Chapter 4a
Progression and treatment evaluation in diseases affecting salivary glands
Abstract

A general overview of existing tools for evaluation of treatment for diseases affecting salivary glands is given. 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.
Introduction

Many diseases and conditions can affect salivary glands resulting in a reduced or increased salivary flow. Treatment for these and other disorders can affect salivary secretion as well. Frequent causes of long-lasting reduced salivary flow are drugs, systemic conditions like Sjögren’s syndrome (SS) and radiation injury to salivary gland tissue. The sensation of a dry mouth (xerostomia) is not always accompanied by a reduced salivary secretion (hyposalivation). In about one third of the patients with xerostomia there is no good correlation between actual mouth dryness and level of salivary secretion. The discrepancy between salivary secretion status and level of complaints is even more striking in drooling. Usually, salivary secretion is normal or even reduced, but swallowing of saliva is impaired. Well-known causes of the inability to empty the mouth of saliva are an infantile swallowing pattern, a disturbed sensibility of the oral tissues and anatomic limitations due to trauma and ablative surgery. Thus, many factors have to be considered when selecting a salivary evaluation tool for the subset of patients or healthy subjects.

Notwithstanding the above, salivary research provides powerful tools to diagnose diseases affecting salivary glands, to assess disease progression, and to evaluate treatment. In progressive diseases like SS, salivary secretion generally diminishes with time. (figure 1) This progression is not so obvious when monitoring whole saliva, but becomes much clearer when measuring gland specific saliva.(1) While sialometry is a robust tool for evaluating disease progression, analysis of salivary composition (sialochemistry) differentiates between salivary gland diseases, and measures the disease activity (table 1)(2) and the effect of intervention treatment.(3) Additional tools are sialography (imaging of the extent of destruction of the ductal system), salivary scintigraphy (imaging of the glandular secretory activity), salivary gland biopsy (glandular pathology underlying the observed changes), and the imaging of anatomical structures with CT, MRI, or ultrasound.

The six above-mentioned variables (sialometry, sialochemistry, sialography, salivary scintigraphy, biopsy, and imaging) are gland-specific and measure disease progression and/or activity. Other essential information might come from the pattern of complaints, medical history, the clinical picture, serology and questionnaires. Serological parameters and subjective questionnaire responses can add important information on the disease progression and treatment outcome.

This chapter discusses the main tools for evaluation of disease progression and treatment including applications to clinical research and practice.
Tools to measure salivary gland function and disease activity

**Sialometry**
Saliva collection provides sound clinical information. Accurate measures of salivary flow rate and composition are essential for many diagnostic, therapeutic, and research protocols. Saliva collection is a noninvasive tool of assessing a variety of disease characteristics and levels of certain drugs and hormones. Whole saliva is a mixture of not only salivary secretions, but also fluids, debris, and cells not originating in the salivary glands. Therefore, the analysis of individual gland saliva is usually a more reliable procedure for diagnosing diseases of the salivary glands than analysis of whole saliva. However, for certain diagnostic procedures whole saliva might be more useful, for example, when assessing specific roles of saliva in the oral cavity or when whole saliva is used as a diagnostic fluid for conditions relying on leakage of serum products or gingival crevicular fluid into saliva.

In healthy subjects and patients in whom both glands are affected simultaneously (e.g. SS) flow rates of the left and right parotid gland are similar. Therefore, sorting out discrepancies between the observed flow of the left and right parotid gland assures the reliability of the samples collected. This is a very powerful internal control of the reliability of the saliva sample collected and outweighs the effect of repeated sampling of a parotid gland to get a reliable baseline sample. Increasing the number of collections has been shown to have a negligible effect on the reliability of baseline parotid flow rates for clinical trials. Consequently, one reliable baseline sample is sufficient for clinical studies evaluating the progression of disease or the effect of a therapy. Moreover, salivary flow rates are not constant and exhibit a considerable amount of variability. Therefore, salivary collections should be performed under well-defined conditions and, for repeated collections, at the same time of the day to minimize intrapatient variability. Nevertheless, even if the circadian rhythm is ruled out and the samples are indeed collected under well-defined conditions, the measured increase or decrease of salivary flow has to exceed about one-quarter to one-third of the parotid flow rate at baseline before an observed effect related to a given therapy can be assessed as a ‘real’ effect in an individual patient. This information is additional to subjective assessments of such an effect.

**Sialochemistry**
Saliva is an attractive diagnostic fluid because salivary testing provides several key advantages including low cost, noninvasiveness, and easy sample collection and processing. Human saliva collection is less invasive than phlebotomy and is clinically relevant because many, if not all, blood components are reflected in saliva. Amongst others, sodium, potassium, chloride, calcium, phosphate, urea, total protein and a number of enzymes (e.g., amylase, lysozyme and lactoferrin) can be detected in saliva and have diagnostic potential. A new method to assess the protein composition in health and disease is salivary proteomics - the identification of the entire spectrum of proteins in human saliva. Saliva also harbours diagnostic RNA biomarkers (detection of RNA biomarkers).

**Sialography**
Through retrograde infusion of oil- or water-based iodine contrast, the architecture of the salivary duct system is visualized radiographically. It is a low morbidity, well-accepted technique. Sialography should not, however, be performed in patients with a history of iodine allergy. The sialographic procedure can be performed in 10 - 15 minutes.
Inflammation appears on sialograms as diffuse collections of contrast fluid at the terminal acini of the ductal tree. This condition, known as sialectasia can be classified into punctate (less than 1 mm), globular (uniform and 1-2 mm), cavitary (coalescent and >2 mm) and destructive (normal ductal structures are no longer visible). Sialectasia is thought to result from progressive acinar atrophy and dilatation, which, in turn, is caused by increasing intraluminal pressure resulting from the presence of periductal lymphocytic infiltrates with secondary duct narrowing. So, these four grades of sialectasia are thought to represent increasing glandular damage, caused by chronic salivary gland inflammation.

**Figure 1**

Relationship between disease duration (time from first complaints induced by or related to oral dryness until referral) and mean (SEM) salivary flow rates in patients with (A) primary SS (pSS) and in those with (B) secondary SS (sSS). Normal values are derived from historic controls (n=36). SM/SL, submandibular/sublingual glands; UWS, unstimulated whole saliva. *Significant difference versus patients with early-onset SS (<1 year oral complaints; p<0.005) by the Mann-Whitney U test. †Significant difference versus patients with early-onset SS (p<0.05) by the Mann-Whitney U test (Pijpe et al. (1), reprinted with permission).
Table 1 Salivary gland parameters and clinical data of some disorders affecting the salivary glands (Van den Berg et al., 2007) (2). SS is an autoimmune disorder affecting the exocrine glands including the salivary glands. Sialosis is a salivary condition characterized by persistent swelling of the parotid glands related to a metabolic disorder as diabetes, alcohol abuse, anorexia and bulimia. Sodium retention syndrome is characterized by mostly unilateral, incidental, short-lasting (hours) swelling of the parotid gland often related to cardiovascular disorders (hypotension, hypertension).

<table>
<thead>
<tr>
<th>SS (pSS/sSS)</th>
<th>Sialosis</th>
<th>Sodium retention syndrome</th>
<th>Medication induced xerostomia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sialometry</td>
<td>UWS ≤1.5 ml in 15 min</td>
<td>Normal, increased or decreased</td>
<td>Normal or decreased</td>
</tr>
<tr>
<td>Sialochemistry</td>
<td>Na and Cl increased</td>
<td>K increased</td>
<td>Na decreased</td>
</tr>
<tr>
<td>Sialography</td>
<td>Sialactasia</td>
<td>Thin duct system, enlarged gland</td>
<td>Usually normal, but a thin duct system and enlarged gland may be present</td>
</tr>
<tr>
<td>Complaints</td>
<td>Mouth dryness in rest and during eating or speaking</td>
<td>Persistent, bilateral swelling of the parotid glands</td>
<td>Often mouth dryness. Recurrent, short lasting (usually at most some hours), mostly unilateral swellings of the parotid gland</td>
</tr>
<tr>
<td>Schirmer's test</td>
<td>≤5mm/5min</td>
<td>Unknown, but reduction is not uncommon</td>
<td>Unknown, but reduction is not uncommon</td>
</tr>
<tr>
<td>Associated Diseases</td>
<td>sSS: associated with another connective tissue/autoimmune disease</td>
<td>Endocrine disorder</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td></td>
<td>sSS: metabolic disorder Dysfunction ANS</td>
<td>Metabolic disorder Dysfunction ANS</td>
<td>Disorder of the fluid or electrolyte balance</td>
</tr>
</tbody>
</table>

**Salivary scintigraphy**

Salivary scintigraphy is based on the ability of parotid and submandibular glands to trap the radionuclide isotope technetium-sodium (Tc99m) pertechnetate. This ability is due to the fact that Tc99m substitutes for chloride in the active sodium/potassium/chloride cotransport in the striated ducts. After intravenous injection of Tc99m, scintigraphy may reveal functional abnormality of the salivary glands through photographically recording with a gamma scintillation camera, the radiation from salivary isotope accumulation and excretion.

Improvements of salivary scintigraphy include salivary single-photon emission computed tomography (SPECT) and human immunoglobulin G (HIG) scintigraphy. Salivary SPECT creates a three-dimensional image with a rotating gamma camera without marking an ROI (region of interest) as it uses a single pixel as the ultimate ROI.

Scintigraphy is a valuable tool to measure activity of the glands, and it can be performed in the same gland at different time periods to assess progression. Unfortunately, the diagnostic accuracy is low.

**Computer tomography and magnetic resonance imaging**

Magnetic resonance imaging (MRI) depicts more accurately because soft tissue contrast resolution is better in MRI than computer tomography (CT). Detailed knowledge of the anatomy of the parotid gland and surrounding structures is necessary for evaluating and diagnosing lesions. Bilateral imaging and comparison between right and left glands is essential. CT and MRI are of less value as diagnostic tools for salivary gland disorders as Sjögren’s syndrome, sialadenosis and bacterial or viral sialadenitis.

**Ultrasound**

Ultrasound has no known contraindications and is a quick and well-accepted, non-invasive procedure. With color Doppler sonography, the complex vascular anatomy can be

---

**Figure 2**
Flow rate of parotid and submandibular/sublingual saliva (SM/SL) as a function of time after start of radiotherapy (conventional fractionation schedule, 2Gy per day, 5 days per week, total dose 60-70 GY). The parotid, submandibular and sublingual glands are located in the treatment portal. Initial flow rates were set to 100% (Adapted from Burlage et al. (12)).
accurately recorded. Its potential in routine salivary diagnostics is restricted as tissue penetration depth is limited and proper interpretation of salivary sonograms requires a great deal of experience.

**Histopathology**
The labial and parotid glands are accessible for histopathological evaluation, and biopsies from these glands are often performed routinely. In SS, a disease affecting the salivary glands in which biopsies most often are taken as a routine procedure, the parotid and labial gland biopsies are diagnostically comparable. However, a parotid biopsy is preferred, due to lower morbidity than labial biopsies in which sensory loss may occur, easier access to larger tissue samples, and earlier detection of lymphomas. In addition, repeated biopsies can be taken from the same parotid gland, making parotid biopsies an important tool in treatment evaluation (the outcomes can even be compared with saliva samples obtained from the same gland).

**Cytology**
A cytological puncture (ultrasound guided) can distinguish salivary gland disorders from lymph nodes disorders, and inflammation from malignancy.

**Figure 3**
Increase and decrease (mean values of 5 patients) in stimulated submandibular/sublingual flow rate, IgM-RF, B cells, VAS score for dry mouth during the night and multidimensional fatigue (MFI) score for fatigue following rituximab (re)treatment (baseline is 100%). Baseline values (week 0 first treatment) were stimulated submandibular/sublingual flow rate 0.09 ml/min (SD 0.07), IgM-RF 339 (SD 329), B cells 0.19 10⁹/l (SD 0.09), VAS score for dry mouth during the night 85 (SD 12), MFI score for fatigue 16 (SD 3). (modified after Meijer et al.(13)).
Subjective evaluation

VAS
A Visual Analogue Scale (VAS) is a line of, for example, 100 mm on which the patient can mark the severity of the complaint. For SS, VAS scores are available for oral dryness, oral dryness during the day, oral dryness at night, difficulty swallowing dry food without any additional liquids, difficulty swallowing any food without any additional liquids, difficulty speaking without drinking liquids, dry eyes (sensation of sand or gravel in the eyes).

MFI
The Multidimensional Fatigue Index (MFI) is a 20-item self-report instrument designed to objectively measure fatigue, including the dimensions of general fatigue, physical fatigue, mental fatigue, reduced motivation and reduced activity. This validated questionnaire detects expected differences in fatigue between groups, within groups and between conditions. (7) A higher score (range 4-20) indicates a higher level of fatigue. Fatigue is a complaint not uncommon to patients suffering from salivary gland disorders, particularly patients with salivary gland disorders related to an autoimmune disease or as a result of cancer treatment.

SF-36
The 36-item short form (SF-36) is constructed to survey health status and was designed for use in clinical practice and research, health policy evaluations and, general population surveys. The SF-36 includes one multi-item scale that assesses eight health concepts. The questionnaire has been developed for self-administration by persons 14 years of age and older or for administration by a trained interviewer. A higher score indicates a higher level of well-being. (8) Health status can severely be impaired in patients suffering from salivary gland disorders particularly in patients with salivary gland disorders related to an autoimmune disease or as a result of cancer treatment.

Dry mouth questionnaires
Objective salivary gland function is not always consistent with the subjective perception. Whether the patient reports sipping liquids to aid in swallowing dry foods, dry mouth when eating a meal, or difficulties swallowing any foods is highly predictive of salivary gland function and, therefore, clinically useful in patients who report oral dryness. (9)

Serological parameters
In systemic diseases affecting the salivary glands, serological parameters can be useful in evaluating activity and progression of the disease and in evaluating treatment. For example, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) are general parameters in peripheral blood for inflammation and are elevated in most autoimmune diseases. IgM-Rf (rheumatoid factor) and IgA correlate with B cell activity and are elevated in SS. IgM-Rf is also elevated in patients with rheumatoid arthritis and some other conditions. Antinuclear antibodies (ANA) and anti-Ro/SSA and anti-La/SSB can be detected in SS (anti-SSB is the most specific antibody).
Application of these tools in clinical research and clinical practice

The clinical application of the above-mentioned variables in treatment evaluation will be illustrated for patients with a reduced salivary flow due to head and neck radiotherapy and SS.

**Radiotherapy**

Xerostomia is a common and disturbing side effect of head and neck radiotherapy, leading to considerable morbidity, including severe oral discomfort, problems with speaking, dysphagia, and an increased incidence of caries and mucosal infections. Although new radiation techniques enabled significant sparing of the parotid glands, the amount of normal salivary gland tissue irradiated may still be substantial resulting in clinically relevant radiation-induced xerostomia. Therefore, protection against radiation-induced salivary gland damage may further improve the therapeutic gain.(10;11)

Although salivary gland tissue is a well-differentiated tissue and, theoretically, should be relatively radioresistant, studies have shown a rapid decline in parotid and submandibular/sublingual salivary flow, even after low doses of radiotherapy (figure 2). In humans, it has been reported that the TD_{50} (i.e., the dose to the whole organ leading to a complication probability of 50%) for parotid glands varies from 28.4 Gy to 31 Gy at 6 weeks increasing to 39 Gy at 1 year after completion of radiotherapy.

**Sjögren’s syndrome**

SS is a chronic lymphoproliferative autoimmune disease with disturbances of T lymphocytes, B lymphocytes and exocrine glandular cells. SS can be primary (pSS) or secondary (sSS), the latter being associated with another autoimmune disease (e.g. rheumatoid arthritis and systemic lupus erythematosus). The main symptoms of SS are xerostomia, dry eyes (keratoconjunctivitis sicca), increased caries activity (exocrine glands) and fatigue and arthralgia (systemic features). The disease can have a great impact on the quality of life of the patients. There are no causal treatment options, and treatment used today is mainly symptomatic. Dry eyes are treated with eyedrops or gel, and sometimes anti-inflammatory or immunosuppressive medication is indicated. Dry mouth is treated with saliva-stimulating medication (pilocarpine) or with saliva substitutes. Currently, drug trials are evaluating biological agents with promising first results. (figure 3)

Salivometry and sialochemistry

Salivary flow rates have diagnostic and prognostic value in SS. Since the amount and composition of saliva reflects the effects of the autoimmune process in the salivary glands, analysis of saliva may also be valuable in diagnosis, prognosis and evaluation of treatment. SS is characterized by a high sodium and high chloride concentration and a low phosphate concentration in parotid gland saliva.

Salivometry and sialochemistry, easily performed and tolerated, are valuable in measuring disease progression (figure 1) and treatment outcome. For example, rituximab significantly increased salivary secretion (figure 3) and nearly normalized salivary sodium concentration.

A pilot study of ten SS patients and ten age- and sex-matched controls demonstrated that pSS patients’ saliva contains proteomic and genomic diagnostic biomarker candidates. Proteonomics of saliva may also be useful in diagnosis, disease progression, and treatment evaluation, but further research is necessary to precisely assess its value.
Histopathology
In SS, widely accepted criteria for histologic confirmation is focal lymphocytic sialoadenitis in labial salivary glands and lymphoepithelial lesions in parotid salivary glands.
Moreover, repeated salivary gland biopsies might offer an objective method for evaluating treatment, in addition to serological and functional parameters. The parotid gland is the primary site to study changes after systemic therapy since SS lymphoproliferation occurs especially in these glands. Repeated parotid biopsies in SS patients treated with rituximab show redifferentiation of lymphoepithelial lesions into regular ducts, which is in line with the sialochemical changes in parotid saliva.

Subjective evaluation
Fatigue is one of the most disabling complaints in SS, and it leads to a substantial decrease in health related quality of life. By using the MFI, patients with pSS reported more fatigue than healthy controls on all the dimensions of the MFI, and when controlling for depression significant differences remain on the dimensions of general fatigue, physical fatigue, and reduced activity. VAS scores have been used to assess subjective sicca complaints and have been validated for patients with xerostomia. After rituximab treatment, in patients with early pSS, assessment of mouth dryness, arthralgia, physical functioning, vitality and most domains of the MFI significantly improved.

Serological parameters
Polyclonal expansion and secretory hyperactivity of B cells is an early event in pSS. This is demonstrated in the blood by increased amounts of different autoantibodies and by increased amounts of total Ig (primarily IgG). The more serious systemic complications occur mainly in patients with increased IgM-Rf levels, and levels of circulating IgM-Rf correlate positively with the number of extraglandular disease manifestations. Other researchers also reported an association between a high B cell autoreactivity (production of ANA, anti-Ro/SSA and anti-La/SSB) and the development of complications or more severe manifestations like neuropathy, kidney and pulmonary involvement. Rituximab treatment resulted in pSS patients in a rapid decrease in peripheral B cells, accompanied by a decrease in IgM-Rf levels.

Conclusion
Salivary research provides powerful tools to diagnose diseases affecting the salivary glands, to assess disease progression and to evaluate treatment. Important gland-specific parameters are sialometry, sialochemistry, and histopathology. More general tools are subjective questionnaires (e.g. VAS, MFI, SF-36) and serological parameters.
Reference List


