Clinical assessments in Sjögren's syndrome: The oral component

How much saliva is enough?

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- the Department of Oral and Maxillofacial Surgery, University Hospital Groningen
- the Department of Internal Medicine, divisions of Clinical Immunology and Rheumatology, University Hospital Groningen

In collaboration with:
- the Department of Ophthalmology, University Hospital Groningen
- the Department of Oral Biology, division of Oral Biochemistry, Faculty of Dentistry Amsterdam (ACTA)
- the Department of Pathology and Laboratory Medicine, University Hospital Groningen

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Wouter Warner Iwe Kalk

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Promotores:	Prof. dr J.L.N. Roodenburg
	Prof. dr C.G.M. Kallenberg
Co-promotor:	Dr A. Vissink
Referenten:	Dr F.K.L. Spijkervet
	Dr H. Bootsma
Beoordelingscommissie:	Prof. dr L. Kater
	Prof. dr M.H. van Rijswijk
	Prof. dr I. van der Waal

LIST OF ABBREVIATIONS

ACE	: angiotensin-converting enzyme
ANOVA	: analysis of variance
BUT	: Break-up time
CREST	: calcinosis, Raynaud, esophagusstenosis, sclerodactyly, teleangiactasia
CRP	: C-reactive protein
ESR	: erythrocyte sedimentation rate
ENT	: ear, nose and throat
IgA	: immunoglobulin class A
IgG	: immunoglobulin class G
IgM	: immunoglobulin class M
LR	: likelihood ratio
MCTD	: mixed connective tissue disease
nonSS	: negative for Sjögren's syndrome
NPV	: negative predictive value
Par	: parotid
PBC	: primary biliary cirrhosis
PPV	: positive predictive value
pSS	: primary Sjögren's syndrome
ROC	: receiver-operating characteristic
SLE	: systemic lupus erythematosus
SM/SL	: submandibular/sublingual
SS	: Sjögren's syndrome
SS-A antibody	: Sjögren's syndrome autoantibody, type A (Ro)
SS-B antibody	: Sjögren's syndrome autoantibody, type B (La)
sSS	: secondary Sjögren's syndrome

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CHAPTER

1

General introduction

GENERAL INTRODUCTION

The condition that is now known as Sjögren's syndrome (SS) has been studied for over a century. Still it poses the clinician a great challenge as its manifestations extend beyond the clinical scope of any of the involved specialists hence requiring multidisciplinary treatment and research. Classified as an autoimmune disorder of the exocrine glands, SS is generally regarded as the second most common rheumatic disorder, exceeded in incidence only by rheumatoid arthritis.[1,2] The occurrence of SS, however, is not well established due to a lack of uniform diagnostic criteria and differences in diagnostic techniques.

Having SS has a great impact on the patient's physical, mental, and social well being. Physically, patients may suffer from exhausting fatigue, persistent daily discomfort of dry eyes and mouth and many other complaints related to either general autoimmune processes, or exocrine gland inflammation throughout their body. Mentally, patients have to cope with a chronic, disabling disease that is mostly invisible (to friends and relatives), is poorly known, and is often initially misdiagnosed, or remains undiagnosed for other reasons for periods of five to ten years. Socially, patients are impaired because they cannot take part in many activities due to either ocular problems (sun-, wind- and smoke intolerance), oral problems (difficulties with speaking, eating, wearing a denture) and a great lack of energy. Many patients have to reduce their level of employment or even resign from their job due to complaints related to SS. The diagnostic procedure itself of SS is also very demanding for the patient, because many tests are currently required for a proper diagnosis.[3] Large variability in disease expression, an insidious onset of the disease, and its resemblance with many other conditions necessitates a variety of tests for establishing the diagnosis. With respect to our understanding of SS, it is striking that we are still dealing with a syndrome instead of a disease; after a century of experience with this relatively common disorder, there is still much to discover. A true organ-specific autoantibody with pathognomonic significance for example, as ultimate proof of the autoimmune nature of SS, has not yet been identified.[4,5] Pathognomonic signs that would otherwise disclose SS are also lacking, rendering clinicians dependent upon a variety of tests that, combined, reach a certain probability for the presence of this syndrome. Since the tear and salivary glands are both well accessible for clinical evaluation, an oral and an ocular component of SS can be distinguished diagnostically. Current treatment modalities for SS are predominantly symptomatic.[6-11] To date, objective methods to estimate disease activity are lacking[12], which renders the evaluation and introduction of new therapeutic agents difficult.

Clinicians are thus confronted with a very common debilitating disease that provokes diffuse sicca complaints, as well as general complaints. The disorder is often difficult to recognise and requires quite some effort to be diagnosed properly. After the diagnosis has been established, there is generally no other treatment than alleviating the symptoms.[13,14]

The main objective in this thesis is to optimise current diagnostics and to obtain clinical outcome parameters in SS. In 200 patients, the oral component of SS has been studied extensively during the past three years by a multidisciplinary research team, in order to improve and simplify the process of diagnosing SS, and to obtain methods to evaluate drugtherapy. All studied patients were diagnosed in accordance to the revised European classification criteria for SS. Specific objectives were to shorten the diagnostic delay, reduce the diagnostic work-up, and, in addition, to find clinical parameters which assess disease activity and progression. An early diagnosis of SS has two main advantages. Firstly, that the complaints can be related properly to the underlying disease, which is often very important for the patient. Secondly, an early diagnosis allows clinicians to consider preventive measurements for ocular and oral damage that may be necessitated by impaired function of tear- and salivary glands. With improved and simplified procedures, the diagnostic work-up will become more concise resulting in better general acceptance by patients and clinicians. This will yield clinically and scientifically relevant advantages, for it facilitates clinical diagnostics and it optimises external validity of research results. With valid outcome parameters of SS, specific disease effects of drug therapy can be monitored, thus clinical trials can be evaluated more accurately.

The oral tests that were selected for evaluation in this thesis each assess the disorder differently, by analysis of saliva, serum, and glandular duct-architecture. The selected diagnostic tests are: sialometry and sialochemistry assessing salivary gland function (chapter 3); measurement of serum salivary isoamylase activity assessing turnover of salivary gland cells (chapter 4); and sialography visualising specific alterations of salivary gland duct architecture (chapter 5). After the different tests for the oral component of SS have been evaluated individually, a comparison was made between the tests for the oral and the ocular component (chapter 6). Two rather unusual cases are presented in the next chapter (chapter 7), demonstrating the risk of misdiagnosing SS in relation to current diagnostic criteria. In chapter 8, conclusions of the individual studies are combined and placed into a wider context.

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CHAPTER

2

Background information

BACKGROUND INFORMATION

In this chapter, additional background information is given on Sjögren's syndrome and its oral component, in order to provide a solid basis of understanding for the topics dealt with in this thesis. Relevant anatomy and physiology of the salivary glands is presented in the first paragraph for better understanding of the different salivary gland investigations that will be discussed in following chapters. In the next paragraph, an historical overview is given on the syndrome, in order to explain old nomenclature and to place acquired insights into their own perspective. The historical overview is followed by a presentation of current insights on the salivary immunopathology in SS. In the last paragraph, clinical symptoms and signs in SS are presented as observed during the history taking and physical examination. Clinical examination forms the basis for further diagnostic investigations. If experienced in this area, one can skip this chapter and proceed to the next chapters.

ANATOMY AND PHYSIOLOGY OF THE SALIVARY GLANDS

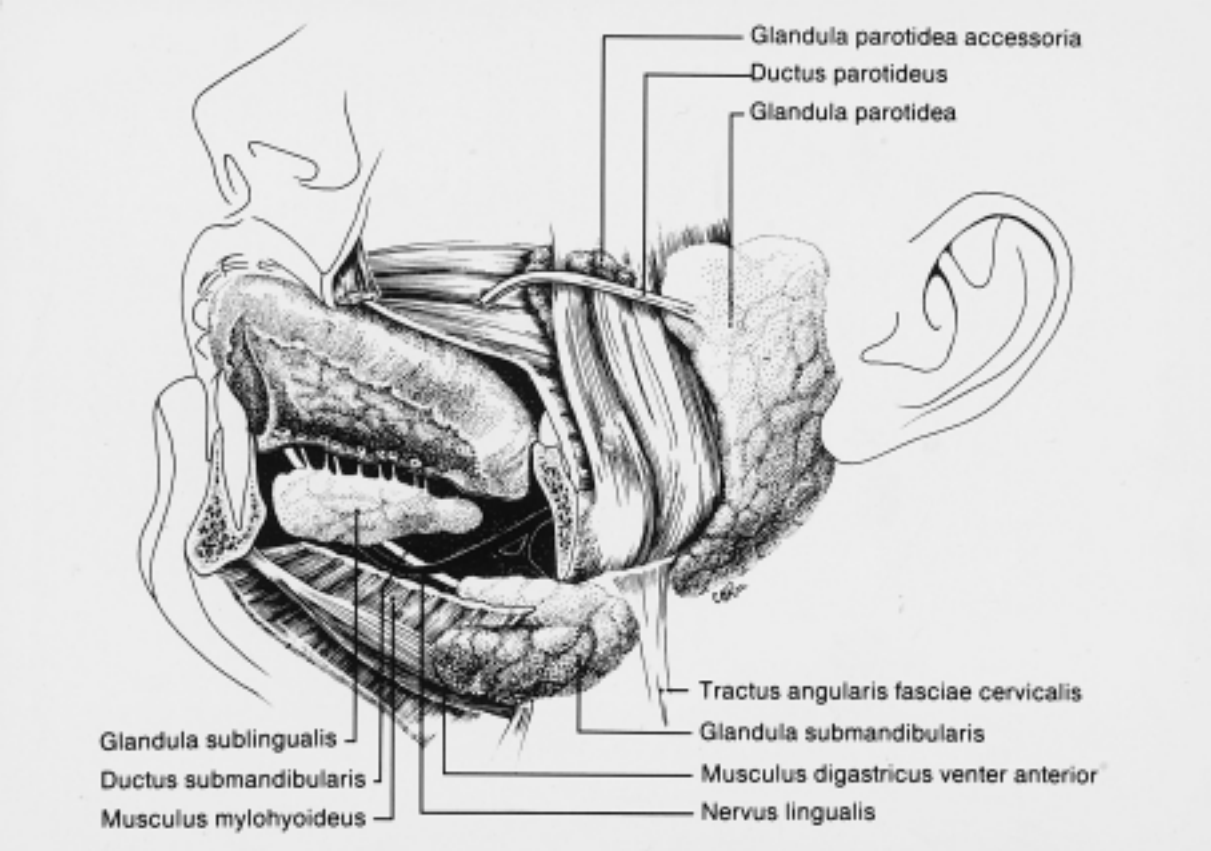
The salivary glands are divided clinically into major and minor salivary glands and functionally into serous, mucous and seromucous salivary glands. The major salivary glands include three pairs of glands, which communicate with the mouth: the parotid-, the submandibular- and the sublingual glands.[1,2]

The parotid gland is the largest of the salivary glands, weighing 14 to 28 grams. This serous gland lies anterior and inferior to the ears. A surgical distinction is often made between the superficial and deep portions of the parotid gland, which are divided by the facial nerve. Anatomically, no capsule or fascia separates these portions. The terminal acini are interconnected with the interlobular ducts through intralobular ducts. Interlobular ducts join to form interlobar ducts. Generally two or three interlobar ducts join at the anterior part of the gland, to form the main parotid duct, named Stensen's duct. After leaving the gland, the main parotid duct crosses over the masseter muscle. At the anterior border of this muscle, it curves medially, passing through the fat of the cheek and the buccinator muscle. It then runs obliquely in the cheek between the buccinator muscle and the mucosa to terminate at the parotid papilla situated adjacent to the second upper molar. The main duct is about 50 mm long, and has a diameter of 1 to 3 mm with the narrowest part at its orifice.

The submandibular gland is the second largest of the salivary glands. This seromucous gland is located in the upper neck and the floor of the mouth and

wraps around the posterior free margin of the mylohyoid muscle. Its size is about half the size of the parotid gland and it weighs 10 to 15 grams. Submandibular saliva enters the mouth through the main submandibular duct, named Wharton's duct. The origin of this main duct is similar to the description given of the main parotid duct, with intralobular, interlobular, and interlobar branches. After leaving the hilus of the gland, the submandibular main duct curves around the posterior margin of the mylohyoid muscle and runs forward and upward across the floor of the mouth in the sublingual space. The duct terminates at the submandibular papilla lateral to the frenulum of the tongue. The main duct is about 70 mm long.

The sublingual glands are the smallest of the major salivary glands, weighing 2 to 3 grams. This mucous gland is located beneath the tongue above the mylohyoid muscle. Most often, each sublingual gland drains through several small ducts that open individually into the floor of the mouth, which are named ducts of Rivinus. Sometimes some of the anteriorly located ducts coalesce into a common duct, named Bartholin's duct. This duct may terminate near the submandibular papilla or may join Wharton's duct.



The minor salivary glands are usually located submucosally, surrounded by connective tissue, or located between muscle fibres. About 450 to 750 glands are present in the mouth, each weighing less than 10 mg. The serous portion of the

secretion of the minor salivary glands varies per region but generally decreases from the oral to the pharyngeal region, so that the pharyngeal glands are almost purely mucous.[3] The individual glands open directly onto the mucosa. By excreting a steady flow of protective fluid, they play an important role in creating and regulating the local environment.

Gustatory, olfactory, and optic stimuli are known to induce saliva secretion mediated through neural pathways, whereas local muscle activity stimulates saliva secretion mechanically. Oral pain, excitement and anger are also known to induce salivary secretion, whereas emotional stress has a depressive effect. Both sympathetic (predominantly ß-adrenergic) and parasympathetic (cholinergic) stimulation evokes salivary secretion. Salivary secretion totals about 500 to 600 ml per day, though secretion rates may vary considerably between individuals. Fluctuation of secretion during the day is also considerable, being low in the morning, reaching a maximum between noon and 6 PM and declining towards a minimum at night (10 ml/8hrs).[2,4] The resting, or unstimulated, salivary secretion originates mostly from the submandibular glands, while the parotid glands physiologically respond to stimulation by strongly increasing its relative contribution to the total of saliva secretion. The relative saliva viscosity of the three main salivary glands differs greatly. After stimulation, submandibular saliva has been found to be twice as viscous as parotid saliva, but four times less viscous than sublingual saliva.[5] The viscosity is directly related to the percentage of mucous cells. A decrease of physiological saliva secretion is named hyposalivation, whereas the subjective perception of oral dryness is named xerostomia. These terms, however, are often used improperly as being identical.

HISTORICAL OVERVIEW OF SJÖGREN'S SYNDROME

Although the association of dry eyes and dry mouth was described by Hadden in 1888[6], the coincidence of lacrimal and salivary gland enlargement by Mikulicz in 1888[7], the systemic nature of ocular dryness by Cougerot in 1925[8], and the relation between filamentary keratitis and arthritis by Mulock Houwer in 1927[9], it was not until 1933 that a full description of the condition was given by the Swedish ophthalmologist Henrik Sjögren.[10] Surprisingly, in his own country the value of his thesis was largely underestimated for many years.

Confusion arose regarding the relationship between Sjögren's syndrome (SS) and the disease reported by Mikulicz. In 1927, Schaffer and Jacobson divided the disease reported by Mikulicz into Mikulicz's disease, enlargement of the lacrimal

and parotid glands due to lymphocytic infiltration of unknown etiology, and Mikulicz's syndrome, enlargement of the same glands as a result of systemic disorders such as leukaemia, lymphosarcoma, tuberculosis and sarcoidosis.[11] In 1953, Morgan and Castleman concluded that Mikulicz's disease, as defined by Schaffer and Jacobson, and SS are identical, sharing the same pathological abnormalities.[12] In the same period Godwin introduced the term 'benign lymphoepithelial lesion'.[13] It was reported that the histopathological changes of lymphocytic infiltration, acinar atrophy, and cystic or solid duct alterations were seen in both diffusely enlarged salivary glands of patients with xerostomia, as well as in localised salivary gland nodules of patients who were otherwise asymptomatic. As these histopathological changes are identical to the changes seen in SS it was recommended that this condition is regarded as SS if clinical features are present, and as benign lymphoepithelial lesion if no other symptoms are present other than the salivary gland enlargement.[13,14]

In 1965, Bloch and Buchanan characterised SS as the triad of keratoconjunctivitis sicca (with or without lacrimal gland enlargement), xerostomia (with or without salivary gland enlargement), and a connective tissue disease, most frequently rheumatoid arthritis.[15] The diagnosis of SS requires at least two of the three characteristic features. The presence of a connective tissue disease differentiates between primary and secondary SS. Patients lacking the connective tissue disease but presenting the first two features are said to have primary SS or (formerly) sicca syndrome. This classical triad has been recognised universally and has provided a basis for the more recently developed sets of criteria.[16-22]

Salivary Immunopathology

Currently, SS is considered to be a disorder of altered immunoregulation in which there is a lymphocyte-mediated destruction of exocrine glands, which in turn leads to diminished or absent glandular secretion and mucosal dryness. Two types of pathological appearance must be considered in the salivary glands in SS: the lymphoepithelial lesion, occurring primarily in the major salivary glands, especially the parotid glands, and focal lymphocytic sialadenitis, occurring in the minor salivary glands.

The lymphoepithelial lesion represents both proliferation of intraparotid lymphoid tissue and infiltration of lymphocytes aggregating around the salivary ducts. The proliferating cells replace the glandular epithelium and may cause clinical enlargement of the gland. With time, metaplastic and hyperplastic ductal epithelium

obliterates the ductal lumen followed by acinar atrophy. These pathological changes may aggravate until the involved salivary gland becomes totally effaced by lymphocytes, leaving only islands of residual deformed ducts, termed epimyoepithelial islands.[13] In 4-5% of the cases, lymphocytic infiltrates may undergo malignant transformation, leading to the development of malignant lymphoma.[23-26] Due to a close resemblance with MALT-tissue, these lymphomas of the salivary glands have collectively been termed lymphomas of MALT-type.[27] They differ from other lymphomas in that they resemble a chronic inflammatory process and may remain localised for long periods.[28] Their clinical course may be relatively indolent in SS.[29-32]

The characteristic histopathological feature of minor salivary glands in SS is focal lymphocytic sialadenitis. It consists of a primary lymphocytic infiltrate in glands which appearance is otherwise normal and is characterised by focal aggregates of 50 or more lymphocytes adjacent to normal appearing acini, present in all or most of the glands in the specimen.[33] Various grading systems have been proposed for estimating the relative number of mononuclear cells infiltrating minor salivary gland tissue.[34-36] The T-cells account for about 80% of the total infiltrate, with the remaining 20% composed of B-cells and plasma cells.[37] It has been suggested that an initial predominance of T-cells is gradually reduced by an accumulation of B-cells and plasma cells.[38,39] There is a predominance of IgG and IgM bearing plasma cells, reflecting the local chronic inflammatory processes, reducing the number of IgA bearing plasma cells that normally represent at least 70 percent of the plasma cells in the minor salivary glands.[37,40]

Immunologically, the prevailing abnormality appears to be a polyclonal B-lymphocyte hyperreactivity, directly or indirectly related to alterations in immunoregulatory T-lymphocytes.[41] This B-lymphocyte hyperreactivity is reflected in serum by a polyclonal hyperglobulinemia and the presence of several autoantibodies.

Etiologically, different theories have been proposed over the years. One theory holds a genetic abnormality of the immune system responsible.[42] This may involve an abnormality of the B-lymphocytes in which there is spontaneous B-lymphocyte activation, or possibly an abnormality of the T-lymphocytes in which excessive T-helper function or decreased T-suppressor function permits or induces B-lymphocyte over-activation and production of antibodies. Another possibility is that the disorder results from an antigenic challenge.[42,43] The acquired antigenic stimulus may be a viral disease that alters surface autoantigens, which in turn stimulates B-lymphocyte activation and the production of autoantibodies. A third possibility is a combination of the two aforementioned theories, in which there is an interaction of

an acquired exogenous stimulus with a certain genetic susceptibility.[44] Furthermore, abnormal apoptosis, and interaction of sex steroid hormones have been proposed to induce initial autoimmune responses.[45-48]

ORAL EXAMINATION

In the full expression of SS, oral and ocular symptoms and signs predominate, but due to its diffuse exocrinopathic involvement, several or all exocrine gland systems may be involved. Dryness from exocrine gland dysfunction is, therefore, certainly not limited to the eyes and the mouth, but may extend to all mucosa-covered surfaces in the body, e.g. in the vagina, nasal cavity, oesophagus, pharynx, vocal cords, airways and lungs. In addition to mucosal dryness, skin dryness is also common.[49,50] Symptoms in SS are not restricted to dryness, as the systemic autoimmune character of the syndrome often induces general complaints; most patients suffer from chronic fatigue, whereas frequently arthralgia and myalgia are experienced as well. Internal organs such as the kidneys, the lungs, the bladder, and even the cerebrum may also be involved in SS.[51-58]

Oral symptoms in SS may consist of sensation of oral dryness, impaired speech, eating difficulties, pain and swelling. It is not known how much saliva is necessary for allowing normal oral function, for oral comfort, and for the maintenance of oral health. The symptoms occur either alone or in combination. Oral dryness in SS results from the lack of saliva that normally moistens the mouth and lubricates the oral mucosa. Often, the first complaints of oral dryness arise at night due to decreased resting salivary secretion rate. With time, oral dryness is also perceived during daytime due to a decrease of both resting as well as stimulated salivary secretion rate. Predilection sites for the sensation of oral dryness are the anterior part of the palate and the dorsum of the tongue, due to a relatively low number of submucosal minor salivary glands present in these areas. The speech difficulties might relate either to impaired tongue movement in a dry and sticky mouth, as well as to hoarseness from dry and irritated vocal cords in case of generalised mucosal dryness. Eating difficulties may include impaired taste due to a lack of saliva (necessary to dissolve food components) and to atrophy of the tongue epithelium.[59] The impaired taste is usually limited to decreased taste acuity and rarely presents as a complete loss of taste as temporarily may occur after radiotherapy. Eating difficulties may also include masticatory problems due to a sticky and painful oral cavity, poor dentition or functional denture-problems, and swallowing difficulties.

Impaired swallowing may either relate to problems with food bolus formation and translocation and mucosal lubrication (oesophageal dryness) as well as to oesophageal motility disorders.[60,61] Oral pain may either result from hypersensitivity of the oral mucosa, dental caries, opportunistic mucosal infection, or (acute) sialadenitis. Hypersensitivity of the oral mucosa occurs quite frequently in SS patients as a result of inadequate moistening of the mouth. This renders the mucous membranes more vulnerable to mechanical trauma and to chemical irritants from food and beverage. Depending on its progression, dental caries causes pain that varies from mild and localised pain reactions to cold or heat to unbearable pulsating pain at one site of the mouth. Opportunistic mucosal infections usually present with a mild pain if painful at all, whereas acute sialadenitis can be very painful, accompanied with symptoms of malaise and feeling ill.
Several aspects of the symptoms may be especially informative, such as their duration, whether they are chronic or recurrent and whether they relate to predisposing factors. Furthermore, information about the general health and medical history of the patients has to be obtained. The patient should explicitly be asked about previous surgery or radiotherapy in the head and neck region. Drug intake should also be noted as many drugs may suppress salivary gland function.[62] Examples of commonly prescribed xerogenic drugs are antihistaminics, antihypertensives and psychotropic drugs.

Examination of the salivary glands includes meticulous inspection of the head and neck area as well as intraoral inspection. Obviously, swelling in the area of the major salivary glands must be noted. The appearance of the oral mucosa, especially on the tongue, may reveal salivary gland dysfunction. It should be determined whether or not physiological pooling of saliva is present sublingually. The dental status and oral hygiene must be noted. The orifices of Stensen's and Wharton's duct must be carefully inspected.
Palpation provides information on the size, consistency and tenderness of the salivary glands and any associated masses. A unilateral or bilateral parotid or other salivary gland swelling occurs frequently, either recurrent or chronic. Tear gland swelling is uncommon. Other exocrine glands are targeted as well, though their involvement is clinically less visible due to their localisation and their small size. Chronic swelling of salivary glands, especially if profound, may indicate the presence of a malignant lymphoma. The head and neck region must therefore also be examined for presence of enlarged lymph nodes. External pressure on the salivary glands may provoke an increased flow of saliva that should be inspected for signs of inflammation. In cases of gross ductectasis in the parotid gland, a spurt of

saliva will be seen. In contrast, a sudden release of saliva during massage of the submandibular gland is normal. If possible, palpation should be done bimanual. Size and consistency of the glands and their main excretory ducts can be optimally assessed by inserting two fingers intraorally while the other hand provides gentle support from outside the mouth.

The intraoral symptoms and signs of SS are not specific, being shared with other conditions in which salivary gland function is diminished. The superficial location of the salivary glands, however, easily permits inspection and palpation. A detailed history together with a thorough examination provides valuable information for a differential diagnosis. It may further reveal problems secondary to salivary gland dysfunction, which need treatment. Subsequently, additional diagnostic procedures, as described in the next chapters, can be performed in order to differentiate between SS and other conditions with similar symptoms and signs.

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CHAPTER

3

Salivary gland function

SUMMARY

Aim - The frequent occurrence of xerostomia in Sjögren's syndrome (SS) as well as the easy accessibility of saliva supports the use of sialometry and sialochemistry in the diagnosis of SS. Collection and analysis of whole saliva (oral fluid) is currently the routine technique for sialometry, despite the fact that it is rather inaccurate and impure. The aim of this study was to assess the value of glandular sialometry and sialochemistry as diagnostic instruments in SS.

Methods - In a group of 100 consecutive patients referred for diagnostics of SS, glandular secretory flow rates and a spectrum of salivary components (sodium, potassium, chloride, calcium, phosphate, urea, amylase, and total protein) were assessed. The patients were classified as positive or negative for SS according to the revised European classification criteria.

Results - Patients with SS differed clearly from the patients tested negative for SS, showing lower submandibular/sublingual (SM/SL) flow rates and a markedly changed salivary composition of parotid- and SM/SL saliva. Besides changes in salivary flow rate and composition, distinct sialometrical profiles were observed, characteristic for either early or late salivary manifestation of SS, or for the xerogenic side effect from medication.

Conclusions - It is concluded that glandular sialometry and sialochemistry are not only useful instruments to differentiate SS from other salivary gland disease in clinical practice, but also have great potential as diagnostic criteria for SS, revealing distinct sialometrical and sialochemical changes as well as profiles. Being *simple*, *safe* (noninvasive) and *sensitive* (early disease detection) glandular sialometry and sialochemistry encompass three major advantages compared to other oral tests for SS.

SIALOMETRY AND SIALOCHEMISTRY: DIAGNOSTIC TOOLS IN SJÖGREN'S SYNDROME

W.W.I. Kalk[1], A. Vissink[1], F.K.L. Spijkervet[1], H. Bootsma[2], C.G.M. Kallenberg[3], A.V. Nieuw Amerongen[4]

Departments of [1]Oral and Maxillofacial Surgery, and Internal Medicine, divisions of [2]Rheumatology and [3]Clinical Immunology, University Hospital Groningen. Department of [4]Oral Biology, division of Oral Biochemistry, Faculty of Dentistry Amsterdam (ACTA)

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INTRODUCTION

Sjögren's syndrome (SS) is considered an autoimmune exocrinopathy resulting in, amongst many other manifestations, tear and salivary gland dysfunction. Since the etiopathogenesis of SS remains unclear, the diagnosis of SS is still based on the presence of characteristic signs and symptoms. A variety of diagnostic tests is currently in use, but none of these tests detects changes pathognomonic for SS. Therefore, different combinations of test criteria have been proposed for the diagnosis of SS.[1-7] Since lacrimal and salivary gland dysfunction are key manifestations of SS, it seems logical to apply dysfunction of these glands for the diagnosis of SS.[8-12] Most combinations of test criteria, however, emphasise histopathologic, serologic and radiologic features rather than gland function itself.

Dysfunction of salivary glands is assessed by measuring salivary flow rate (sialometry) and by chemical analysis of saliva (sialochemistry).

Sialochemistry has been proposed as an instrument in the differential diagnosis of various salivary gland diseases, including SS[13-16], as many salivary gland diseases are well documented in the literature regarding their sialochemical manifestations.[17-19]

Sialometry can be used as a diagnostic tool mainly in two ways: collection of whole saliva (*i.e.* combined secretions of all salivary glands), and collection of glandular salivas (*i.e.* gland specific saliva).[20] In the assessment of the secretory capacity of a patient, at first glance measurement of the total of secretions accumulating in the mouth (oral fluid) seems to be the most appropriate method, reflecting the overall capacity of all salivary glands. Collection of whole saliva is the method most frequently used because it is very easy to practice, taking only a few minutes, without the need for a collecting device. For analytical purposes, however, whole

saliva is of limited value, as it detects neither dysfunction of any of the separate salivary glands, nor gland specific sialochemical changes.[9,16,21] Another argument against the use of whole saliva is that does it not necessarily represent the sum of individual gland secretions but may include contamination of sputum, serum, food debris and many other nonsalivary components. Nevertheless, only a reduced secretion rate of unstimulated whole saliva is currently considered to be of diagnostic value in SS.[4,6-7,22] By contrast, the collection of glandular salivas may reveal preferential involvement of salivary glands, such as selective hyposecretion of the submandibular/sublingual salivary glands, which has been frequently observed in SS.[2,23-26] In addition, sialochemistry of the collected glandular saliva samples may reveal several characteristic changes in electrolytes and proteins (enzymes) in SS, reflecting the effect of autoimmune attack on the secretory cells in individual salivary glands.[27]

Previous studies examined the value of glandular sialometry and sialochemistry in subjects with SS compared to healthy subjects.[13,25-26] In clinical practice, however, SS needs to be differentiated from other salivary gland diseases and conditions mimicking SS.[28] In the present study, the potential value of glandular sialometry and sialochemistry as diagnostic tools in SS was explored by comparative examination of glandular secretory flow rates and a spectrum of salivary components, assessed in a non-selected group of patients referred for evaluation of SS who were subsequently diagnosed as positive or negative for SS.

PATIENTS AND METHODS

Patients

One hundred consecutive patients referred to the outpatient clinic of the Department of Oral and Maxillofacial Surgery of the University Hospital Groningen in the period from September 1997 until March 1999 participated in this study. Patients, suspected of Sjögren's syndrome (SS), were referred by rheumatologists, internists, neurologists, ophthalmologists, ENT-specialists, general practitioners and dentists. Reasons for referral included mouth-dryness, eye-dryness, swelling of the salivary glands, arthralgia and fatigue. The diagnostic work-up for SS was carried out in all patients and included the following aspects: subjective complaints of oral and ocular dryness (table 3.1.1), sialography, histopathology of salivary gland tissue, serology (SS-A- and SS-B antibodies) and eye tests (Rose Bengal staining and Schirmer tear test). In addition to these diagnostic tests, the duration of oral symptoms was assessed, defined as the time from first complaints induced by or

related to oral dryness until referral. 'Short duration' was defined as less than one year, and 'long duration' as more than two years of oral symptoms. Sialometry, as proposed in the European criteria was not used in the diagnostic work-up, in order to avoid any incorporation bias when investigating sialometry as diagnostic tool for SS. Instead, parotid sialography was used to fulfil the criteria regarding the oral component.

According to the revised European classification criteria for Sjögren's syndrome[6,29-31] patients were categorised as primary SS (group A), secondary SS (group B), or negative for SS (group C). The usage of xerogenic drugs - *i.e.* antihypertensives, beta-blockers, antihistaminics, and psychotropics - was relatively frequent in all patients (A: 30%, B: 60%, C: 55%).

Table 3.1.1. *Ocular and oral symptoms according to the European criteria for the classification of Sjögren's syndrome (Vitali et al, 1993, 1996).*

I. Ocular symptoms (definition: a positive response to at least one of the following 3 questions)
- Have you had daily, persistent, troublesome dry eyes for more than 3 months?
- Do you have a recurrent sensation of sand and gravel in the eyes?
- Do you use tear substitutes more than three times a day?

II. Oral symptoms (definition: a positive response to at least one of the following 3 questions)
- Have you had daily feeling of dry mouth for more than 3 months?
- Have you had persistently swollen salivary glands as an adult?
- Do you frequently drink liquids to aid in swallowing dry food?

Saliva collection and chemical analysis

All salivary assessments were performed in absence of acute sialadenitis. If clinical signs of acute inflammation were present, salivary assessment was postponed until clinical signs had subsided for at least six weeks.

Glandular salivas were collected in a standardised manner. In brief, patients were instructed not to eat, drink or smoke during ninety minutes preceding the sialometrical assessment. All assessments were performed at a fixed time of the day, in this study between one and three p.m., in order to minimise fluctuations related to a circadian rhythm of salivary secretion and composition. All assessments were performed by the same observer. Glandular salivas were collected in preweighed plastic tubes from both individual parotid glands by using modified Lashley cups (Carlson-Crittenden cups), and simultaneously from the submandibular/sublingual (SM/SL) glands by syringe aspiration.[32-33] Saliva from the SM/SL glands was collectively aspirated, as separate aspiration is rather difficult in clinical practice due to the close anatomical relationship between the orifices of both glands and the frequent presence of communicating ducts between the submandibular and sublingual main ducts. Unstimulated salivary secretions were collected during five minutes, followed by collection of stimulated secretions during ten minutes. The

salivary glands were stimulated with citric acid solution (2% weight/volume) applied with a cotton swab to the lateral borders of the tongue at 30-second intervals. Mixing of the acid solution applied to the tongue and SM/SL saliva pooling anteriorly in the floor of the mouth (orifices of the SM/SL glands) was carefully avoided. Lag phase, defined as the time from first acid application on the tongue until first visible saliva secretion (in the tubes connected to the cups) was recorded for both parotid glands.

After weighing the saliva samples in order to calculate flow rates (assuming specific gravity of saliva is 1,0 g/cm^3), sialochemical analysis was performed. The following salivary components were quantified: sodium, potassium, chloride, calcium, phosphate, urea, total protein and amylase. Sodium and potassium ions were measured flame photometrically with lithium ions as a standard (3000 ppm). Chloride ions were measured by titration with silver ions. Calcium ions were measured spectrophotometrically at 577 and 600 nm after complexing with *o*-cresolphthalein.[34] Inorganic phosphate was measured at 340 and 383 nm after addition of molybdate and reduction with bisulphite in the presence of *p*-methylaminephenolsulphate.[35-36] Urea was measured at 340 nm after addition of urease/glutamate dehydrogenase.[37] Total protein was measured at 604 nm after addition of pyrogallol. Amylase was quantified by the method of Pierre and Nadj.[38]

Sialometrical analysis

To compare secretory capacities of the major salivary glands, and unstimulated with stimulated flow rates, secretory flow rates were defined (table 3.1.2). Stimulated flow rates were considered as being reduced when below mean minus SD of controls (group D).

Table 3.1.2 *Definitions for sialometrical analysis. ND: not defined.*

	extremely low	low	(sub)normal
Unstimulated			
Parotid flow rate (mL/min/gland)	ND	≤0.03	>0.03
SM/SL flow rate (mL/min/SM/SL-glands)	ND	≤0.03	>0.03
Stimulated			
Parotid flow rate (mL/min/gland)	≤0.05	0.05-0.10	≥0.10
SM/SL flow rate (mL/min/SM/SL-glands)	≤0.05	0.05-0.20	≥0.20

Statistical analysis

Data were submitted for statistical analysis using the Statistical Package for the Social Sciences (SPSS), version 8.0. The following statistical procedures were applied: test for association according to Spearman, chi-square-statistic, Mann-Whitney *U* test and ANOVA (multiple comparison according to Scheffé). In the

results section it is stated which statistical test was applied in a specific situation. A significance level of 0.05 was pre-defined in all cases.

RESULTS

Studied group

By applying the revised European classification criteria for Sjögren's syndrome (SS) on the studied cohort, patients were categorised as primary SS (group A), secondary SS (group B) and as negative for SS (group C). The latter were, based upon additional clinical and laboratory tests, diagnosed as having sialoadenosis (n=10), sodium retention dysfunction syndrome (n=12), medication induced xerostomia (n=9), or as having no alternative disease directly related to salivary gland pathology (n=11).

Group A, patients with primary SS, comprised 3 men and 30 women (male /female ratio: 1/10; mean age of 51 years; SD 16; range 21 to 84). Group B, patients with secondary SS, comprised four men and 21 women (male/female ratio: 1/5; mean age of 54 years; SD 12; range 25 to 78). Group C, patients tested negative for SS, comprised two men and 40 women (male/female ratio: 1/20; mean age of 55 years; SD 17; range 20 to 81) (table 3.1.3). A fourth group, group D, comprised 36

Table 3.1.3 *Group characteristics.*

	pSS (group A)	sSS (group B)	non-SS (group C)
N	33	25	42
Age (mean) at referral	51	54	55
Sex (male/female)	3/30	4/21	2/40
Xerogenic medication	10 (30%)	15 (60%)	23 (55%)
Chronic fatigue	21 (63%)	19 (76%)	29 (69%)
Salivary gland swelling[1]	17 (51%)	7 (28%)	8 (19%)
Connective tissue disease	0 (0%)	*RA:* 14 (56%) *SLE:* 4 (16%) *CREST:* 1 (4%) *Vasculitis:* 1 (4%) *PBC:* 1 (4%) *Polymyositis:* 1 (4%) *Overlap:* 2(8%)	*RA:* 7 (17%) *SLE:* 2 (5%) *Scleroderma :* 1 (2%)
Positive salivary gland biopsy	32 (97%)	24 (96%)	0 (0%)
Positive serology			
SS-A	28 (85%)	13 (52%)	3 (7%)
SS-B	15 (45%)	8 (32%)	1 (2%)
Positive eye-test(s)[2]	25 (76%)	17 (68%)	18 (43%)
Positive parotid sialogram[3]	28 (100%)	16 (76%)	3 (8%)
Subjective complaints[4]			
dry eyes	24 (73%)	20 (80%)	28 (76%)
dry mouth	32 (96%)	23 (92%)	31 (74%)

1. present at first visit
2. according to European classification criteria: at least one positive eye-test (Vitali *et al*, 1993)
3. sialectasia present, percentages based on the number of patients with available information
4. according to definition by European criteria listed in table 3.1.1

non-medicated healthy subjects without a history of salivary gland diseases (male/female: 16/20; mean age of 39 years; SD 12; range 23 to 58). This group served as historical controls for sialometry and sialochemistry, assessed with identical methods as used in this study.[26]

Sialometry

Lag phase was increased significantly in groups A, B and C compared to group D (table 3.1.4) and was inversely related to flow rate ($r_{parotid}$ −0.51, P<0.01). Mean stimulated parotid flow rate in groups A, B and C was reduced as compared to normal (group D)(figure 3.1.1, table 3.1.4). Patients in group B had significantly

Table 3.1.4 *Salivary flow rate (mean ± SD) of SS-positive patients (groups A and B: primary and secondary Sjögren's syndrome, respectively), SS-negative patients (group C), and healthy controls (group D). Significant difference between SS-positive and SS-negative patients is marked with *, significant difference between patients and healthy controls is marked with #. Statistical test used: ANOVA.*

N	Group A 33	Group B 25	Group C 42	Group D 36
Unstimulated				
Parotid flow rate (mL/min/gland)	0.02±0.04#	0.02±0.04#	0.04±0.06	0.05±0.06
SM/SL flow rate (mL/min/SM/SL-glands)	0.05±0.09*#	0.02±0.03*#	0.12±0.13	0.12±0.12
Lag Phase (s)	212±212*#	119±180#	52±83#	9±54
Stimulated				
Parotid flow rate (mL/min/gland)	0.12±0.13#	0.24±0.25#	0.19±0.15#	0.52±0.42
SM/SL flow rate (mL/min/SM/SL-glands)	0.24±0.28*#	0.26±0.28#	0.42±0.28	0.46±0.24

higher stimulated parotid flow rate compared to patients in group A (figure 3.1.1, table 3.1.4). Unstimulated and stimulated submandibular/sublingual (SM/SL) flow rates were reduced in groups A and B as compared to SM/SL flow rates in groups C and D (figure 3.1.1, table 3.1.4).

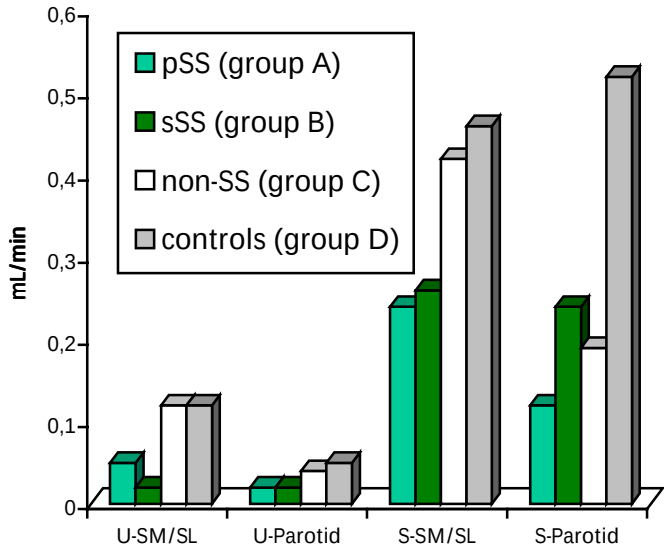

Figure 3.1.1 *Mean salivary flow rates of the unstimulated (U) and stimulated (S) SM/SL and parotid glands. Note the difference of stimulated SM/SL flow rates between SS-positive patients (groups A and B), and SS-negative patients (group C). Data (mean ± SD) are listed in table 3.1.4.*

Sialochemistry

Sialochemical results listed in table 3.1.5 are limited to stimulated saliva samples, as the volume of unstimulated saliva samples was insufficient for full sialochemical assessment in the majority of the patients studied (A: 75%, B: 76%, C: 36%, D: 0%). In eight percent of the patients in this study, sialochemistry was not performed due to absence of measurable salivary secretion (A: 18%, B: 8%, C: 0%, D: 0%).

Table 3.1.5 *Composition of stimulated glandular salivas (mean ± SD) of SS-positive patients (groups A and B: primary and secondary Sjögren's syndrome, respectively), SS-negative patients (group C), and healthy controls (group D). Data are based on the number of patients wih available information. Significant difference between SS-positive and SS-negative patients is marked with *, significant difference between patients and healthy controls is marked with #. Statistical test used: ANOVA (multi comparison according to Scheffé). Full sialochemical analysis of stimulated saliva was not possible in all patients, due to an extremely low stimulated secretion rate in some patients. ND: not determined.*

	Parotid glands (mean of both sides)				Submandibular/Sublingual glands			
	A	B	C	D	A	B	C	D
Sodium (mmol/L)	26±23*#	23±22*	4±4#	14±12	20±15*#	16±11*#	6±6#	11±6
Potassium(mmol/L)	23±6	23±9	30±21#	24±6	21±21	18±7	20±6#	17±6
Chloride(mmol/L)	30±14#	37±28*#	18±6	16±12	27±15#	35±35*#	16±5	16±6
Calcium (mmol/L)	1.3±1.0	1.0±0.2	1.3±0.8	0.8±0.6	1.9±0.9	1.9±0.5	2.2±1.6	1.7±0.6
Phosphate (mmol/L)	4.5±2.4	4.2±1.6	5.8±2.9	ND	2.3±1.2*	2.5±1.2*	3.9±1.7	ND
Urea (mmol/L)	5.6±2.0	4.9±2.4	6.1±2.5	3.8±1.2	2.9±1.8	3.8±2.3	4.0±1.9	2.5±0.6
Total protein (g/L)	1.2±0.5#	1.6±1.3	1.2±0.6#	0.6±0.6	0.6±0.3	0.8±0.5	0.7±0.4	0.8±0.6
Total protein (g/min)	0.1±0.1	0.3±0.5	0.2±0.2	0.3±0.3	0.2±0.2	0.3±0.6	0.3±0.3	0.4±0.3
Amylase (10^3U/L)	519±344	618±474	842±486#	590±510	117±97	162±293	138±121	ND
Amylase (10^3U/min)	59±65	180±295	152±142	307±264	45±60	27±60	58±70	ND

In groups A and B, mean sodium and chloride concentrations in parotid saliva were increased six fold and two fold respectively, compared to the concentrations in group C (figure 3.1.2). In groups A and B, the mean phosphate concentration in

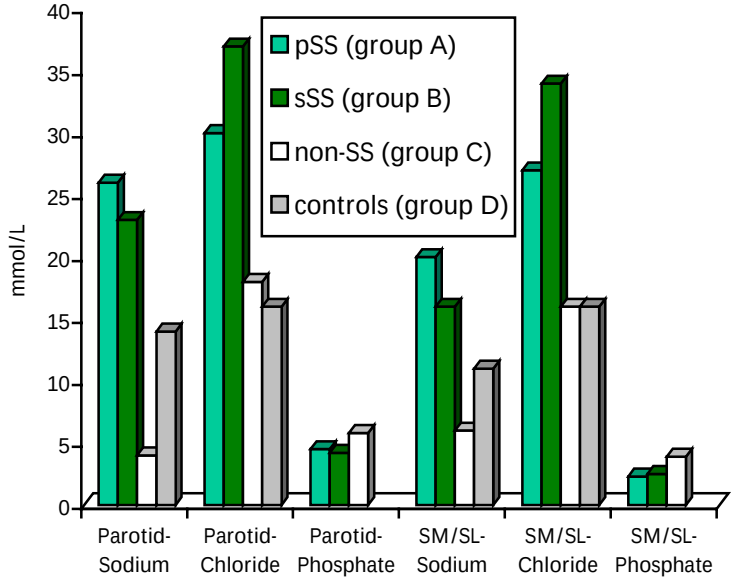

Figure 3.1.2 *Mean concentrations of sodium, chloride and phosphate in stimulated saliva. Note the increase of sodium and chloride and decrease of phosphate in the SS-positive patients (groups A and B). Data (mean ± SD) are listed in table 3.1.5.*

SM/SL saliva was decreased to two-third of the concentration in group C (figure 3.1.2). The total amount of amylase being secreted (U/min) was markedly less in groups A, B and C compared to group D. Total protein concentration in parotid saliva was increased in groups A, B and C compared to group D, but did not differ significantly between groups A, B and C. In groups A and B the sialochemical electrolyte changes in SM/SL saliva paralleled the changes in parotid saliva. With regard to calcium and urea concentrations, no significant differences were observed between the four groups. Salivary composition did not differ significantly between the patients of groups A and B. In group C, significant changes in salivary composition were observed, compared to healthy controls (group D). Increases in potassium and amylase concentration and a decrease in sodium concentration in parotid and SM/SL saliva were observed. The above listed sialochemical differences observed when comparing groups A and B to group C were also observed when comparing groups A and B to group D. However, the former differences (A and B versus C) were more clear cut due to the decreased mean salivary sodium concentration in group C contrasting with the sodium increase in groups A and B. A relatively large spread was present (large SD) in most sialochemical parameters, which is caused by the fact that the concentration of many salivary constituents is related to salivary flow rates.[18,19]

Early salivary manifestation in SS

In about a fifth of the patients in groups A and B (A: 18%, B: 20%), sialometry showed normal flow rates, accompanied by markedly changed salivary composition, including increased sodium and chloride concentrations. This combination of normal flow rates and changed salivary composition was not

Table 3.1.6 *Presence of characteristic sialometrical profiles in SS-positive patients (groups A and B: primary and secondary Sjögren's syndrome, respectively) and SS-negative patients (group C). Number of patients (percentage) within the groups that apply such profiles is given.*

	A	B	C
N	33	25	42
Sjögren-related profiles			
1. Normal SM/SL and parotid flow rates with changed composition	6 (18)	5 (20)	0 (0)
2. Low stimulated SM/SL flow rate with normal parotid flow rate	5 (15)	6 (24)	1 (2)
3. Extremely low stimulated SM/SL flow rate	3 (9)	4 (16)	0 (0)
4. Extremely low SM/SL and parotid flow rates	10 (30)	4 (16)	1 (2)
Miscellaneous			
1. Low unstimulated with normal stimulated SM/SL and parotid flow rate	4 (12)	2 (8)	9 (21)

observed in groups C and D (table 3.1.6). About a fifth of the patients in groups A and B (A: 15%, B: 24%) showed low stimulated flow rate of the SM/SL glands accompanied by (sub)-normal flow rate of the parotid glands. This selective

hyposecretion was observed in one of the patients in group C and in none of the individuals in group D (table 3.1.6). These profiles are characteristic for early salivary manifestation of SS, since both occurred almost exclusively in the SS groups (A and B) and were related to short duration (less than 1 year) of oral symptoms (tables 3.1.6, 3.1.7).

Table 3.1.7 *Relation between presence of characteristic sialometrical profiles and duration of oral symptoms in SS-positive patients (groups A and B; n=58). Statistical test used Mann-Whitney U test.*

Mean duration of oral symptoms	Months		Statistics	
Profile:	present	absent	*U*	P
Sialometrical profiles				
Early salivary manifestations				
1. Normal SM/SL and parotid flow rates with changed composition	5	32	74	<0.01
2. Low stimulated SM/SL flow rate with normal parotid flow rate	11	30	138	0.09
Late salivary manifestations				
3. Extremely low stimulated SM/SL flow rate	63	12	43	<0.01
4. Extremely low SM/SL and parotid flow rates	74	16	35	<0.01

Late salivary manifestation in SS

Extremely low stimulated flow rate of exclusively the SM/SL glands was found in about a tenth of the Sjögren patients (A: 9%, B: 16%) whereas extremely low flow rates of all major salivary glands were found in a quarter (A: 30%, B: 16%). Extremely low flow rates of all salivary glands were observed in one of the patients in group C and in none of the individuals in group D (table 3.1.6). These profiles proved, retrospectively, characteristic for late salivary manifestation of SS, since both occurred almost exclusively in the SS groups (A and B) and were related significantly to long duration (more than two years) of oral symptoms (tables 3.1.6, 3.1.7).

Changes unrelated to SS

A combination of low unstimulated flow rates and (sub) normal stimulated flow rates of all salivary glands was found in a tenth of the patients in groups A and B (A: 12%, B: 8%), in a fifth of the patients in group C (21%) and was not observed in group D (table 3.1.6). The presence of this combination of low unstimulated and normal stimulated flow rates related significantly to the use of psychotropic drugs (chi-square 5.0, $P<0.05$). The low unstimulated flow rates originate from a suppressive drug-effect on the SM/SL glands, which are physiologically the most active glands in the unstimulated condition. Unstimulated SM/SL flow rate related significantly in the studied groups to the use of psychotropic drugs ($r_{spearman}$ -0.29, $P<0.01$) and the use of any xerogenic drug ($r_{spearman}$ -0.27, $P<0.01$).

Secretory flow rate as function of time

Mean duration of oral symptoms before patients attended our outpatient clinic for salivary assessment was 32 months for group A (median 14, range 0 to 168) and 29 months for group B (median 12, range 0 to 120). All glandular secretory flow rates related inversely with duration of oral symptoms in groups A and B (table 3.1.8). On average, the reduction of stimulated SM/SL flow rate preceded the reduction of stimulated parotid flow rate, as shown by frequent occurrence of selective or relatively strong hyposecretion of the SM/SL glands in groups A and B (table 3.1.6).

Table 3.1.8 *Relation between secretory flow rate and duration of oral symptoms in SS patients (groups A and B, n=58).*

	Duration of oral symptoms	
	$r_{spearman}$	P
Unstimulated		
Parotid flow rate	-0.47	<0.01
SM/SL flow rate	-0.52	<0.01
Lag phase	0.63	<0.01
Stimulated		
Parotid flow rate	-0.64	<0.01
SM/SL flow rate	-0.72	<0.01

DISCUSSION

The results from this sialometrical and sialochemical study demonstrate a variety of potentially clinically applicable differences between patients with a positive diagnosis of Sjögren's syndrome (SS) and patients tested negative for SS. Since salivary data of patients with primary SS did not differ significantly from patients with secondary SS, patients with primary and secondary SS are addressed in the discussion as one group.

Our data confirm that results from previous studies, concerning reduced submandibular/sublingual (SM/SL) flow rate in SS[2,23-26], also apply in a clinical setting, when SS patients are compared to patients with clinical conditions resembling SS (non-SS). A possible explanation for this markedly reduced flow rate is early involvement of the SM/SL glands in SS. Though the underlying mechanism is not yet understood, it seems that measuring SM/SL flow rate may well contribute to an early diagnosis of SS. By contrast, parotid flow rate showed a comparable decrease in both SS-positive and SS-negative patients (figure 3.1.1), which is in accordance to the literature. As a consequence, it can be supported that measurement of parotid flow as a single test is of no use in diagnosing SS in clinical practice.[9,21,25,39]

Our findings of significant changes in salivary concentration of sodium, chloride and phosphate in SS patients as compared to non-SS patients are in agreement with the

findings in many other studies comparing SS patients to healthy controls.[13,18,25-26,40-42] These sialochemical changes can be used to determine whether salivary gland biopsy is indicated[16], but may also serve to differentiate SS from other salivary gland disease. As the observed sialochemical changes are not pathognomonic for SS, it is sometimes difficult to differentiate changes due to SS (chronic inflammation) from those associated with acute inflammation of the salivary glands.[9] However, both conditions can be differentiated by the presence of a much higher salivary protein concentration in the acute inflammation, due to protein leakage from serum.[43] In case of an acute exacerbation of a chronic inflammation in SS, much of the increase in sodium and chloride and the decrease in phosphate will persist after the acute inflammation has subsided, and, hence, sampling on a longitudinal basis may be required.[18]

In addition to the diagnostic potential of sialochemical changes in SS, the changes observed in the group of SS-negative patients are useful as well in the differential diagnosis of salivary gland diseases by clearly demonstrating the presence of other common salivary gland diseases.
The observed increases of potassium and amylase concentrations indicated the presence of a subset of patients with sialoadenosis, whereas the decrease of sodium concentration indicated presence of patients with sodium retention dysfunction syndrome, both non-inflammatory salivary gland diseases. Sialoadenosis is a parenchymatous salivary gland disorder due to secretory and metabolic disturbances of the acinar parenchyma, which presents clinically with xerostomia and the presence of a bilateral chronic or recurrent painless swelling of the salivary glands, particularly the parotid glands.[17-18,44-47] Sodium retention dysfunction syndrome presents clinically with xerostomia and recurrent unilateral painless swelling of a parotid gland for a few hours. It has been suggested to be related to impaired gland perfusion, which may occur due to homeostatic mechanisms of the blood supply in favour of other organ.[48]

In order to understand the etiology of the observed sialochemical changes in SS, the process of saliva production needs to be studied closely. Under normal circumstances, primary saliva is secreted into the acinar lumen and subsequently transported to the oral cavity through the salivary ducts by contraction of epimyoepithelial cells and other hydrostatic forces. As primary saliva traverses the striated ducts, salivary composition is modified considerably: phosphate is thought to be slightly concentrated, whereas sodium and chloride are extensively reabsorbed at low flow rate.[9,19]

In SS, however, a common defect in the major salivary glands is suggested by the parallel sialochemical changes observed in SM/SL and parotid saliva. Since the resorptive and secretory processes are flow dependent, observed increases in sodium and chloride concentrations and decrease in phosphate concentration would even be more striking if corrected for the low salivary flow rate in SS patients.[18-19] In spite of the low flow rate, duct cells seem unable to actively reabsorb sodium and chloride and to concentrate phosphate in SS.[13] One might hypothesise that duct cells are impaired in their function by the periductal lymphocytic infiltration, which is present in the major salivary glands affected by SS.[25,49] Perhaps, locally produced autoantibodies directed against duct cells cause impairment of electrolyte transport in duct cells. The unaltered levels of potassium and calcium in SS do not necessarily oppose this theory of ductal dysfunction, but may indicate that their transport differs from the normal active ductal transport of sodium, chloride and phosphate.

The observation of sialometrical profiles, characteristic for either early or late salivary manifestation of SS, or the side effect of drugs might be very useful in diagnosing SS. The early profiles are important for detecting the presence of SS shortly after disease onset when other symptoms still may be inconspicuous. Furthermore, the early and late profiles seem useful for staging the disease with regard to its oral component, comparable with the use of sialography for staging glandular changes in SS.[50] The profile characteristic for xerogenic drug usage is useful to reveal the presence of a suppressive drug-effect on the secretory function of salivary glands. In case of drug induced xerostomia often a normal stimulated salivary flow rate and composition is observed, whilst unstimulated flow rate is substantially reduced. Because drugs are the most common inducer of oral dryness, it is strictly necessary to explore drug-effect as cause of this symptom beside other systemic causes.

The applicability of sialometry and sialochemistry as diagnostic instruments varies under certain clinical conditions. In case of relatively normal salivary gland function, as may be present in an initial phase of SS - when autoimmune inflammation has not yet resulted in significant loss of secreting cells - sialometry is of little use as diagnostic instrument. In this situation, however, sialochemistry is often useful, since sialochemical changes - reflecting autoimmune attack on secretory cells - usually precede salivary gland dysfunction in SS. In case of severe salivary gland dysfunction, as may be present in a progressed phase of SS - when autoimmune inflammation has resulted in massive loss of active secretory cells - sialochemistry may not be possible to use as diagnostic instrument due to lack of saliva. In this

situation, however, sialometry is highly diagnostic for SS.[28] Therefore, it is advisable to combine sialometry and sialochemistry as diagnostic instruments, and assess their joint diagnostic value in an early as well as a progressed phase of SS.

In conclusion, glandular sialometry and sialochemistry is useful to differentiate SS from other salivary gland disease, revealing not only separate changes in salivary flow rate and composition but also characteristic sialometrical profiles. Currently, sialometrical and sialochemical results, if obtained at all, are taken into account when deciding whether additional (more invasive) diagnostic procedures are required.[16] In order to transform sialometry and sialochemistry from a method to differentiate salivary gland diseases into an applicable diagnostic tool in incipient through advanced stages of SS, cut-off values for the relevant variables need to be formulated and analysed in addition to this survey.[28] Several sialometrical and sialochemical variables have the potency to differentiate SS from non-SS to such an extent that an optimal combination of variables might result in a test with high diagnostic value. Therefore, if applied and interpreted properly, this method may be an excellent tool to diagnose the oral component of SS, being *simple*, *safe* (noninvasive) and *sensitive* (early disease detection). If proven sufficiently accurate in addition, it may subsequently be a valuable supplement to or even replace currently applied oral tests within the international test criteria for SS.

Perhaps in the future, other sialochemical variables might be added to the list of markers for SS, such as cytokines, interleukins, hyaluronic acids and certain proteins which are currently under investigation.[51-54] However, some of these markers lack the direct relationship to loss and dysfunction of exocrine gland tissue, which is the major outcome of SS, but merely reflect complex inflammatory processes or co-processes in the disease. Therefore, these markers may have more use in the understanding of the immunopathogenesis of SS rather than in the diagnosis of SS. Furthermore, sialometry probably has the potency to be used as a parameter for disease progression of SS (at least regarding the oral component). However, in order to clarify the prognosis of salivary gland function and other aspects of disease progression in SS, a long-term prospective study, as previously suggested, is still required.[40]

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SUMMARY

Aim - Analysis of salivary variables has frequently been proposed as a diagnostic tool for Sjögren's syndrome (SS). Since univocal salivary reference values are lacking, it is currently rather difficult to use sialometry and sialochemistry for diagnosing SS, unless major changes have occurred in salivary secretion and composition. Therefore, the main objective is to define reference values of several salivary variables, which offers a new and noninvasive possibility to diagnose SS.

Methods - In this study, cut-off points were selected from Receiver-Operating Characteristic (ROC) curves of gland specific sialometrical and sialochemical variables, which have proven to be potentially relevant for diagnosing SS in a previous study, *i.e.* sodium-, chloride- and phosphate concentration in stimulated parotid and submandibular/sublingual (SM/SL) saliva, unstimulated and stimulated SM/SL flow rates, and lag phase of parotid secretion, respectively. By combining the most discriminative variables, two different diagnostic approaches for SS were applied in a group of one hundred patients and, subsequently, evaluated in a second group of twenty patients.

Results - In the first approach, variables were combined by applying their cut-off points into sets of criteria for a positive diagnosis of SS, in the second approach by constructing a logistic regression model that predicts the true state of a patient (SS or non-SS). From both approaches the tests with highest likelihood ratio combined with the smallest number of rejected cases were selected for clinical use. The most accurate test reached a sensitivity of 0.85 and a specificity of 0.96 by combining the stimulated SM/SL flow rate and parotid sodium- and chloride concentration as salivary variables. The selected tests proved equally accurate in the second group of patients.

Conclusions - Since the proposed noninvasive diagnostic tools can be easily applied, do not need a laboratory other than for routine blood testing, and are very accurate, we feel that gland-specific sialometry and sialochemistry may eventually replace other, more invasive, diagnostic techniques for diagnosing SS.

SIALOMETRY & SIALOCHEMISTRY: A NONINVASIVE APPROACH FOR DIAGNOSING SJÖGREN'S SYNDROME

W.W.I. Kalk[1], A. Vissink[1], B. Stegenga[1], H. Bootsma[2], A.V. Nieuw Amerongen[3], C.G.M. Kallenberg[4]

Departments of [1]Oral and Maxillofacial Surgery, and Internal Medicine, divisions of [2]Rheumatology and [4]Clinical Immunology, University Hospital Groningen. [3]Department of Oral Biology, division of Oral Biochemistry, Faculty of Dentistry Amsterdam (ACTA)

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INTRODUCTION

Salivary gland dysfunction is one of the key manifestations in Sjögren's syndrome (SS). Assessment of salivary gland function is, therefore, of potential diagnostic significance.[1-12] Methods for determining salivary gland (dys)function include salivary flow rate measurements (sialometry) and analysis of salivary composition (sialochemistry), for which whole saliva (oral fluid) is most frequently used. The accuracy of these techniques, however, can be improved to a considerable extent by using glandular saliva rather than whole saliva. Several distinct alterations in flow rate and composition of glandular saliva have been reported in SS-patients, not only when compared with healthy controls[2,9-10,12], but also when compared with patients with clinical conditions resembling SS (§3.1).[13] These alterations are not observed or less obvious when using whole saliva.

The use of glandular saliva for diagnosing SS is hampered by the lack of univocal salivary reference values. As a result, sialometry and sialochemistry of glandular saliva can only be diagnostic for (the oral component of) SS when major changes in salivary secretion and composition have occurred. The aim of the present study was to define thresholds (cut-off points) for potentially relevant sialometrical and sialochemical variables for diagnosing SS (§3.1)[13], and to construct and evaluate an easily applicable diagnostic approach for SS.

PATIENTS AND METHODS

Patients

Between September 1997 and August 1999, 120 patients suspected of Sjögren's Syndrome (SS) were referred to the outpatient clinic of the Department of Oral and Maxillofacial Surgery of the University Hospital Groningen by rheumatologists, internists, neurologists, ophthalmologists, ENT-specialists, general practitioners and dentists. Reasons for referral included mouth-dryness, eye-dryness, swelling of the salivary glands, arthralgia and fatigue. The diagnostic work-up for SS, carried out in all patients, included: subjective complaints of oral and ocular dryness (§3.1, table 3.1.1), sialography, histopathology of salivary gland tissue, serology (SS-A- and SS-B antibodies) and eye tests (Rose Bengal staining and Schirmer tear test). In this study, the revised European classification criteria for Sjögren's syndrome were used as reference standard for the diagnosis of SS, categorising patients as primary and secondary SS and non-SS patients.[14,15]

The first one-hundred patients – referred between September 1997 and March 1999 – participated in a previous study, in which sialometrical and sialochemical variables of potential diagnostic value have been identified (§3.1).[13] In the present study these patients served as 'observation group' to define cut-off points and to construct diagnostic models. The subsequent twenty patients – referred between April 1999 and August 1999 – served as 'test group' to evaluate these diagnostic models.

The 'observation group' consisted of 58 SS-patients (33 primary SS and 25 secondary SS; male/female ratio: 1/8, mean age 53 years, SD 14, range 21 to 84) and 42 patients testing negative for SS (male/female ratio: 1/20, mean age 55 years, SD 17, range 20 to 81)(§3.1, table 3.1.3). The latter were diagnosed as having sialoadenosis (n=10), sodium retention dysfunction syndrome (n=12), medication-induced xerostomia (n=9), or as having no alternative disease directly related to salivary gland pathology (n=11).

The 'test group' consisted of 7 SS-patients (2 primary, 5 secondary SS; male/female ratio: 1/6, mean age 62 years, SD 10, range 46 to 76) and 13 non-SS patients (male/female ratio: 0/13, mean age 55 years, SD 11, range 36 to 76). The latter were diagnosed as having sialoadenosis (n=3), sodium retention dysfunction syndrome (n=3), medication induced xerostomia (n=2), whereas 5 patients remained without an alternative diagnosis.

The usage of xerogenic drugs, i.e. antihypertensives, beta-blockers, antihistamines, and psychotropics, was relatively frequent in all patients ('observation group': SS 45%, non-SS 55%; 'test group': SS 57%, non-SS 54%).

Saliva collection and chemical analysis

All salivary assessments were performed prior to the diagnostic work-up and were performed by the same observer. Techniques of glandular saliva collection and analysis have been described in detail in a previous study (§3.1).[13]

Sialometrical and sialochemical variables studied

In a previous study the following variables have been demonstrated to be relevant for diagnosing SS: sodium-, chloride- and phosphate concentration in stimulated parotid and submandibular/sublingual (SM/SL) saliva, unstimulated and stimulated flow rates of the SM/SL glands, and lag phase of parotid secretion (lag phase defined as the time between the start of salivary gland stimulation and first visible saliva secretion (§3.1, tables 3.1.4 and 3.1.5).[13]

Potassium concentration and amylase activity in parotid saliva were excluded as diagnostic variables in SS, although they differed significantly between SS-positive and SS-negative patients. The reason is that the observed differences appeared to result from the presence of patients with a non-inflammatory salivary gland disease (sialoadenosis) in the group of SS-negative patients (§3.1).[13] The relevant sialometrical and sialochemical variables were submitted for further statistical analysis.

Immunological assessment

In addition to the detection of SS-A and SS-B autoantibodies as part of the diagnostic work-up, additional blood tests were performed that reflect inflammatory and/or immunological activity. The purpose of this blood testing was to search for readily available variables that might increase the diagnostic potential of sialometrical and sialochemical variables for SS. The following variables were assessed: erythrocyte sedimentation rate, C-reactive protein level, full blood count, white blood count differentiation and level of immunoglobulins (IgG, IgA, IgM).

Statistical analysis

Data were submitted for statistical analysis using MedCalc version 5.0 in order to calculate Receiver-Operating Characteristic (ROC) curves[16] and the Statistical Package for the Social Sciences (SPSS) version 9.0 for the remaining statistical procedures, including independent sample T-test and (multiple linear) logistic regression analysis. A significance level of 0.05 was pre-defined in all cases.

By selecting diagnostic indicators and combining these into a model, two different diagnostic approaches were applied, one by univariate and one by multivariate analysis. The univariate analysis consisted of selecting cut-off points from ROC-

curves of the relevant diagnostic indicators and combining these into a definition for a positive diagnosis of SS. The multivariate analysis (in which the diagnosis of SS is a descriptive of a set of jointly relevant diagnostic indicators) consisted of the construction of a logistic regression model, including variables stepwise backward by likelihood ratio.[17] It predicts the true state (SS or non-SS) of a patient.
Diagnostic indicators and tests were evaluated by ROC-curve and likelihood ratio. The ROC-curve provides an index of diagnostic accuracy of a test, whereas the likelihood ratio expresses its usefulness by measuring the change in certainty of diagnosis (post-test probability = likelihood ratio x pre-test probability).

RESULTS

Variables of inflammation and immune activation in SS

The inflammatory nature of Sjögren's syndrome (SS) was reflected by significant changes of the following blood variables: erythrocyte sedimentation rate (ESR), levels of C-reactive protein (CRP) and immunoglobulins (total, IgG and IGA)(table 3.2.1). The level of serum IgG was the most discriminating inflammatory variable for SS, with raised values (>15 g/L) in 93% of the SS-patients and in 20% of the SS-negative patients.

Table 3.2.1 *Results (mean ± SD) of blood tests in SS- non-SS patients ('observation group'). Significant differences marked with *. Statistical test used: independent sample T-test. CI-diff: 95% confidence interval of the difference (note: if zero is not included in the interval the difference is significant).# If group mean is above normal range, the prevalence of raised values is given (%).*

	SS	non-SS	CI-diff
N	58	42	
Hemoglobin(mmol/L) *(N:7.5-9.9)*	8.0±0.7	8.4±0.7	[-0.7;-0.2]*
MCV(fL) *(N:80.0-96.0)*	88.8±4.7	89.3±4.3	[-2.6;1.5]
Leukocyte count(10^9/L) *(N:4.0-11.0)*	6.2±1.7	8.0±2.4	[-2.7;-0.9]*
Neutrophils(%) *(N:45-75)*	65±10	66±10	[-5;5]
Lymphocytes(%) *(N:25-50)*	25±8	26±9	[-6;3]
Thrombocyte count(10^9/L) *(N:150-300)*	254±89	253±74	[-36-36]
ESR(mm/h)# *(N:0-6)*	40±33 (91%)	15±19 (35%)	[13;37]*
CRP(mg/L)# *(N:0-5)*	12±18 (66%)	3±6 (28%)	[3;15]*
Immunoglobulins(g/L)			
Total# *(N: -18)*	29.9±10.6 (94%)	18.0±4.5 (33%)	[8.3;15.5]*
IgG# *(N:8.5-15.0)*	22.8±8.1 (93%)	13.4±3.5 (20%)	[6.7;12.1]*
IgA# *(N:0.9-4.5)*	4.1±3.7 (23%)	2.7±1.4 (8%)	[0.3;2.6]*
Igm# *(N:0.6-2.6)*	2.8±3.3 (29%)	1.9±0.8 (20%)	[-0.2;2.0]

Sialometrical and sialochemical variables: cut-off points for SS

Cut-off points for a positive diagnosis of SS were selected from ROC-curves of the potentially relevant sialometrical and sialochemical variables (figure 3.2.1, table 3.2.2).

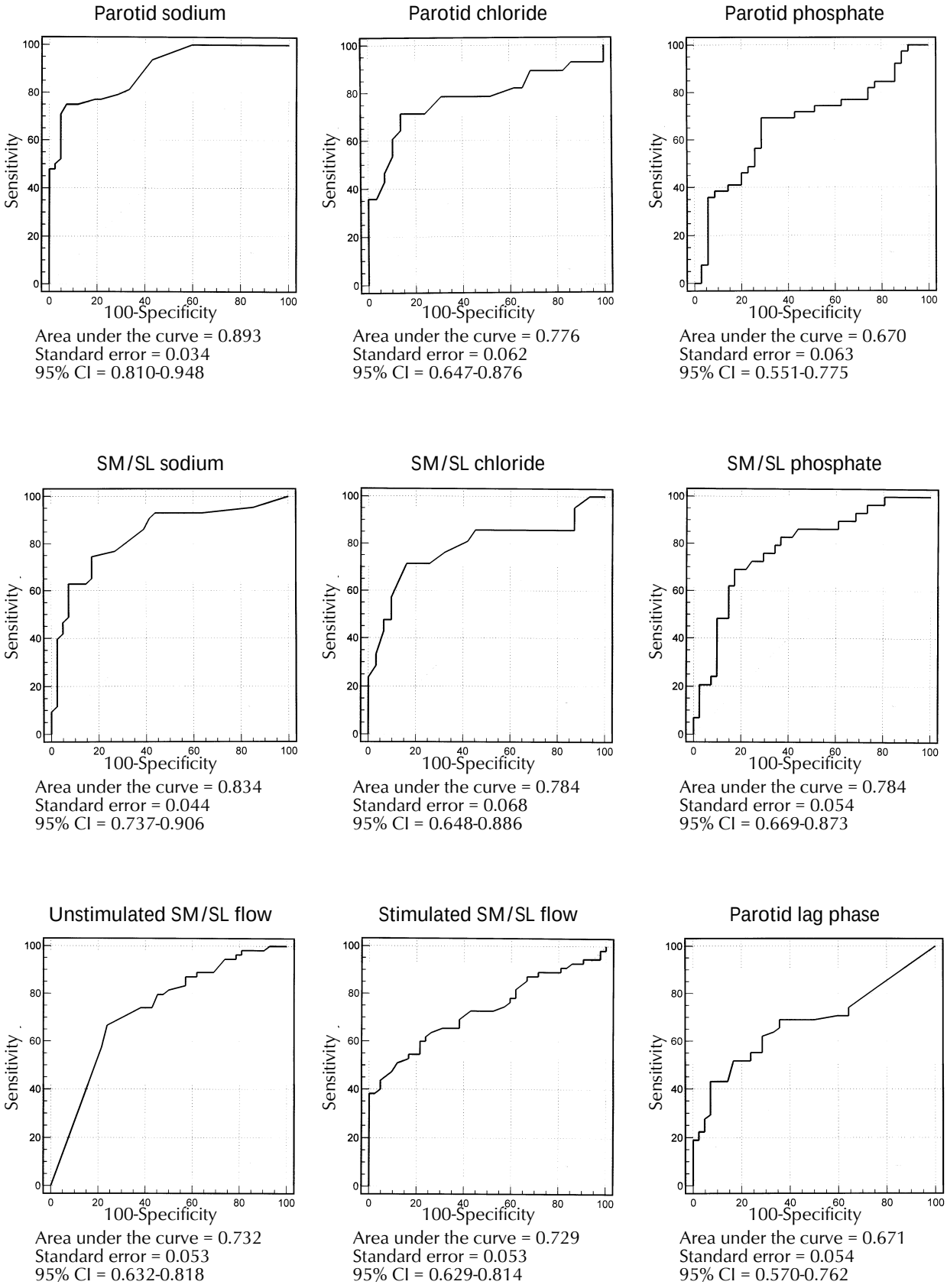

Figure 3.2.1 *Non-parametric ROC-curves of sialochemical and sialometrical variables in identifying SS in patients referred for diagnostics. Fifty-eight patients had SS; forty-two did not.*

The cut-off points were selected with emphasis on specificity (up to 1.00) in order to compensate for specificity loss, which will inevitably occur when parameters are combined as test for SS.

Table 3.2.2 *Cut-off points of sialometrical and sialochemical variables for a positive diagnosis of SS. Variables are ranked by likelihood ratio.*

Test variable	Cut-off point	specificity	sensitivity	LR	PPV	NPV
Sialometry						
Stimulated SM/SL flow[1)]	<0.05 ml/min	100%	38%	∞	100%	55%
Parotid lag phase	>2.20 min	93%	42%	6	89%	54%
Unstimulated SM/SL flow	≤0.01 ml/min	76%	67%	3	78%	64%
Stimulated SM/SL flow[2)]	≤0.20 ml/min	76%	62%	3	77%	60%
Sialochemistry (stimulated)						
Parotid sodium[1)]	≥20 mmol/L	100%	48%	∞	100%	62%
Parotid sodium[2)]	≥10 mmol/L	95%	71%	14	94%	74%
Parotid chloride	≥30 mmol/L	93%	46%	7	87%	64%
SM/SL chloride	>20 mmol/L	90%	57%	6	80%	76%
SM/SL sodium	>10 mmol/L	85%	63%	4	82%	69%
SM/SL phosphate	≤2.50 mmol/L	85%	55%	4	73%	73%
Parotid phosphate	≤4.75 mmol/L	71%	67%	2	72%	66%

1) restricted cut-off point, with highest specificity; 2) widened cut-off point, with increased sensitivity and decreased specificity; LR: likelihood ratio; PPV: positive predictive value; NPV: negative predictive value; SM/SL: submandibular/sublingual

Diagnostic approach I – combined cut-off points as a test for SS

Parotid and SM/SL variables were combined as a test for SS, which increased the sensitivity up to 0.92, however, at the expense of specificity (table 3.2.3). By using sialochemical variables only, 8% of the patients could not be diagnosed due to missing data (insufficient saliva could be collected for full sialochemical analysis).

Table 3.2.3 *Sialometrical and sialochemical variables as tests for SS.*

	Criteria for classifying SS test (cut-off point approach)	specificity	sensitivity	LR	N	PPV	NPV
	Parotid electrolytes						
1.	Parotid(stimulated) sodium ≥20 mmol/L or Parotid(stimulated) chloride ≥30 mmol/L	95%	56%	11	90	93%	66%
2.	Parotid(stimulated) sodium ≥20 mmol/L or Parotid(stimulated) chloride ≥30 mmol/L or Parotid(stimulated) phosphate ≤4.75 mmol/L	69%	81%	3	90	75%	76%
	SM/SL electrolytes						
3.	SM/SL(stimulated) sodium >10 mmol/L or SM/SL(stimulated) chloride >20 mmol/L or SM/SL(stimulated) phosphate ≤2.50 mmol/L	71%	81%	3	84	74%	78%
	Parotid and SM/SL electrolytes						
4.	Parotid(stimulated) sodium ≥20 mmol/L or Parotid(stimulated) chloride ≥30 mmol/L or Parotid(stimulated) phosphate ≤4.75 mmol/L or SM/SL(stimulated) sodium >10 mmol/L or SM/SL(stimulated) chloride >20 mmol/L or SM/SL(stimulated) phosphate ≤2.50 mmol/L	62%	92%	2	92	74%	87%
	Sialometrical variables						
5.	Stimulated SM/SL flow <0.05 ml/min or Parotid lag phase >2.20 min	93%	57%	8	100	92%	61%

LR: likelihood ratio; N: number of cases included in the analysis out of total (observation group N=100). Cases are rejected when data are missing in all variables used in the criteria; PPV: positive predictive value; NPV: negative predictive value; SM/SL: submandibular/sublingual

To improve the diagnostic potential of the tests, sialochemical variables were combined with sialometrical variables. This resulted in four tests with high specificity and moderate sensitivity (table 3.2.4). By combining sialometrical and sialochemical variables, all patients could be classified (*i.e.* no loss of cases due to missing data).

Table 3.2.4 *Sialometrical and sialochemical variables combined as tests for SS.*

	Criteria for classifying SS test (cut-off point approach)	specificity	sensitivity	LR	N	PPV	NPV
	Sialometrical and sialochemical variables						
6.	Stimulated SM/SL flow <0.05 ml/min or Parotid(stimulated) sodium ≥20 mmol/L	100%	66%	∞	100	100%	68%
7.	Stimulated SM/SL flow <0.05 ml/min or Parotid(stimulated) sodium ≥20 mmol/L or Parotid(stimulated) chloride ≥30 mmol/L	95%	71%	14	100	95%	70%
8.	Stimulated SM/SL flow <0.05 ml/min or Parotid(stimulated) sodium ≥20 mmol/L or Parotid lag phase >2.20 min	93%	74%	11	100	93%	72%
9.	Stimulated SM/SL flow <0.05 ml/min or Parotid(stimulated) sodium ≥20 mmol/L or Parotid(stimulated) chloride ≥30 mmol/L or Parotid lag phase >2.20 min	90%	78%	8	100	92%	75%

LR: likelihood ratio; N: number of cases included in the analysis out of total (observation group N=100). Cases are rejected when data are missing in all variables used in the criteria; PPV: positive predictive value; NPV: negative predictive value; SM/SL: submandibular/sublingual

Table 3.2.5 *Logistic regression models (formulas) for sialometrical and sialochemical variables combined as tests for SS. The probability of a subject having SS is represented in logistic regression formula as 'P'. A 'P'-value below 0.50 is considered positive for SS. For comparison with cut-off point approach see tables 3.2.3 and 3.2.4.*

	Formulas for classifying SS test (logistic regression approach)	specificity	sensitivity	LR	N	PPV	NPV
	Parotid electrolytes						
10.	Parotid sodium(X_1) and parotid chloride(X_2) (Parotid phosphate removed by analysis) Formula: LNR(P) = 2.8603 – 0.2044X_1 – 0.0547 X_2	93%	75%	11	57	91%	79%
	SM/SL electrolytes						
11.	SM/SL chloride(X_1) and SM/SL phosphate(X_2) (SM/SL sodium removed by analysis) Formula: LNR(P) = 0.9882 – 0.1411X_1 + 0.6928 X_2	90%	72%	7	49	81%	85%
	Parotid and SM/SL electrolytes						
12.	Parotid sodium(X_1) and parotid chloride(X_2) and SM/SL phosphate(X_3) (SM/SL sodium, SM/SL chloride and parotid phosphate removed by analysis) Formula: LNR(P) = 1.6479 – 0.2274X_1 - 0.1268X_2 + 1.3265X_3	96%	80%	20	43	92%	90%
	Sialometrical variables						
13.	Stimulated SM/SL flow(X_1) and parotid lag phase(X_2) Formula: LNR(P) = - 0.1546 + 1.1286X_1 - 0.0050X_2	60%	67%	2	100	68%	58%
	Sialometrical and sialochemical variables						
14.	Parotid sodium(X_1) and stimulated SM/SL flow(X_2) (Parotid chloride and lag phase removed by analysis) Formula: LNR(P) = 0.6765 – 0.2353X_1 + 3.3929X_2	93%	57%	8	100	92%	61%

LR: likelihood ratio; N: number of cases included in the analysis out of total (observation group N=100). Cases are rejected when data are missing in any of the variables in the formula; PPV: positive predictive value; NPV: negative predictive value; LNR = ^{E}log; SM/SL: submandibular/ sublingual

Diagnostic approach II – a logistic regression model as test for SS

Alternatively, logistic regression models were constructed representing a diagnostic index for SS.[17] *Sialochemical* variables were included in a logistic regression model stepwise backward by likelihood ratio, which resulted in tests for SS with high specificity (average 0.93) and moderate sensitivity (average 0.76)(tests 10-12, table 3.2.5). However, about 50% of the patients could not be classified due to missing data (lack of saliva).

When both *sialometrical and sialochemical* variables were included in a logistic regression model 10% of the patients could not be classified due to missing data. However, no improvement of test accuracy (specificity, sensitivity) was observed.

Salivary test enhancement by including serum IgG

By stratifying for elevated serum IgG (N ≤15 g/L) and accordingly widening the salivary cut-off points (for the cut-off point approach), or including serum IgG in the logistic model, the sensitivity of the sialometrical/sialochemical tests for SS increased on average by 15% (tables 3.2.6, 3.2.7). The calculated likelihood ratio, however, remained unchanged.

Table 3.2.6 *Diagnostic potential of sialometrical/sialochemical tests for SS, after stratifying for elevated serum IgG (N≤15 g/L). Adjusted (widened) salivary cut-off points are applied if serum IgG is elevated in order to improve the sensitivity.*

Criteria for classifying SS test (cut-off point approach)	specificity	sensitivity	LR	N	PPV	NPV
Sialometrical variables and IgG						
19. IgG≤15: Stimulated SM/SL flow <0.05 ml/min IgG>15: Stimulated SM/SL flow <0.20 ml/min	95%	53%	11	100	94%	61%
Sialochemical variables and IgG						
20. IgG≤15: Parotid(stimulated) sodium ≥20 mmol/L IgG>15: Parotid(stimulated) sodium ≥10 mmol/L	100%	69%	∞	90	100%	74%
Sialometrical/sialochemical variables and IgG						
21. IgG≤15: Parotid(stimulated) sodium ≥20 mmol/L or Stimulated SM/SL flow <0.05 ml/min IgG>15: Parotid(stimulated) sodium ≥10 mmol/L or Stimulated SM/SL flow <0.20 ml/min	95%	83%	17	100	96%	80%
22. IgG≤15: Parotid(stimulated) sodium ≥20 mmol/L or Parotid(stimulated) chloride ≥30 mmol/L or Stimulated SM/SL flow <0.05 ml/min IgG>15: Parotid(stimulated) sodium ≥10 mmol/L or Parotid(stimulated) chloride ≥30 mmol/L or Stimulated SM/SL flow <0.20 ml/min	90%	86%	9	100	93%	83%
23. IgG≤15: Parotid(stimulated) sodium ≥20 mmol/L or Parotid(stimulated) chloride ≥30 mmol/L or Stimulated SM/SL flow <0.05 ml/min or Parotid lag phase >2.20 min IgG>15: Parotid(stimulated) sodium ≥10 mmol/L or Parotid(stimulated) chloride ≥30 mmol/L or Stimulated SM/SL flow <0.20 ml/min or Parotid lag phase >2.20 min	93%	57%	8	100	92%	61%

LR: likelihood ratio; N: number of cases included in the analysis out of total (observation group N=100). Cases are rejected when data are missing in all variables used in the criteria; PPV: positive predictive value; NPV: negative predictive value; SM/SL: submandibular/sublingual

Table 3.2.7 *Logistic regression models (formulas) for sialometrical/sialochemical variables and serum IgG as tests for SS. The probability of a subject having SS is represented in logistic regression formula as 'P'. A 'P'-value below 0.50 is considered positive for SS. For comparison with cut-off point approach see table 3.2.6.*

Formulas for classifying SS test (logistic regression approach)	specificity	sensitivity	LR	N	PPV	NPV
Sialometrical variables and IgG						
15. Stimulated SM/SL flow(X_1) and serum IgG(X_2) Formula: LNR(P) = 5.2645 + 3.3610X_1 – 0.3968X_2	85%	84%	6	100	88%	81%
Sialochemical variables and IgG						
16. Parotid sodium(X_1) and serum IgG(X_2) Formula: LNR(P) = 6.5479 – 0.1596X_1 - 0.3193X_2	90%	82%	8	90	90%	82%
Sialometrical/sialochemical variables and IgG						
17. Stimulated SM/SL flow(X_1), Parotid sodium(X_2) and parotid sodium(X_2) Formula: LNR(P) = 5.5999 + 5.3278X_1 - 0.2138X_2 - 0.3501X_3	93%	83%	12	90	92%	84%
18. Stimulated SM/SL flow(X_1), parotid sodium(X_2), parotid chloride(X_3) and parotid sodium(X_2) Formula: LNR(P) = 6.9853 + 5.7582X_1 - 0.2423X_2 – 0.3755 X_3	96%	85%	21	53	96%	87%

LR: likelihood ratio; N: number of cases included in the analysis out of total (observation group N=100). Cases are rejected when data are missing in any of the variables in the formula; PPV: positive predictive value; NPV: negative predictive value; LNR = ^{E}log; SM/SL: submandibular/ sublingual

Evaluation

Both diagnostic approaches were evaluated by applying them in a separate group of patients, the 'test group'. The outcomes on salivary flow, salivary composition and blood tests were comparable to those in the SS and non-SS patients in the observation group (tables 3.1.4, 3.1.5, 3.2.1, 'test group'-data not shown). The test definitions (cut-off points) and test formulas (logistic regression) with the highest likelihood ratio combined with the lowest number of rejected cases (due to missing data) were considered as the most useful clinically and were, therefore, evaluated. The selected tests are listed in table 3.2.8. These tests were also evaluated after including serum IgG.

The selected test definitions and test formulas had on average equal sensitivity and specificity in the 'test group' compared to the 'observation group'. By using the selected test formulas (logistic regression) 15% of the patients in the test group could not be diagnosed due to missing data. However, by using the selected test definitions (cut-off point) all patients could be classified.

Table 3.2.8 *Evaluation of sialometrical/sialochemical tests for SS on a 'test group'. The probability of a subject having SS is represented in logistic regression formula as 'P'. A 'P'-value below 0.50 is considered positive for SS. For comparison with test results in the 'observation group' see tables 3.2.4-3.2.7.*

Criteria (cut-off points)/ Formulas test (logistic regression) for classifying SS	specificity	sensitivity	LR	N	PPV*	NPV*
Cut-off points: sialometrical/sialochemical variables (for comparison see 3.2.4)						
7. Stimulated SM/SL flow <0.05 ml/min or Parotid(stimulated) sodium ≥20 mmol/L or Parotid(stimulated) chloride ≥30 mmol/L	86%	83%	6	20	71%	92%
Logistic regression: sialometrical/ sialochemical variables (for comparison see 3.2.5)						
14. Parotid sodium(X_1) and Stimulated SM/SL flow(X_2) (Parotid chloride and lag phase removed by analysis) Formula: LNR(P) = 0.6765 – 0.2353X_1 + 3.3929X_2	100%	67%	∞	17	100%	85%
Cut-off points: Sialometrical/sialochemical variables and IgG (for comparison see table 3.2.6)						
17. IgG≤15: Parotid(stimulated) sodium ≥20 mmol/L or Stimulated SM/SL flow <0.05 ml/min IgG>15: Parotid(stimulated) sodium ≥10 mmol/L or Stimulated SM/SL flow <0.20 ml/min	86%	83%	6	20	71%	92%
Logistic regression: Sialometrical/sialochemical variables and IgG (for comparison see table 3.2.7)						
22. Stimulated SM/SL flow(X_1), Parotid sodium(X_2) and parotid sodium(X_2) Formula: LNR(P) = 5.5999 + 5.3278X_1 - 0.2138X_2 - 0.3501X_3	83%	91%	13	17	83%	91%

LR: likelihood ratio; N: number of cases included in the analysis out of total (test group N=20). Cases are rejected when data are missing; PPV: positive predictive value; NPV: negative predictive value; * prevalence of SS in test group 35%; LNR = log; SM/SL: submandibular/ sublingual

DISCUSSION

Many sialometrical and sialochemical variables can contribute to the diagnosis of Sjögren's syndrome (SS). Some have greater diagnostic potential than others, as can be determined by the likelihood ratio, as well as by the shape of a Receiver-Operating Characteristic (ROC) curve. High potential is indicated by a high likelihood ratio and by a ROC-curve that approaches the upper left corner of the diagram.

Due to the nature of the disease it is not always possible to collect sufficient saliva for full sialochemical analysis, while the salivary flow rate can obviously be determined at any level of glandular dysfunction. On the other hand, when the disease is still incipient, sialometry may not reveal any loss of glandular function, while the salivary composition may already have changed significantly. Therefore, a combination of at least one sialometrical and one sialochemical variable is preferred for a diagnostic test to cover all stages of the disease. Although the use of a

combination of variables has the advantage of an increased sensitivity, the number of variables to be combined is limited by the extent of loss of specificity.
Variables can be combined by applying their cut-off points into a set of criteria for a diagnosis of SS, but also by using a logistic regression model that predicts the true state of a patient (SS or non-SS) based upon the selected variables. The univariate method – the cut-off point approach – has the advantage that the sensitivity and specificity of the test can be adjusted to its purpose (e.g. screening, diagnosis) by selecting the proper cut-off points. The multivariate method – the logistic regression approach – has the advantage of using the full (joint) discriminative potency of the variables included and correcting for their mutual influences. This method, however, has the limitation that if any variable is missing the test cannot be carried out, since all variables are required in the formula. This may frequently occur, as sialochemistry is often impaired in xerostomic patients by lack of saliva. This problem of having only small amounts of saliva for sialochemical analysis may be less important if only few variables are selected for assessment (only the variables required for the diagnostic test), opposed to the wide selection of variables which we needed to assess in our study.

The limitation as well as the strength of the logistic regression model is reflected by the results from this study. The diagnostic approach with a logistic regression model was frequently inapplicable (rejected cases varying from 10 to 50%), whereas the approach by combined cut-off points was far more universally applicable (rejected cases varying from 0 to 10%). The impaired applicability of the logistic regression model was counterbalanced by a higher likelihood ratio (likelihood ratio of 21 versus 17 of cut-off point approach). Both approaches (logistic regression and cut-off point) proved adequate for diagnosing SS using only two or three salivary variables. The logistic regression approach, having the highest likelihood ratio, is the best option for diagnosing individual patients, while the cut-off point approach, being more universally applicable, may have greater value for diagnosing series of patients.

From both methods, the tests that combined the highest likelihood ratio with the lowest number of rejected cases were selected for clinical use (table 3.2.8: tests 7, 14, 17 and 22). The selected tests appeared to be equally accurate on a separate group of patients, indicating their general applicability. In clinical practice only two salivary variables are required for diagnosing SS, *i.e.* the sodium concentration in stimulated parotid saliva and the stimulated secretory flow rate of the SM/SL glands. With these variables, the logistic regression formula (table 3.2.5: test 14) accurately

predicts the presence or absence of SS. In cases of missing data, the cut-off point criteria (table 3.2.4: test 7) can be used as an alternative to diagnose the patient.

Since SS is a chronic disease with over-activation of the immune system, it is not surprising to find that serum IgG is the most discriminating immunological variable. This finding is in agreement with the literature.[18-21] By including this serological variable, the diagnostic approach of SS by sialometry and sialochemistry may be further improved, because the presence of raised serum IgG is accompanied by an increase of prior probability for SS. Since only the sensitivity of the test is optimised (no remarkable increase of likelihood ratio was observed), we conclude that adding serum IgG to the method of choice (table 3.2.6,3.2.7: tests 17 and 22) may be worthwhile in (patient) populations with low prevalence of SS, but not in general.

Until now, sialometry and sialochemistry have been useful methods that contribute to the differentiation of salivary gland diseases. By defining cut-off points and constructing proper models, glandular sialometry and sialochemistry have become clinically applicable methods that, when combined, form a reliable diagnostic technique for SS. Since the collection of saliva takes only few minutes and is noninvasive, and the analysis requires no laboratory other than for routine blood testing, we feel that glandular sialometry and sialochemistry may eventually replace other, more invasive, techniques for diagnosing SS.

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SUMMARY

Aim - The high incidence of Sjögren's syndrome (SS) and the variety of conditions that often mimic SS prompt for a simple screening test for SS, which can be used by dentists and general practitioners.
Methods - Based upon a noninvasive diagnostic technique, which was recently proposed to assess the oral component of SS, we have designed a test-strip that can be used for screening for SS using a drop of saliva.
Results - Changes in the composition of saliva characteristic for SS (altered chloride, phosphate and sodium concentration) can be visualised within five minutes. These changes proved to have a sensitivity of 92 percent, and a specificity of 62 percent or higher depending on the type of saliva used.
Conclusion - Appropriate and early referral, resulting from proper use, will benefit patients as well as clinicians confronted with SS.

A NEW SCREENING DEVICE: DETECTION OF SJÖGREN'S SYNDROME ON A DROPLET SALIVA

W.W.I. Kalk[1], A. Vissink[1], F.K.L. Spijkervet[1], B. Stegenga[1], J.L.N. Roodenburg[1], H. Bootsma[2], H.W. Frijlink[3], C.G.M. Kallenberg[4]

Departments of [1]Oral and Maxillofacial Surgery, and Internal Medicine, divisions of [2]Rheumatology and [4]Clinical Immunology, University Hospital Groningen, and [3]Research Group Pharmaceutical Technology and Biopharmacy, University of Groningen

Patent pending

INTRODUCTION

The diagnosis of Sjögren's syndrome (SS) is quite difficult to obtain, as many investigations are required in different medical fields.[1] Recently, a noninvasive diagnostic technique has been reappraised to assess the oral component of SS by using glandular saliva.[2] This technique requires a trained observer, equipment to collect saliva from the salivary glands and a laboratory to analyse the saliva samples. Therefore, usage of this technique is restricted to the secondary healthcare. However, the high incidence of SS (estimated at 0.5 - 2 percent of the population)[3,4] and the variety of conditions that often mimic SS prompt for a simple screening test for SS, that can also be used in the primary healthcare (by dentists and general practitioners).

Based upon previous salivary studies[2,5], we have, therefore, designed a test-strip that can be used for screening patients for SS using a drop of saliva only. With a high sensitivity, changes in the composition of saliva characteristic for SS can be visualised on a test-strip. Its use can be comparable with the detection of cystitis with a urine-strip, or dysglycaemia with a glucose-strip. Changes in salivary composition often precede other symptoms of SS[5], which favours the use of the saliva test-strip for detecting incipient SS. Therefore, this device seems highly suitable for usage by dentists, and general practitioners who observe in most cases the patients' first symptoms, *e.g.* dry mouth, dry eyes, unexplained fatigue. By screening patients with clinical evidence for SS with the test-strip, dentists and general practitioners are able to support their decision whether or not a patient needs to be referred for further diagnostics.

In this paper, preliminary details are discussed regarding technical methods for detection, indication for use, clinical procedure and interpretation of the proposed test-strip. Manufacturing of the test-strip and subsequent clinical evaluation is subject of current studies.

TECHNICAL INFORMATION OF THE SALIVA TEST-STRIP

Salivary variables measured

Three variables are measured in parotid and submandibular/sublingual (SM/SL) saliva for detection of Sjögren's syndrome (SS). These are the electrolyte chloride, phosphate and sodium. In a previous study cut-off points have been determined for these electrolytes (§3.2, table 3.2.2).[2] Changes of these electrolytes in SS may result from chronic inflammation of the salivary glands affected by SS or, more specifically, from the presence of periductal infiltrates.[5-7]

Chemical detection methods

The detection methods given here are examples and not limiting. In literature, many alternative methods have been described in detail. Chloride could be detected by reacting with silver ions, decolourising red-brown silverchromate (Merckoquant® Chloride, Merck). Phosphate could be detected by reacting with molybdate ions in a solution acidified with sulphuric acid, which is reduced to phosphomolybdenum blue (PMB)(Merckoquant® Phosphate, Merck). Sodium could be detected by reaction with uranyl potassium ferrocyanide to form uranyl sodium acetate, thereby changing the colour from red-brown to greenish.[8]

Accuracy of test result

The accuracy of the test by using the saliva test-strip is an estimate, based upon a previous study using laboratory techniques for sialochemical analysis (§3.2).[2] The test-strip can be used for parotid saliva detection only, as well as for parotid- and SM/SL saliva detection. When only a drop of parotid saliva is applied on the strip, the test has an estimated sensitivity of 56 and specificity of 95 percent by reading chloride and sodium concentrations; when both parotid and SM/SL saliva drops are applied the test reaches a sensitivity of 92 and a specificity of 62 percent (table 3.3.1).

Table 3.3.1 *Salivary electrolyte as tests for SS, based upon an unselected group of 100 patients* (§3.1).

Criteria for classifying SS	specificity	sensitivity	LR	N	PPV	NPV
Parotid electrolyte Parotid(stimulated) sodium ≥20 mmol/L or Parotid(stimulated) chloride ≥30 mmol/L	95%	56%	11.2	90	11.2%	99.5%
SM/SL electrolyte SM/SL(stimulated) sodium >10 mmol/L or SM/SL(stimulated) chloride >20 mmol/L or SM/SL(stimulated) phosphate ≤2.50 mmol/L	71%	81%	2.8	84	2.8%	99.7%
Parotid and SM/SL electrolyte Parotid(stimulated) sodium ≥20 mmol/L or Parotid(stimulated) chloride ≥30 mmol/L or Parotid(stimulated) phosphate ≤4.75 mmol/L or SM/SL(stimulated) sodium >10 mmol/L or SM/SL(stimulated) chloride >20 mmol/L or SM/SL(stimulated) phosphate ≤2.50 mmol/L	62%	92%	2.4	92	2.4%	99.9%

LR: likelihood ratio; N: number of cases included in the analysis out of total (observation group N=100). Cases are rejected when data are missing in all variables used in the criteria; PPV: positive predictive value; NPV: negative predictive value; predictive values are based upon estimated disease prevalence in the first echelon (1%); SM/SL: submandibular/sublingual

CLINICAL PROCEDURE

Indication for use

The test-strip is mainly designed for usage in the first echelon (general practitioners and dentists). The test should be used to support the decision whether or not referral to a specialist is required among patients with clinical symptoms or signs suspect for SS. In addition to first-echelon-usage, the test-strip could also be used in a hospital setting by internists and rheumatologists, to quickly screen among certain patients for oral signs of SS, comparable with the use of the Schirmer test-strip for ocular signs. Combined use of both screening tests further increases the sensitivity for SS, however at the cost of loss of specificity. It is not clear yet whether or not combined testing has additional value. The saliva test-strip should not be used as a screening device on a healthy population. When applied to individuals without symptoms and signs, the prevalence of SS in the tested group is about one percent, causing a positive predictive value that is far too low to be of any clinical relevance (table 3.3.1). Moreover, the high salivary flow rates that may be present among some of the healthy individuals may cause false positive test results, as salivary electrolyte transport is flow dependent (high flow rates decrease exchange of salivary constituents in the ductal system of the salivary glands).

Procedure

Before saliva samples can be taken from the patient's mouth, the salivary glands need to be stimulated during two minutes. This can be achieved by asking the

patient to chew (mint or citric) flavoured gum during the two minutes before sampling. This stimulation is required to prevent incorrect outcomes, resulting from changed salivary composition from stasis of saliva in the salivary ducts. After the chewing gum is removed from the mouth, the mouth must be rinsed with water. The inside of a cheek is exposed, and dried with a cotton swab followed by expressing a drop of saliva out at the opening of the Stensen's duct. This opening is located at the inner cheek adjacent to the second upper molar. The drop of saliva is harvested with a saliva pipette, and applied on the strip at the parotid site. After some seconds required for a chemical reaction the first test result can be read (by a marked change of colour). To complete the test, a drop of SM/SL saliva is harvested with another saliva pipette, after drying the floor of the mouth with a cotton swab and expressing a drop of saliva out at the opening of the Wharton's duct. This opening is located under the tongue paramedian in the floor of the mouth. The drop of saliva is applied on the strip at the SM/SL site, after which the final test result can be read.

Interpretation

The sensitivity of 92 percent is sufficient for excluding presence of SS by a negative test result, with a relatively high level of certainty (see negative predictive values, table 3.3.1). Therefore, a negative test result (both SM/SL and parotid saliva drops applied) makes a diagnosis of SS very unlikely, and reduces the need for referral. A positive test result (from parotid and/or from SM/SL saliva) does not prove the presence of SS, but indicates that there is a significant chance that SS is present, and prompts for referral to a specialist for further diagnostic testing.

CONCLUSION

Due to the chronic nature of Sjögren's syndrome (SS), initially, only subtle changes in the exocrine glands, that are difficult to detect with routine diagnostics, take place. Chronic inflammation of the salivary glands causes a decrease of salivary secretion only after months to years.[5] Also radiographic alterations, as demonstrated with sialography, develop very slowly from disease onset.[9] The inflammatory reaction in the salivary glands itself can be detected, however, by changes in the salivary composition. Dentists and general practitioners can now detect such changes simply by using a saliva test-strip. By applying two drops of saliva on a test-strip, clarity is achieved whether or not a patient is likely to suffer from SS and needs referral to a specialist. Specialists can subsequently confirm the diagnosis, by using

more invasive diagnostic procedures with the highest specificity possible (e.g. salivary gland biopsy, parotid sialography).

If the test-strip is used on patients with either symptoms or signs suggestive of SS, any diagnostic delay, as often is the case with SS, can be greatly reduced. Furthermore, specialists can then be confronted with a group of referred patients that has a high prevalence of SS and thus efficiency is optimised.

An early diagnosis in SS has two main advantages. First, the complaints can be related properly to the underlying disease, which is often very important for the patient. The feeling of being misunderstood, as if complaints are being under appreciated, during the period in which no diagnosis has been obtained yet, may cause great (additional) distress to the patient. Second, an early diagnosis allows dentists to consider preventive measurements for the dentition (e.g. fluoride application, referral to an oral hygienist), that may be necessitated by an increased caries risk. Likewise, ophthalmologists may prevent irreversible ocular surface damage by appropriate treatment from the onset.

In conclusion, the diagnostic work-up for SS can be greatly improved by the use of the saliva test-strip in the first echelon. Appropriate and early referral, resulting from proper use, will benefit patients as well as clinicians confronted with SS.

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CHAPTER

4

Salivary enzyme in serum

SUMMARY

Aim - Organ damage that directly results from an autoimmune attack in Sjögren's syndrome (SS) can hypothetically be demonstrated by an increase of organ-specific enzymes in serum, as an alternative to currently practised techniques that demonstrate loss of function or change in architecture. This assumption could be true for the salivary glands, containing large amounts of amylase, and almost invariably involved in SS. The objective of this study is to determine the clinical value of measurement of serum isoamylase activity as a clinical parameter in SS.

Methods - In a group of 100 consecutive patients referred for diagnostics of SS serum activity of salivary (S) and pancreatic (P) isoamylase were assessed. The patients were either diagnosed as positive or negative for SS according to the revised European criteria.

Results - SS patients showed significantly higher serum activities for salivary- and total (salivary and pancreatic) amylase compared to non-SS patients. The optimum threshold for detecting SS, selected from a Receiver-Operating Characteristic (ROC) curve, had a specificity of 89%, but a limited sensitivity of 35%. Further data analysis revealed that in SS patients S-type isoamylase serum activity had a biphasic course (increase-decrease) related to the duration of oral complaints, explaining the limited sensitivity. In addition, S-type isoamylase serum activity correlated positively with the sialochemical variables sodium and chloride concentration, which both are known to be related to inflammation of the salivary glands.

Conclusions - This prospective clinical study shows that measurement of isoamylases in serum has limited diagnostic value for SS, but does have potential use for assessing disease progression.

THE PERFORMANCE OF SERUM SALIVARY ISOAMYLASE AS A CLINICAL PARAMETER IN SJÖGREN'S SYNDROME

W.W.I. Kalk[1], A. Vissink[1], J.C.J.M. Swaanenburg[2], F.K.L. Spijkervet[1], J.L.N. Roodenburg[1], H. Bootsma[3], C.G.M. Kallenberg[4]

Departments of [1]Oral and Maxillofacial Surgery, [2]Pathology and Laboratory Medicine, and Internal Medicine, divisions of [3]Rheumatology and [4]Clinical Immunology, University Hospital Groningen

Rheumatol, in press

INTRODUCTION

Sjögren's syndrome (SS) is considered a systemic autoimmune disease with the exocrine glands as main target-organs. The diagnosis of SS is based on both subjective and objective findings that mainly result from damage to the lacrimal and salivary glands due to chronic inflammation. Since none of these objective findings is pathognomonic for SS, new diagnostic tests that are more accurate or less invasive than the tests currently applied, are widely evaluated.

In general, organ damage can be demonstrated by loss of function, change of architecture, and by increase of organ-specific enzymes which are released in serum. The first two of these three general approaches are currently used for diagnosing SS. Loss of function is assessed by measuring tear- and saliva secretion (Schirmer tear test and sialometry, respectively). Change of architecture is assessed by imaging techniques and salivary gland biopsy.

So far, measurement of serum enzymes as an estimate for organ damage is not routinely used in patients suffering SS. This seems rather surprising considering that the major salivary glands contain large amounts of amylase while these glands are almost invariably involved in the disease process of SS.[1-5] Besides the pancreatic gland, the salivary glands are the only human organs that contain large amounts of the digestive enzyme amylase. Consequently, increase of serum amylase activity is highly suggestive for either pancreatic or salivary gland disorders. By determining the particular isoenzymes pancreatic (P) and salivary (S) isoamylase it is possible to differentiate between pancreatic and salivary gland disorders. It has been suggested that hyperamylasemia reflects (initial) salivary gland damage, e.g. immediately after exposure to ionising radiation, whereas decreased activity of S-type isoamylase may indicate progressive salivary gland destruction.[6]

The objective of this study is to determine the clinical value of serum measurement of isoamylases in SS.

PATIENTS AND METHODS

Patients

One hundred consecutive patients referred to the outpatient clinic of the Department of Oral and Maxillofacial Surgery of the University Hospital Groningen in the period from January 1998 until January 2000 were included in this study. Patients suspected of Sjögren's syndrome (SS) were referred by rheumatologists, internists, neurologists, ophthalmologists, ENT-specialists, general practitioners and dentists. Reasons for referral included mouth-dryness, eye-dryness, swelling of the salivary glands, arthralgia and fatigue. The diagnostic work-up for SS was carried out in all patients and included the following aspects: subjective complaints of oral and ocular dryness (table 3.1.1), sialometry and sialochemistry[7], sialography, histopathology of salivary gland tissue, serology (SS-A- and SS-B antibodies) and eye tests (Rose Bengal staining and Schirmer tear test). In addition to these diagnostic tests, serum isoamylases were measured. Also, the duration of oral symptoms, defined as the time from first complaints induced by or related to oral dryness until referral, was assessed.

In this study, the revised European classification criteria for Sjögren's syndrome were used as reference standard for the diagnosis of SS, categorising patients as primary and secondary SS and non-SS patients.[8-11]

Serum isoamylase assessment

None of the patients had clinical evidence of acute pancreatitis, gall bladder disease, or acute parotitis for at least six weeks prior to the blood collection, and none were known to suffer from alcohol abuse. These conditions are considered exclusion criteria for this study.

The S-isoamylase activity was calculated from the total amylase and P-isoamylase activities. Total amylase and P-isoamylase were determined according to the instructions of the manufacturer (Roche, prod.nr. 1660675). P-isoamylase activity was determined after inhibition of S-isoamylase activity with two different monoclonal antibodies (Roche, prod.nr. 1660764).

Statistical analysis

Data were submitted for statistical analysis using MedCalc version 5.0 in order to calculate Receiver-Operating Characteristic (ROC) curves[12] and the Statistical Package for the Social Sciences (SPSS) version 9.0 for the remaining statistical procedures. These included independent sample T-test, one way analysis of variance (ANOVA) and Pearson's correlation test. A significance level of 0.05 was pre-defined in all cases.

RESULTS

Studied group

By applying the revised European classification criteria for Sjögren's syndrome (SS), 37 patients were categorised as SS (22 primary SS and 15 secondary SS; male/female ratio: 1/18, mean age 57 years, SD 13, range 24 to 84) and 63 patients as non-SS (male/female ratio: 1/31, mean age 54 years, SD 12, range 20 to 84)(table 4.1.1). The latter were, based upon additional clinical and laboratory tests,

Table 4.1.1 *Group characteristics.*

	SS	non-SS
N	37	63
Age (mean) at referral	54	55
Sex (male/female)	2/35	2/61
Xerogenic medication	20 (54%)	38 (60%)
Connective tissue disease	0 (0%)	*RA:* 17 (27%) *SLE:* 3 (5%) *Scleroderma :* 1 (2%) *MCTD :* 1 (2%)
Positive salivary gland biopsy	35 (95%)	0 (0%)
Positive serology: SS-A	21 (57%)	5 (8%)
SS-B	9 (24%)	0 (0%)
Positive eye-test(s)[2]	29 (78%)	31 (49%)
Positive parotid sialogram[3]	30 (97%)	3 (5%)
Subjective complaints[4]: dry eyes	20 (80%)	28 (69%)
dry mouth	32 (86%)	52 (82%)

1. according to European classification criteria: at least one positive eye-test (Vitali *et al*, 1993)
2. sialectasia present, percentages based on the number of patients with available information
3. according to definition by European criteria listed in table 3.1.1

diagnosed as having sialoadenosis (n=13), sodium retention dysfunction syndrome (n=18), medication induced xerostomia (n=13), or as having no alternative disease directly related to salivary gland pathology (n=19).

Mean isoamylase concentrations

The mean total amylase activity in the group of 37 SS patients (184 ± 92 U/L) significantly exceeded the corresponding activity for the 63 non-SS patients (146 ±

58 U/L). This difference was mainly due to an increase in S-type isoamylase activity (table 4.1.2). With regard to the P-type isoamylase, no significant difference was observed between SS- and non-SS patients.

Table 4.1.2. *Serum activity of amylase iso-enzymes in SS- and non-SS patients. Significant differences of mean amylase iso-enzyme activity between SS- and non-SS patients are marked with * (independent T-test).*

N	**SS** 37	**non-SS** 63
Mean total amylase (U/L)	184* (SD 92)	146 (SD 58)
Mean S-type isoamylase (U/L)	97* (SD 88)	70 (SD 45)
Mean P-type isoamylase (U/L)	87 (SD 29)	76 (SD 32)
Patients with S-type isoamylase level above normal (N≤105U/L)	13 (35%)	9 (14%)
Patients with S-type isoamylase level below mean-SD	8 (21%)	18 (28%)
Patients with P-type isoamylase level above normal (N≤115U/L)	5 (14%)	8 (13%)

Reference value for amylase iso-enzymes

When applying the normal reference values (thresholds) for the isoamylases (according to our hospital laboratory), 35% of the SS patients showed S-type activity above the reference range compared to 14% of the non-SS patients (N: ≤105U/L) (table 4.1.2). P-type isoamylase was above reference range in 14% of the SS patients and in 13% of the non-SS patients (N: ≤115U/L).

Through analysis with a Receiver-Operating Characteristic (ROC) curve of the S-type isoamylase activity in SS- and non-SS patients, the optimum threshold for differentiating SS from non-SS was found to be similar to the normal threshold at 105 U/L (figure 4.1.1). In our studied population, this threshold has a likelihood ratio of 3.2 with a specificity of 89% and a sensitivity of 35% for a diagnosis of SS (table 4.1.3). The total and P-type amylase activities were also evaluated by ROC-curve analysis (figure 4.1.1). These variables proved less specific but more sensitive for SS than S-type isoamylase, however, with rather poor likelihood ratios (table 4.1.3).

Table 4.1.3. *Thresholds (cut-off points) of serum iso-enzymes and total amylase activity for a positive diagnosis of SS, optimised by ROC- curve analysis. For ROC-curves see figure 4.1.1.*

Serum enzymes	threshold	specificity	sensitivity	LR
S-type isoamylase	>105 U/L	89%	35%	3.2
P-type isoamylase	>73 U/L	56%	70%	1.6
Total amylase	>148 U/L	64%	65%	1.8

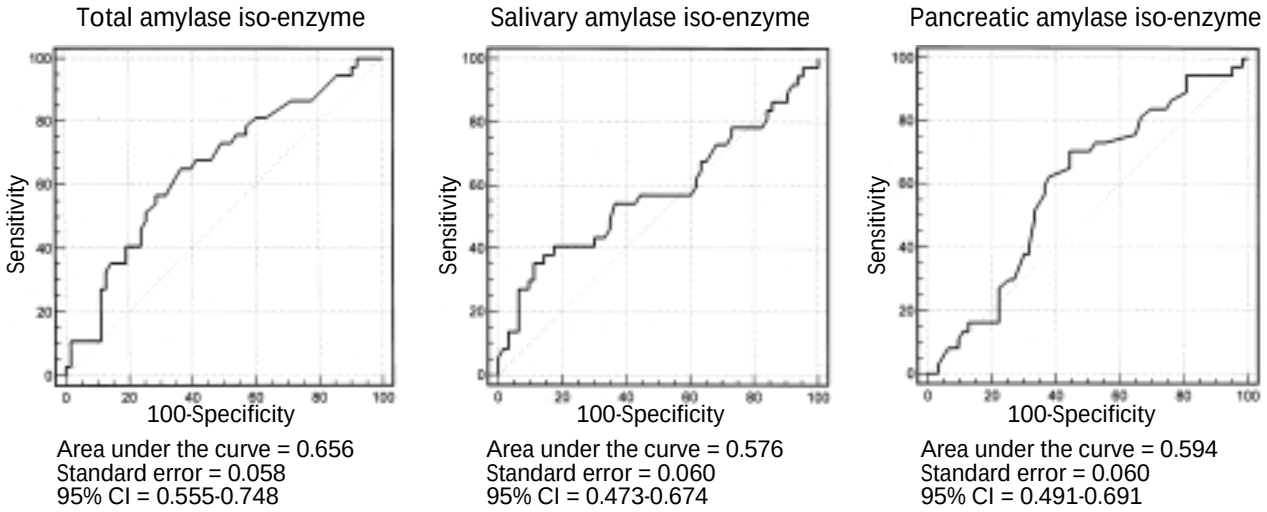

Figure 4.1.1 *Non-parametric ROC-curves of serum amylase iso-enzymes in identifying SS in patients referred for diagnostics. Thirty-seven patients had SS; sixty-three did not. Note the flat shape of the curves indicating poor diagnostic accuracy.*

Isoamylase activity versus duration of oral symptoms in SS

Mean duration of oral symptoms before referral was 29 months for both SS- and non-SS patients (range: SS 0-156, non-SS 0-240 months). In SS patients, serum S-type isoamylase activity and duration of oral symptoms related inversely ($r_{pearson}$ -0.33, p=0.05). The SS patients with high S-type isoamylase activity (above normal range) had a much shorter duration of oral symptoms (mean 11 months, range 0-30), than the SS patients with normal S-type isoamylase activity (mean 35 months, range 0-156), whereas the SS patients with low S-type isoamylase activity (below mean minus SD) had a much longer duration of oral symptoms (mean 49 months, range 12-108) (figure 4.1.2). These observed differences proved statistically significant by a one-way analysis of variance (ANOVA).

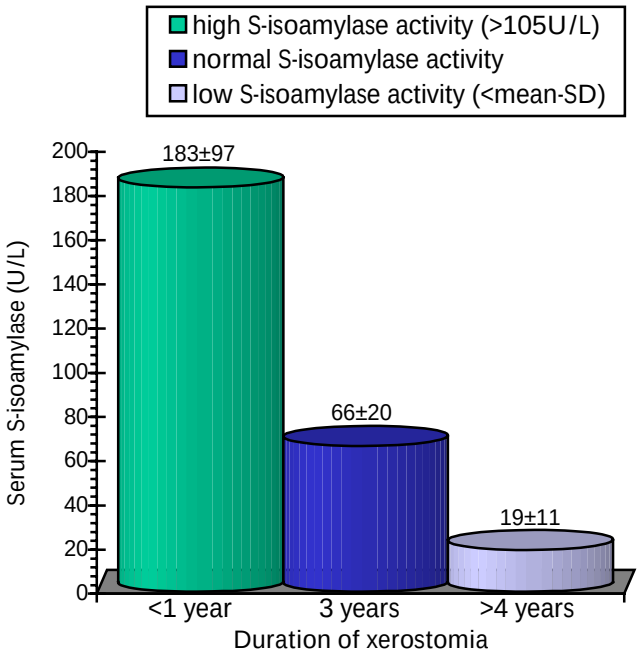

Figure 4.1.2. *S-isoamylase (U/L, mean ± SD) versus duration of xerostomia*

Isoamylase activity versus other salivary gland diseases

Two-thirds of the non-SS patients were diagnosed with another condition affecting the salivary glands, including sialoadenosis, sodium-retention dysfunction syndrome or medication induced hyposalivation. Patients with the non-inflammatory salivary gland diseases sialoadenosis or sodium-retention dysfunction syndrome had a tendency to a higher mean S-type isoamylase activities in serum (84 and 81 U/L, respectively) than patients with medication induced hyposalivation or without another condition affecting the salivary glands (63 and 54 U/L, respectively). These differences, however, did not reach significance probably due to rather low numbers.

Relation of isoamylase activity with sialometry and sialochemistry in SS

The activity of S-type and total amylase in sera of SS patients correlated significantly with the sialochemical variables sodium and chloride (also after correction for salivary flow rates), which are related to inflammation of the salivary glands (table 4.1.4).[7] Furthermore, the SS patients with S-type isoamylase activity above normal range had on average 30 to 50 percent higher salivary flow rates for all major salivary glands, compared to the SS patients with normal S-type isoamylase activity. So, there is a tendency of high S-type isoamylase activity in SS patients with (relatively) high salivary flow rates and high salivary sodium and chloride concentrations.

Table 4.1.4. *Relation between S-type and total amylase activity in serum and sialochemical variables in SS patients (n=37).*

	S-type isoamylase		Total amylase	
	$r_{spearman}$	P	$r_{spearman}$	P
Parotid saliva				
Sodium	0.46	<0.01	0.37	<0.01
Chloride	0.35	<0.01	0.34	<0.01
SM/SL saliva				
Sodium	0.48	<0.01	0.42	<0.01
Chloride	0.36	<0.01	0.29	0.02

DISCUSSION

The results from this prospective clinical study show that serum isoamylase-measurement has limited clinical value with regard to the diagnosis of Sjögren's syndrome (SS). It does have, however, potential for monitoring disease progression.

Serum measurement of total amylase seems to lack sufficient specificity for detecting SS, as it includes both the salivary and pancreatic fractions of amylase. Measuring only salivary isoamylase results in better discrimination between SS- and non-SS patients. Since most recordings in SS-patients were still within reference

range, optimising the threshold of S-type isoamylase for the differentiation between SS and non-SS by analysing data with a ROC-plot was tried. However, the sensitivity (and the likelihood ratio) of the optimum threshold for SS remained too low to be of clinical value for diagnosing SS.

The observations that high serum S-type isoamylase activity in SS patients corresponded with a relatively short duration of oral symptoms (less than one year), and relatively high salivary flow rates (and high salivary sodium and chloride concentrations), confirm the assumption that (initial) salivary gland damage is reflected by high serum S-type isoamylase activity.[6] In a progressed phase of oral manifestation the enzyme leakage in serum seems to extinguish, as low serum S-type isoamylase activity corresponded in SS patients to long duration of oral symptoms (on average four years) and decreased salivary flow rates. In a previous study it was demonstrated that sialometry and sialochemistry are useful to stage the oral manifestation of SS (§3.1).[13] An early stage of SS was characterised by either normal (secretory) gland function with changed salivary composition or by selective dysfunction of the submandibular/sublingual (SM/SL) salivary glands, whereas a progressed stage was characterised by extreme dysfunction of the SM/SL glands or by extreme dysfunction of all major salivary glands. Such staging seems also possible with S-type isoamylase activity in serum. High activity may indicate an early stage and low activity a progressed stage.

The biphasic course, in terms of time, of serum S-type isoamylase in SS patients may well explain its low sensitivity for SS. Only the patients who recently developed oral complaints from SS seem likely to show increased serum activity of this salivary enzyme. The remaining SS patients may be beyond this initial phase of intracellular enzyme leakage and, therefore, cannot be recognised enzymatically anymore.

Interestingly, leakage of salivary isoamylase in serum correlated significantly to the disturbance of the sialochemical variables sodium and chloride, which are characteristic for salivary gland inflammation in SS.[7] As leakage into serum of intracellular enzymes is thought to result from increased cell death, it might be hypothesised that the amount of serum amylase leakage in SS corresponds with the inflammatory activity of the disease at the glandular level.[14] Perhaps, salivary isoamylase in serum may even be informative regarding the prognosis of salivary gland function. High serum iso-amylase activity may indicate an active disease at the glandular level (thus relatively rapid deterioration of secretory functions), normal activity may indicate a more stable situation, whereas low activity an end situation with little change to be expected in secretory function.

In conclusion, measurement of S-type isoamylase in serum has limited diagnostic value for SS. Measuring S-type isoamylase activity may be very useful for assessing disease progression, rather than for diagnosing SS. A long-term prospective study is warranted in order to verify such considerations regarding outcome variables in SS.

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CHAPTER

5

Salivary imaging

SUMMARY

Aim - Sialography is commonly used for the purpose of diagnosing Sjögren's syndrome, though its invasive nature is often regarded as a serious drawback for routine usage. The aim of this study was to evaluate the morbidity and acceptability of parotid sialography using oil-based contrast fluid.

Methods - Twenty-four consecutive sialographic procedures were evaluated by assessing the morbidity and patient's acceptance of the procedure with a standardised questionnaire, and by recording relevant physical parameters during the procedure.

Results - There was good acceptance of the sialographical procedure, and the morbidity was low. No signs of overfilling or fausse route were observed in any of the sialograms. On average, 0.74 ml contrast fluid was infused at a velocity of 0.01 ml/s. The whole procedure was completed within 12 minutes.

Conclusions - Parotid sialography appears less invasive than is often thought. It has a low morbidity and is well accepted by the patients.

MORBIDITY FROM PAROTID SIALOGRAPHY

W.W.I. Kalk, A. Vissink, F.K.L. Spijkervet, J.M. Möller, J.L.N. Roodenburg

Department of Oral and Maxillofacial Surgery, University Hospital Groningen

Oral Surg Oral Med Oral Pathol Oral Radiol Endod, in press

INTRODUCTION

Introduced early in the previous century, sialography has proven a useful technique throughout the years. Through retrograde infusion of oil- or water-based iodine contrast, the architecture of the salivary duct system is visualised radiographically. There is debate ongoing in the literature regarding the current value of contrast sialography for the clinical differentiation of salivary gland disorders. Due to the availability of new imaging procedures, such as MRI, CT scanning, scintigraphy and ultrasonography, its diagnostic indication has significantly been narrowed and limited to imaging of the ductal system.[1-6] For the purpose of diagnosing Sjögren's syndrome, sialography is still commonly used, as it has been proven to reveal characteristic changes of the salivary gland ductal system with rather high sensitivity and specificity.[7-10]

Following the introduction of water-based contrast fluids in the 1950's[11], these fluids have been advocated in sialography for their better tolerance in the human body having a physicochemical composition closer to body fluids.[12] A comparative study between oil-based and water-based contrast revealed no adverse reactions with both contrast media.[13] Nevertheless, invasiveness and poor tolerance is by some clinicians considered a drawback for routine usage of oil-based sialography, especially in SS patients.[14] To date, however, the subjective experiences of the patients have not been described. The aim of this study was to evaluate the morbidity and acceptability of oil-based contrast sialography.

PATIENTS AND METHODS

Patients

Twenty-four consecutive patients who had undergone parotid contrast sialography for the diagnosing of Sjögren's syndrome in the period from October 1999 until April 2000 at the Department of Oral and Maxillofacial Surgery of the University

Hospital Groningen participated in this study. The studied group comprised 22 women and 2 men (mean age of 57 years; SD 9; range 41 to 75). The right parotid gland was used 21 times and the left parotid gland three times. Iodine allergy was applied as an exclusion criterion for the study, since iodine is present in the contrast fluid used. No patients had to be excluded from the study.

Technical procedure for sialography

All sialograms were obtained in absence of acute sialadenitis. If clinical signs of acute inflammation were present, sialography was postponed until clinical signs had subsided for at least six weeks. Parotid sialograms were made unilaterally (preferably of the right gland) in a standardised manner. Reasons for using the left parotid gland included probing difficulties and asymmetrical parotid gland swelling. All sialograms were made by the same clinician. After cannulation of the parotid main duct, the gland was filled through retrograde infusion of an oil-based contrast fluid (Lipiodol U.F.®), under low pressure[15] using a 2-ml Cornwall® syringe (Becton Dickinson). The patient's sensation of a sudden increase of pre-auricular pressure was used to estimate the proper filling level of the gland, after which two pictures (posteroanterior and lateral) were made. Premature leakage of contrast fluid was prevented through main duct ligation under local anaesthesia. A General Electric G1000 was used as X-ray apparatus; pictures were made with an additional filter with 58 kV during 0.18 s. After removal of the ligature and massaging the parotid gland patients were advised to stimulate salivary gland secretion with citric flavoured gum or candy during the first hours, in order to enhance wash out of remaining contrast fluid.

Evaluation of the procedure

The sialographical procedure was evaluated by an independent investigator using the following two methods: assessment of relevant physical parameters during the procedure and a standardised patient questionnaire. There was no time interval between the making of the sialogram and the subsequent evaluation, with the exception of the duration of pain sensation after the procedure and the overall judgement, which were recorded at a recall visit after three weeks. As physical parameters, the duration of the whole procedure, the duration of contrast infusion and the total amount of infused contrast fluid were recorded. The velocity of contrast infusion was in addition calculated. The questionnaire contained multiple choice questions about presence and severity of pain during the sialographical procedure, duration of pain following sialography, and the patients' acceptance of

the procedure. Pain severity was graded on a visual analogue scale (0 representing no pain, 10 representing severe pain).

RESULTS

Physical parameters of sialography

The duration of the whole procedure averaged 12 minutes (mean 716 s, SD 186 s, range 552 – 955 s). The volume of infused contrast fluid averaged 0.74 ml (SD 0.08 ml, range 0.50 - 0.90 ml), whereas the mean velocity of infusion was calculated at 0.01 ml/s (SD 0.001ml/s, range 0.008 - 0.013 ml/s). There was no evidence of leakage of contrast medium into the oral cavity until the ligature was removed. Radiographically, all sialograms showed good filling of the gland; no fausse route was observed.

Morbidity and acceptability

Twenty-four sialographical procedures were evaluated in twenty-four patients. Nineteen of 24 patients experienced no pain during infusion of the contrast fluid, whereas 5 patients experienced little pain. The pain severity during the procedure averaged 4.0 in latter patients.

Sixteen of 24 patients experienced 'no discomfort' during the sialographical procedure, 7 patients found it 'a little unpleasant' and one patient found it 'very unpleasant'. To the question, which part of the procedure was experienced as the most unpleasant sensation, surprisingly, patients stated most often it was the opening of the mouth (n=7), instead of the placement of the ligature (n=2) or the infusion of contrast fluid (n=2).

Fifteen patients had no pain at all after the procedure and 5 felt soreness for one day. Two patients stated that a sore feeling had lasted about a week whereas two had a sore feeling nearby the parotid region lasting about two weeks.

To estimate the subjective acceptability of the sialographical procedure, the patients were requested to judge the procedure using a number between 0 and 10, with 0 indication 'very bad experience' and 10 'no problems at all'. The judgement averaged 8.7.

DISCUSSION

Most of the patients experienced no pain or discomfort during sialography (79% and 67%, respectively). The average patient judgement of the procedure was very high.

The mild pain that was felt by 5 of the patients during the contrast infusion was most likely related to the perception of raised intraluminal pressure or distension of the parotid capsule from minor glandular enlargement.

Soreness in the parotid region for a short period after parotid sialography, as occurred in a few patients, may relate to presence of some residual contrast fluid in the gland, especially in cases of sialectasia. Another explanation for the temporary soreness might be the presence of a subclinical sialadenitis prior to sialography, rendering the gland more sensitive to contrast fluid. The two patients who felt pain for longer than a week both have been examined by their general practitioner, who concluded that other reasons than sialography had been responsible for their complaints (sinusitis and headache, respectively).

Though sialography is considered the imaging procedure of choice for diagnosing Sjögren's syndrome[16], it should not be performed in case of iodine allergy to prevent local and systemic allergic reactions. Alternative positive contrast materials for iodine, such as barium-sulphate suspensions, are not suitable for sialography due to a large particle size. Therefore, if confronted with patients with a history of iodine allergy, other imaging techniques such as scintigraphy or ultrasonography should be used instead to visualise salivary gland pathosis. Regarding the use of CT and MRI techniques in diagnosing SS, conflicting results have been reported in literature.[2,3,5]

Though the use of oil-based contrast fluid has often been associated in literature with non-allergic adverse tissue responses or even with damage to the gland[14], we have experienced no complications whatsoever during or after sialography. We feel that, if lipiodol is restrained to the ductal lumen during the procedure, no adverse tissue effects can be expected. Lipiodol remains, due to its hydrophobic nature, much better within the salivary ducts, than water-based contrast fluids that pass more easily through the ductal epithelium.[17] As a result, the clearance of contrast fluid differs substantially between oil- and water based contrast. Oil-based fluid leaves the gland with saliva secretion via the main salivary duct, whereas water-based fluid diffuses across the ductal epithelium and is cleared subsequently from the circulation by kidneys and liver. Therefore, one might actually expect less adverse reactions from oil-based contrast than from water-based contrast in the normal situation.[18-20] Only if a iatrogenic fausse route is induced during contrast infusion, is a less favourable tissue response to be expected from oil-based contrast,

since in such situation it remains in the gland parenchyma for a long time inducing a chronic granulomatous inflammation, as opposed to quick clearance of water-based contrast in the same situation. If uncertain about the ductal probing or if inexperienced with contrast sialography, it seems wise therefore to use water-based contrast fluids. This way, adverse tissue reaction from a possible fausse route or overfilling is minimised.

The use of oil-based contrast, whose hydrophobic nature impairs the fluid's ability to mix with saliva or to pass through epithelial membranes of the salivary ducts, renders much sharper X-ray images than water-based alternatives.[13,17,21] Therefore, we prefer the use of lipiodol or other oil-based contrast fluids, yielding optimum quality of sialographical images with, in our hands, no adverse side effects on the salivary glands of any kind.

Given its low morbidity and its subjective acceptability, sialography of the parotid gland appears, if performed properly, to be less invasive than is often thought. With some practice, this diagnostic imaging technique can be applied in ten to fifteen minutes, differentiating between a variety of salivary gland disorders including Sjögren's syndrome.

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SUMMARY

Aim - Despite the availability of many new imaging procedures, sialography has, after decades of use, maintained its status as the imaging procedure of choice for evaluating the oral component of Sjögren's syndrome (SS). In this study, the clinical value of sialography as a diagnostic tool in SS was explored by assessing its diagnostic accuracy, observer bias and staging potential.

Methods - One hundred parotid sialograms were interpreted independently in a blind fashion by two trained- and two expert-observers. Sialograms were derived from a group of consecutive patients, referred for diagnostics of SS. Patients were categorised as SS and non-SS by the revised European classification criteria.

Results - Trained observers reached a sensitivity of 95 and a specificity of 33 percent for SS by sialogram, whereas expert-observers reached a sensitivity of 87 and a specificity of 84 percent. There was only 'fair' inter-observer agreement between trained- and expert-observers, whereas both expert-observers showed 'good' agreement with one another, according to Cohen's kappa. Intra-observer agreement was 'good' to 'very good' for all observers. The four different gradations of sialectasia, i.e. punctate, globular, cavitary and destructive, showed a weak but significant correlation with the duration of oral symptoms.

Conclusions - This study markedly shows that the diagnostic value of parotid sialography for diagnosing SS greatly depends upon the skills of the observer, implying that sialography lacks general applicability as a diagnostic tool in SS and requires specific expertise. Nevertheless, given its potentially high sensitivity and specificity in diagnosing SS as well as its useful staging potential, sialography still has its use in the evaluation of the oral component of SS.

PAROTID SIALOGRAPHY FOR DIAGNOSING SJÖGREN'S SYNDROME

W.W.I. Kalk[1], A. Vissink[1], F.K.L. Spijkervet[1], H. Bootsma[2], C.G.M. Kallenberg[3], J.L.N. Roodenburg[1]

Departments of [1]Oral and Maxillofacial Surgery, and Internal Medicine, divisions of [2]Rheumatology, and [3]Clinical Immunology, University Hospital Groningen

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INTRODUCTION

Sjögren's syndrome (SS) is considered a systemic autoimmune disease with the exocrine glands as main target-organs. As a result, the presence of this disease may cause structural damage and secretory dysfunction of the tear- and salivary glands. The tear- and salivary gland involvement with its inherent morbidity is often addressed as the ocular- and the oral component of SS, respectively.

The oral component of SS can be evaluated in many ways. Generally, two different procedures are practised, *i.e.* assessment of salivary gland function and salivary gland imaging. Salivary gland function is assessed through measurement of salivary secretion rate (sialometry) and analysis of salivary composition (sialochemistry).[1-3] Salivary gland imaging is currently performed by several procedures including magnetic resonance imaging (MRI), computer tomography (CT) scanning, ultrasonography, scintigraphy and sialography.[4-9] Despite the availability of new imaging procedures, the oldest procedure of all, sialography has maintained its status as the method of choice for exploring the ductal system of the salivary gland to diagnose SS.[10]

Sialography reveals the architecture of the salivary duct system radiographically by insertion of a contrast fluid. This radiographic demonstration of salivary glands in vivo was first performed in 1913.[11] Four decades ago the sialographic changes seen on sialograms were accurately described and, with regard to chronic sialadenitis, classified into punctate, globular, cavitary and destructive sialectasia (dilatation) of the acinar and ductal system.[12-14] These four sialectatic changes are thought to represent increasing glandular damage, respectively, caused by chronic salivary gland inflammation.[14] SS is by far the most frequent cause of such chronic salivary gland inflammation. Therefore, by observing sialectasia on a sialogram, the presence (and progression) of SS with regard to its oral component can be determined.

It has been demonstrated that SS-related sialographic findings such as sialectasia are more closely related to SS-related clinical symptoms (stimulated parotid salivary flow, incidence of keratoconjunctivitis sicca) than is the periductal lymphocytic infiltration of the labial glands.[15] In addition, superior sensitivity[16-18] and/or specificity for SS have been frequently ascribed to sialography as compared to labial gland biopsy.[19-23] However, the subjective nature of reading and interpreting a sialogram causes a certain observer bias, as is the case with diagnostic-imaging tests in general. The amount of observer bias may have a substantial impact on the clinical value of a particular diagnostic test.
In this study the clinical value of sialography as a diagnostic tool in SS was explored by assessing its diagnostic accuracy, observer bias and staging potential in 100 sialograms.

PATIENTS AND METHODS

Patients

In order to study the clinical value of sialography for diagnosing Sjögren's syndrome (SS) 100 parotid sialograms were interpreted independently by four observers. Two observers had large general experience in judging sialograms, whereas two observers were in addition especially experienced in the judging of sialograms with respect to the diagnosis SS. The observers with general experience are termed 'trained observers' and the observers with specific SS expertise are termed 'expert-observers'. Sialograms were derived from a non-selected group of 100 consecutive patients referred to the outpatient clinic of the Department of Oral and Maxillofacial Surgery of the University Hospital Groningen in the period from December 1997 until August 1999.
Patients suspected of SS were referred by rheumatologists, internists, neurologists, ophthalmologists, ENT-specialists, general practitioners and dentists. Reasons for referral included mouth-dryness, eye-dryness, swelling of the salivary glands, arthralgia and fatigue. The diagnostic work-up for SS, carried out in all patients, included the following aspects: subjective complaints of oral and ocular dryness (table 3.1.1), sialometry and sialochemistry, histopathology of salivary gland tissue, serology (SS-A- and SS-B antibodies) and eye tests (Rose Bengal staining and Schirmer tear test). Sialography was excluded for diagnostic use in this study, in order to avoid an incorporation bias. In addition to the diagnostic tests, the duration of oral symptoms and the serum level of immunoglobulin class G (IgG) were assessed in order to be studied in relation to the sialographical stage. Duration of

oral symptoms was defined as the time from first complaints induced by or related to oral dryness until referral.
In this study, the revised European classification criteria for SS[24,25] were used as reference standard for the diagnosis of SS, categorising patients as primary SS, secondary SS, or non-SS patients.

Exclusion criteria

Iodine allergy was applied as an exclusion criterion for the study, since iodine is present in the contrast fluid used. Furthermore, the exclusion criteria of the European classification criteria for SS were applied. Psoriatic arthritis and HIV-infection were excluded as both diseases may cause sialographical pictures resembling SS.[26-33] No patients had to be excluded from the study.

Technical procedure for sialography

All sialograms were obtained in absence of acute sialadenitis. If clinical signs of acute inflammation were present, sialography was postponed until clinical signs had subsided for at least six weeks. Parotid sialograms were made unilaterally (preferably of the right gland) in a standardised manner. All sialograms were made by the same clinician. After cannulation of the parotid main duct the gland was filled through retrograde infusion of an oil-based contrast fluid (lipiodol U.F.®) using a 2-ml Cornwall syringe® (Becton and Dickinson). The patient's sensation of a sudden increase of pre-auricular pressure was used to estimate the proper filling level of the gland. Premature leakage of contrast fluid into the mouth was prevented through main duct ligation under local anaesthesia. A General Electric G1000 was used as X-ray apparatus; posteroanterior (6˚ mediolateral) and lateral pictures were made with an additional filter with 58 kV during 0.18 s. After removal of the ligature and massaging the parotid gland patients were advised to stimulate salivary gland secretion with citric flavoured gum or candy during the first hours in order to enhance wash out of the remaining contrast fluid. The whole procedure was completed within 15 minutes.

Evaluation of the sialograms

Four observers each examined independently 100 sialograms in a random order with no information from the patients other than the reason for referral (clinical suspicion of SS) and the amount of inserted contrast fluid. Of the 100 sialograms, 25 were judged a second time by all observers without being aware of it, in order to determine intra-observer variability. All sialograms were examined in the presence of

an independent investigator who made sure that each set of sialograms was examined within 2 minutes without being revised afterwards.
Before the observers examined the sialograms, a calibration session took place in which all observers agreed upon the criteria to be applied when describing the sialograms. Four different pathological descriptions were agreed upon. The observers had to determine whether or not these patterns were present in each sialogram. These patterns were sialectasia (subdivided into punctate, globular, cavitary and destructive), thin appearance of the ducts with or without gland enlargement, irregular and widened main ducts, and presence of a space-occupying lesion, respectively.

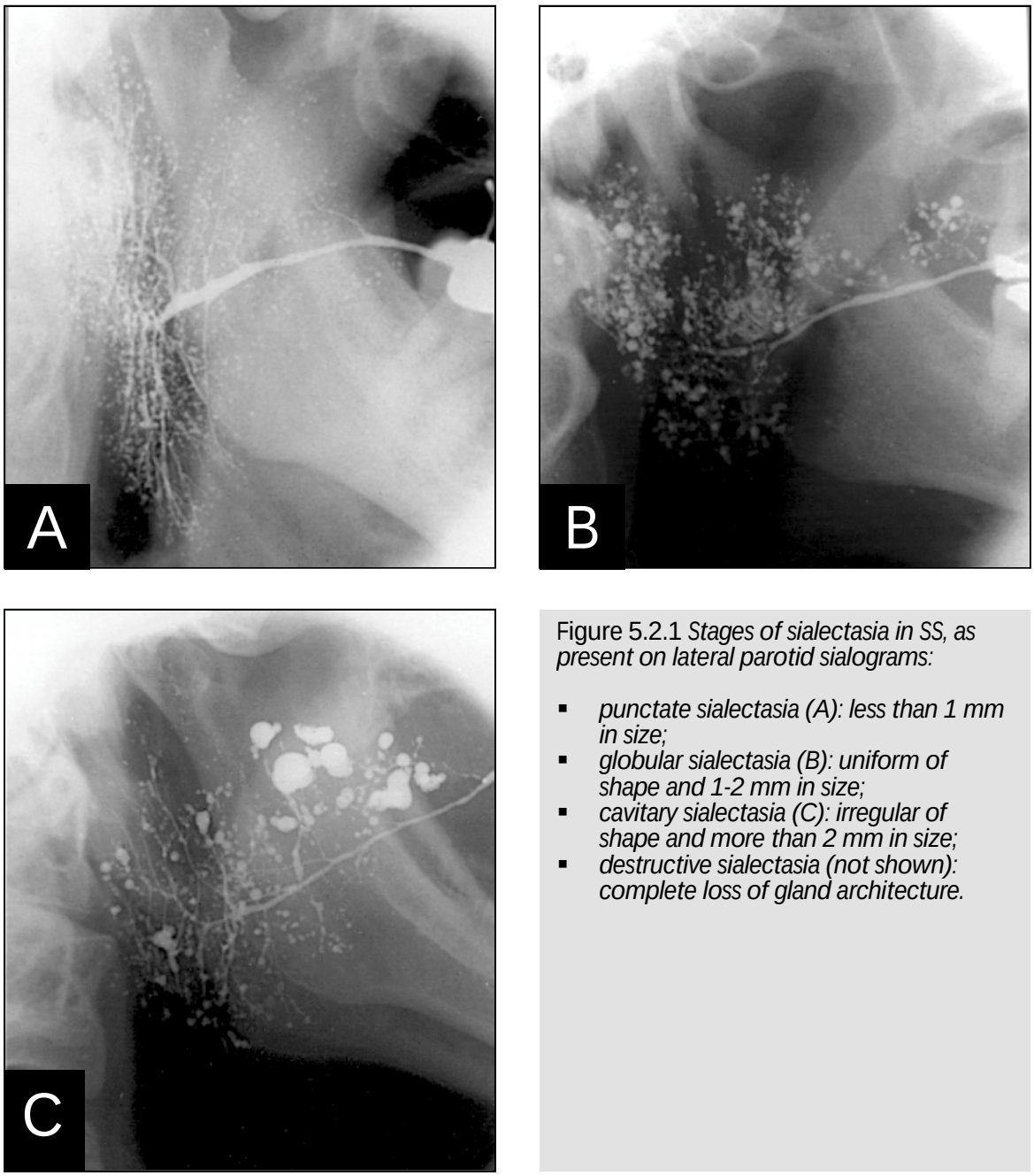

Figure 5.2.1 *Stages of sialectasia in SS, as present on lateral parotid sialograms:*

- *punctate sialectasia (A): less than 1 mm in size;*
- *globular sialectasia (B): uniform of shape and 1-2 mm in size;*
- *cavitary sialectasia (C): irregular of shape and more than 2 mm in size;*
- *destructive sialectasia (not shown): complete loss of gland architecture.*

If present, sialectasia (dilatations) were graded according to the description by Blatt: punctate if less than 1 mm in size; globular if uniform and 1-2 mm in size; and cavitary if irregular and more than 2 mm in size (figure 5.2.1). A destructive pattern was defined as complete destruction of the gland architecture, simulating an invasive neoplastic process.[12] Sialectasia were considered the only descriptions suggestive for a diagnosis of SS. Presence of thin ducts was regarded as possibly consistent with sodium retention dysfunction syndrome or with sialoadenosis.[34,35] Irregular and widened main ducts consistent with sialodochitis (salivary duct inflammation) were considered the prevalent feature in chronic-recurrent sialadenitis.[36-39] A space-occupying lesion on a sialogram was considered suggestive for a tumour compressing the gland.
A consensus judgement whether or not a sialogram is in accordance with the diagnosis SS was based upon the majority of the individual descriptions of the observers.

Statistical analysis

Data were submitted for statistical analysis using the Statistical Package for the Social Sciences (SPSS), version 9.0. The following statistical procedures were applied: Cohen's kappa as measure of inter- and intra-observer agreement (observer bias)[40,41], and Pearson's and Spearman's coefficients as correlation tests. In the results section it is stated which statistical test was applied in a specific situation. A significance level of 0.05 was pre-defined in all cases.

RESULTS

Study group

By applying the revised European classification criteria for Sjögren's syndrome (SS) on the studied cohort, 39 patients were categorised as SS (20 primary- and 19 secondary SS; male/female ratio: 1/7; mean age of 54 years; SD 15; range 21 to 84) and 61 patients as non-SS (negative for SS) (male/female ratio: 1/14; mean age of 54 years; SD 15; range 20 to 81). The latter were, based upon additional clinical and laboratory tests, diagnosed as having sialoadenosis (n=18), sodium retention dysfunction syndrome (n=18), medication induced xerostomia (n=11), or as having no alternative disease directly related to salivary gland pathology (n=14). Mean duration of oral symptoms before referral was 35 months for SS- and 30 months for non-SS patients (range: SS 0-180 months, non-SS 0-240 months).

Test accuracy for SS

By determining the presence of sialectasia as diagnostic indicator for SS, the sensitivity and specificity differed greatly between the trained- and expert-observers. Trained observers reached a sensitivity of 95 and a specificity of 33 percent, whereas expert-observers reached a sensitivity of 87 and a specificity of 84 percent (table 5.2.1). The large difference in specificity between trained- and expert-observers was mainly due to their decision when doubting between 'no abnormality' and 'punctate sialectasia'. Examples of sialograms that gave rise for doubt are illustrated in figures 5.2.2 and 5.2.3.

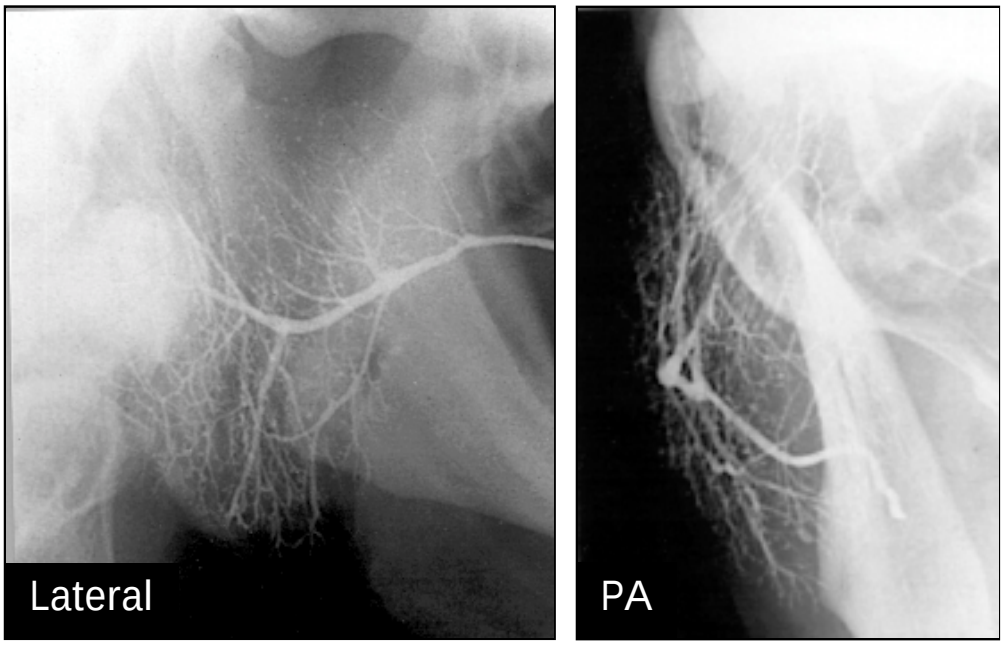

Figure 5.2.2 *Example of a parotid sialogram of a SS-patient, which could give rise for doubt. Note the presence of initial sialectasia on both projections. All observers judged this sialogram as positive for SS (sialectasia present).*

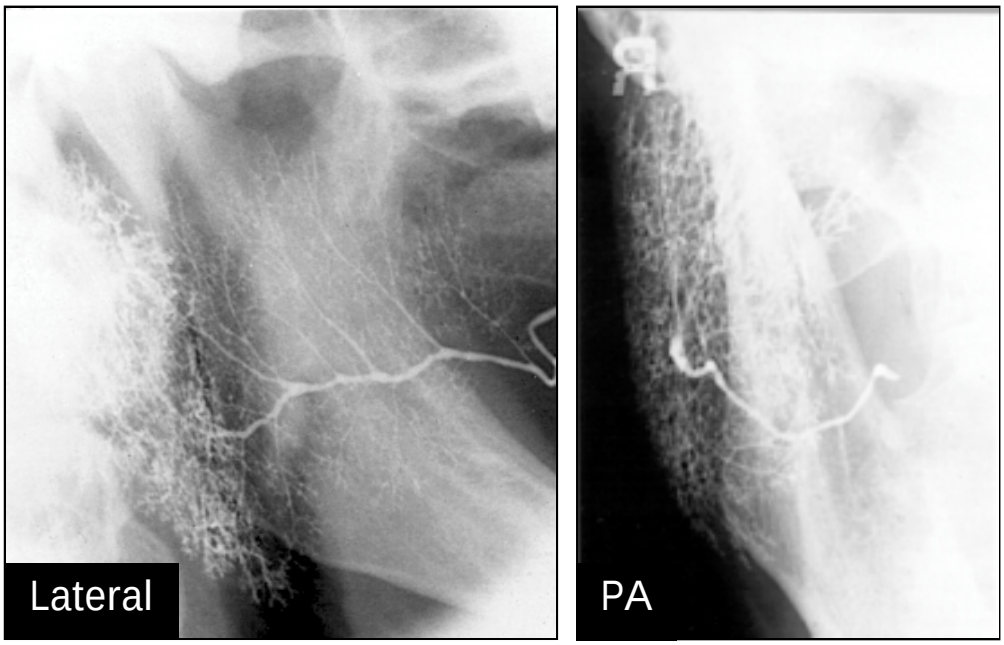

Figure 5.2.3 *Parotid sialogram of a non-SS patient. Note the small radiodensities that could be easily misinterpreted. Experienced-observers judged this sialogram as positive, whereas expert observers as negative for SS (no sialectasia present).*

Table 5.2.1. *Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and likelihood ratio (LR) of the four observers (expert: A, B; trained: C, D) for the diagnosis of SS on a group of 100 patients by presence of sialectasia on the sialogram. Consensus judgement was based upon the majority of individual judgements for each sialogram. Note the large differences between expert- and trained observers regarding specificity and likelihood ratio.*

N=100	A	B	C	D	consensus
Sensitivity	87%	82%	95%	92%	92%
Specificity	71%	84%	33%	23%	71%
PPV	65%	76%	47%	43%	67%
NPV	90%	88%	91%	82%	94%
LR	3.0	5.0	1.4	1.2	3.1

Expert-observers, reached a high specificity by choosing 'no abnormality' in case of doubt (observers A and B), whereas trained observers, who chose for 'punctate sialectasia' in the same situations, suffered from a major drop in specificity and gained only slight improvement of sensitivity (observers C and D). Consequently, the likelihood ratios also greatly differed between trained- and expert-observers, varying from 1.2 (not very useful as a test) to 5.0 (very useful as a test). Consensus judgement based upon the majority of individual judgements reached an intermediate sensitivity and specificity for SS of 92 and 71 percent, respectively, and a likelihood ratio of 3.1. In 18 of the 61 non-SS patients, sialectasia was present on the sialogram, according to consensus judgement (table 5.2.2).

Table 5.2.2. *Judgements of 100 sialograms regarding the presence and grade of sialectasia by four individual observers (expert: A, B; trained: C, D) and by consensus. For each descriptive category the number of cases accordingly judged is given. Consensus judgement is based upon the majority of individual judgements for each sialogram. Note the large variability between expert- and trained observers regarding false positivity: the trained observers judged many sialograms from non-SS patients as punctate.*

N=100	A		B		C		D		consensus	
Sialectasia	SS	non-SS	SS	non-SS	SS	non-SS	SS	non-SS	SS	non-SS
none	5	43	7	51	2	20	3	14	3	43
punctate	13	5	15	1	11	24	12	36	14	5
globular	11	6	10	3	4	5	15	5	11	7
cavitary	4	3	3	0	15	8	7	5	5	5
destructive	6	4	4	6	7	4	2	1	6	1

Observer agreement

Inter- and intra-observer agreement was calculated for the four pathological conditions that were determined on the sialograms by each observer. With regard to the presence of SS (sialectasia as indicator), there was only 'fair' inter-observer agreement between trained- and expert-observers, whereas both expert-observers showed 'good' agreement with one another, according to Cohen's kappa. The intra-observer agreement was 'good' to 'very good' (table 5.2.3). The determination of other sialographical alterations that occur in sialoadenosis, sialodochitis and salivary

gland tumour all suffered from rather low inter-observer agreement, varying from 'poor' to 'moderate' (data not shown).

Table 5.2.3. *Inter- and intra-observer agreement between the observers (expert: A, B; trained: C, D) regarding the judgement of presence of sialectasia on a sialogram. Note there is fair inter-observer agreement between trained- and expert-observers, moderate agreement between both trained observers, and good agreement between both expert-observers. Observer agreement is expressed by Cohen's kappa (as adjusted by Landis & Koch, 1977).*
< 0.200: poor agreement
0.200-0.400: fair agreement
0.400-0.600: moderate agreement
0.600-0.800: good agreement
>0.800: very good agreement

Inter-observer agreement

N=100	A	B	C	D
A	-			
B	0.762	-		
C	0.386	0.339	-	
D	0.322	0.258	0.588	-

Intra-observer agreement

N=25	A	B	C	D
	0.824	0.874	0.839	0.762

Staging potential

The different gradations of sialectasia (figure 5.2.1) showed a weak but significant correlation with the duration of oral symptoms in SS patients ($r_{pearson}$ 0.29, $p<0.05$). According to consensus judgement of the sialograms, the observation of punctate sialectasia corresponded with an average duration of oral symptoms of 15 months, whereas globular-, cavitary- and destructive sialectasia corresponded with increasing duration of 39, 44 and 59 months, respectively.
No relation was observed between the serum level of immunoglobulin class G (IgG) and the presence or gradation of sialectasia in SS patients.

Volume lipiodol

The gradation of sialectasia related significantly to the amount of lipiodol that was infused into the parotid gland with sialography ($r_{pearson}$ 0.26, $p<0.01$). On average, 10% more lipiodol fluid had been infused in the parotid gland of SS patients compared to non-SS patients (0.81±0.20 ml versus 0.73±0.10 ml lipiodol, T-test: $p<0.05$). In agreement with this, on average 20% less lipiodol had been infused if thin ducts were observed (0.66±0.08 ml versus 0.79±0.15 ml lipiodol, T-test: $p<0.01$).

Ductal changes

The presence of widened or irregular main ducts, consistent with sialodochitis, was not diagnostic for SS (sensitivity 28%, specificity 62%, likelihood ratio 0.7), and was neither related to salivary flow rates nor to duration of oral complaints. The observation of thin ducts with or without salivary gland enlargement, regarded as possibly consistent with sialoadenosis or sodium retention dysfunction syndrome,

did not relate significantly to any changes of salivary composition (*e.g.* sodium, potassium, amylase, total protein), nor to salivary flow rate.

DISCUSSION

It has become clear from this study that it is possible to achieve both a sensitive and specific test result with parotid contrast sialography for diagnosing SS (likelihood ratio up to 5.0). This diagnostic accuracy, however, is very much dependant on the observer involved, which implies that the technique lacks general applicability and requires specific expertise.

The four different gradations of sialectasia showed a weak but significant relation to the duration of oral symptoms in SS patients, suggesting that sialectasia slowly worsens (increases in number and size) during the disease course of SS. Previous studies have already shown that, in SS patients, increasing gradations of sialectasia correspond with lower salivary flow rates[3,15,42], as well as that salivary flow rates deteriorate with increasing duration of oral symptoms (§3.1).[43] We therefore suggest that SS can be subdivided into different sequential stages according to the type of sialectasia on the sialogram, with a corresponding degree of hyposalivation.

Though the use of oil-based contrast fluid has often been associated in literature with high rates of complications, we have experienced none of the complications associated with oil-based contrast fluids during or after the one hundred sialograms performed. Since the use of oil-based contrast fluid does result in superior image quality, we prefer oil-based contrast fluid above water-based alternatives for use in sialography, in case of well-trained clinicians. Otherwise, a water-based contrast fluid is advisable. In case of iodine allergy, sialography should not be performed to prevent local and systemic allergic reactions. Alternative positive contrast materials other than iodine that are currently in use are not suitable for sialography. Therefore, in cases of iodine allergy other imaging techniques such as scintigraphy or ultrasonography should be used instead to visualise salivary gland involvement in SS. Conflicting results have been reported in literature regarding the use of CT and MRI techniques in diagnosing SS.[4,5,8]

Though some studies have reported abnormal parotid sialographic findings as a fairly common finding in control subjects (up to 40%)[8,44,45], sialography is generally considered a very specific diagnostic test for SS.[20-23] However, sialectasia, may also occur singly as result of chronic-recurrent parotitis, a condition unrelated to SS.[38,39] The latter may perhaps account for at least some of the sialectasia we observed in 30% of the non-SS patients. Furthermore, some of the observed sialectasia in non-SS

patients probably has to be attributed to observer error, since the number of false positive cases varied markedly between trained- and expert-observers. The observer's decision, especially when in doubt about recognising initial sialectasia at the beginning of SS, reflects crucially upon the test specificity, *i.e.* the number of false positive cases (tables 5.2.1 and 5.2.2, figures 5.2.2 and 5.2.3). Other imaging procedures, however, may well suffer from the same human factor, *i.e.* subjectivity and varying expertise with interpreting the image.
Since diagnostic testing for SS is performed in the second echelon, there is an increased prior chance for SS compared to the general population. Furthermore, the diagnosis SS is based upon several diagnostic tests. Both the raised prior chance for SS and the combined-test approach require diagnostic tests with emphasis on specificity. For this reason, it is recommended that one chooses negatively when in doubt about the presence of sialectasia on a sialogram (as illustrated in figures 5.2.2 and 5.2.3), thereby increasing the specificity of the test result. The diagnostic accuracy of sialography might be further improved with a digital subtraction technique that eliminates osseous background structures, and thus offers interference-free visualisation of glandular structures.[10,22] Such enhancement of image quality might not only reduce the number of false positive test-results, but also significantly improve the inter-observer agreement. Negative aspects of this procedure are its sensitivity to patient movement (swallowing, tongue movement) during contrast injection and the need for advanced X-ray equipment.

In conclusion, reading and interpreting a sialogram requires certain expertise with regard to the recognition and correct interpretation of first stage sialectasia, restricting its use as a diagnostic tool for incipient SS to expert-observers. In cases of doubt, one should therefore consider sending the (digitised) sialogram to an expert centre. Despite limited general applicability, sialography still has its unique value in the evaluation of SS. Its costs are low and, if interpreted properly, it is highly diagnostic. Furthermore, it has a relatively low degree of invasiveness and it is a relatively simple and quick procedure (§5.1).[46] The time-relation of the progression of sialectasia renders sialography an especially valuable tool in SS with regard to the assessment of disease progression.

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CHAPTER

6

Oral and ocular comparison

SUMMARY

Aim - Sjögren's syndrome (SS) is considered an autoimmune exocrinopathy with inherently severe sicca complaints. Dysfunction of exocrine glands manifests itself clinically predominantly in the lacrimal and salivary glands. Little is known, however, about the relationship between lacrimal and salivary gland involvement in SS. Furthermore, it is of interest which eye test contributes the most to the diagnosis of SS. Therefore, the aim of this study was to determine the performance of different tear tests and to disclose how these tests relate to common serologic and salivary tests in SS.

Methods - In patients suspected of SS, the tear break-up time (BUT), and a possible new test, the tear mucus score, were evaluated in addition to the routine tests, Rose Bengal score and Schirmer test. Eighty consecutive patients were included in this study, categorised into primary SS (pSS), secondary SS (sSS), and negative for SS (non-SS) in accordance to the revised European classification criteria for SS.

Results - A corresponding change of tear- and saliva quality and secretion rate was noted in both pSS and sSS patients. Also a clear correlation was found in SS patients, between the Rose Bengal score and observations with parotid sialography. Hyperglobulinemia and presence of SS-B antibodies in serum of SS patients both correlated significantly to increased Rose Bengal scores. The Rose Bengal score was also significantly increased with the duration of subjective eye-dryness, and a decreased tear-gland function as estimated by the Schirmer test. The BUT and mucus score both performed insufficiently in diagnosing SS.

Conclusions - From the observed relationship between the ocular and the oral component, we conclude that, *theoretically*, a positive evaluation of one of these components (either ocular or oral), in addition to positive serology or histopathology for SS, could be sufficient to diagnose the syndrome *for clinical purposes*. Furthermore, we conclude that hyperglobulinemia and especially positive SS-B serology may warrant close monitoring of the eyes since these serum findings appear to relate to the severity of ocular surface damage. Of all eye tests, the Rose Bengal score still remains the test of choice having the highest specificity for SS. It also appears applicable for monitoring disease progression of SS, relating to duration of subjective complaints and to tear-gland dysfunction.

ORAL AND OCULAR MANIFESTATIONS IN SJÖGREN'S SYNDROME.

W.W.I. Kalk[1], Kh. Mansour[2], A. Vissink[1], F.K.L. Spijkervet[1], H. Bootsma[3], C.G.M. Kallenberg[4], A.V. Nieuw Amerongen[5], J.L.N. Roodenburg[1]

Departments of [1]Oral and Maxillofacial Surgery, [2]Ophthalmology, and Internal Medicine, divisions of [3]Rheumatology, and [4]Clinical Immunology, University Hospital Groningen. Department of [5]Oral Biology, division of Oral Biochemistry, Faculty of Dentistry Amsterdam (ACTA)

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INTRODUCTION

Sjögren's syndrome (SS) is considered a systemic autoimmune disease with the exocrine glands as main target-organs. Since the tear- and salivary glands are almost invariably affected, the oral and ocular sicca components form a significant part of this syndrome. The diagnosis of SS is based largely upon subjective and objective findings confirming damage or dysfunction of tear- and salivary glands, in accordance with one of the international sets of diagnostic criteria.[1]

Very little is known, however, about the differential involvement of the eyes and the mouth in SS. Furthermore, it is of interest which eye test contributes the most to the diagnosis of SS. Therefore, our main objective was to determine the performance of different eye tests and to disclose how these tests relate to serologic and salivary tests used for diagnosing SS.[2-5] The eye tests studied were the tear break-up time (BUT), tear lactoferrin level, and a possible new test, the mucus score, in addition to the routine diagnostic tests, *i.e.* the Schirmer test and Rose Bengal score.

PATIENTS AND METHODS

Patients

Eighty consecutive patients were included in this study. All patients attended the outpatient clinics of the Department of Oral and Maxillofacial Surgery and the Department of Ophthalmology of the University Hospital Groningen in the period August 1997 through April 2000. Patients suspected of Sjögren's syndrome (SS)

were referred to both departments by rheumatologists, internists, neurologists, ENT-specialists, general practitioners and dentists. Reasons for referral were not limited to ocular- or oral manifestations such as eye-dryness, mouth-dryness, and swelling of the salivary glands, but also included arthralgia and fatigue. The diagnostic work-up for SS, carried out in all patients, included the following aspects: subjective complaints of oral and ocular dryness (table 3.1.1), sialometry and sialochemistry, sialography, histopathology of salivary gland tissue, serology (SS-A- and SS-B antibodies) and eye tests (Rose Bengal score, Schirmer tear test).
In addition, as part of the diagnostic work-up for eye-dryness, tear break-up time (BUT), tear fluid lactoferrin level, and a possible new test, the tear mucus score, were measured for purpose of evaluation.
Furthermore, the duration of oral and ocular symptoms was recorded, defined as the time between referral and first complaints induced by or related to oral and ocular dryness, respectively. In addition to the assessment of autoantibodies, serum immunoglobulin levels were measured.
As reference standard for the diagnosis of SS, the revised European classification criteria for Sjögren's syndrome were used in this study, categorising patients as primary and secondary SS (pSS, sSS) and as negative for SS (non-SS).[2-5]
The usage of xerogenic drugs, *i.e.* antihypertensives, beta-blockers, antihistamines, and psychotropics, was relatively frequent in all patients (primary SS: 50%, secondary SS: 56%, non-SS 69%).

Eye tests

The Schirmer test was carried out with a filter-paper strip (Whatman no. 41) of 0.5 x 30 mm. The strip is placed in the lower fornix between the medial and lateral third of the eyelid of the unanaesthetised eye. After 5 minutes, the amount of wetting is measured from the extrafornical position of the strip. A value of 5 mm or less per 5 minutes was considered diagnostic for SS, according to the European classification criteria for SS.[3,6]
The Rose Bengal score, a quantified version of the original Rose Bengal test, was used to quantify the degree of staining. The test was performed by placing a 1% Rose Bengal solution in the inferior fornix of both eyes and asking the patient to make one or two full blinks. Afterwards the intensity of staining of both medial and lateral bulbar conjunctiva and of the cornea was scored, each section up to three points (1: sparsely scattered, 2: densely scattered, 3: confluent), so that a maximum score of nine could be obtained.[3,7,8] A score of 4 or more was considered diagnostic for SS, according to the European classification criteria for SS.

The tear break-up time (BUT) is defined as the interval between a complete blink and the appearance of the first randomly distributed dry spots. A 1% fluorescein solution is installed in the inferior fornix of both eyes. The patient is asked to blink a few times, afterwards the interval in seconds between the last blink and the first break in the tear film is measured.[3,7]

The tear fluid lactoferrin level was measured using a lactoplate kit (JDC, Culemborg, The Netherlands). A paper disc was placed in the lateral part of the inferior fornix of each eye and removed when completely damp (usually within five minutes). Each disc was then transferred to its corresponding reagent gel in the lactoplate kit and left for three days at room temperature. The diameter of the precipitate ring was then measured and the lactoferrin concentration calculated using the table provided in the kit.[3,9]

The tear mucus score was measured by semi-quantitative clinical assessment. After evaluation of the Rose Bengal score, the debris in the cul-du-sac, which was then stained with Rose Bengal, was considered a clinical indication for mucus content. A scale from 0 to 3 was used whereby 0 means no stained mucus at all in the cul-du-sac or on the cornea, 1 means mucus pellets in the cul-du sac, 2 means mucus flocks in the cul-du-sac or the cornea and 3 means mucus flocks as well as mucus threads in the cul-du sac and on the cornea. This score was carried out every time by two observers and the mean value was noted.

Salivary tests

The methods of saliva collection and analysis (sialometry and sialochemistry) and sialographical procedure have been described in detail in previous publications (§3.1 and §5.1).[10,11]

Statistical analysis

Data were submitted for statistical analysis using the MedCalc version 5.0 in order to calculate Receiver-Operating Characteristic (ROC) curves[12] and the Statistical Package for the Social Sciences (SPSS) version 9.0 for the remaining statistical procedures. These included independent sample T-test and Spearman's correlation test. A significance level of 0.05 was pre-defined in all cases. ROC curves express the diagnostic accuracy of a test variable, by plotting the sensitivity of the test against the specificity at all possible thresholds. A flat curve indicates poor accuracy, whereas a curve that approaches the left corner of the diagram indicates high accuracy.

RESULTS

Study group

By using the revised European classification criteria for Sjögren's syndrome, 32 patients could be classified as pSS (male/female ratio: 2/30, mean age 53 years, SD 14, range 24 to 84), 25 patients as sSS (male/female ratio: 6/19, mean age 58 years, SD 13, range 27 to 78) and 23 patients as negative for SS (non-SS) (male/female ratio: 2/21, mean age 48 years, SD 12, range 20 to 70)(table 6.1.1).

Table 6.1.1 *Group characteristics.*

	pSS	sSS	non-SS
N	32	25	23
Age (mean) at referral	53	58	48
Sex (male/female)	2/30	6/19	2/21
Positive salivary gland biopsy	30 (94%)	24 (96%)	0 (0%)
Positive serology	24 (75%)	11 (46%)	3 (13%)
SS-A	24 (75%)	11 (46%)	2 (9%)
SS-B	13 (41%)	8 (13%)	1 (4%)
Positive eye-test(s)[1]	22 (71%)	24 (96%)	11 (48%)
Schirmer tear test (≤5mm/min)	16 (50%)	17 (64%)	10 (43%)
Rose Bengal score (≥4)	19 (63%)	19 (79%)	6 (26%)
Positive oral test(s)[1]	31 (97%)	23 (92%)	14 (61%)
Parotid sialography[2]	28 (100%)	16 (76%)	3 (8%)
Sialometry (UFWS≤1.5ml/15min)[3]	23 (72%)	19 (83%)	13 (57%)
Subjective complaints[1]			
dry eyes	27 (84%)	23 (92%)	17 (74%)
dry mouth	28 (87%)	23 (92%)	18 (78%)

1. according to European classification criteria: at least one positive eye-/oral test, for subjective complaints see table 3.1.1 (Vitali *et al*, 1993)
2. sialectasia present, percentages based on the number of patients with available information
3. UFWS=unstimulated flow of whole saliva

Diagnostic performance of the evaluated tear tests

The BUT performed poorly as a diagnostic test for SS with a specificity of only 4 percent, when the original threshold of 10 seconds was used. ROC-plot analysis revealed an optimum threshold at 3 seconds (≤3 s), with a sensitivity of 76% and a specificity of 74% for SS (likelihood ratio 2.9)(figure 6.1.1).

The tear mucus score had a sensitivity of 60% and a specificity of 74% (likelihood ratio 2.3), when scores of >1.8 (on a scale of 0-3) were considered diagnostic for SS (figure 6.1.1). If a threshold of >2.0 was applied the test gained specificity, however at the cost of a serious loss of sensitivity (sensitivity 22%, specificity 95%, likelihood ratio 4.2). ROC-plot analysis further showed that the diagnostic performance of the BUT and mucus score are superior to the performance of the Schirmer test, with more rounded curves toward the upper left corner and larger areas under the curve. However, none of the tear-tests could compare to the diagnostic value of the Rose

Bengal score (figure 6.1.1). It must be noted, however, that the estimates of diagnostic value of the Rose Bengal score and the Schirmer test are unavoidably somewhat flattered by an incorporation bias, for both tests were also used to support the diagnosis of the studied patients.

The diagnostic performance of the tear lactoferrin concentration test and its relation to other tests could not be properly determined due to a low sample size. This was caused by the fact that the manufacturer withdrew the diagnostic kit for lactoferrin from the market during the study at which time only patients 22 were tested.

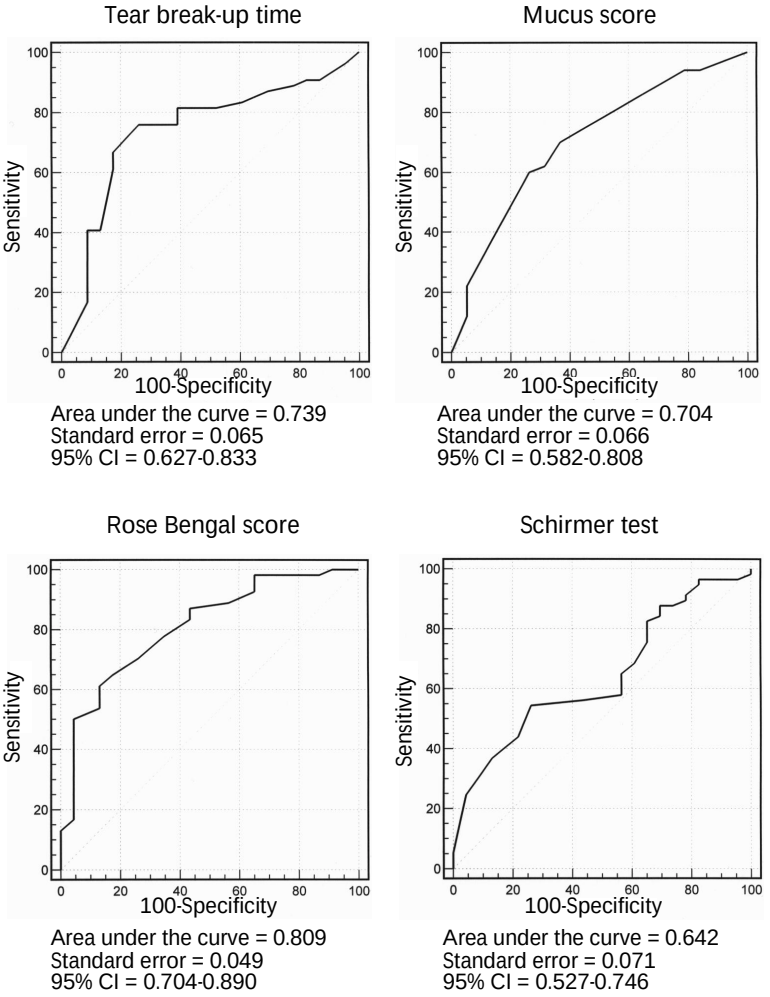

Figure 6.1.1 *Non-parametric ROC-curves of tear tests evaluated for its use in identifying SS in patients referred for diagnostics. Fifty-seven patients had SS; twenty-three did not. Note the relative rounded curve (towards the upper left corner of the diagram) of the Rose Bengal test, indicating good diagnostic performance.*

Subjective manifestation: onset and duration

A three to four year diagnostic delay was observed in the SS patients studied, estimated by the duration of their subjective complaints prior to diagnosis.

Table 6.1.2 *Onset and duration of symptoms of eye- and mouth dryness in SS patients (primary, secondary, and total), and non-SS patients.*

	pSS	sSS	SS	non-SS
N	32	25	57	23
Onset of first complaints				
Eye dryness before mouth dryness	16%	40%	26%	13%
Eye dryness only	3%	8%	5%	9%
Mouth dryness before eye dryness	31%	20%	26%	13%
Mouth dryness only	6%	8%	7%	13%
Simultaneous onset	34%	24%	30%	39%
Neither eye- nor mouth dryness	10%	0%	5%	13%
Duration at first visit				
Eye dryness (months, median)	38	50	43	31
Mouth dryness (months, median)	44	34	39	31

No preponderance of either oral- or ocular manifestations was found at the onset of SS; one fourth of the SS patients reported mouth dryness as the first complaint, another one fourth reported eye dryness as the first complaint, whereas one third reported a simultaneous onset of both mouth- and eye dryness (table 6.1.2).

Ocular manifestation

Rose Bengal scores were significantly increased and BUT were significantly decreased in pSS and sSS patients, compared to non-SS patients. Furthermore, Schirmer values were significantly decreased and mucus scores significantly increased in sSS patients, compared to non-SS patients (table 6.1.3). According to the European classification criteria, 71% of the primary SS patients and 96% of the secondary SS patients tested positive for the ocular component. Noteworthy, 48 percent of the non-SS patients also tested positive for the ocular component (table 6.1.1).

Table 6.1.3. *Ocular tests (mean ± SD) of primary SS (pSS), secondary SS (sSS) and SS-negative patients (non-SS). Statistical test used: independent sample T-test. Significant differences between pSS and sSS patients marked with #. Significant difference between SS and non-SS patients (pSS versus non-SS and sSS versus non-SS) is marked with *.*

	pSS	sSS	non-SS
N	32	25	23
Schirmer value (mm/5min)	8.2±8.2#	4.5±4.7*	10.2±8.8
Rose Bengal score	5.0±2.4*	5.7±2.1*	2.7±2.0
Break-up time	3.4±3.9*	2.7±3.3*	5.7±3.6
Mucus score	1.6±0.8#	2.0±0.7*	1.2±0.8

Oral manifestation

Stimulated secretory flow rates of the SM/SL-glands were significantly decreased in both pSS and sSS patients compared to non-SS patients (table 6.1.4). In addition, the salivary inorganic composition in pSS and sSS patients differed significantly compared to non-SS patients with increased sodium and chloride concentrations and decreased phosphate concentration in stimulated parotid saliva (table 6.1.4).

Table 6.1.4 *Oral tests: salivary flow rate and inorganic composition (mean ± SD) of primary SS (pSS), secondary SS (sSS), and non-SS patients Significant difference between SS and non-SS patients is marked with *. Statistical test used: independent sample T-test. SM/SL = submandibular/ sublingual*

	pSS	sSS	non-SS
N	32	25	23
Unstimulated flow rates			
Parotid (mL/min/gland)	0.02±0.03	0.01±0.03	0.03±0.07
SM/SL (mL/min/SM/SL-glands)	0.07±0.12	0.02±0.03*	0.10±0.11
Whole saliva	0.11±0.18	0.05±0.08*	0.16±0.22
Lag phase (s)	218±237*	144±178	62±137
Stimulated flow rates			
Parotid (mL/min/gland)	0.13±0.15	0.15±0.19	0.19±0.12
SM/SL (mL/min/SM/SL-glands)	0.25±0.30*	0.24±0.35*	0.42±0.25
Whole saliva	0.25±0.30*	0.55±0.68	0.79±0.44
Composition stimulated saliva			
Parotid sodium (mmol/L)	19±18*	18±22*	3±3
Parotid chloride (mmol/L)	26±15	33±27*	19±8
Parotid phosphate (mmol/L)	4.9±1.8*	4.1±1.9*	6.5±2.4

According to the European classification criteria, 72% of the pSS and 83% of the sSS patients tested positive for the oral component when applying the sialometrical criterion only (unstimulated flow rate of whole saliva ≤1.5 ml/15min). Fifty-seven percent of the non-SS patients also tested positive for this sialometrical criterion (table 6.1.1). When applying the radiographic criterion only (presence of sialectasia on a sialogram), 100% of the primary- and 76% of the secondary SS patients tested positive, whereas only 8% of the non-SS patients did.

Serologic manifestation

Serum SS-A and/or SS-B antibodies were present in 75 percent of the pSS and 46 percent of the sSS patients compared to 13 percent of the non-SS patients (table 6.1.1). In addition, increased serum levels of immunoglobulins were observed in pSS and sSS patients; significance was reached in pSS patients only (table 6.1.5).

Table 6.1.5 *Serum immunoglobulin levels (g/L, mean ± SD) of primary SS (pSS), secondary SS (sSS) and SS-negative patients (non-SS). Statistical test used: independent sample T-test. Significant difference between SS and non-SS patients is marked with *. No significant differences were observed between pSS and sSS patients.*

	pSS	sSS	non-SS
N	32	25	23
Ig-total	26.5±9.5*	22.4±6.5	19.0±6.5
IgG	20.1±7.5*	17.1±5.5	14.1±4.9
IgA	3.3±1.8	3.6±1.7	2.9±1.3
IgM	3.1±3.8	1.7±0.8	2.0±1.2

Correlation between manifestations of tear- and salivary gland dysfunction in SS

The Schirmer test, as estimate of tear gland secretion, correlated significantly to the stimulated secretion of the submandibular/sublingual (SM/SL) salivary glands in SS patients ($r_{spearman}$ 0.29, $p<0.01$). In addition, the tear mucus score correlated significantly to the sodium concentration in stimulated parotid saliva ($r_{spearman}$ 0.26, $p<0.05$).

Correlation between imaging of ocular and oral pathology in SS

The Rose Bengal score correlated highly significantly to the severity of radiographic changes observed with parotid sialography (iodine-contrast imaging) ($p<0.01$, $r_{spearman}$ 0.39) when radiographic changes are graded by their size and shape as punctate-, globular-, cavitary- or destructive sialectasia.[13]

Correlation between ocular and serologic manifestations in SS

The total level of immunoglobulins in serum of SS patients as well as the presence of SS-B antibodies correlated positively to the Rose Bengal score (Ig: $r_{spearman(Ig\text{-}conc)}$ 0.22, $p<0.01$; SS-B: T-test, $p<0.05$).

Progression of ocular manifestation in SS

The Rose Bengal score was the only ocular manifestation that worsened significantly with increasing duration of subjective eye-dryness (table 6.1.6).

Table 6.1.6 *Correlation between tear function tests and their relation to duration of subjective eye-dryness in SS patients. Note the significant correlation of the Rose Bengal score with the duration of eye dryness and with all other tear-tests. NS = not significant.*

N	Duration $r_{spearman}$	Schirmer test $r_{spearman}$	Rose Bengal $r_{spearman}$	BUT $r_{spearman}$	Mucus score $r_{spearman}$
Duration	xxxx				
Schirmer test	-0.03, NS	xxxx			
Rose Bengal	0.24, p<0.05	-0.60, p<0.01	xxxx		
BUT	-0.05, NS	-0.52, p<0.01	-0.70, p<0.01	xxxx	
Mucus score	0.04, NS	-0.57, p<0.01	0.71, p<0.01	-0.66, p<0.01	xxxx

DISCUSSION

In this study, tear- and salivary gland function were extensively investigated in Sjögren's syndrome (SS) patients, in order to compare exocrine disease manifestations regarding onset, severity and progression. Obviously, SS patients manifested decreased values for tear and saliva secretion, altered quality and composition of tear and saliva fluid, as well as marked pathosis with imaging techniques (ocular staining and parotid sialography) as compared to non-SS patients. In addition, the majority of SS patients had a positive serology for SS-A/B autoantibodies and high immunoglobulin levels in serum. From all studied tear tests, the Rose Bengal score remained the test of choice regarding diagnostic accuracy. When analysing the independent results from tear, saliva, and blood tests, remarkably clear coherence was found within the group of SS patients.

The BUT performed insufficiently in diagnosing SS. Despite the use of improved thresholds by ROC-plot analysis, the BUT reached only moderate specificity and sensitivity as a test for SS. The low potential for discriminating between SS- and non-SS patients of the BUT is in accordance to results from the study of Vitali and co-workers.[3]

The evaluated new tear test, the tear mucus score, also performed insufficiently in diagnosing SS. Although the mucus score correlated highly significantly to all other eye-tests (Rose Bengal score, Schirmer test, BUT), it had a poor sensitivity for SS, which impaired its use as single test for diagnosing SS. However, since the observation of raised mucus scores appeared very specific for SS (score>2: specificity 95%), and the test requires nothing but simply observing the tear mucus

after staining with Rose Bengal, it seams worth including the mucus score into the routine inspection of the dry eye.
Concerning the diagnostic performance of the tear- and salivary tests as currently included in the European classification criteria for SS, no unequivocal conclusions can be drawn from this study, because the same criteria were used in this study to support the diagnoses by which patients were categorised. Therefore, the calculated sensitivity and specificity from these tests are flattered by an incorporation bias. Nevertheless, it can be concluded from this study that both the Schirmer criterion (wetting≤5mm/5min) and the sialometrical criterion (unstimulated flow rate of whole saliva≤1.5 ml/15min) as proposed in the European classification criteria seem to produce many false positive test results (about half of the non-SS patients in our study tested positive for these criteria). When further evidence from future studies supports that these secretory tests are indeed rather non-specific for SS, the thresholds of these tests should be critically reconsidered, or, the tests should be replaced by tests with better diagnostic performance in the international criteria for SS. We consider measurement of stimulated SM/SL flow an excellent alternative for the currently applied salivary flow test (measurement of unstimulated whole saliva), since it proved a very specific diagnostic test for the oral component of SS (§3.2).[14]

Previous studies have reported sialographical alterations (punctate, globular, cavitary sialectasia) to be related to a decrease in salivary gland function[15-17] and to the duration of complaints of oral dryness.[11] Comparable to these observations, the Rose Bengal score correlated significantly to decreased tear gland function (Schirmer test), altered tear-film quality (BUT, mucus score), and to the duration of subjective complaints of eye-dryness. Since both sialography and Rose Bengal staining appear to relate to time, that is duration of subjective complaints, ánd to glandular function, these two diagnostic techniques may have valuable use for monitoring disease progression of SS.

A significant relation between tear- and saliva secretion was found in SS, expressed by correlating lacrimal- and SM/SL-gland secretion in SS patients. This correspondence in secretory dysfunction may be somehow related to the fact that the lacrimal- and SM/SL-glands share the same seromucous exocrine nature; in contrast, the parotid gland is a serous exocrine gland. Also a significant correlation was observed in SS patients between tear and saliva quality, expressed by correlating tear mucus score and parotid sodium concentration. There is, however, no proper explanation for this observed correlation, other than that both tests relate to SS. In previous studies, the SM/SL secretion and the parotid sodium

concentration were proven the most powerful predictors for SS of various sialometrical and sialochemical variables.[14,17-20]
In addition to the correlations between disturbances in tear and saliva secretion and quality in SS, also a clear correlation was noted between the Rose Bengal score and observations with parotid sialography, which is in accordance to scarce data from literature.[16] The Rose Bengal test, disclosing ocular surface damage resulting from tear gland involvement in SS, correlated highly significantly to parotid sialography, disclosing ductal damage resulting from salivary gland involvement in SS. The observed correlation between the Rose Bengal score and the severity of sialectasia on a sialogram does not necessarily reflect an etiological connection, but might be the logical result from the fact that both relate to duration and severity of exocrine malfunction.

The tear tests did not only correlate to salivary tests, but also to serologic tests: the presence of SS-B autoantibodies in serum and/or hyperglobulinemia appeared to be connected to more severe ocular surface damage, as measured by Rose Bengal staining. This suggests that especially SS patients with these findings in serum might be at risk for developing profound ocular surface damage and, hence, may require close monitoring by an ophthalmologist.

The significant correspondence between the oral and ocular component in SS is a striking finding, because this actually suggests that, theoretically, the evaluation of only one component complemented by serological or histopathological confirmation could be sufficient to diagnose the syndrome *for clinical purposes*. Thereby, subjecting patients to less diagnostic procedures and, hence, achieving a quicker diagnosis with less discomfort. An exception to this theoretical reduction of diagnostic work-up is formed by patients, who have tested positively for one component and suffer from subjective complaints that indicate involvement of the other component as well. In such cases, additional evaluation is still required, in order to assess whether there is a need for preventive measures or (symptomatic) treatment.
The concept of reducing the diagnostic testing to only one sicca component should be subject of further studies, because it leaves the classical diagnostic triad of Bloch and Buchanan (1965).[21] For research purposes, it is evidently preferable to perform full diagnostic testing on both components, yielding maximum external validity.

In brief, from this study it is concluded that the Rose Bengal score remains the tear test of choice for diagnosing SS. It was observed that tear tests correlate strikingly

with salivary tests. Therefore, *theoretically*, a positive evaluation of one component (either ocular or oral) complemented by serological or histopathological confirmation might be sufficient to diagnose SS, since both components appear to be related. Consequently, further diagnostic testing could be based upon clinical indication only. Furthermore, it is concluded that SS-B autoantibodies in serum and/or hyperglobulinemia may warrant close monitoring of the eyes, since it appears to relate to more severe ocular surface damage.

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CHAPTER

7

Unusual cases

SUMMARY

A case of primary sialoangiectasia, which in this case was initially misdiagnosed as Sjögren's syndrome, is described. Other diseases, including HIV-infection, psoriatic arthritis and acute parotitis, may cause glandular changes similar to the changes found in the syndrome. Therefore, sialography always must be combined with other methods of assessment of the oral cavity when suspicion is high for Sjögren's syndrome. Properly applied, sialography provides essential information regarding the severity of glandular damage and the progression of the disease.

PRIMARY SIALOANGIECTASIA: A DIAGNOSTIC PITFALL IN SJÖGREN'S SYNDROME. CASE REPORT

W.W.I. Kalk[1], A. Vissink[1], F.K.L. Spijkervet[1], H. Bootsma[2]

Departments of [1]Oral and Maxillofacial Surgery, and [2]Internal Medicine, division of Rheumatology, University Hospital Groningen

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INTRODUCTION

Sialography is still a frequently applied tool in the diagnosis of Sjögren's syndrome (SS), disclosing changes in the ductal and acinar system of the salivary glands as a result of disease activity. Several other methods are used to examine the oral component of SS, including salivary flow measurement (sialometry), salivary composition analysis (sialochemistry), salivary gland scintigraphy, ultrasonography, and histopathology. Despite controversy regarding the value of these methods, sialography has been used for decades and has been proposed as a criterion for the oral component of SS in 5 of the 9 sets of diagnostic criteria (revised criteria included) that are currently in use worldwide.[1-3]

The first radiographic demonstration of salivary glands in vivo was performed in 1913. After the use of lipiodol was introduced in 1926, the procedure was named sialography.[4] It was not until 1964 that the sialographic changes on the sialogram were classified by Blatt into punctate, globular, cavitary and destructive sialectasia (dilatation) of the acinar and ductal system; through use of this system, the progression of glandular damage caused by chronic inflammation could be graded.[5]

In 1965, results from the famous study of Bloch and co-workers demonstrated the high sensitivity of this diagnostic tool; 36 out of 37 patients with SS showed such sialectasia.[6] In addition, more recent studies on sialography have shown that the demonstration of sialectasia is highly specific for SS. In a study that compared sialography and salivary gland biopsy in a series of 150 patients with SS, it was concluded that sialography has been unduly overlooked as a diagnostic tool and should be reconsidered as a useful and noninvasive procedure.[7] In another study, sialography was shown to be a better indicator of the presence of SS than labial salivary gland biopsy, flow measurement of whole saliva, and scintigraphy.[8] In a

review article on the effectiveness of sialography, the method appeared to be less sensitive but more specific than flow measurements of whole saliva, and more sensitive but less specific than labial salivary gland biopsy.[9]
Not surprisingly, the presence and severity of sialectatic changes in the parotid gland is paralleled by the degree of hyposalivation; this is in contrast to only a weak or absent relationship between a positive labial salivary gland biopsy and the degree of hyposalivation.[10-12] Furthermore, in comparison with labial salivary gland biopsy, sialography has the advantage of being a relatively noninvasive technique.
Although it was stated that (non-obstructive) sialectatic changes in the salivary glands are pathognomonic for SS[5], 3 other diseases, unrelated to SS, have been reported to cause similar glandular changes; they are HIV-infection, psoriatic arthritis, and acute (bacterial) parotitis.[13-15] Sialectasia has also been identified in 9% of control subjects with supposedly normal parotid glands; this condition is called primary (idiopathic) sialoangiectasia.[16-18] A congenital developmental disorder might cause this condition.[16,19]
The following case report describes a patient with primary sialoangiectasia who was originally misdiagnosed, mainly on the basis of sialography, as having SS.

CASE REPORT

In October 1997, a 56-year-old homosexual man was referred to the Department of Oral and Maxillofacial Surgery and the Department of Ophthalmology by his rheumatologist for confirmation of a diagnosis of primary Sjögren's syndrome. Eleven years earlier, the diagnosis of primary Sjögren's syndrome had been made at another institution on the basis of clinical complaints, parotid gland sialography demonstrating globular sialectasia, and a positive Schirmer's test. At that time, serologic findings were negative for rheumatoid arthritis factor, antinuclear antibodies, SSA-antibodies, and SSB-antibodies, and labial salivary gland biopsy showed no lymphocytic infiltration. The patient's medical history included tuberculosis during infancy, recurrent peri-anal herpetic infections since 1986, mild psoriasis since 1987 and a neuropathy of the phrenic nerve since 1996. The patient had stopped working as a male nurse 9 years earlier because of complaints attributed to his diagnosis of primary Sjögren's syndrome.
On admission at our outpatient department, the patient complained of chronic fatigue, arthralgia, tendinomyalgia, and ocular dryness. The patient had no complaints of oral dryness, nor did he have a history of recurrent parotitis. Physical examination showed a patient with general adiposity and a diffuse symmetrical,

nontender enlargement of both parotid glands. No palpable lymph nodes were found in the head and neck region.

Intraoral examination showed a normal oral mucosa, salivary pooling in the floor of the mouth, and a well-preserved dentition. Sialometrical analysis showed normal resting and 2%-citric-acid-stimulated flows of whole saliva. Gland-specific sialometry showed normal secretion values for the submandibular and sublingual salivary glands and slightly decreased values for the parotid glands. Sialochemical analysis revealed normal values for electrolytes, total protein, and amylase in acid stimulated parotid and submandibular saliva (table 7.1.1). Sialography of the right parotid gland, repeated to examine the disease progression, showed globular sialectasia (figure 7.1.1). This sialographic image was similar to the one that was made eleven years before; no progression was noted. An incisional biopsy of the right parotid gland showed fatty serous glandular tissue without pathological changes.

Table 7.1.1. *Findings of sialometry (salivary flow rates) and sialochemistry (saliva composition). SM/SL = submandibular / sublingual.*

Salivary gland	Right parotid	Left parotid	SM/SL
Flow			
Unstimulated flow (ml/min)	0.05	0.02	0.40
Stimulated flow (ml/min)	0.16	0.24	1.26
Chemistry			
Sodium (mmol/L)	7	5	6
Potassium (mmol/L)	23	21	17
Chloride (mmol/L)	17	16	17
Total protein (g/L)	1.41	1.25	1.88
Amylase (10^3 U/L)	275	320	-

Laboratory investigations revealed a normal full blood count, a normal white blood count differentiation and erythrocyte sedimentation rate, and normal levels of C-reactive protein, amylase, lactic-acid dehydrogenase, alkaline phosphatase, transaminases, and immunoglobulins (IgG 12.8, IgA 2.4, IgM 0.9 g/L). Immunologic tests showed negative serology for rheumatoid arthritis factor, antinuclear antibodies, SSA-antibodies, and SSB-antibodies. Furthermore, the patient tested negative for HIV.

Ophthalmologic examination in the Department of Ophthalmology showed a negative Schirmer's test (8/10 mm, right and left eyes), a negative Rose Bengal staining of both eyes, and a positive break-up time (3 seconds).

Physical examination in the Department of Rheumatology showed tender points (11/18) according to the American College of Rheumatology criteria in the absence of signs of arthritis.

Because the patient did not fulfil any of the nine currently proposed sets of diagnostic criteria for SS, the diagnosis primary Sjögren's syndrome was rejected.

Some of the patient's complaints - i.e. tendinomyalgia and fatigue – might be related to the diagnosis of psoriasis; others remained unexplained, however. The sialographic findings were defined as primary sialoangiectasia.

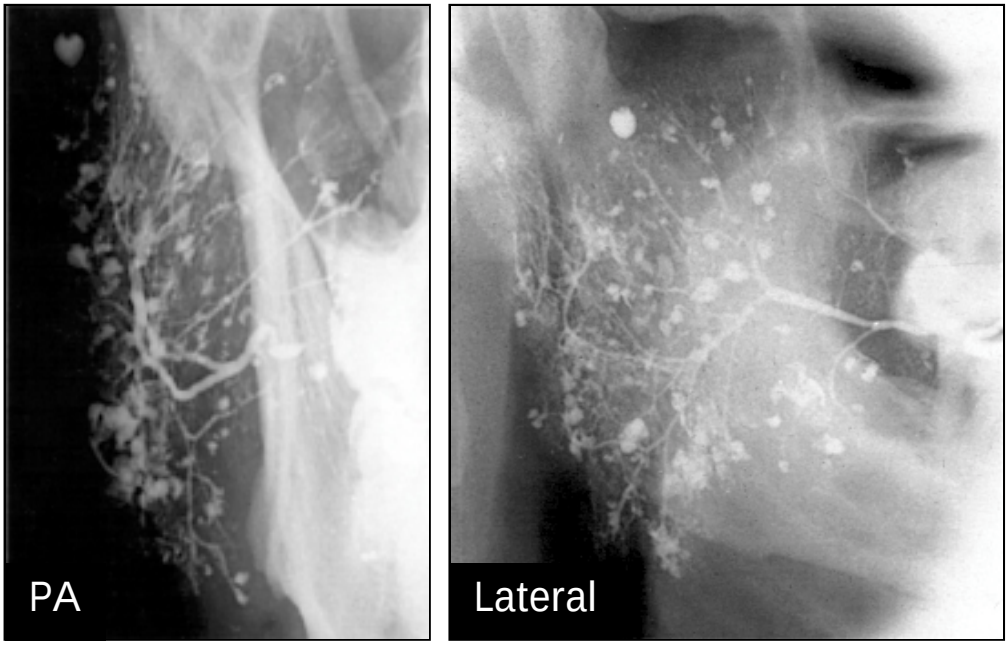

Figure 7.1.1 *Postero-anterior and lateral sialogram of the right parotid gland.*

Note the presence of globular to cavitary sialectasia present on both projections.

DISCUSSION

A patient is presented showing evident sialectatic changes on the sialogram, supporting the diagnosis of SS as a possible explanation for his complaints of chronic fatigue, arthralgia, tendinomyalgia, and ocular dryness. Despite these findings, it seems that this patient had not had Sjögren's syndrome for the past 11 years, for both the serologic findings and the histopathologic findings were negative for SS, as were the findings of sialometry and sialochemistry. Moreover, the sialogram showed no further glandular destruction with time. In addition, the absence of oral complaints argues against the diagnosis of SS. With a decreased break-up-time and globular sialectasia on the sialogram as the only objective signs, sufficient evidence for the diagnosis of primary Sjögren's syndrome is lacking.

The complaints of chronic fatigue, arthralgia, tendinomyalgia, and ocular dryness and the abnormal sialographic findings make a striking combination. After the diagnosis of SS was ruled out, the question of whether these symptoms and signs share another common cause was raised. An HIV-infection would have been a logical explanation for several reasons. It is known that HIV can cause swelling of the parotid glands and sialectasia of the ductal and acinar system (HIV-salivary gland disease), mimicking changes that are seen in SS.[14,15-20-24] The chronic fatigue, the

arthralgia, the tendinomyalgia and the ocular dryness all are common manifestations of AIDS related complex.[25-31] Moreover, as a homosexual the patient belonged to a high-risk group for infection with HIV. However, although HIV-infection was in theory a logical explanation, the patient tested negative for HIV.

Other diseases that might have caused the observed sialectasia were also ruled out. No signs of arthritis were found during rheumatologic examination; the patient therefore could not be classified as having psoriatic arthritis, a diagnosis that has been associated with sialectasia in the parotid glands.[13] Furthermore, the patient did not have a history of acute parotitis, which is another possible cause for sialectasia in the parotid glands. The patient did have a history of tuberculosis during infancy, a disease that is known to cause specific changes in the salivary glands[16,32]; however these changes, described as complicated patterns that are difficult to distinguish from malignant tumors, are not compatible with the sialographic findings observed in the reported case.

Apparently, none of the diseases known to cause sialectasia in the salivary glands can be held responsible for the sialographic findings in the reported case. Therefore, as a diagnosis of exclusion, the observed sialectasia was defined as primary or idiopathic sialoangiectasia.

Because the sialographic findings in the reported case initially led to a misdiagnosis, the value of sialography as a pathognomonic diagnostic tool is questionable. Sialography is certainly not a pathognomonic diagnostic tool in SS, because other conditions can cause similar sialograms, as previously mentioned. However, to assess the oral component of SS, sialography provides relevant information regarding disease activity and progression by showing ductal and acinar changes in the salivary glands that are related to the degree of hyposalivation. If sialography is combined with other methods in assessment of the oral component, the risk of an unfortunate misdiagnosis caused by primary sialoangiectasia is eliminated. Although it is less invasive than salivary gland biopsy and although it is competitive with respect to diagnostic value (sensitivity and specificity), sialography should not be used to replace the histopathologic analysis for confirmation of the diagnosis of SS.

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SUMMARY

A 32-year-old woman is demonstrated who presented with sarcoidosis and Sjögren's syndrome. Diagnoses of both diseases were based on current internationally accepted criteria. Furthermore, histopathological findings characteristic for both diseases were present in salivary gland biopsies. As sarcoidosis is considered an exclusion criterion for Sjögren's syndrome in current sets of diagnostic criteria we propose that these criteria should be reconsidered with respect to the exclusion of sarcoidosis.

SIMULTANEOUS PRESENTATION OF SARCOIDOSIS AND SJÖGREN'S SYNDROME. CASE REPORT

A.A. van de Loosdrecht[1], W.W.I. Kalk[2], H. Bootsma[3], J.M.L. Henselmans[4], J. Kraan[5], C.G.M. Kallenberg[6]

Department of Internal medicine, divisions of [1]Hematology, [3]Rheumatology, [5]Pulmonology and [6]Clinical Immunology, and Departments of [2]Oral and Maxillofacial Surgery and [4]Neurology, University Hospital Groningen

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INTRODUCTION

Coexistence of sarcoidosis and autoimmune diseases has been described in several case reports.[1-5] Simultaneous presentation of sarcoidosis and Sjögren's syndrome has been suggested in several reports[1,4-18], but internationally accepted criteria for the diagnosis of Sjögren's syndrome[19-25] were not met. We present a case in which both disease entities, sarcoidosis and Sjögren's syndrome, presented simultaneously and were diagnosed based on accepted criteria. Although sarcoidosis is generally considered an exclusion criterion for the diagnosis of Sjögren's syndrome, this exclusion criterion needs critical reconsideration as demonstrated by the following case report.

CASE REPORT

A 32-year-old woman was referred to the outpatient clinic because of pretibial painful red nodules. She had a 6-month history of migratory pain and swelling of several joints. She had daily complaints of progressive oral dryness, thirst, impaired swallowing and blurred vision with a feeling of dry eyes for more than three hours a day. She did not feel dyspneic. Past medical, family, occupational and environmental history were non-contributory. She used an oral contraceptive and ibuprofen. Physical examination, which was otherwise not remarkable, showed edema of the ankles and erythema nodosum on both legs. The parotid glands were not enlarged or painful.

Laboratory investigations revealed an ESR of 44 mm/h (n: <10), a CRP level of 42 mg/l (n: < 3). Haemoglobin concentration, leukocyte and thrombocyte count, glucose, electrolytes, creatinine level, liver and thyroid function, Ca, total protein, protein electrophoresis and 1.25$(OH)_2$-vitamin D_3 were all within the normal range. Urine analysis showed no erythrocytes. Creatinine clearance and urinary Ca-excretion were normal, slight proteinuria of 300 mg of protein/24 h was present. Serum lysozyme was elevated at 9.7 mg/l (n: 0.6-2.6), angiotensin-converting-enzyme (ACE) was 134 U/l (n: <45). Antinuclear antibody (ANA), antibodies to extractable nuclear antigens (ENA) and anti-dsDNA (Farr-assay) were all negative. Antibodies against SS-A were positive. IgM-rheumatoid factor was elevated at 105 kilo-U/l (n: <15). Levels of IgG were 19.2 g/l (n: 8.5-15.0); IgA 5.4 g/l (n: 0.9-4.5) and IgM 2.2 g/l (n: 0.6-2.6). No cryoglobulins were found. A tuberculin test with purified protein derivate (PPD) was negative.

A chest radiograph showed extensive mediastinal and bilateral hilar lymphadenopathy with tracheal displacement to the left and increased interstitial lining. Pulmonary function tests (lung volumes and diffusing capacity for carbon monoxide) were normal. Radiographs of hand and feet showed bilateral cystic lesions and small erosion of the left basisphalanx (hand) of the third generation.
Ophthalmologic examination demonstrated a normal Schirmer's tear test of 10 and 9 mm/5 min of the right and left eye, respectively (n: ≥5.5 mm/5 min) but a decreased tear film break-up time of 2 seconds for both eyes (n: ≥10 s before the tear film breaks) and a positive Rose Bengal staining (score of 5 and 3 according to Van Bijsterveld, of the right and left eye, respectively; n: ≤3.5), consistent with keratoconjunctivitis sicca.[26]

Sialometrical analysis showed a reduced unstimulated and stimulated (2% citric acid) flow of whole saliva (unstimulated flow n: >0.1 ml/min; stimulated flow n: > 0.7 ml/min).[27] In detail, the reduced unstimulated flow of whole saliva was caused by a very low secretion of all individual salivary glands (<0.01 ml/min), whereas the reduced stimulated flow of whole saliva was mainly caused by very low secretion of the parotid glands (0.02 ml/min/gland; n ≥0.05-0.5 ml/min).[28-30] Sialochemistry revealed elevated sodium concentrations and a normal amylase content in both whole, parotid and submandibular/sublingual saliva. Sialography of the right parotid gland showed punctate sialectasia, characteristic for Sjögren's syndrome (figure 7.2.1).[31]

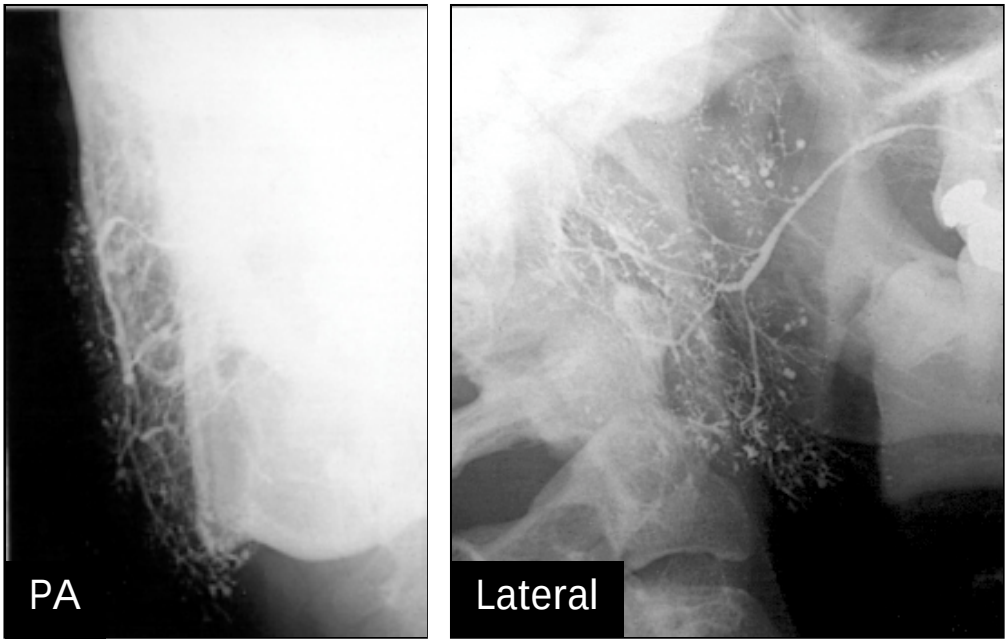

Figure 7.2.1 *Postero-anterior and lateral sialogram of the right parotid gland.*

Note the presence of punctate sialectasia on both projections.

To differentiate between Sjögren's syndrome and sarcoidosis as cause of the observed salivary gland dysfunction, an incisional parotid biopsy was taken.

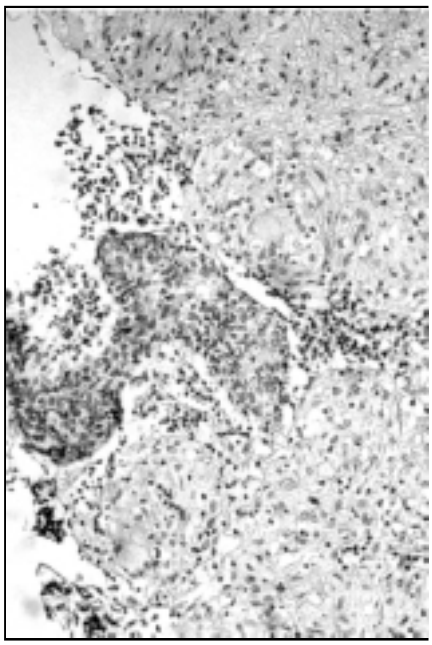

Figure 7.2.2 *Microscopic view of parotid gland tissue.*

Note the presence of epitheloidcell granuloma and epimyo-epithelial islands (magnification 175x; staining hematoxylin eosin)

Histopathological examination of parotid tissue showed epithelioid cell granuloma as well as periductal lymphocytic infiltration and epimyo-epithelial islands (figure 7.2.2). Immunohistochemical staining showed a polyclonal plasmacytic infiltrate and a shift in the relative number of IgA bearing plasma cells in favour of IgG bearing plasma cells. These histopathological findings, which are in part suggestive for Sjögren's syndrome, asked for an additional labial salivary gland biopsy, in order to meet the current international standard for histological confirmation of Sjögren's syndrome.[32] This revealed extensive epithelioid cell granuloma and lymphocytic infiltration with a positive focus score of 1 (n: <1; focus defined as ≥50 mononuclear cells/4 mm^2) (figure 7.2.3). Since Sjögren's syndrome is associated with an increased incidence of malignant lymphoma, (a relative risk ratio of 44), the extensive mediastinal lymphadenopathy prompted us for additional histologic examination.[33,34] Cervical mediastinoscopy of the lymphnodes revealed epithelioid

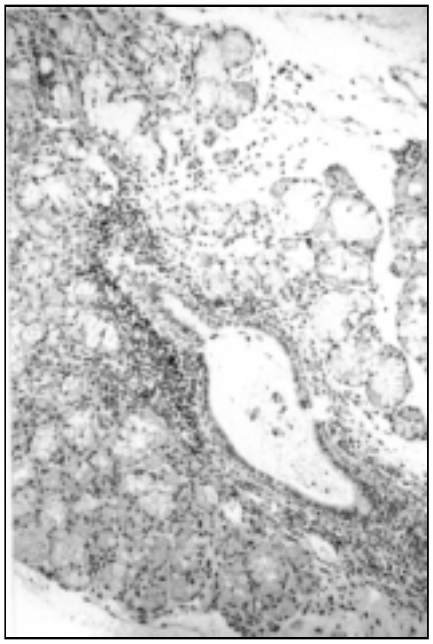

Figure 7.2.3
Microscopic view of labial salivary gland tissue.

Note the presence of a lymphocytic focus in an acinus that is not affected by granuloma formation (magnification 100x; staining hematoxylin eosin)

cell granuloma, with a negative auramine staining, and without evidence of malignant lymphoma.

The patient was diagnosed as having sarcoidosis, presenting as Löfgren's syndrome, with coexisting Sjögren's syndrome. Six month after the first presentation she developed a transient nontender bilateral cervical and supraclavicular lymphadenopathy. During a two years follow-up, laboratory investigations revealed a normalisation of CRP (<3 mg/l) and ACE-activity (31 U/l). IgM-rheumatoid factor was elevated to 210 k-U/l and stabilised to 115 k-U/l with persistence of SS-A antibodies. Repeated sialometrical analysis showed persisted reduction of unstimulated and stimulated flow of whole saliva with further reduction of the submandibular/sublingual salivary secretion. Sialochemistry revealed persisted elevation of sodium concentration compatible with chronic inflammation. Finally, a chest radiograph showed a major reduction in mediastinal and hilar lymphadenopathy. Until now, she has been treated symptomatically for her sicca complaints.

DISCUSSION

A patient is described with migratory symmetrical polyarthralgia, erythema nodosum, extensive generalised lymphadenopathy, and sicca syndrome, in which two disease entities, sarcoidosis and Sjögren's syndrome presented simultaneously.
The diagnosis of sarcoidosis (Löfgren's syndrome) was confirmed by the presence of epithelioid cell granuloma in mediastinal lymph nodes, parotid and labial salivary glands, according to recommendations for the clinical evaluation of granulomatous diseases.[35] Currently, seven sets of international criteria for the diagnosis of Sjögren's syndrome are being used which apply the exclusion criterion of sarcoidosis. Nevertheless, the present case demonstrates that sarcoidosis and Sjögren's syndrome can coexist. The diagnosis of Sjögren's syndrome was based on signs and symptoms of keratoconjunctivitis sicca and xerostomia, presence of SS-A antibodies and histological conformation of Sjögren's syndrome in both parotid and labial

salivary glands. Although the reduced stimulated secretory function of the parotid glands observed in our patient could also be caused by sarcoidosis, the observed elevation of salivary sodium concentration and the punctate sialectasia on the sialogram are distinctive features in Sjögren's syndrome and have not been described previously in association with sarcoidosis.[36-39] The normalisation of ACE-activity, the persistence of sialometrical and sialochemical abnormalities and SS-A antibodies during a two years follow-up support the remaining presence of Sjögren's syndrome that initially presented in coexistence with sarcoidosis. Erythema nodosum could be caused either by sarcoidosis, by leukocytoclastic vasculitis associated with Sjögren's syndrome, as well as by hypergammaglobulinemic purpura.

Histopathological examination of salivary gland specimens revealed periductal lymphocytic infiltration as well as epimyoepithelial islands, besides clear evidence for sarcoidosis. These epimyoepithelial islands and periductal lymphocytic infiltration are both common features of major salivary glands in Sjögren's syndrome.[40-42] Only occasionally, epimyoepithelial islands were found in association with sarcoidosis.[40,43] The decreased percentage of IgA bearing plasma cells further supports coexisting Sjögren's syndrome.[44] However, it is unknown whether an increased percentage of IgG and/or IgM bearing plasma cells may occur in salivary glands of patients with sarcoidosis.

The possibility of a common immunopathogenic pathway for sarcoidosis and Sjögren's syndrome is suggested by several observations. The presence of SS-A antibodies, which are rarely found in healthy individuals or in patients without connective tissue diseases has been associated with sicca complaints in systemic lupus erythematosus (SLE) and with Sjögren's syndrome. The presence of SS-A antibodies has further been shown to define a subset of patients with Sjögren's syndrome with lymphadenopathy and polyclonal hyperglobulinemia.[45] Furthermore, the HLA-DR3 phenotype is associated with primary Sjögren's syndrome, a subgroup of patients with SLE with the presence of SS-A antibodies as well as with a subgroup of patients with sarcoidosis with a specific antigen-driven overexuberant cellular immune response. In our patient sarcoidosis with coexisting Sjögren's syndrome was diagnosed in the presence of SS-A antibodies. However, the HLA-DR3 phenotype was absent in our case.

Several reports have shown overlap between sarcoidosis and autoimmune disorders including rheumatoid arthritis, SLE, systemic sclerosis, CREST syndrome and spondylarthropaties.[1-5] In the majority of cases sarcoidosis is associated with SLE in which predominance exists for pulmonary symptoms. In addition, some cases have

been reported suggestive for sarcoidosis and coexisting Sjögren's syndrome.[1,4-18] The incidence of sarcoidosis and Sjögren's syndrome may be much higher than is suggested by this relatively small number of case reports since presence of sarcoidosis is one of the exclusion criteria for diagnosing Sjögren's syndrome. In defining exclusion criteria for diseases with similar clinical presentations there might be a risk of underdiagnosis of overlap syndromes. We emphasise that in a subset of patients both disease entities might be present as illustrated in this case with possibly common immunopathogenic mechanisms. Furthermore, the presence of Sjögren's syndrome may be easily underdiagnosed since extensive epithelioidcell granuloma may dominate the histopathological specimen, especially in minor salivary gland biopsy.

In conclusion, a patient with sarcoidosis and coexisting Sjögren's syndrome is described suggesting shared immunopathogenic mechanisms. Careful use of exclusion criteria for Sjögren's syndrome is warranted to define subgroups of patients with overlapping clinical syndromes. We propose that the exclusion criteria currently applied in diagnostic criteria sets for Sjögren's syndrome need to be reconsidered with regard to the exclusion of sarcoidosis.

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CHAPTER

8

General discussion

GENERAL DISCUSSION

Diagnosing Sjögren's syndrome (SS) has remained difficult throughout the years. After Bloch and Buchanan stated their classical diagnostic triad in 1965, several new international diagnostic sets of criteria were proposed for diagnosing the disorder. To date, many diagnostic tests in different fields are considered necessary before it can be properly concluded whether or not someone is affected by the syndrome. Due to scarce availability of methods to monitor disease activity and progression of SS, the evaluation and introduction of new therapeutic agents is also very difficult[1], rendering patients largely dependent upon symptomatic treatment. The main objective in this thesis was therefore to optimise current diagnostics and to obtain potential clinical outcome parameters in SS.

Three main issues are being discussed in the next paragraphs. After evaluating how the separate diagnostic conclusions from the different studies could be combined into an optimised diagnostic work-up, consideration is given to which type of histopathological confirmation is most ideal for SS. After closing the diagnostic issue, the focus will be directed at possibilities to estimate the level of disease activity and status of progression by using the clinical outcome parameters observed in the studies.

AN OPTIMISED DIAGNOSTIC WORK-UP

By studying various aspects of the oral component of SS, possibilities were explored to optimise the current diagnostic work-up, with respect to accuracy, invasiveness, and clinical applicability. Through perfection and modification of existing procedures, a reduction of diagnostic work-up was pursued. Strengths and weaknesses of common diagnostic tests were revealed by close examination of specific disease-induced alterations in the salivary glands, to support subsequent adjustments and modifications.

From the studies on salivary gland function, it was concluded that disturbances of salivary secretion and composition are actually very useful for the clinical differentiation of SS (chapter 3.1). The additional determination of relevant cut-off points (chapter 3.2), made it possible to specifically diagnose the oral component of SS with sialometry and sialochemistry, by measuring the stimulated submandibular saliva secretion and the electrolyte concentrations in parotid saliva, respectively. Sialometry becomes a major diagnostic tool for SS when salivary function progressively declines throughout the disease course, whereas salivary chemistry

has already diagnostic potential for SS shortly after onset. Combined, sialometry and sialochemistry therefore form a diagnostic tool that potentially covers all stages of the disease. The collection of saliva for chemical analysis does require certain skills and equipment, which impairs wide usage. For this reason, it was proposed to detect the alterations of salivary composition on a test-strip (chapter 3.3), which can be easily used as screening device in the first instance by dentists and general practitioners when suspecting a patient suffers from SS. Such primary diagnostic testing, preferably having high sensitivity for the disease, optimises referral of SS patients to specialists by reducing diagnostic delay and increasing the prior probability of referred patients for having SS. An increase of prior probability of SS will theoretically reduce the need for multiple testing in the second echelon; fewer tests will be needed to obtain sufficient evidence for a positive diagnosis. Estimated results of the proposed test-strip were preliminarily presented, though the device has not yet been developed or thoroughly evaluated. As soon as this will have been accomplished, second echelon testing can be adjusted.

From the study on salivary isoamylase (chapter 4), an intracellular enzyme of parotid gland cells, it was concluded that its serum activity is unfortunately not very useful in diagnosing SS, displaying a biphasic activity pattern throughout the disease course. An initial raise of serum enzyme activity returns to normal after 2 to 3 years, which narrows its window of diagnostic use.

Concerning parotid sialography, it became clear that the procedure is well accepted by the patients (chapter 5.1). The use of oil-based contrast for sialography is preferred, yielding optimum image quality without adverse side effects. Furthermore, it was concluded from a large series of sialograms judged by multiple observers, that the reading and interpreting of a sialogram requires specific expertise with regard to the recognition and correct interpretation of first stage sialectasia, thereby restricting its use as diagnostic tool for (incipient) SS to expert-observers (chapter 5.2). This does not mean that sialography, which was observed as being potentially highly diagnostic for SS, should be replaced by other diagnostic procedures, but merely implies that in cases of doubt about presence of first stage sialectasia, one should consider sending the sialogram to an expert centre.

From a comparison between tests for the ocular and oral component (chapter 6), it was observed that there is significant correspondence between diminished tear and salivary gland function, tear and saliva quality, and ocular and oral imaging in SS. This implies that for clinical use, theoretically, one positive component, in addition to positive serologic and/or histopathologic findings, could be sufficient to diagnose the syndrome. Thereby, subjecting patients to less diagnostic procedures and, hence, achieving a quicker diagnosis with less discomfort. This concept, however, is

rather controversial and will probably meet strong resistance, because it leaves the classical triad of Bloch and Buchanan (1965).[2] Therefore, further research is needed providing additional evidence to support this concept, before it can be translated into a reduced diagnostic work-up. For research purpose, however, it is evidently preferable to perform full diagnostic testing on both components, yielding maximum external validity.
During the period of study, two patients were encountered with very unusual clinical presentations (chapter 7). Both cases demonstrated the substantial risk of misdiagnosing SS, stressing the need for accurate diagnostic procedures and caution when interpreting test results. One case report shows that seemingly apparent results of a single test may lead to a false diagnosis, despite controversy with the remaining test results. It thus stresses the need for accurately performing ánd interpretating all of the tests required by diagnostic criteria. The other case report demonstrates the risk of missing the diagnosis SS if exclusion criteria are applied too strictly in the clinics. Criteria that exclude certain diseases with similarities in order to improve the validity of scientific research may clinically hamper proper diagnoses when a patient actually has two diseases at the same time. Furthermore, it may prevent new insights regarding possible relations between coinciding diseases with clinical similarities.

By combining the separate conclusions from the different studies the following can be stated. The number of diagnostic tests required for a valid diagnosis of SS probably can be reduced by simple first echelon screening, increasing the prior probability of referred patients for having SS. The number of tests could also be reduced if the relationship between the ocular and the oral component becomes generally established in near future. However, by reducing the number of tests, inherent loss of specificity must be compensated for by using highly specific diagnostic tests in the diagnostic work-up. To increase diagnostic specificity, it is recommended to measure stimulated submandibular saliva instead of whole saliva with sialometry, and to use oil-based instead of water-based contrast fluid with sialography. Consulting an expert centre in case of doubt about presence of first stage sialectasia will also significantly improve the specificity of sialography.

THE TYPE OF HISTOPATHOLOGICAL CONFIRMATION

The diagnosis of all SS patients studied was based upon a standardised diagnostic work-up according to the revised European classification criteria for SS.[3] All tests

were performed in accordance with the classification criteria except for one: instead of using labial salivary glands for histopathologic confirmation of the syndrome, parotid gland specimens were used. The deliberate choice of parotid tissue instead of minor salivary gland tissue was based upon differences in diagnostic potential, differences in sample size, and upon the advantage of single gland examination. In the literature, the parotid gland has been proven to have unique value for assessing disease activity and progression of SS but lacking surplus diagnostic value compared to minor salivary glands[4], or opposed to this, to be superior to minor salivary glands when it comes to the diagnosing of several conditions, including sarcoidosis, lymphomas and SS.[5,6] Furthermore, the labial salivary gland biopsy suffers from relevant false positivity as well as false negativity.[7-12] Another substantial difference between parotid specimen, obtained from an incisional biopsy, and minor salivary gland specimen is the large difference in size; during a labial salivary gland biopsy, only a few small glands are harvested, whereas a parotid biopsy yields a much larger tissue sample for microscopic examination. Consequently, sample size errors are far more likely to occur after minor salivary gland biopsy as compared to major salivary gland biopsy. Furthermore, it has great appeal from a research point of view, because it allows studying different disease-induced processes on the same type of gland. By performing parotid biopsies, it is possible to study microscopic aspects in relation to radiographic ductal architecture, saliva production and excretion, and intracellular enzyme loss from the very same gland. Such a single gland examination renders the research on different glandular processes and co-processes much more sensitive. As the submandibular gland shows the most diagnostic alterations regarding salivary flow rates in SS, this gland appears the gland of choice for single gland examination. However, its surgical access is rather complicated, requiring general anaesthesia. For this reason, the parotid gland, which easily allows an incisional biopsy, offers a good alternative for single gland examination.

However, several unfounded assumptions have led to scarce diagnostic usage of the incisional parotid biopsy: the surgical procedure is assumed to be difficult and rather invasive, and having a substantial risk for damaging the facial nerve. None of these assumptions appears valid though. The incisional parotid biopsy, as described by Kraaijenhagen[13], involves a quick and simple procedure under local anaesthesia, the invasiveness of which is very low[6,14], comparable with the labial salivary gland biopsy, according to preliminary results of a morbidity study, which is currently in progress (unpublished data). The assumption of a risk of facial nerve damage during an incisional parotid biopsy is also not evidence based, because the facial nerve is located more than 2 cm below the level at which parotid tissue is harvested during this procedure, as demonstrated in a large cadaver study.[14] For reasons of

convenience, biopsies are most often taken from the sublabial salivary glands. Different specialists are skilled to perform a labial salivary gland biopsy, whereas few are capable of taking a biopsy from one of the major salivary glands.
Due to being unpopular the parotid biopsy still lacks proper diagnostic validation, despite its showing marked pathological changes in various diseases including SS. Probably for the same reason, it is not included in international classification criteria sets. In order to validate the parotid gland biopsy properly, and to establish its true morbidity and diagnostic potential, a prospective study is currently in progress in which both parotid and labial salivary gland biopsies are performed in every patient.

DISEASE ACTIVITY AND PROGRESSION

Disease activity involves reversible processes, whereas disease progression involves irreversible processes. Disease activity may, therefore, change during flares and remissions, provoked spontaneously or by treatment.[1] By studying various aspects of the oral component of SS, possibilities were explored to measure disease activity as well as to stage disease progression. As a diagnostic delay is very common in SS, the duration of complaints relating to the syndrome is perhaps a better measure of disease duration than is the period from diagnosis. From the different aspects studied regarding oral (and ocular) involvement, several outcome parameters were studied, showing retrospectively a significant relation with the duration of subjective oral (or ocular) complaints prior to the parameter assessments.
Salivary secretion rates declined significantly for all major salivary glands throughout the disease course, with the submandibular glands tending to show the first decline (chapter 3.1). It was also observed that different sialometrical profiles could be discerned matching the disease duration. Shortly after disease onset (within one year after the onset of first complaints), SS patients showed either a combination of normal salivary flow rates with a changed salivary composition, or a selective decrease of the submandibular secretion rates with normal parotid secretion rates. After 5 to 6 years, patients were observed to show extremely low secretion rates of the submandibular glands either exclusively or in combination with extremely low secretion rates of the parotid glands as well.
The serum leakage of intracellular salivary isoamylase was inversely related to disease duration (chapter 4). Increased serum enzyme activity corresponded with disease duration of less than 1 year, which declined to normal activity after 2 to 3 years, and further declined to decreased activity after 5 years. The serum activity also corresponded significantly with salivary sodium and chloride concentrations,

which reflect salivary gland inflammation in SS. As leakage into serum of intracellular enzymes is thought to result from increased cell death, the serum salivary isoamylase activity may therefore correspond with inflammatory activity of SS at the glandular level. It might even be informative regarding the prognosis of salivary gland function; high serum isoamylase activity could indicate an active disease at the glandular level with inherently relatively rapid deterioration of secretory functions, normal activity could indicate a more stable situation, whereas low activity an end situation with little change to be expected in secretory function. However, decrease of serum activity throughout the disease course may not only reflect diminished glandular disease activity, *i.e.* diminished glandular cell turnover, but also diminished glandular size, which complicates the interpretation of serum enzyme activity.
The four different gradations of sialectasia on parotid sialograms, disclosing ductal damage, showed a weak but significant relation to disease duration in SS patients (chapter 5.2). This suggests that sialectasia slowly worsens - increases in number and size - during the disease course of SS. Previous studies have shown that increasing gradations of sialectasia correspond with lower salivary flow rates.[16-18] Comparable to these observations, the Rose Bengal staining of the eyes, disclosing corneal damage, was shown to relate significantly to tear gland function, tear quality, and to disease duration (chapter 6). The Rose Bengal staining also correlated significantly to the gradation of sialectasia.

FINAL REMARKS

The diagnostic process of SS can be optimised by modification of current diagnostic procedures, by first-echelon screening with tear- or saliva-strips (latter not yet available), and perhaps in near future by reducing the diagnostic process by one component if proven to be justified. After establishment of the diagnosis, it appears SS can be subdivided into two or three different sequential stages of disease progression according to the type of sialectasia and/or to the Rose Bengal score, with a corresponding degree of oral and ocular dryness, respectively. Such sequential stages may consist of an early- (<1 year), an intermediate- (1-4 years) and a progressed stage of SS (>4 years). Furthermore, it appears that glandular disease activity could be estimated by measuring serum salivary isoamylase activity, which might even predict the rate of exocrine function-loss. It is required to validate all retrospectively acquired 'time relations', to differentiate reversible processes, *i.e.* disease activity, from irreversible processes, *i.e.* disease progression, and to further define the different disease stages with long-term prospective studies. If validated

markers of disease progression and -activity could eventually be agreed upon they would constitute important tools for disease monitoring and clinical trials.

Follow-up and ongoing studies in our clinic encompass a histopathology- and morbidity study on salivary gland biopsies, long-term prospective studies on clinical disease course, and randomised clinical trials with new therapeutic agents in SS patients.

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CHAPTER

9

Summary

SUMMARY

This thesis comprehends seven clinical studies related to the oral component of Sjögren's syndrome (SS), aiming to improve current diagnostics and to obtain clinical outcome parameters.

In chapter 1, it is described that the oral component of SS has been studied in depth in 200 patients by a multidisciplinary research team, in order to improve and simplify the process of diagnosing SS, and to obtain methods to evaluate the effects of drug-therapy. All studied patients were diagnosed in accordance to the revised European classification criteria for SS.

Chapter 2 provides additional background information on SS and its oral component, in order to provide a solid basis of understanding for the topics dealt with in this thesis. Relevant anatomy and physiology of the salivary glands is given for better understanding of the various salivary gland investigations performed in this study. Furthermore, an historical overview is given on the syndrome, in order to explain old nomenclature and to put acquired insights into a proper perspective. Subsequently, current insights on the salivary immunopathology in SS are presented. A summary of clinical symptoms and signs in SS, as observed during the history taking and physical examination, concludes the chapter.

In chapter 3, it is studied and discussed how sialometry and sialochemistry can contribute to the diagnostic process of SS.
The frequent occurrence of xerostomia in SS and the easy accessibility of saliva both support the use of sialometry and sialochemistry in the diagnosis. Despite the fact that it is rather inaccurate and impure, collection and analysis of whole saliva is currently in use as the routine technique for sialometry in the diagnostic process of SS. In chapter 3.1, the value of glandular sialometry and sialochemistry was studied as diagnostic instruments in SS. In a group of 100 consecutive patients referred for diagnostics of SS, glandular secretory flow rates and a spectrum of salivary components were assessed. Patients diagnosed with SS differed clearly from the patients tested negative for SS, showing lower flow rates of exclusively the submandibular/sublingual (SM/SL) glands, and a markedly changed composition of parotid- and SM/SL saliva. Besides changes in salivary flow rate and composition, distinct sialometrical profiles were observed, characteristic for either early or late salivary manifestation of SS, or for the xerogenic side effect from medication. It was concluded that glandular sialometry and sialochemistry are not only useful

instruments to differentiate SS from other salivary gland disease in clinical practice, but also have great potential as diagnostic criteria for SS, revealing distinct sialometrical and sialochemical changes as well as profiles. Being simple, safe (noninvasive) and sensitive (early disease detection) glandular sialometry and sialochemistry encompass three major advantages compared to other oral tests for SS.

Since univocal salivary reference values are lacking, it is currently rather difficult to use sialometry and sialochemistry for diagnosing SS, unless major changes have occurred in salivary secretion and composition. In chapter 3.2, cut-off points were selected from Receiver-Operating Characteristic (ROC) curves of gland specific sialometrical and sialochemical variables, which have proven to be potentially relevant for diagnosing SS in the preceding study (chapter 3.1). By combining the most discriminative variables, two different diagnostic approaches for SS were applied in a group of one hundred patients and, subsequently, evaluated in a second group of twenty patients. In the first approach, variables were combined by applying their cut-off points into sets of criteria for a positive diagnosis of SS, in the second approach by including the variables into a logistic regression model that predicts the true state of a patient (SS or non-SS). From both approaches the tests with highest likelihood ratio combined with the smallest number of rejected cases were selected for clinical use. The most accurate test reached a sensitivity of 0.85 and a specificity of 0.96 by combining the stimulated SM/SL-flow rate and parotid sodium and chloride concentration as salivary variables. The selected tests proved equally accurate in the second group of patients. Since the proposed noninvasive diagnostic tools can be easily applied, do not need a laboratory other than for routine blood testing, and are very accurate, we feel that gland-specific sialometry and sialochemistry may eventually replace other, more invasive, diagnostic techniques for diagnosing SS.

The estimated high incidence of SS and the variety of conditions that often mimic SS prompt for a simple screening test for SS, which can also be used by dentists and general practitioners. Based upon a noninvasive diagnostic technique, which was proposed in the previous study to assess the oral component of SS, a test-strip is designed (chapter 3.3) that can be used for screening for SS using a drop of saliva. Changes in the composition of saliva characteristic for SS (altered chloride, phosphate and sodium concentration) can be visualised within a few minutes. These changes proved to have a sensitivity of 92 percent, and a specificity of 62 percent or higher depending on the type of saliva used. The manufacturing and subsequent clinical evaluation of the test-strip is subject of current studies. Appropriate and early

referral, resulting from proper use, will benefit patients as well as clinicians confronted with SS.

Organ damage that directly results from an autoimmune attack in SS can hypothetically be demonstrated by measuring an increase of organ-specific enzymes in serum, as an alternative to currently practised techniques that demonstrate loss of organ-function or change in architecture. This assumption could be true for the salivary glands, containing large amounts of amylase, and almost invariably involved in SS. In chapter 4, the clinical value of measurement of serum isoamylase activity as a clinical parameter in SS is determined. In a group of 100 consecutive patients referred for diagnostics of SS serum activity of salivary (S) and pancreatic (P) isoamylase were assessed. SS patients showed significantly higher serum activities for salivary- and total (salivary and pancreatic) amylase compared to non-SS patients. The optimum threshold of S-isoamylase for detecting SS (105U/L), selected from a Receiver-Operating Characteristic (ROC) curve, had a specificity of 89%, but a limited sensitivity of 35%. Further data analysis explained this low sensitivity by disclosing a biphasic course of S-type isoamylase serum activity in SS patients (increase-decrease), related to the duration of oral complaints. In addition, data analysis revealed that S-type isoamylase serum activity correlated positively with the sialochemical variables sodium and chloride concentration, which both are known to be related to inflammation of the salivary glands. This prospective clinical study shows that measurement of isoamylases in serum has limited diagnostic value for SS, but does have potential use for assessing disease progression.

In chapter 5, characteristics of sialography are studied with regard to its use as diagnostic instrument in SS.
Sialography is commonly used for the purpose of diagnosing SS, though its invasive nature is often regarded as a drawback for routine usage. The aim of this study was to evaluate the morbidity and acceptability of parotid sialography using oil-based contrast fluid (chapter 5.1). Twenty-four consecutive sialographic procedures were evaluated by assessing the morbidity and the patient's acceptance of the procedure with a standardised questionnaire, and by recording relevant physical parameters during the procedure. There was good acceptance of the sialographical procedure, and the morbidity was low. No signs of overfilling or fausse route were observed in any of the sialograms. On average, 0.74 ±0.08 ml contrast fluid was infused at a velocity of 0.01 ml/s. The whole procedure was completed within 12 minutes. From this study, it appears that parotid sialography appears less invasive than is often thought, given its low morbidity and its good acceptance by the patients.

Despite the availability of many new imaging procedures, sialography has, after decades of use, maintained its status as the imaging procedure of choice for evaluating the oral component of SS. In chapter 5.2, the clinical value of sialography as a diagnostic tool in SS was explored by assessing its diagnostic accuracy, observer bias and staging potential. One hundred parotid sialograms were interpreted independently in a blind fashion by two trained- and two expert-observers. Sialograms were derived from a group of consecutive patients, referred for diagnostics of SS. Patients were categorised as SS and non-SS by the revised European classification criteria. Trained observers reached a sensitivity of 95 and a specificity of 33 percent, whereas expert-observers reached a sensitivity of 87 and a specificity of 84 percent. There was only 'fair' inter-observer agreement between trained- and expert-observers, whereas both expert-observers showed 'good' agreement with one another, according to Cohen's kappa. Intra-observer agreement was 'good' to 'very good' for all observers. Furthermore, the four different gradations of sialectasia, *i.e.* punctate, globular, cavitary and destructive, showed a weak but significant correlation with the duration of oral symptoms. This study markedly shows that the diagnostic value of parotid sialography for diagnosing SS greatly depends upon the skills of the observer. This implies that sialography lacks general applicability as a diagnostic tool in SS and requires specific expertise, especially for doubtful cases. Nevertheless, given its potentially high sensitivity and specificity in diagnosing SS as well as its useful staging potential for SS, sialography still has its use in the evaluation of its oral component.

Dysfunction of exocrine glands manifests itself clinically predominantly in the lacrimal and salivary glands (chapter 6). Little is known, however, about the relationship between lacrimal and salivary gland involvement in SS. Furthermore, it is of interest which tear test contributes the most to the diagnosis of SS. Therefore, the aim of this study was to determine the performance of different tear tests and to disclose how these tests relate to common serologic and salivary tests in SS, as used in the revised European classification criteria. In patients suspected of SS, the tear break-up time (BUT), and a possible new test, the tear mucus score, were evaluated in addition to the routine tests, Rose Bengal score and Schirmer test. Eighty consecutive patients were included in this study, categorised into primary SS (pSS), secondary SS (sSS), and negative for SS (non-SS). A corresponding change of tear- and saliva quality and secretion rate was noted in both pSS and sSS patients. Also a clear correlation was found in SS patients, between the Rose Bengal score and observations with parotid sialography. Hyperglobulinemia and presence of SS-B antibodies in serum of SS patients both correlated significantly to increased Rose

Bengal scores of the eyes. The Rose Bengal score was also significantly increased with longer duration of subjective eye-dryness, and with a decreased tear-gland function as estimated by the Schirmer test. The BUT and mucus score both performed insufficiently in diagnosing SS. From the observed relationship between the ocular and the oral component, we conclude that, theoretically, a positive evaluation of one of these components (either ocular or oral), in addition to positive serology or histopathology for SS, could be sufficient to diagnose the syndrome for clinical purposes. Furthermore, it is concluded that hyperglobulinemia and especially positive SS-B serology may warrant close monitoring of the eyes since these serum findings appear to relate to the severity of ocular surface damage. Of all tear tests, the Rose Bengal score still remains the test of choice having the highest specificity for SS. It also appears applicable for monitoring disease progression of SS, relating to duration of subjective complaints and to tear-gland dysfunction.

In chapter 7, two unusual cases are described that stress the importance of accurate diagnostic procedures in SS.
A case of primary sialoangiectasia, which in this case was initially misdiagnosed as SS, is described in chapter 7.1. Other diseases, including HIV-infection, psoriatic arthritis and acute parotitis, may cause glandular changes similar to the changes found in the syndrome. Therefore, sialography always must be combined with other methods of assessment of the oral cavity when suspicion is high for SS.
A patient is demonstrated (chapter 7.2) who presented with sarcoidosis and SS. Diagnoses of both diseases were based on current internationally accepted criteria. Furthermore, histopathological findings characteristic for both diseases were present in salivary gland biopsies. As sarcoidosis is considered an exclusion criterion for SS in current sets of diagnostic criteria we propose that these criteria should be reconsidered with respect to the exclusion of sarcoidosis.

In chapter 8, general conclusions are drawn. The diagnostic process of SS can be optimised by modification of current diagnostic procedures, by first-echelon screening with tear- or saliva-strips (latter not yet available), and perhaps, in near future by reducing the diagnostic process by one component if proven to be justified. After establishment of the diagnosis, it appears SS can be subdivided into two or three different sequential stages of disease progression according to the type of sialectasia and/or to the Rose Bengal score, with a corresponding degree of oral and ocular dryness, respectively. Such sequential stages may consist of an early- (<1 year), an intermediate- (1-4 years) and a progressed stage of SS (>4 years). Furthermore, it appears that glandular disease activity could be estimated by

measuring serum salivary isoamylase activity, which might even predict the rate of exocrine function-loss. In addition to these transversal prospective studies, it is required to validate all retrospectively acquired 'time relations', to differentiate reversible processes, *i.e.* disease activity, from irreversible processes, *i.e.* disease progression, and to further define the different disease stages with long-term prospective studies. If validated markers of disease progression and -activity could eventually be agreed upon they would constitute important tools for disease monitoring and clinical trials.

Follow-up and ongoing studies in our clinic encompass a histopathology- and morbidity study on salivary gland biopsies, long-term prospective studies on clinical disease course, and randomised clinical trials with new therapeutic agents in SS patients.

CHAPTER

10

Samenvatting

SAMENVATTING

In dit proefschrift wordt een zevental klinische studies naar het syndroom van Sjögren (SS) beschreven, waarin de nadruk ligt op de klinische bepaling van aanwezigheid, activiteit en progressie van de aandoening.

In hoofdstuk 1 wordt beschreven hoe de orale component van SS bij tweehonderd patiënten is onderzocht door een multidisciplinaire onderzoeksgroep, teneinde de diagnostiek naar SS te verbeteren en methoden te vinden om de effecten van geneesmiddelen te evalueren. Alle deelonderzoeken betreffen prospectief opgezette klinische studies waarin patiënten volgens de herziene Europese diagnostische criteria voor SS zijn gediagnostiseerd.

Hoofdstuk 2 voorziet in aanvullende informatie over SS en zijn orale component, als toelichting op in dit proefschrift behandelde onderwerpen. Relevante anatomie en fysiologie van de speekselklieren wordt beschreven ten behoeve van de diverse speekselklier onderzoeken die aan bod komen in de volgende hoofdstukken. Verder wordt een historisch overzicht gegeven over het syndroom, waarin oude naamgevingen worden toegelicht en verworven inzichten in het juiste perspectief worden geplaatst. Aansluitend worden huidige inzichten weergegeven over de immunopathologie in SS. Een samenvatting van bij SS voorkomende klachten en symptomen besluit het hoofdstuk.

In hoofdstuk 3 wordt ingegaan op de wijze waarop sialometrie en sialochemie optimaal kunnen bijdragen aan de diagnostiek naar SS.
De waarde van sialometrie en sialochemie als diagnostische technieken bij SS werd onderzocht (hoofdstuk 3.1) door van zowel de parotis als submandibularis/ sublingualis (SM/SL) klieren speeksel secretiesnelheden en -samenstelling te bepalen bij honderd achtereenvolgende patiënten. Deze patiënten werden door diverse specialisten onder de klinische verdenking van SS verwezen. Patiënten mét SS verschilden duidelijk van de patiënten die negatief werden gediagnostiseerd, met een veel lagere secretie snelheid van SM/SL speeksel en een veranderde samenstelling van parotis en SM/SL speeksel. Ook werden diverse sialometrische profielen waargenomen, welke karakteristiek waren voor een vroege fase van SS, een voortgeschreden fase van SS of voor de bijwerking van medicatie. Geconcludeerd werd dat klierspecifieke sialometrie en sialochemie zeer bruikbare diagnostische instrumenten zijn waarmee in de algemene klinische praktijk het

syndroom op eenvoudige wijze kan worden onderscheiden van andere speekselklier aandoeningen.
Door het ontbreken van eenduidige referentiewaarden voor speeksel is het echter niet goed mogelijk om sialometrie en sialochemie te gebruiken bij het diagnostiseren van SS, tenzij er reeds grote veranderingen zijn opgetreden in de speekselsecretie of samenstelling. Om deze reden werden in een vervolgstudie (hoofdstuk 3.2) afkappunten geselecteerd van ROC-curves van klier-specifieke sialometrische en sialochemische variabelen, welke in het voorgaande onderzoek als potentieel bruikbaar naar voren kwamen voor het diagnostiseren van SS. Door de meest discriminerende speekselvariabelen te combineren, werden twee verschillende diagnostische modellen toegepast in een groep van 100 patiënten, en aansluitend geëvalueerd in een tweede groep van 20 patiënten. Bij het eerste diagnostische model werden variabelen gecombineerd door hun afkappunten toe te passen in criteria voor een positieve diagnose, in het tweede model door variabelen in een logistisch regressie model op te nemen dat de 'ware staat' van de patiënt voorspelt (SS of non-SS). Van beide modellen werden de testen, die de hoogste likelihood-ratio combineerden met het kleinste aantal niet te diagnostiseren patiënten, geselecteerd voor klinische toepassing. De meest nauwkeurige test behaalde een sensitiviteit van 85 procent en een specificiteit van 96 procent door gestimuleerde SM/SL secretiesnelheid te combineren met parotis chloride en natrium concentraties. De geselecteerde testen bleken even nauwkeurig in de tweede groep van 20 patiënten. Omdat de hier voorgestelde niet-invasieve technieken op eenvoudige wijze kunnen worden toegepast, geen bijzonder laboratorium vereisen anders dan voor routine bloedonderzoek, en uiterst betrouwbaar zijn, is het waarschijnlijk dat klier-specifieke sialometrie en sialochemie uiteindelijk andere, meer invasieve technieken, zullen vervangen bij het diagnosticeren van SS.
De geschatte hoge incidentie van SS en een keur van op SS gelijkende aandoeningen vragen om een eenvoudige screenende test die ook door huisartsen en tandartsen kan worden gebruikt (hoofdstuk 3.3). Op basis van een niet invasieve methode om de orale component van SS te bepalen is daarom een test-strip ontworpen, waarmee snel gescreend kan worden op SS met slechts een druppel speeksel. De veranderingen in de speekselsamenstelling die kenmerkend zijn voor SS (veranderde chloride, fosfaat en natrium concentraties), kunnen hiermee binnen enkele minuten zichtbaar worden gemaakt. Deze test heeft een sensitiviteit van 92 procent en een specificiteit van 62 procent of hoger, afhankelijk van het type speeksel dat voor de test wordt gebruikt. De vervaardiging en aansluitende klinische evaluatie van de test strip is thans onderwerp van studie. Adequate en vroege

verwijzing van patiënten, door correct gebruik van de test-strip, zal patiënten alsook clinici ten goede komen.

Orgaan schade, die ontstaat als direct gevolg van lokale auto-immuun ontsteking bij SS, kan theoretisch worden aangetoond door het meten van toegenomen orgaanspecifieke enzymen in het serum, als alternatief op de thans toegepaste technieken die verlies van orgaanfunctie of -architectuur aantonen. Deze theorie kan mogelijk klinisch worden toegepast op de speekselklieren, aangezien deze vrijwel altijd betrokken zijn bij SS en grote hoeveelheden amylase bevatten. Teneinde dit na te gaan werd de klinische waarde bepaald van de serum-amylase activiteit als diagnosticum voor SS (hoofdstuk 4). Bij een groep van 100 patiënten, verwezen voor diagnostiek onder de klinische verdenking van SS, werd de serum activiteit van speeksel (S) en pancreas (P) amylase iso-enzymen gemeten. Van de verwezen patiënten hadden SS patiënten significant hogere waarden voor S- en totaal (P en S) amylase activiteit in serum, vergeleken met patiënten die negatief werden gediagnostiseerd. Het optimale afkappunt van S-amylase voor het aantonen van SS, bepaald met behulp van een ROC-curve, had een specificiteit van 89%, maar slechts een beperkte sensitiviteit van 35%. Uit verdere data analyse bleek dat de beperkte sensitiviteit wordt verklaard door een bifasisch verloop in tijd van serum S-amylase activiteit bij de SS patiënten. Ook bleek uit de analyse dat de S-amylase activiteit in serum significant correleert met chloride en natrium concentraties in speeksel, welke beide gerelateerd zijn aan ontstekingsactiviteit in de speekselklieren. Deze prospectieve klinische studie toont dat S-amylase activiteit slechts beperkte diagnostische waarde heeft voor SS, doordat de klier-specifieke enzymen alleen initieel stijging vertonen in serum. Mogelijk heeft S-amylase meer waarde als parameter voor ziekte monitoring.

In hoofdstuk 5 wordt de waarde van sialografie onderzocht als diagnosticum bij SS. Sialografie wordt regelmatig gebruikt om SS te diagnostiseren, hoewel een vermeend invasief karakter van deze röntgencontrast techniek veelal als nadeel wordt beschouwd. Het doel van de studie in hoofdstuk 5.1 was het evalueren van de invasiviteit van sialografie, bij gebruik van een contrast vloeistof op oliebasis. Bij 24 aansluitende sialografie procedures werd de morbiditeit en de mate van acceptatie door de patiënten gemeten door middel van gestandaardiseerde vragenlijsten en protocollair fysisch onderzoek tijdens de procedure. Er was een hoge mate van acceptatie door de patiënten van de procedure en de morbiditeit was zeer gering. Technische complicaties, zoals overvulling of een fausse route, werden niet op de sialogrammen gezien. Gemiddeld werd 0,74 ml contrast

vloeistof ingebracht met een snelheid van 0,01 ml/s, waarbij de hele procedure gemiddeld minder dan 12 minuten duurde. Uit deze studie blijkt dat parotis sialografie minder invasief is dan vaak wordt verondersteld, gezien de zeer geringe morbiditeit en de hoge mate van acceptatie door de patiënten.
Ondanks de komst van diverse nieuwe radiologische technieken, heeft sialografie na vele jaren zijn positie behouden als voorkeurstechniek om de speekselklier betrokkenheid bij SS te visualiseren. In hoofdstuk 5.2 wordt de klinische en diagnostische waarde van sialografie ten aanzien van SS onderzocht. Honderd geanonimiseerde sialogrammen werden door 2 geoefende- en 2 expert beoordelaars individueel beoordeeld. De sialogrammen waren afkomstig van een groep van honderd achtereenvolgende patiënten, die door diverse specialisten onder de klinische verdenking van SS werden verwezen. De geoefende beoordelaars behaalden een sensitiviteit van 95 procent en specificiteit van 33 procent, terwijl de expert beoordelaars een sensitiviteit van 87 procent en een specificiteit van 84 procent behaalden. Er was slechts redelijke overeenstemming tussen de geoefende- en expert- beoordelaars, doch goede overeenstemming tussen beide expert beoordelaars, berekend met Cohen's kappa. De vier verschillende sialografische stadia, te weten punctaat, globulair, cavitair en destructief, vertoonden een zwakke doch significante correlatie met de duur van monddroogheid (gerelateerde) klachten. Deze studie toont op markante wijze dat bij sialografie de diagnostische waarde voor SS in hoge mate wordt bepaald door de vaardigheden van de beoordelaar. Dit betekent dat sialografie specifieke expertise vereist en beperkt algemeen toepasbaar is. Desondanks heeft sialografie, gezien zijn potentieel hoge sensitiviteit en specificiteit bij het diagnostiseren en zijn mogelijke toepassing bij het stadiëren van SS, specifieke waarde bij het evalueren van het syndroom.

Dysfunctie van exocriene klieren bij SS manifesteert zich klinisch voornamelijk in de traan- en speekselklieren. Er is echter nog weinig bekend over de relatie tussen de traan- en speekselklier betrokkenheid bij SS (hoofdstuk 6). Het doel van deze studie was het meten van de diagnostische waarde voor SS van diverse traantesten, en het bepalen hoe deze testen relateren aan de gebruikelijke bloed- en speeksel onderzoeken, zoals toegepast in de herziene Europese criteria. Bij patiënten met een klinische verdenking op SS werden de traanfilm stabiliteit (break-up time of BUT), de traan lactoferrine concentratie en, een mogelijk nieuwe test, de traan mucus score als testen geëvalueerd naast de routine traantesten, te weten de Bengaal's rood score en de Schirmer test. Tachtig achtereenvolgende patiënten werden in de studie geïncludeerd, onderverdeeld in primaire SS (pSS), secundaire

SS (sSS) en negatief voor SS (non-SS). Er werd een overeenkomende verandering van traan- en speeksel kwaliteit en secretiesnelheid gezien in zowel pSS als sSS patiënten. Ook werd een duidelijke correlatie gezien tussen de Bengaal's rood score en de mate van afwijkingen bij sialografie. In het bloed correleerde hyperglobulinemie en aanwezigheid van SS-B antistoffen significant bij SS patiënten met een hoge Bengaal's rood score in de ogen. Verder was de Bengaal's rood score bij SS patiënten significant hoger naarmate de klachten van oogdroogheid langer bestonden en naarmate de oogdroogheid ernstiger was, zoals gemeten met de Schirmer test. De BUT en de mucus score presteerden beide matig als diagnostische traantest voor SS. Op basis van de waargenomen overeenkomst tussen de oogheelkundige en orale component, concluderen wij dat, *theoretisch*, een positieve evaluatie van één component, in aanvulling op positieve serologie of histopathologie voor SS, zou kunnen volstaan om de diagnose *voor klinische doeleinden* te stellen. Verder concluderen wij dat hyperglobulinemie en met name positieve SS-B serologie bij SS patiënten vraagt om nauwgezette oogheelkundige controle, aangezien deze bloedbevindingen lijken te relateren aan ernstigere oogschade. Van alle traantesten blijft de Bengaal's rood score de test van voorkeur gezien zijn hoge specificiteit voor SS. Deze test lijkt tevens geschikt voor het monitoren van progressie van SS, aangezien de scores relateren aan de duur van de klachten en aan de mate van traanklier dysfunctie.

In hoofdstuk 7 worden twee patiënten beschreven met ongebruikelijke ziekte manifestaties, die beiden het belang van nauwgezette diagnostiek bij SS benadrukken.
Een patiënt met primaire sialoangiectasie, die aanvankelijk ten onrechte werd gediagnostiseerd als SS, wordt in hoofdstuk 7.1 beschreven. Andere ziekten, waaronder HIV-infectie, arthritis psoriatica en acute parotitis kunnen sialografisch speekselklier afwijkingen veroorzaken die identiek zijn aan de afwijkingen bij SS. Daarom kan niet worden volstaan met het alleen verrichten van sialografie, maar moet bij sterke verdenking op SS deze techniek worden gecombineerd met andere onderzoeken van de speekselklieren.
In hoofdstuk 7.2 wordt een 32-jarige patiënte beschreven, die zich presenteerde met sarcoïdose en SS. De diagnose van beide ziekten was gebaseerd op huidige internationaal geaccepteerde criteria. Tevens waren in speekselklier biopten histopathologische kenmerken van beide ziekten aanwezig. Omdat sarcoïdose in de diverse internationale diagnostische criteria voor SS thans als een exclusie criterium wordt beschouwd, stellen wij voor deze criteria te heroverwegen ten aanzien van de exclusie van sarcoïdose.

In hoofdstuk 8 worden algemene conclusies uit de diverse deelonderzoeken gecombineerd en in een breder perspectief geplaatst. Het diagnostisch onderzoek naar SS kan worden verbeterd door modificatie van huidige diagnostische technieken, door in de eerste lijn al te testen met speeksel- of traan strips (speeksel-strips nog niet beschikbaar), en mogelijk in de nabije toekomst door de diagnostiek terug te brengen tot één component, zodra rechtvaardiging hiervan is bevestigd. Nadat de diagnose is vastgesteld lijkt het dat SS kan worden onderverdeeld in verschillende opeenvolgende stadia van ziekteprogressie op basis van het sialografische stadium en/of de Bengaal's rood score, met een bijbehorende mate van mond- en oogdroogheid, respectievelijk. Dergelijke opeenvolgende stadia kunnen worden geformuleerd als een vroeg stadium (<1jaar), een tussen stadium (1-4 jaar), en een voortgeschreden stadium (>4 jaar). Verder lijkt het mogelijk de ziekte activiteit te bepalen aan de hand van iso-amylase activiteit in het bloed. Mogelijk heeft het zelfs prognostische waarde ten aanzien van de snelheid waarmee de exocriene klieren hun secretoire functie verliezen. Het is wel noodzakelijk dat alle retrospectief verkregen inzichten over ziekte progressie worden bevestigd, dat reversibele ziekteprocessen (ziekte activiteit) worden onderscheiden van irreversibele ziekteprocessen (ziekte progressie), en dat de verschillende ziekte stadia verder worden gedefinieerd met behulp van prospectief longitudinaal onderzoek. Als uiteindelijk overeenstemming kan worden bereikt over gevalideerde markers van ziekte activiteit en -progressie, zouden deze markers belangrijke hulpmiddelen worden bij het monitoren van de ziekte alsook bij klinische trials.

Vervolgstudies in onze kliniek bestaan uit een histopathologie- en morbiditeit studie van speekselklier biopten, een prospectieve longitudinale studie naar klinisch ziektebeloop en gerandomiseerde klinische trials met nieuwe therapeutische middelen bij SS patiënten.

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CURRICULUM VITAE

Wouter Kalk was born in 1971 in Zaandam. He attended the secondary school at the Zaanlands Lyceum and subsequently the Elshof College, where he passed his exam (Gymnasium) in 1989. He attended Medical School at the University of Groningen from 1989 until 1995. While in Medical School, he worked as clinical assistant at the Department of Anatomy. His medical school thesis work, on donor-site morbidity from autogenous bone harvesting, was performed from 1994 until 1995 at the Department of Oral and Maxillofacial Surgery at the University Hospital Groningen under the supervision of Prof. G. Boering, Dr. G.M. Raghoebar, and Dr. J. Jansma. After receiving his medical degree in May 1995, he worked as a medical officer in the Royal Navy, stationed at the operational unit of the Dutch Marines. During a two-year employment he attended extreme weather survival courses, as well as a medical trauma training at the University Hospital Rotterdam, after which he returned to the University of Groningen in 1997 to study Dentistry. While in Dental School, the research described in this doctoral thesis was performed under the supervision of Prof. J.L.N. Roodenburg, Prof. C.G.M. Kallenberg, Dr. A. Vissink, Dr. F.K.L. Spijkervet and Dr. H. Bootsma. Furthermore, during this period, he taught and examined practical skills at the Medical School. After receiving his dental degree in June this year, Wouter Kalk started his oral and maxillofacial surgery residency at the University Hospital Groningen (department head: Prof. L.G.M. de Bont). Recently, he has been assigned as medical advisor for the NVSP, the Dutch association for Sjögren' syndrome patients.

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Stellingen

behorend bij het proefschrift

Clinical assessments in Sjögren's syndrome: The oral component

How much saliva is enough?

Wouter W.I. Kalk
19 september 2001

1. Eén druppel kan het verschil bepalen.
   Dit proefschrift.

2. Het testen in de eerste lijn verhoogt de doelmatigheid van het diagnostisch onderzoek naar het syndroom van Sjögren.
   Dit proefschrift.

3. Niet de invasiviteit maar de interpretatie is de belangrijkste tekortkoming van sialografie.
   Dit proefschrift.

4. Het syndroom van Sjögren kent verschillende ziektestadia, welke deels subklinisch verlopen.
   Dit proefschrift.

5. Duidelijk samenhangende pathologie van traan- en speekselklieren bij het syndroom van Sjögren tornt aan de diagnostische trias van Bloch uit 1965.
   Dit proefschrift.

6. Hoewel aanvullende diagnostiek veelal in waarde wordt overschat, is dit bij het syndroom van Sjögren essentieel voor een valide diagnose.
   Dit proefschrift.

7. Het syndroom van Sjögren is huilen zonder tranen.

8. Zonder een uniforme set van diagnostische criteria blijft de incidentie en prevalentie van het syndroom van Sjögren onbekend.

9. De relatieve onbekendheid van het syndroom van Sjögren is goeddeels gebaseerd op Goethe's dictum: 'Was man weisst sieht man'.

10. Onvoorwaardelijk vertrouwen op de aanwezigheid van een wetenschappelijke oplossing of verklaring is een modern geloof.

11. Elk geneeskundig handelen tijdens of voor de reproduktieve fase van de mens, uitgezonderd antenatale diagnostiek, draagt bij aan de ondermijning van de menselijke evolutie, ergo geneeskunde betreft korte termijn geluk.

12. Tandheelkunde is niet bij elke patiënt 'natte vinger werk'.

13. Arts en tandarts hebben complementaire kennis.

14. In saliva veritas.
    I.D. Mandel