiënten met een positieve uitslag op het echografisch onderzoek en de aanwezigheid van SSA antistoffen voldeden aan de 'American-European Consensus Group' (AECG), 'American College of Rheumatology' (ACR) en 'American College of Rheumatology/European League Against Rheumatism' (ACR-EULAR) criteria.

Het onderzoek dat beschreven is in **hoofdstuk 3E** gaat in op een belangrijke omissie in de studies die zijn geïncludeerd in de in hoofdstuk 3A beschreven meta-analyse**.** In geen van deze studies bleek als controle patiënten te zijn meegenomen met ziekten die effecten van pSS op speekselklieren kunnen nabootsen, zoals sarcoidose, amyloidose, en infecties met humaan immunodeficiëntie virus en hepatitis C virus. In een pilot onderzoek hebben wij daarom gekeken naar de echografische karakteristieken van de grote speekselklieren van dergelijke patiënten. Hoewel echografisch onderzoek van deze speekselklieren een beeld kan geven dat gelijkt op het echografisch beeld zoals wordt gezien bij patiënten met pSS, lijkt echografisch onderzoek van de grote speekselklieren toch voldoende onderscheidend om patiënten met pSS te kunnen onderscheiden van deze systemische ziekten.

Behandeling

In **hoofdstuk 4A** wordt beschreven dat een behandeling met rituximab resulteert in een afname van het lymfocytaire infiltraat in het parenchym van de gl. parotidea van patiënten met pSS en dat tevens het B-cel compartiment en het aantal kiemcentra kleiner werden. Daarnaast werd een afname van de hoeveelheid en ernst van de lymfoepitheliale laesies gezien. Deze bevindingen duiden erop dat een behandeling met rituximab leidt tot herstel van het speekselklierparenchym. Een belangrijke nevenbevinding was dat patiënten die klinisch goed reageren op behandeling met rituximab, een hoger aantal CD20+ B-cellen/mm^2 parotisparenchym hebben in een biopt dat voorafgaand aan de behandeling was genomen dan patiënten die niet goed reageren op deze behandeling. Met andere woorden, op basis van het aantal B-cellen/mm^2 parotisparenchym kan worden voorspeld of een patiënt al dan niet goed zal reageren op een behandeling met rituximab. Het aantal B-cellen/mm^2 parotisparenchym op baseline zou dus een biomarker kunnen zijn voor het instellen van een gepersonaliseerde behandeling aan pSS patiënten.

Dezelfde conclusie kon worden getrokken wanneer gekeken werd naar het percentage B-cellen in het parotisparenchym op baseline, in plaats van de absolute aantallen B-cellen (**hoofdstuk 4B**).

In **hoofdstuk 4C** wordt benadrukt dat het belangrijk is om gestandaardiseerde richtlijnen te ontwikkelen voor het beoordelen van de histopathologie van speekselklierweefsel van patiënten met pSS. Door histopathologische kenmerken in de speekselklierbiopten van patiënten met pSS te meten en kwantificeren op basis van dergelijke richtlijnen, kan zowel de ziekteprogressie beter worden gemonitord als een gepersonaliseerde behandeling van patiënten worden ingesteld.

Online patiënten educatie

In **hoofdstuk 5A** wordt een studie beschreven waarin een online zoekopdracht is uitgevoerd in vier veelgebruikte zoekmachines: Google™, Bing™, YAHOO!® en Ask®. De termen "dry mouth" en "xerostomia" werden afzonderlijk ingevoerd om de online zoekopdracht na te bootsen. Uit dit onderzoek bleek dat het grootste gedeelte van de online beschikbare informatie over xerostomie in een voor een leek moeilijk te begrijpen taal is geschreven. Bovendien waren de gevonden sites beperkt te bereiken en was de inhoud van deze sites weinig betrouwbaar. Met andere woorden er is een reëel risico dat pSS patiënten op basis van beschikbare informatie op internet verkeerd worden geïnformeerd over hun ziekte.

Naast informatie op websites, zijn er ook YouTube video's beschikbaar over pSS. In **hoofdstuk 5B wordt** beschreven dat slechts ongeveer de helft van de YouTube video's over pSS nuttig waren. De inhoud van deze video's kan mogelijk bijdragen aan het verbeteren van de interactie tussen patiënten en artsen, en daarmee patienten aanmoedigen om zowel zelf hun zorg te organiseren als wel beter de keuzes in behandelmogelijkheden samen met hun arts te kunnen maken. Er waren echter ook misleidende video's beschikbaar die onduidelijk en/of onjuiste informatie gaven over de oorzaak van pSS en over medicijnen met onbekende ingrediënten. Alle video's werden beoordeeld door middel van de 'global quality score (GQS)'. Dit is een 5-puntsschaal waarmee de kwaliteit van de informatie en het nut van de video voor een patiënt kan worden bepaald. De overheid en nieuwsdiensten bleken de beste bron te zijn voor video's. Deze video's hadden een hoge GQS, waren betrouwbaar en gaven duidelijke informatie. De GQS van video's die via universitaire kanalen en professionele organisaties waren geplaatst bleek even hoog te zijn als die van de overheid en nieuwsdiensten. Op basis van ons onderzoek kan aan patienten worden aangeraden dat YouTube video's over pSS van de in het onderzoek gevonden betrouwbare bronnen goede informatie verschaft.

Conclusie

Het in dit proefschrift beschreven onderzoek laat zien dat echografisch onderzoek van de grote speekselklieren een betrouwbare beeldvormende techniek is bij de diagnostiek naar pSS. Voorzichtigheid is echter geboden wanneer deze techniek wordt gebruikt om patiënten in de tijd te volgen of om het effect van een bepaalde behandeling te evalueren. Om dan een goed inzicht te krijgen in de veranderingen die zijn opgetreden wordt aangeraden om het echografisch onderzoek telkens door dezelfde echografist te laten uitvoeren. Voorts werd aangetoond dat een positieve echografische uitslag in combinatie met de aanwezigheid van SSA-antistoffen voorspellend is voor de classificatie van een patiënt als lijdend aan pSS. Met betrekking tot de histopathologische karakteristieken van het parotisklierbiopt, kan op basis van deze karakteristieken worden voorspeld of een pSS patiënt goed zal reageren op een behandeling met rituximab. Op deze wijze kan de behandeling worden gepersonaliseerd. Tot slot moet worden opgemerkt dat de online beschikbare informatie over xerostomie en pSS van sterk wisselende kwaliteit is.

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Curriculum Vitae

Konstantina Delli graduated from the Faculty of Dentistry, Aristotle University – Military School of Officers Corps, Thessaloniki, Greece in 2005. Subsequently, she served as a dental officer/staff member at the Department of Restorative Dentistry, 251 Hellenic Air Force VA General Hospital. In 2012, she completed the 3-year full-time postgraduate program (MSc) in Oral Medicine and Pathology at the University of Athens, Greece. During the same period, she was assigned a researcher position at the Department of Oral Surgery and Stomatology, University of Bern, Bern, Switzerland, where she obtained her Doctoral degree (Dr med dent) in 2012. Since December 2012, she has joined the Department of Oral and Maxillofacial Surgery, University Medical Center Groningen, the Netherlands, where she combined PhD research activities with treatment of patients with Oral Medicine associated conditions. Her main clinical and research interests are diseases of the oral mucosa, salivary gland pathophysiology and e-health.

Address for correspondence

K. Delli
University Medical Center Groningen
Department of Oral and Maxillofacial Surgery
P.O. Box 30.001
9700RB Groningen
The Netherlands

work e-mail: k.delli@umcg.nl
private e-mail: kondelli@yahoo.gr

Distinctions

1. Sjögren's award 2016 granted by the Dutch association of patients with Sjögren's syndrome.

2. Best original article during the year 2015 in *Oral Diseases* for the article: 'Diagnostic properties of ultrasound of major salivary glands in Sjögren's syndrome: a meta-analysis.'

3. Travel grant 2015 for The 13th International Symposium on Sjögren's Syndrome in Bergen, Norway.

4. Travel grant 2013 for The 12th International Symposium on Sjögren's Syndrome in Kyoto, Japan.

5. Best scientific poster award 2013 at the 89th Congress of the European Orthodontic Society in Reykjavik, Iceland.

List of publications

1. **Delli K**, Arends S, van Nimwegen JF, Dijkstra PU, Stel AJ, Spijkervet FKL, Bootsma H, Vissink A, on behalf of the EULAR US-pSS Study Group. Ultrasound of the major salivary glands is a reliable imaging technique in patients with clinically suspected primary Sjögren's syndrome. Accepted for publication in European Journal of Ultrasound/ Ultraschall in der Medizin
2. **Delli K**, Doff JJ, Vissink A, Spijkervet FKL. [Localized (juvenile) spongiotic gingival hyperplasia: an underdiagnosed disease.] Ned Tijdschr Tandheelkd 2017 Feb. [Epub ahead of print]
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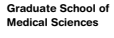
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