Chapter 7

Summary and general discussion
Summary

Sjögren’s syndrome (SS) is a systemic autoimmune disease characterised by chronic inflammation of the salivary and lacrimal glands, resulting in complaints of xerostomia and keratoconjunctivitis sicca in about 95% of the patients. These symptoms are frequently accompanied by extraglandular manifestations, and 85% of the patients suffer from severe fatigue. Furthermore, the presence of SS has a large impact on health related quality of life (HR-QoL), employment and disability.

Yet, no causal systemic treatment is available in SS and therefore only symptomatic treatment can be given. Currently, biological agents have been introduced in various systemic autoimmune diseases including rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). However, no biological agent has been approved thus far for the treatment of SS, but several phase II and III studies have recently been completed or are currently being conducted. The effect of treatment with biological agents is aimed at reducing disease activity and to slow down progression of SS.

In the research described in this thesis the impact of SS on quality of life has been evaluated, the different approved and experimental treatment options have been reviewed, existing and new tools to evaluate treatment were assessed and treatment results with anti-CD20 monoclonal antibodies (rituximab) are presented.

Chapter 2 describes HR-QoL, employment and disability in patients with primary (pSS) and secondary (sSS) SS, compared to data available from the general Dutch population. A questionnaire was sent to the total cohort of SS patients within the University Medical Center Groningen that is seen for scheduled follow-up. 195 out of 235 patients (83%) responded. The results revealed that SS has a large impact on HR-QOL, employment and disability as reflected by lower Short Form-36 (SF-36) scores (measuring subjective well-being), lower employment rates and higher disability rates in SS patients when compared to the general Dutch population. In addition, physical functioning, bodily pain and general health were worse in sSS than in pSS patients. The results of this trial underscore the necessity for the development of causal treatment for SS.

Therefore, in chapter 3 an overview is given of the trials performed in SS with biological agents up to 2006 and future perspectives are presented. The gain in knowledge regarding the cellular mechanisms of T and B lymphocyte activity in the pathogenesis of SS and the current availability of various biological agents (anti-TNF-\(\alpha\), IFN-\(\alpha\), anti-CD20, and anti-CD22) have resulted in new possibilities for therapeutic intervention. In SS, various phase I and II studies have been performed to evaluate these biologicals. Currently, B cell directed therapies, and especially the use of anti-CD20 monoclonal antibodies, have been shown to be more promising than T cell related therapies. In the near future a large role for treatment with biologicals for SS is expected. Larger phase II and III trials are necessary to confirm these first promising results.

In general, evaluation of a new treatment modality requires well defined and usable tools to evaluate the effect of treatment. Chapter 4a gives a general overview of existing tools for evaluation of treatment for diseases affecting salivary glands. Assessments of salivary gland function (sialometry, sialochemistry) and histopathological examination of salivary gland biopsies provide powerful tools to diagnose diseases affecting the salivary glands, to
assess disease progression and to evaluate treatment. More general tools are subjective questionnaires (e.g., visual analogue scale (VAS) scores, Multidimensional Fatigue Inventory (MFI) score and SF-36) and serological parameters.

Chapter 4b describes the development of a new evaluation tool, the genomic and proteonomic profile of whole saliva. In the study described in this chapter, the profiles for SS patients were compared to healthy age and sex matched controls. This preliminary study indicated that both glandular and whole saliva from pSS patients contain molecular signatures that reflect damaged glandular cells and an activated immune response. Whole saliva was shown to be more useful in SS diagnostics than parotid and submandibular/sublingual saliva. The candidate proteonomic and genomic biomarkers found in whole saliva may improve the clinical detection of pSS once they have been further validated in a larger group of patients.

The evaluation tools described in chapter 4 were used in evaluating treatment with rituximab, described in chapter 5. In chapter 5a a study is described assessing the efficacy and safety of (re)treatment of SS patients with rituximab after extended follow-up (mean follow-up 57 weeks) of B cell depletion therapy. Included were 8 early pSS patients and 7 pSS patients with a mucosa-associated lymphoid tissue (MALT)-type lymphoma (MALT/pSS). Rituximab was effective for 6-9 months in pSS patients and, probably, even longer in MALT/pSS patients. Retreatment of 5 pSS patients resulted in a comparable beneficial effect as observed after the first course. Development of serum sickness-like disorder in 27% of pSS patients indicated that higher doses of corticosteroids might be needed during rituximab treatment.

In chapter 5b the results of histopathological evaluation of parotid tissue after rituximab treatment were correlated with clinical results of parotid function in order to evaluate rituximab treatment on a more fundamental level. Sequential parotid biopsies before and 12 weeks after rituximab treatment in pSS patients demonstrated histopathological evidence of reduced glandular inflammation and redifferentiation of lymphoepithelial duct lesions to regular striated ducts as a putative morphological correlate of increased parotid flow and normalization of salivary sodium content. These histopathological findings underline the efficacy of B cell depletion and prove the potential for glandular restoration in SS. This study was performed as a pilot in the 5 pSS patients that received retreatment described in chapter 5b. Analysis of larger groups of patients biopsied before and after rituximab treatment are necessary to confirm these first results.

Based on these promising results, a randomized double-blind placebo-controlled trial was performed (chapter 5c). In this trial 30 pSS patients were included, of which 20 were treated with rituximab, while 10 patients received placebo. All 30 patients received an additional dose of corticosteroids in order to prevent the development of side effects. In this trial, B cell depletion led to improvement of objective and subjective parameters of disease activity. Salivary function improved, fatigue diminished, extraglandular manifestations improved. Most improvements were seen 12 to 36 weeks after treatment. These promising results suggest that a larger phase III trial should be performed in order to receive approval for rituximab treatment of SS.

Although SS is considered to be a T lymphocyte mediated disease, there are more and more signs that the role of the B cells should not be underestimated. The description of the cases described in chapter 6 has deepened our insight into the B cell component of SS.

In this chapter, we retrospectively evaluated 8 patients with the combination of SS and
localized cutaneous amyloidosis. The databases of 3 amyloidosis centres (Italy; University of Pavia, Germany; University of Heidelberg and the Netherlands; Medical Center Groningen) were searched in order to find this rare combination. It was likely that AL amyloid was the actual type in all 8 patients, which is an immunoglobulin light chain associated amyloid, locally produced by a light chain-restricted plasma cell population in the skin. The combination of cutaneous amyloid and SS appeared to be a distinct disease entity reflecting a particular and benign part of the polymorphic spectre of lymphoproliferative diseases related to SS.

General discussion

Sjögren syndrome: is there a need for treatment and which treatment is available?

SS is known to affect patients’ physical, psychological and social functioning (3), but the impact of SS on health-related quality of life (HR-QOL), and especially on employment and disability, has not been studied extensively before. However, this information is necessary to interpret the burden of the disease and also to gain insight into the necessity for treatment. Therefore, the analysis described in chapter 2 was performed. Comparable to other autoimmune diseases, SS has a large impact on HR-QOL, employment and disability as reflected by lower SF-36 scores and employment rates, and higher disability rates when compared with the general Dutch population. The impact on socioeconomic status described in chapter 2 justifies further research on biologicals in the treatment of SS, even though these treatments are expensive and intensive. In addition, the overview of the reports on biological treatment for SS (chapter 3) revealed that anti-CD20 (rituximab) is the most promising biological agent so far (4-6) The results of some of therapies targeting TNF-α (infliximab, etanercept and adalimumab) and IFN-α were also promising in phase I and II studies, but in larger placebo controlled randomized trials results were disappointing. So, although the first results with rituximab seem promising, also regarding this biological larger placebo controlled trials are needed to confirm these promising results (see section on rituximab treatment). Moreover, as rituximab is a chimeric anti-CD20 agent that has the inherent hazard of inducing serum sickness, humanized anti-CD20 (ocrelizumab) that more recently has become available might, in potential, be an even more promising B cell therapy. Another promising B cell directed therapy is anti-CD22 (epratuzumab). This agent seemed to be effective in a small open-label trial, although to a lesser extent than rituximab as it only partially depletes B cells (7). Other potential targets for biological therapy include cytokines such as IL-6 and BlyS (BAFF), interferons, adhesion molecules and chemokines. No trials in SS have yet been performed with these biological therapies, however.

Which evaluation tools are useful?

With the increasing number of trials performed aiming to treat SS, there is a growing need for more specific assessment parameters to monitor treatment effects, both subjectively and objectively. For studies on intervention in SS, especially evaluation of the parotid gland might be of use. Assessment of parotid secretory function (sialometry), composition of parotid saliva (sialochemistry) and histological examination of parotid gland tissue (repeated incisional biopsies) are routinely used in our setting to evaluate the effect of an intervention therapy as a function of time. Also scintigraphy, functional MRI, PET scans and ultrasound can be used repeatedly in evaluating the parotid gland. The diagnostic accuracy of the latter tools is lower and these are therefore less often used in our setting for treatment
evaluation. More general tools, but very valuable in evaluating intervention in SS, are subjective questionnaires (e.g. VAS scores, MFI scores and SF-36) and serological parameters such as rheumatoid factor and immunoglobulin levels, and B cell counts in the case of B cell depletion therapy.

Furthermore, both glandular and whole saliva are easy to obtain and the first results from studies on genomics and proteomics (chapter 4b) showed valuable results. As a continuation of this study, a validation paper reported on the discovery of highly specific autoantibody biomarkers for pSS using protein microarray technology.(8) If the genomics and proteomics can be used in the future as diagnostic tools for SS and as tools for monitoring the effect of treatment, for example rituximab treatment, in depth saliva analysis might even replace more invasive diagnostic tools such as parotid biopsies, PET and scintigraphy.

What about rituximab treatment?

Based on the promising results described in the review (chapter 3) and in the open label phase II study (chapter 5a and 5b), a randomised, placebo-controlled trial with rituximab was performed (chapter 5c). The results of the latter trial confirmed the promising results of the phase II trials, but, also some criticism was raised related to the treatment of early pSS patients without extraglandular manifestations with this biological. Because the long term (side-)effects of treatment with biological agents in SS are not known yet, some SS experts suggest to use treatment only for those SS patients with severe extraglandular manifestations(9;10). However, we observed that patients with remaining glandular function at the time of diagnosis benefit more from rituximab treatment than patients without any function left. Thus, in our opinion patients with active disease, as reflected by high levels of IgG and rheumatoid factor, increasing complaints of fatigue, and/or sicca complaints and/or swelling of the parotid gland (but still having glandular function), are the preferred patients to be treated with rituximab. Besides this group of early patients, also patients with severe extraglandular manifestations may benefit significantly from treatment. Of course, the long-term side effects of rituximab treatment have to be thoroughly investigated in larger phase III trials before implementation of this biological as therapy for SS.

In contrast to patients with lymphoma or RA treated with rituximab, serum sickness or serum sickness-like adverse events are more frequently reported in SS patients, with a rate between 6% and 27%. (chapter 3) This initially unexpected finding may be due to the use of different co-medication. Patients with RA and systemic lupus erythematosus (SLE) usually receive higher doses of steroids or concomitantly immunosuppressive drugs as compared with SS patients, which may prevent certain adverse events. In addition, RA and SLE patients often have been treated with a wide range of medication (including biological agents) before receiving treatment with rituximab, whereas SS patients are far more medication-naïve at the time of rituximab treatment. We also observed in the trial described in chapter 5c, as well as in our pilot trial, that patients who developed serum sickness were more likely to have an active, early and progressive form of the disease.(6) It is possible that such patients are more prone to develop serum sickness; however, such patients might also be the ones that most likely benefit from rituximab therapy. Another possibility is that SS patients may be more prone to develop and deposit immune complexes because of hypergammaglobulinaemia and/or cryoglobulinemia.(4) Consequently, because of the inherent risk of developing serum sickness (like) disease, we decided to increase the steroid dose in the trial described in chapter 5c. Of the 30 included patients, only
one patient developed serum sickness-like disease (5%), which is considerably lower than the incidence reported in our open-label study (27%).(6) Furthermore, HACA (human antichimeric antibodies) development, which occurred in 27% of patients in our open-label trial, was not found in the only patient who developed serum sickness-like disease. Based on these findings, we would recommend administering 100 mg methylprednisolone immediately prior to each infusion of rituximab. The oral regimen of prednisolone in the days following each infusion differs between different trials and should be explored in future trials. The administration of higher doses of prednisolone in the days following infusion, such as is performed during lymphoma treatment, should also be considered, because most lymphoma patients are, as SS patients, medication-naive at the time of rituximab treatment, and no serum sickness has been reported in these patients.

Retreatment with rituximab resulted in a positive effect comparable to that of the first treatment with this biological (chapter 5a). Therefore, offering patients maintenance therapy with rituximab infusions every 6 to 9 months may be a reasonable approach. Advantages of maintenance therapy might be a reduction or even arrest of disease progression and better quality of life for a long period. A threat might be the, so far unknown, long term side effects of repeated B cell depletion. The timing of retreatment could be based on return of symptoms, however, retreatment just before return of symptoms would even be better. A prediction model based on the results of our placebo controlled trial, showed that levels of rheumatoid factor could be a good predictor for return of subjective symptoms such as dry mouth and fatigue (unpublished results). However, these preliminary results were based on 20 pSS patients and, therefore, in future trials, attention should be paid to the correlation between objective and subjective symptoms. We even like to pose that such a correlation might provide a base for selecting the most optimal retreatment schedule. Probably, for each patient an individual time scheme has to be made because we observed that the time period in which rituximab reduced SS related symptoms/complaints differed considerably between patients.

The dose of rituximab that patients should receive during maintenance treatment should also be investigated. Based on the positive results after 2 infusions of 375mg/m² (which is in total about 1000 mg) as reported by Devauchelle et al.(5), probably even only one infusion of 1000 mg could be sufficient. Another issue concerns the question which group of patients should be offered retreatment. In RA patients, results of trials on retreatment of non-responders to first treatment are not conclusive. Thurlings et al. (11) reported that only responders to the first treatment benefit from retreatment, while Vital et al. reports that retreatment of non-responders before circulating plasma cells return to baseline levels enhances B cell depletion and results in a better clinical response.(12) With respect to SS, criteria for defining responders versus non-responders should first be formulated.

Table 1 Number of patients who actually received placebo or rituximab and the estimation of the patients and the physicians.

<table>
<thead>
<tr>
<th></th>
<th>True</th>
<th>False</th>
<th>True</th>
<th>False</th>
<th>True</th>
<th>False</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>16</td>
<td>4</td>
<td>18</td>
<td>2</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td>Rituximab (20)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>7</td>
<td>2</td>
<td>8</td>
<td>1</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Total (29)</td>
<td>23 (79%)</td>
<td>6 (21%)</td>
<td>26 (90%)</td>
<td>3 (10%)</td>
<td>25 (86%)</td>
<td>4 (14%)</td>
</tr>
</tbody>
</table>
and validated and results of retreatment of both responders and non-responders should be evaluated in future trials.

As a general rule, a placebo effect should not be underestimated in clinical trials with a long follow up period. In order to obtain some insight into a placebo effect in a clinical trial with only 30 patients (chapter 5c) all patients were asked after 24 weeks by mail if they thought they received placebo or rituximab and the reason why they thought to have received the active drug or placebo. One patient did not respond and was therefore excluded from this analysis. Both study coordinators (physicians of the departments of rheumatology and oral and maxillofacial surgery), who regularly assessed the patients and who were blinded for the study medication, also guessed whether the patient had used rituximab or placebo. In 23 out of 29 patients estimation of treatment was correct for both physicians. The physicians correctly scored treatment modality of 25 and 26 patients out of the 29 patients, respectively (Tables 1 and 2).

In conclusion, both the blinded patients and doctors could quite accurately estimate if a patient received placebo or rituximab. Therefore, the placebo effect in this particular study is small which gives us an additional hint that rituximab is an effective treatment for SS.

Role of B cells
The classical view on the role of B cells in immunity is focused on the production of antibodies and autoantibodies in the case of autoimmune diseases. However, over the past years the role of B cells seems to have acquired much more dimensions such as regulating T cell subsets and dendritic cells through cytokine production, activation of T cells and antigen presentation to T cells. As other autoimmune diseases, SS is long considered to be a T-lymphocyte mediated disease, however, in the light of these new developments the role of B cells might be more prominent than thought in the past. The promising results of B cell depletion therapy in SS also support the theory that there is a role for B cells in the pathogenesis of SS. E.g., cutaneous nodular amyloidosis in SS seems to be the result of a benign clonal proliferation of plasma cells in the skin that is part of the spectre of lymphoproliferative diseases associated with SS. Despite its rare occurrence, 16 cases of cutaneous amyloidosis have been reported in patients with SS, which is about 25% of the reported cases of cutaneous amyloidosis These cases and the description of the cases described in chapter 6 support the role of the B cell in SS.

Future perspectives
Today, SS is diagnosed more and more in an early stage of the disease. Screening might become much easier if, in the future, e.g., the proteonomic profile can be used for diagnosis. Only one drop of saliva might be sufficient for diagnostics and/or treatment evaluation.

Today no causal treatment is available, however, so far, the performed trials revealed that B cell depletion with rituximab is probably the most effective therapy available to date. Also our randomized double-blind placebo-controlled trial (chapter 5c) with rituximab treatment showed promising results. A trial investigating retreatment of all patients involved in that trial is in progress. Focus of that study will be a longer follow up period (64 weeks), the effect of retreatment and the effect of treatment in patients who have received initially a placebo. A histopathological study of parotid gland biopsies before and after rituximab treatment of the patients described in chapter 5c has also been initiated and hopefully confirms our clinical findings and the results of our pilot study on histopathological effects of rituximab treatment (chapter 5b).
Besides the already performed phase II trials, larger phase III trials are needed before approval can be obtained for rituximab treatment in SS patients. In these larger phase III studies, additional attention should be paid to the long term side effects, possibility of retreatment, and the oral dose of prednisolone during the days after each infusion. We also like to pose that rituximab treatment is especially effective for patients with active disease, extraglandular manifestations and/or remaining salivary gland secretory potential. To confirm these hypotheses, in future larger trials less strict inclusion criteria related to baseline salivary gland function and a larger number of patients are needed. In order to define treatment protocols, criteria regarding responders/non-responders have to be implemented. Studies regarding disease activity scores are currently being performed and are also important for future treatment protocols.

In addition to phase III rituximab trials, also other types of B cell depletion therapies should be investigated including completely humanized anti-CD20, anti-CD22 and anti-BAFF. To our opinion, there is a large role in the future for biologicals in the treatment of SS which could add substantially to a good quality of life of SS patients.

Table 2 Number of correct estimations. Maximum score is 3: patient and both physicians scored correct.

<table>
<thead>
<tr>
<th>Number of correct estimations</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>1 (3%)</td>
<td>2 (7%)</td>
<td>4 (14%)</td>
<td>22 (76%)</td>
</tr>
</tbody>
</table>
Reference List
