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, 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). 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 and interpreting 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. 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, 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. Furthermore, the labial salivary gland biopsy suffers from relevant false positivity as well as false negativity. 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, involves a quick and simple procedure under local anaesthesia, the invasiveness of which is very low, 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. 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. 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.\textsuperscript{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.

\section*{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.

REFERENCES