Chapter 1

General Introduction
Sjögren’s syndrome (SS) is a chronic inflammatory and lymphoproliferative disease with autoimmune features, characterized by progressive lymphocytic infiltration of the exocrine glands, notably the lacrimal and salivary glands. SS can be divided in primary and secondary SS, the latter being associated with another connective tissue disease, such as rheumatoid arthritis or systemic lupus erythematosus. SS is a worldwide disease and its prevalence is estimated at 0.5-1% of the population with a female preference (female to male ratio nine to one). SS is therefore the second most common autoimmune disease (second to rheumatoid arthritis). The main clinical features of SS are a progressive dryness of the eyes (keratoconjunctivitis sicca) and dryness of the mouth (xerostomia). Various extraglandular manifestations may develop in addition, such as neuropathy, arthritis, vasculitis, and renal or lung involvement. B cell activation is a key feature of SS, leading to the production of autoantibodies and hypergammaglobulinemia. Furthermore, 5 percent of patients with SS develop malignant B cell lymphoma during follow-up. The classic lesion of the exocrine glands in SS is the lymphoepithelial lesion in the major salivary glands and a focal lymphocytic sialoadenitis in the minor salivary glands. These lymphoepithelial lesions develop as a result of hyperplasia of ductal basal cells within a lymphocytic infiltrate. The infiltrate in the minor salivary glands is characterized by focal aggregates of 50 or more lymphocytes adjacent to normal appearing acini. T cells account for about 80% of the total infiltrate, with the remaining 20% composed of B cells and plasma cells. Immunologically, the prevailing abnormality is a polyclonal B lymphocyte hyperreactivity, reflected by polyclonal hypergammaglobulinemia and the presence of several autoantibodies (rheumatoid factor, antinuclear antibodies amongst which anti-Ro/SSA, anti-La/SSB-antibodies) in serum. The pathogenesis of SS is still largely unknown. It has become apparent that disturbances of the immune system play a central role. Whether this is a primary abnormality or a result of infectious (viral) or other extrinsic factors remains uncertain. A recent study implicated a possible role for coxsackievirus in induction and maintenance of primary SS. Glandular persistence of these viruses in salivary gland epithelial cells may lead to chronic lymphocytic sialoadenitis with subsequent formation of follicles. It is not known whether viral infection is primary or secondary in the development of the autoimmune process or whether this process depends on other environmental, hormonal, or hereditary factors. Current data suggest that SS may have distinct subtypes of disease and different degrees of disease activity and severity. The presence of low complement C4 levels and/or palpable purpura distinguishes high-risk patients from patients with uncomplicated disease course. An early and accurate diagnosis of SS can prevent or ensure treatment of many complications. Especially patients with above mentioned risk factors or patients with disease onset might be susceptible to disease progression. Histopathology of the major salivary glands and evaluation of function of individual salivary glands can possibly benefit in recognition of early lymphoma development and in identifying patients who might benefit from intervention therapy.
Table 1. The American-European classification criteria for SS.18

Revised international classification criteria for Sjögren’s syndrome

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

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

III. Ocular signs—that is, objective evidence of ocular involvement defined as a positive result for at least one of the following two tests:
   1. Schirmer’s I test, performed without anaesthesia (<5 mm in 5 minutes)
   2. Rose bengal score or other ocular dye score (>4 according to van Bijsterveld’s scoring system)

IV. Histopathology: in minor salivary glands (obtained through normal-appearing mucosa) focal lymphocytic sialoadenitis, evaluated by an expert histopathologist, with a focus score >1, defined as a number of lymphocytic foci (which are adjacent to normal-appearing mucous acini and contain more than 50 lymphocytes) per 4 mm² of glandular tissue

V. Salivary gland involvement: objective evidence of salivary gland involvement defined by a positive result for at least one of the following diagnostic tests:
   1. Unstimulated whole salivary flow (<1.5 ml in 15 minutes)
   2. Parotid sialography showing the presence of diffuse sialectasias (punctate, cavitary or destructive pattern), without evidence of obstruction in the major ducts
   3. Salivary scintigraphy showing delayed uptake, reduced concentration and/or delayed excretion of tracer

VI. Autoantibodies: presence in the serum of the following autoantibodies:
   Antibodies to Ro(SSA) or La(SSB) antigens, or both

For primary SS: in patients without any potentially associated disease, primary SS may be defined as follows:
   a. The presence of any 4 of the 6 items is indicative of primary SS, as long as either item IV (Histopathology) or VI (Serology) is positive or
   b. The presence of any 3 of the 4 objective criteria items (that is, items III (Ocular signs), IV (Histopathology), V (Salivary gland involvement), or VI (Serology)).

For secondary SS: in patients with a potentially associated disease (for instance, another well defined connective tissue disease), the presence of item I or item II plus any 2 from among items III, IV, and V may be considered as indicative of secondary SS

Exclusion criteria:
   Past head and neck radiation treatment
   Hepatitis C infection
   Acquired immunodeficiency disease (AIDS)
   Pre-existing lymphoma
   Sarcoidosis
   Graft versus host disease
   Use of anticholinergic drugs (since a time shorter than 4-fold the half life of the drug)
**Scope of the thesis**

The main objective of this thesis was to optimize diagnostic procedures in SS with regard to histopathology, analysis of gland specific saliva, and imaging. Next, exocrine and non-exocrine progression in SS was studied in order to gain more insight in progression of salivary gland dysfunction and lymphoma development. Finally, new therapeutic strategies for intervention were evaluated.

**Diagnosis**

In 1933, the Swedish ophthalmologist Henrik Sjögren first described clinical and histopathological findings in patients with a condition of rheumatoid arthritis, dry eyes and dry mouth. In 1965, Bloch et al. characterized SS as the triad of keratoconjunctivitis sicca, xerostomia, and a connective tissue disease. This classical triad has provided the basis for various sets of classification criteria. However, there is no single widely accepted diagnostic set of criteria for the diagnosis of SS. Seven published sets of criteria have been proposed, each with different sensitivity and specificity. Those of European and American groups differed so much that almost ten times the number of cases would be diagnosed when using the European criteria than by use of either set of the American criteria. Recently, the American-European Consensus group suggested a new set of criteria (Table 1). This new set of criteria is less liberate in diagnosing SS than, in particular, the former European criteria.

Although no single test can serve as a gold standard for diagnosing SS, histopathology of the labial salivary gland remains a key-feature in all sets of criteria. A widely accepted criterion for histological confirmation of SS is focal lymphocytic sialoadenitis in the labial salivary gland. However, biopsies of the labial salivary glands may have several disadvantages. Its sensitivity and specificity are often low, and it may be difficult to harvest a sufficient amount of acini in atrophic submucosa. In addition, permanent sensory loss of the mucosa of the lower lip is a common complication of a labial biopsy. Incision biopsy of the parotid gland can probably overcome most of the disadvantages of the labial biopsy.

In chapter 2.1 the diagnostic values of the labial and parotid gland biopsy are compared. First, the parotid biopsy was assessed as a criterion for SS. Next, the potential of the parotid biopsy as a diagnostic tool was compared with the diagnostic potential of the labial biopsy in a prospective single center study on 35 consecutive patients suspected of SS. In addition, both types of biopsies were analyzed with respect to the morbidity of the two biopsy techniques.

Assessment of salivary flow rates (sialometry) is of diagnostic and, possibly, prognostic value in SS. Since the amount and composition of saliva reflect the effects of the autoimmune process in the salivary glands, analysis of saliva (sialochemistry) may also be valuable in diagnosis, assessment of prognosis, and evaluation of treatment. Sialometry and sialochemistry can be used as a diagnostic tool either by collecting whole saliva (the combined secretions of all salivary glands) or by collecting glandular saliva (gland specific saliva).
Chapter 2.2 describes the accuracy and reproducibility of collecting parotid saliva in different patient groups. Day to day variation and the effect of increasing the number of collections were assessed in healthy volunteers, correlation between flow rates of left and right parotid glands were studied in patients with SS, whilst a group of patients with head and neck cancer participated in an assessment of the effect of repeated collections on the reliability of baseline values for clinical studies.

There are a variety of diagnostic imaging techniques for monitoring salivary gland damage in SS. The most commonly used techniques are sialography, digital subtraction sialography, scintigraphy, sonography, computed tomography scanning (CT-scan), magnetic resonance imaging (MRI) and magnetic resonance sialography (MR-sialography). There are large differences between these techniques regarding invasiveness, applicability and costs. It is not known which technique is preferable regarding its diagnostic performance.

Chapter 2.3 systematically reviews the available evidence on the diagnostic accuracy for SS of the above mentioned imaging techniques. A standardized search was conducted up to November 2005 in computerized databases (Medline, Pubmed, Embase, Cinahl, Cochrane) and by reference tracking. Outcomes of studies were weighted according to the number of participants included in that study. A multivariate linear regression analysis was performed to evaluate diagnostic performance of the different imaging techniques for evaluating salivary gland involvement in SS.

Progression
Salivary gland dysfunction is considered a key manifestation in SS. Particularly at the time SS develops, not all salivary glands may manifest dysfunction, rendering whole saliva less valuable as a diagnostic fluid. The collection of glandular saliva, however, reveals sequential involvement of different glands, reflecting the autoimmune process in individual salivary glands. Based on a cross-sectional study in SS patients, different sialometrical and sialochemical profiles, characteristic for either early or late salivary manifestations, have been reported. Patients with short duration of oral symptoms (less than one year) showed either normal flow rates with changed salivary composition, or reduced stimulated flow rate from the sublingual/submandibular glands accompanied by (sub)normal flow rate from the parotid glands. It seems that the parotid gland is the last salivary gland to manifest hyposalivation, which has been confirmed in other studies.

Chapter 3.1 describes the results of a study where loss of function of individual salivary glands in patients with primary and secondary SS was evaluated, in order to get insight into the natural history of the disease. Sixty SS patients were included in this prospective study. Whole and gland specific saliva were collected at baseline and at follow-up, together with sialochemical, laboratory and subjective parameters.

Besides these disabling exocrine symptoms, SS patients can present with severe systemic manifestations and lymphoproliferative disease. Five percent of patients with SS develop malignant B cell lymphoma, 48-75% of which are of MALT-type and most frequently located in the parotid gland. Especially patients with extraglandular manifestations (e.g. palpable purpura, vasculitis, and peripheral neuropathy), persistent bilateral parotid
gland swelling, and monoclonal gammopathy, cryoglobulinemia, reduced C4 levels or CD4+ lymphocytopenia have an increased risk for lymphoma development. \textsuperscript{34,35} Patients with MALT lymphoma and associated SS (MALT-SS) usually have a more severe form of SS than patients with SS without MALT lymphoma. Lymphadenopathy, skin vasculitis, peripheral nerve involvement, fever, anaemia and lymphopenia were observed significantly more often than in the general SS population.\textsuperscript{3} MALT lymphoma in patients with SS is a spectrum from indolent asymptomatic lymphoma with no SS disease activity, up to disseminated lymphoma with severe extraglandular SS manifestations. No clear guidelines are available for the management of patients with MALT-SS. Treatment has always been patient tailored, taking into account the site, the clinical characteristics and the stage of the MALT lymphoma.

In chapter 3.2 twenty-two consecutive cases of MALT-SS lymphomas were studied in order to assess their clinical characteristics, the course of disease and their response to different treatment strategies. Based on these data guidelines have been proposed for management of patients with MALT-SS.

**Intervention**

In SS, there is no evidence-based intervention therapy. The treatment of dryness related symptoms is mainly based on stimulation of the residual capacity of the salivary glands, or on replacement of saliva with a salivary substitute.\textsuperscript{36} Corticosteroids and disease-modifying anti-rheumatic drugs (DMARDs) have no major effect on the disease course,\textsuperscript{37} although prednisone seems effective in selected patients, increasing salivary flow and improving main clinical and histological features.\textsuperscript{38-40} Cyclophosphamide, with or without prednisone, has been shown effective in patients with renal involvement, and in different other severe extraglandular complications, such as systemic vasculitis or polyneuropathy.\textsuperscript{1,41,42} A promising treatment for both MALT lymphoma and SS is rituximab, a chimeric murine/human anti-CD20 monoclonal antibody that binds to the B cell surface antigen CD20. It is considered a promising agent in the treatment of various autoimmune disorders, such as rheumatoid arthritis and systemic lupus erythematosus.\textsuperscript{43-45} Because B cells appear to be involved in the pathogenesis of SS, B cell depletion may lead to a decrease of SS disease activity.

**Chapter 4.1** describes a case-report where rituximab showed a beneficial effect on histological and sialometrical/chemical characteristics in a patient with MALT-SS.

**Chapter 4.2** presents the results of a phase II study evaluating the effect of rituximab in patients with pSS with or without MALT lymphoma. In this prospective study 15 patients with early SS or patients with MALT-SS were treated with rituximab. Patients were evaluated at baseline and at 5 and 12 weeks after the first infusion by immunological, salivary/lacrimal functional and subjective parameters.

In chapter 5, the findings of this thesis are summarized and discussed.
References


