Chapter 5

Summary and General Discussion

Summary and general discussion

Sjögren's syndrome (SS) is a chronic inflammatory and lymphoproliferative progressive autoimmune disease. It is characterized by B cell activation and infiltration of T and B cells in the exocrine glands. Common symptoms are related to diminished lacrimal and salivary gland function. Besides keratoconjunctivitis sicca and xerostomia, patients can present with severe systemic complications, such as vasculitis and nephritis. Furthermore, 5 percent of patients with SS develop malignant B cell lymphoma during follow-up. Diagnosis of SS remains difficult, especially with regard to the oral component. Still is much unknown with regard to loss of salivary gland function and the evolution of malignant lymphoma, and there is no evidence-based intervention therapy. The main objective of the first part of this thesis was to optimize *diagnostic* procedures in SS with regard to histopathology, collecting of saliva, and imaging. Next, *progression* of salivary gland dysfunction and lymphoma development were studied. In the last part of this thesis, the effect of B cell depletion in SS with or without MALT lymphoma was evaluated.

Diagnosis

In **chapter 2.1** the parotid incisional biopsy was validated as an assessment in the diagnosis of primary SS. Next, the parotid and the labial biopsy were compared with regard to diagnostic value and morbidity of the technique in 35 patients suspected for primary SS. The validation study revealed a focus score of ≥1 or lymphocytic infiltrates in combination with benign lymphoepithelial lesions (LELs) as highly diagnostic criteria for primary SS (sensitivity 93%, specificity 95%). In accordance with the focus score in labial biopsies, this parotid focus score was based on the number of focal inflammatory cell aggregates containing 50 or more lymphocytes in each 4 mm^2 of salivary gland. The comparative study of the parotid and labial biopsies showed that the diagnostic potential of labial and parotid biopsies was equal, but that the parotid biopsy was considered superior with respect to its lower morbidity with regard to sensory nerve loss (without morbidity at the motory nerve level). The possible additional value of the parotid biopsy for early detection of malignant lymphoma must be further investigated. On the other hand a new clinical dilemma is introduced: what to do with the "subclinical" lymphoma?

Chapter 2.2 describes the accuracy and reproducibility of measuring stimulated parotid saliva in healthy volunteers, head and neck cancer patients, and patients with SS. Especially in SS patients, there was a strong correlation between flow rates from the left and right parotid gland. Intra-individual variation amounted almost 24% in stimulated parotid flow rates obtained by repeated measurements in healthy volunteers. Conversely, repeated measurement did not result in a significant less variation in baseline values in healthy volunteers and head and neck cancer patients. An increase of the numbers of collections at baseline does not increase reliability of baseline parotid flow rates. From this study it was concluded that large standard deviations (average 24%) have to be taken into account when measuring stimulated parotid flow rate in clinical studies. Increase or decrease in parotid flow rate should surpass this measuring error in order to demonstrate the true effect of treatment or disease progression.

In **chapter 2.3** a systematic review of the literature on the diagnostic accuracy for SS of

the currently available imaging techniques is described. Publications concerning cohort studies regarding diagnostic imaging techniques in SS were included. In order to ensure the methodological quality of the studies to be reviewed, a minimum validity score, at least 10 of the maximum of 19, according to the checklist of the Cochrane Methods Working Group on Meta-analysis of Diagnostic and Screening Tests was used for inclusion. The literature search identified 851 articles of which 54 had a sufficient validity score. The mean diagnostic accuracy of studies analyzing MRI/magnetic resonance sialography (MR-sialography) was 87.8%, for studies analyzing sialography, sonography, and scintigraphy the values were 83.2, 82.5, and 76.1%, respectively. After correction for confounders, studies analyzing sialography report the highest diagnostic accuracy followed by studies analyzing MRI and sonography. Studies analyzing scintigraphy report by far the lowest accuracy after correction for confounders. This study shows that MRI and sonography have proven their value as diagnostic instruments in SS, but sialography remains the best performing diagnostic imaging technique in SS, with an accuracy significantly higher than MRI and sonography.

Progression

Salivary gland dysfunction is one of the key manifestations of SS. **Chapter 3.1** describes a study where loss in function of individual salivary glands was prospectively assessed in patients with primary and secondary SS in relation to disease duration and use of medication. SS patients with short disease duration had significantly higher stimulated flow rates at baseline than patients with longer disease duration. In these patients, submandibular/sublingual flow rates were already more diminished than parotid flow rates. This still reasonable salivary gland function, in patients with short disease duration, showed a progressive decrease over time, regardless of the use of disease modifying antirheumatic drugs (DMARDs) or steroids. In contrast, SS patients with longer disease duration are characterized by both severely reduced parotid and submandibular/sublingual secretions. These observations may have an impact for early diagnosis of SS and for identifying patients who most likely benefit from intervention therapy.

B cell activation is a key feature of SS and these patients are at risk of severe extraglandular manifestations and the development of mucosa-associated lymphoid tissue (MALT) lymphoma. **Chapter 3.2** describes the clinical data of 22 patients with MALT lymphoma located in the parotid gland associated with SS (MALT-SS) and proposes a treatment protocol. Treatment of MALT-SS should target both the autoimmune and the neoplastic nature of the disease. A multidisciplinary approach is necessary for evaluation and treatment of these patients. In selected patients, monoclonal antibody therapy combined with immunosuppression seems promising.

Intervention

Chapter 4.1 decribes a case report on the successful use of rituximab on salivary gland immunohistology and function in a patient with MALT-SS. The patient was treated with four infusions of rituximab (375 mg/m^2) on a weekly basis, which resulted in complete remission of the lymphoma. Histological evaluation (parotid gland biopsies) showed post-treatment improvement of (immuno)histopathological characteristics of SS, with possible regeneration of salivary gland tissue. Salivary analyses showed decrease of inflammatory characteristics and increase of stimulated salivary flow. From these outcomes, a phase II

study was initiated to investigate the safety and efficacy of B cell depletion in the treatment of patients with active primary SS of short duration (early pSS) and patients with primary SS and associated MALT lymphoma (MALT-SS) (**chapter 4.2**). Fifteen patients with primary SS were included in this phase II trial. Significant improvement of subjective complaints and an increase in salivary gland function was observed in patients with residual salivary gland function. Immunological analysis showed a rapid decrease of peripheral B cells, and stable levels of IgG. Human anti-chimeric antibodies (HACAs) developed in 4/15 (27%) patients, all patients with early primary SS. Three of these patients developed a serum sickness-like disorder. Of the patients with MALT-SS, 86% achieved complete remission or stable disease. This phase II study suggests that rituximab is effective in the treatment of patients with pSS. The high incidence of HACAs and associated side-effects observed in this study needs further evaluation.

General Discussion

This thesis comprehends studies on diagnostic procedures, progression, and new intervention therapy in patients with SS.

Diagnosis

The recently suggested diagnostic criteria from the American-European consensus group were proposed to solve the discrepancy in the different diagnostic criteria.[1] An important diagnostic criterion is histopathology of the labial salivary gland. However, biopsies of the labial salivary glands have several disadvantages. The sensitivity and specificity have been reported to be low, harvesting sufficient numbers of salivary glands can be difficult, and sensory loss is a common complication of a labial biopsy. Comparison of the labial biopsy with an incision biopsy of the parotid gland showed that labial and parotid biopsies were comparable with regard to diagnostic value. However, parotid biopsies seem to have some advantages over labial biopsies. Morbidity is low in both biopsy types, but patients encounter permanent skin hypoesthesia after a labial biopsy only. Two other studies compared labial and parotid biopsies, showing that an incisional parotid gland biopsy was superior to or at least comparable with a labial biopsy in the diagnosis of SS.[2,3] Unfortunately, no firm histological criteria were provided for the parotid biopsies in these two studies, rendering the studies irreproducible. This study showed that a focus score of ≥1 or a focus score of <1 in the presence of benign LELs in the parotid gland is highly suggestive of SS, with a diagnostic sensitivity and specificity at least comparable to that of a labial biopsy in the diagnostic work-up of SS. The incision biopsy of the parotid gland should therefore be included in the diagnostic criteria as an alternative to the labial biopsy.

Several diagnostic imaging techniques are available for evaluating salivary gland dysfunction in SS, the most commonly used are sialography, scintigraphy, sonography, and MRI. It is not known which technique is preferable regarding its diagnostic performance. Therefore, the diagnostic accuracy of the currently available imaging techniques was evaluated. A major problem in assessing literature on diagnostics in SS is the change in diagnostic criteria throughout the years. Furthermore, the influence of the applied diagnostic criteria is a very important confounder. After correcting for this bias, studies analyzing sialography had the highest diagnostic accuracy, followed by studies analyzing MRI and sonography. Sialography shows the architecture of the salivary duct system radiographically by infusion of a contrast fluid. Although MRI is preferred in imaging of tumours of the salivary glands,

sialography remains the method of choice for exploring the ductal system of the salivary gland to diagnose SS. It was concluded that there is no longer place for scintigraphy in the diagnostic work-up of SS, unless the diagnostic accuracy of this technique is improved. Two possible improvements of scintigraphy, i.e. SPECT and HIG-scintigraphy, indicated promising diagnostic accuracies, but this must be confirmed in larger studies.

Progression

Gland specific sialometry is important in diagnosing patients with early SS and in the evaluation of clinical trials. The often used unstimulated whole saliva lacks specificity, because patients with short disease duration may still have a reasonable unstimulated parotid flow, masking the clinical complaints related to the already diminished submandibular/sublingual salivary flow. These patients often complain of mouth dryness at night, while they have no problems swallowing dry food or speaking. In the course of disease, there is a progressive decrease in salivary gland function, especially of the parotid glands. It is important to recognize patients with residual salivary gland function at an early stage. In these patients, intervention therapy might prevent further glandular damage, or even lead to regeneration of salivary gland tissue. Also, SS patients with residual salivary gland function may benefit from stimulating agents, such as pilocarpin. These agents are likely to be of no use in patients with longstanding SS, because, due to the progressed destruction of glandular tissue, insufficient secretory tissue is left to result in clinically sufficient moistening of the oral tissues upon stimulation. Collection of whole saliva is the method most frequently used, because it is easy to practice, without the need for a collecting device. For early diagnosis and evaluation of treatment, glandular sialometry and sialochemistry is preferred.

For studies on progression or intervention of SS, especially evaluation of the parotid gland might be of use; function, composition of saliva and histology can be evaluated of the same gland at different time-points. This combination of sialometry, -chemistry and parotid histology might also be of value in studies on pathogenesis. It was showed that left and right parotid salivary flow rates can serve as an intra-individual control (unless there is an exacerbation of one of the parotid glands). It was also suggested that an increase in the order of one-quarter to one-third of the parotid flow rate from baseline level should be used as an objective measure to evaluate a real effect of treatment, because of the large standard deviations for stimulated parotid flow. In patients with SS this variation is rather low, because of their decreased salivary flow rates.

In addition to these exocrine manifestations, SS patients have an increased risk for developing lymphoma. The often cited 44 fold increased risk as compared with the normal population is probably only valid for selected patients with severe disease.[4] Apparently, strong polyclonal B cell activity includes monoclonal components. Interestingly, skin vasculitis, peripheral nerve involvement, lymphadenopathy, fever, anaemia and lymphopenia were observed more frequently in patients with SS and lymphoma than in the general SS-population.[5] This suggests a relation between clonal B cell expansion in the salivary glands and extraglandular (clonal) B cell expansions. This is in line with earlier studies, where high-risk patients could be distinguished from low-risk patients by the presence of palpable purpura and low C4 levels at the first visit.[6] It is important to recognize these patients, as they are characterized by high B cell activity and they are at risk of developing severe extraglandular manifestations,

such as polyneuropathy or malignant lymphoma. These lymphomas are usually of MALT-type and are located in the parotid gland. 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. It is important to differentiate patients with MALT lymphoma with or without high SS-disease activity, because treatment strategy is mainly dependent on SS-activity. Traditional staging systems are not useful in patients with MALT-SS. CT/MRI-scanning of the head and neck region seems sufficient in identifying patients at risk of progression of both MALT and SS. Bone marrow involvement is rare en does not influence prognosis nor treatment.
In patients with asymptomatic or symptomatic MALT lymphoma, but with high SS disease activity (severe extraglandular manifestations, such as vasculitis or polyneuropathy), treatment should consist of a combination of B cell depletion and immunosuppression. Further studies are needed to investigate the effect of rituximab combined with cyclophosphamide and prednisone in these patients. The standardized response criteria for non Hodgkin Lymphoma as proposed by Cheson *et al*.[7] do not seem applicable in these patients, as the involvement of multiple extranodal sites may not reflect truly disseminated disease, nor inferior prognosis.[8] Both the lymphoma and the autoimmune disease must be evaluated in order to assess response to treatment.
Pre- and post-treatment parotid biopsies are a method for evaluating treatment with low morbidity.[9] These repeated biopsies can, however, be very difficult to interpret and histological criteria for the diagnosis of residual disease or complete remission are not clearly defined. Criteria for response to treatment in parotid biopsies from patients with SS need to be standardized.

Intervention

In this thesis, a phase II study is reported where the effect of B cell depletion in SS was studied. Although SS has always been seen as a T cell mediated disease, new insights show an important role for B cells in both local and systemic autoimmunity. Indeed, targeting B cells in SS with rituximab, a chimeric murine/human anti-CD20 monoclonal antibody, effectively depletes B cells. Especially patients with residual salivary gland function showed an increase of this function and improvement of subjective symptoms, supporting our theory that patients with short disease duration are the most likely candidates for intervention therapy.
Analysis of parotid saliva showed normalization of sodium readsorption after rituximab treatment, indicating regeneration or recovery of parotid gland tissue. In healthy subjects most of the sodium is readsorbed from primary saliva during its transport through the ductal system.[10] Higher concentrations of salivary sodium at the same flow rate are related to more severe disease manifestations.[11] The increase in salivary secretion and the normalization of sodium concentration might indicate recovery or regeneration of salivary gland tissue. This regeneration probably occurs in patients with residual functional salivary gland tissue only. Recent data support this theory. Repeated parotid gland biopsies after treatment with rituximab showed redifferentation of the lymphoepithelial duct lesion into a normal striated duct.[12] This response to B cell depletion urges the need for early and aggressive treatment in patients with SS with short disease duration, often characterized by residual salivary gland function and high levels of salivary sodium. Physical functioning is impaired in these early patients, and B cell depletion may lead to an increase of quality of life and preservation of

lacrimal and salivary gland function. Long-term follow-up showed that effect of treatment declined to baseline after 9 to 12 months. Retreatment with rituximab in 5 patients resulted in similar subjective and objective response.[13]
An unexpected finding was the high incidence of adverse events; four of the 15 patients (all patients with early and active primary SS) developed antibodies against the chimeric part of the monoclonal antibodies (HACAs). Three of these patients developed a serum-sickness like disease, the presence of HACAs making a type III hypersensitivity reaction very likely. HACAs were not detected in patients with MALT-SS.
Studies of rituximab in SLE and rheumatoid arthritis do not mention similar adverse events.[14-16] Conversely to our study, patients in the latter studies received high doses of corticosteroids, cyclophosphamide or methotrexate, which may account for the lack of these events. This might also explain why patients with MALT-SS in our study did not develop HACAs or serious adverse events. In addition to the premedication, 3 of the 7 patients with MALT-SS continued to receive prednisone 7.5-15 mg/day in combination with methotrexate or azathioprine, which might have contributed to the lack of serious adverse events in this group. HACAs have been reported at a higher rate in autoimmune disease than in lymphoma patients (27%) compared to 4.3% in RA patients with, 35% in SLE patients, and 0.6% in lymphoma patients.[14,17,18] The combination of high B cell activity (as reflected by high levels of IgM-Rf) and lack of concomitant immunosuppressives might be responsible for the development of serum-sickness like reactions after treatment with rituximab in our SS patients. Based on these preliminary results we would recommend a combination with higher or prolonged concomitant corticosteroid treatment in order to prevent development of serum sickness-like disorders in future studies on rituximab treatment in SS.

Repeated parotid biopsies before and after rituximab treatment in SS demonstrated histological evidence of a significant reduction of the lymphoid infiltration with a decrease in B/T-cell ratio and increase of parenchymal regeneration.[12] This might underlie the observed increase of salivary flow rate. Furthermore, partial and complete redifferentation of lympoepithelial lesions to regular ducts was seen, which might underly the normalization of salivary sodium. These combined clinical-morphological findings support the efficacy of rituximab treatment in SS and greatly improves the understanding of the underlying cellular mechanisms.

Future perspectives

As shown in this study, gland specific sialometry and –chemistry is useful in identifying patients with residual gland function and high disease activity who might benefit from intervention therapy. Although most patients with SS have a benign disease course, some may develop severe extraglandular manifestations and have very disabling disease. It is important to recognize these patients at an early stage, so that they can be closely followed and treated aggressively if necessary. Identification of biomarkers can lead to earlier diagnosis of SS. Signature proteomic and genomic biomarkers in saliva of primary SS are currently investigated in close collaboration with researchers from UCLA School of Dentistry in Los Angeles. In addition, a double blinded randomized placebo-controlled study investigating the effect of rituximab combined with corticosteroids has been initiated. Furthermore, clonal expansions of B cells in parotid gland biopsies will be studied in order to characterize the different phases of lymphoproliferation in SS and to study the influence of B cell depletion on the development of these clonal expansions.

References

1. Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, Daniels TE, Fox PC, Fox RI, Kassan SS, Pillemer SR, Talal N, Weisman MH. Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Ann Rheum Dis 2002; 61:554-558.

2. Marx RE, Hartman KS, Rethman KV. A prospective study comparing incisional labial to incisional parotid biopsies in the detection and confirmation of sarcoidosis, Sjögren's disease, sialosis and lymphoma. J Rheumatol 1988; 15:621-629.

3. Wise CM, Agudelo CA, Semble EL, Stump TE, Woodruff RD. Comparison of parotid and minor salivary gland biopsy specimens in the diagnosis of Sjögren's syndrome. Arthritis Rheum 1988; 31:662-666.

4. Theander E, Henriksson G, Ljungberg O, Mandl T, Manthorpe R, Jacobsson LT. Lymphoma and other malignancies in primary Sjögren's syndrome. A cohort study on cancer incidence and lymphoma predictors. Ann Rheum Dis 2005.

5. Voulgarelis M, Dafni UG, Isenberg DA, Moutsopoulos HM. Malignant lymphoma in primary Sjögren's syndrome: a multicenter, retrospective, clinical study by the European Concerted Action on Sjögren's Syndrome. Arthritis Rheum 1999; 42:1765-1772.

6. Ioannidis JP, Vassiliou VA, Moutsopoulos HM. Long-term risk of mortality and lymphoproliferative disease and predictive classification of primary Sjögren's syndrome. Arthritis Rheum 2002; 46:741-747.

7. Cheson BD, Horning SJ, Coiffier B, Shipp MA, Fisher RI, Connors JM, Lister TA, Vose J, Grillo-Lopez A, Hagenbeek A, Cabanillas F, Klippensten D, Hiddemann W, Castellino R, Harris NL, Armitage JO, Carter W, Hoppe R, Canellos GP. Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. NCI Sponsored International Working Group. J Clin Oncol 1999; 17:1244-1253.

8. Thieblemont C. Clinical presentation and management of marginal zone lymphomas. Hematology (Am Soc Hematol Educ Program) 2005; 307-313.

9. Pijpe J, Van Imhoff GW, Vissink A, Van der Wal JE, Kluin PM, Spijkervet FKL, Kallenberg CGM, Bootsma H. Changes in salivary gland immunohistology and function after rituximab mono-therapy in a patient with Sjögren's syndrome and associated MALT-lymphoma. Ann Rheum Dis 2005; 64:958-960.

10. Mandel ID, Baurmash H. Sialochemistry in Sjögren's syndrome. Oral Surg Oral Med Oral Pathol 1976; 41:182-187.

11. Pedersen AM, Reibel J, Nordgarden H, Bergem HO, Jensen JL, Nauntofte B. Primary Sjögren's syndrome: salivary gland function and clinical oral findings. Oral Dis 1999; 5:128-138.

12. Pijpe J, Bootsma H, Spijkervet FKL, Vissink A, Kallenberg CGM, Ihrler S. Rituximab treatment in Sjögren's syndrome: Clinical-histological evidence of the efficacy of B-cell depletion. The IXth international symposium on Sjögren's Syndrome, Washington, USA, 2006.

13. Meijer JM, Pijpe J, Imhoff GW, Vissink A, Spijkervet FKL, Mansour K, Kallenberg CGM, Bootsma H. Rituximab treatment in patients with primary Sjögren's Syndrome: 1-year follow-up and retreatment. The IXth international symposium on Sjögren's Syndrome, Washington, USA, 2006.

14. Edwards JC, Szczepanski L, Szechinski J, Filipowicz-Sosnowska A, Emery P, Close DR, Stevens RM, Shaw T. Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis. N Engl J Med 2004; 350:2572-2581.

15. Leandro MJ, Edwards JC, Cambridge G, Ehrenstein MR, Isenberg DA. An open study of B lymphocyte depletion in systemic lupus erythematosus. Arthritis Rheum 2002; 46:2673-2677.

16. Leandro MJ, Edwards JC, Cambridge G. Clinical outcome in 22 patients with rheumatoid arthritis treated with B lymphocyte depletion. Ann Rheum Dis 2002; 61:883-888.

17. Looney RJ, Anolik JH, Campbell D, Felgar RE, Young F, Arend LJ, Sloand JA, Rosenblatt J, Sanz I. B cell depletion as a novel treatment for systemic lupus erythematosus: a phase I/II dose-escalation trial of rituximab. Arthritis Rheum 2004; 50:2580-2589.

18. McLaughlin P, Grillo-Lopez AJ, Link BK, Levy R, Czuczman MS, Williams ME, Heyman MR, Bence-Bruckler I, White CA, Cabanillas F, Jain V, Ho AD, Lister J, Wey K, Shen D, Dallaire BK. Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. J Clin Oncol 1998; 16:2825-2833.

Chapter 6

Nederlandse samenvatting