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EXTENDED REPORT

# Sialometry and sialochemistry: a non-invasive approach for diagnosing Sjögren's syndrome

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**Background:** Analysis of salivary variables has frequently been proposed as a diagnostic tool for Sjögren's syndrome (SS). Because univocal salivary reference values are lacking, it is currently rather difficult to use sialometry and sialochemistry for diagnosing SS unless major changes have occurred in salivary secretion and composition.
**Objective:** To define reference values of several salivary variables, which offer a possible new and non-invasive means of diagnosing SS.
**Methods:** Cut off points were selected from receiver operating characteristic curves of gland-specific sialometrical and sialochemical variables, which have proved to be potentially relevant for diagnosing SS in a previous study—that is, sodium, chloride, and phosphate concentration in stimulated parotid and submandibular/sublingual (SM/SL) saliva, unstimulated and stimulated SM/SL flow rates, and lag phase of parotid secretion, respectively. By combining the most discriminating variables, two different diagnostic approaches for SS were applied in a group of 100 patients and subsequently evaluated in a second group of 20 patients. The first approach was to combine variables by applying their cut off points into sets of criteria for a positive diagnosis of SS. The second approach was to construct a logistic regression model that predicts the true state of a patient (SS or non-SS). From both approaches, the tests with highest likelihood ratio combined with the smallest number of rejected cases were selected for clinical use.
**Results:** The most accurate test combined the stimulated SM/SL flow rate and parotid sodium and chloride concentration as salivary variables for diagnosing SS; it had a sensitivity of 0.85 and a specificity of 0.96. The selected tests proved equally accurate in the second group of patients.
**Conclusions:** Because the proposed non-invasive diagnostic tools can be easily applied, do not need a laboratory other than for routine blood testing, and are very accurate, gland-specific sialometry and sialochemistry may eventually replace other, more invasive, diagnostic techniques for diagnosing SS.

Salivary gland dysfunction is one of the key manifestations in Sjögren's syndrome (SS). Assessment of salivary gland function is, therefore, of potential diagnostic importance.[1–12] Methods for determining salivary gland (dys)function include salivary flow rate measurements (sialometry) and analysis of salivary composition (sialochemistry), for which whole saliva (oral fluid) is most frequently used. The accuracy of these techniques, however, can be improved considerably by using glandular saliva rather than whole saliva. Several distinct alterations in flow rate and composition of glandular saliva have been reported in patients with SS, not only when compared with healthy controls,[2 9 10 12] but also when compared with patients with clinical conditions resembling SS.[13] These alterations are not seen or are less obvious when using whole saliva.

The use of glandular saliva for diagnosing SS is hampered by the lack of univocal salivary reference values. As a result, sialometry and sialochemistry of glandular saliva can only be diagnostic for (the oral component of) SS when major changes in salivary secretion and composition have occurred. Our study aimed at defining thresholds (cut off points) for potentially relevant sialometrical and sialochemical variables for diagnosing SS,[13] and constructing and evaluating an easily applicable diagnostic approach for SS.

## PATIENTS AND METHODS

### Patients

Between September 1997 and August 1999, 120 patients suspected to have SS were referred to the outpatient clinic of the department of oral and maxillofacial surgery of the University Hospital Groningen by rheumatologists, internists, neurologists, ophthalmologists, ear, nose and, throat specialists, general practitioners, and dentists. Reasons for referral included mouth dryness, eye dryness, swelling of the salivary glands, arthralgia, and fatigue. The diagnostic investigation for SS, carried out in all patients, included: subjective complaints of oral and ocular dryness,[14] sialography, histopathology of salivary gland tissue, serology (SS-A- and SS-B antibodies), and eye tests (rose bengal staining and Schirmer's tear test). In this study, the revised European classification criteria for Sjögren's syndrome were used as reference standard for the diagnosis of SS,[14 15] categorising patients as primary and secondary SS and non-SS patients.

The first 100 patients—referred between September 1997 and March 1999—participated in a previous study, in which sialometrical and sialochemical variables of potential diagnostic value were identified.[13] In the present study these patients served as the observation group to define cut off points and to construct diagnostic models. The subsequent 20 patients—referred between April 1999 and August 1999—served as a test group to evaluate these diagnostic models.

The observation group consisted of 58 patients with SS (33 primary SS and 25 secondary SS; male/female ratio 1/7, mean (SD) age 53 (14) years, range 21–84) and 42 patients testing negative for SS (male/female ratio 1/20, mean age 55 (17)

**Abbreviations:** ROC, receiver operating characteristic; SM/SL, submandibular/sublingual; SS, Sjögren's syndrome

**Table 1** Group characteristics (observation group). Results are given as No (%)

| Characteristic | pSS (n=33) | sSS (n=25) | | Non-SS (n=42) | |
|---|---|---|---|---|---|
| Age at time of referral (mean) | 51 | 54 | | 55 | |
| Sex (male/female) | 3/30 | 4/21 | | 2/40 | |
| Xerogenic drugs | 10 (30) | 15 (60) | | 23 (55) | |
| Chronic fatigue | 21 (63) | 19 (76) | | 29 (69) | |
| Salivary gland swelling* | 17 (51) | 7 (28) | | 8 (19) | |
| Connective tissue disease | 0 (0) | RA: | 14 (56) | RA: | 7 (17) |
| | | SLE: | 4 (16) | SLE: | 2 (5) |
| | | Scleroderma: | 1 (4) | Scleroderma: | 1 (2) |
| | | CREST: | 1 (4) | | |
| | | Vasculitis: | 1 (4) | | |
| | | PBC: | 1 (4) | | |
| | | Polymyositis: | 1 (4) | | |
| | | Overlap syndrome: | 2 (8) | | |
| Positive salivary gland biopsy | 32 (97) | 24 (96) | | 0 (0) | |
| Positive serology | | | | | |
| Anti-SS-A | 28 (85) | 13 (52) | | 3 (7) | |
| Anti-SS-B | 15 (45) | 8 (32) | | 1 (2) | |
| Positive eye test(s)† | 25 (76) | 17 (68) | | 18 (43) | |
| Parotid sialography‡ | | | | | |
| Sialectasia (positive for SS) | 28 (100) | 16 (76) | | 3 (8) | |
| Subjective complaints§ | | | | | |
| Dry eyes | 24 (73) | 20 (80) | | 28 (67) | |
| Dry mouth | 32 (96) | 23 (92) | | 31 (74) | |

*Present at first visit; †according to European criteria (at least one positive eye test)[14]; ‡according to Blatt,[14a] percentages based on the number of patients with available information; §according to definition by European criteria listed in this table.

years, range 20–81) (table 1). The latter were diagnosed as having sialoadenosis (n=10), sodium retention dysfunction syndrome (n=12), drug induced xerostomia (n=9), or as having no alternative disease directly related to the salivary gland (n=11).

The test group consisted of seven patients with SS (two primary, five secondary SS; male/female ratio 1/6, mean age 62 (10) years, range 46–76) and 13 non-SS patients (male/female ratio 0/13, mean age 55 (11) years, range 36–76). The latter were diagnosed as having sialoadenosis (n=3), sodium retention dysfunction syndrome (n=3), drug induced xerostomia (n=2), and five patients remained without an alternative diagnosis.

The usage of xerogenic drugs—that is, antihypertensive drugs, β blockers, antihistamines, and psychotropic drugs, was relatively common in all patients (observation group: SS 43%, non-SS 55%; test group: SS 4/7 (57%), non-SS 7/13 (54%)).

### Saliva collection and chemical analysis

All salivary assessments were made before the diagnostic investigation and were performed by the same observer. Techniques of glandular saliva collection and analysis have been described in detail in a previous study.[13]

### Sialometrical and sialochemical variables studied

In a previous study the following variables were shown to be relevant for diagnosing SS: sodium, chloride, and phosphate concentration in stimulated parotid and submandibular/sublingual (SM/SL) saliva, unstimulated and stimulated flow rates of the SM/SL glands, and lag phase of parotid secretion (lag phase defined as the time between the start of salivary gland stimulation and first visible saliva secretion) (tables 2 and 3).[13]

Potassium concentration and amylase activity in parotid saliva were excluded as diagnostic variables in SS, although they differed significantly between SS positive and SS negative patients. This was because the observed differences seemed to result from the presence of patients with a non-inflammatory salivary gland disease (sialoadenosis) in the group of SS negative patients.[13] The relevant sialometrical and sialochemical variables were submitted for further statistical analysis.

### Immunological assessment

In addition to the detection of SS-A and SS-B autoantibodies as part of the diagnostic investigation, more blood tests were performed that reflect inflammatory or immunological activity, or both. This blood testing was used to search for readily available variables that might increase the diagnostic potential of sialometrical and sialochemical variables for SS. The following variables were assessed: erythrocyte sedimentation rate, C reactive protein level, full blood count, white blood count differentiation, and level of immunoglobulins (IgG, IgA, IgM).

**Table 2** Salivary flow rate (mean (SD)) of SS positive patients (SS) and SS negative patients (non-SS) in the "observation group". Statistical test used: independent sample *t* test. Significance marked with *. 95% Confidence interval of the difference (CI-diff) given. (Note: if zero is not included in the interval the difference is significant)

| Salivary flow rates | SS (n=58) | Non-SS (n=42) | CI-diff |
|---|---|---|---|
| Unstimulated flow rates | | | |
| Parotid (ml/min/gland) | 0.02 (0.04) | 0.04 (0.06) | –0.04 to 0.01 |
| SM/SL (ml/min/glands) | 0.04 (0.07) | 0.12 (0.13) | –0.12 to –0.04* |
| Stimulated flow rates | | | |
| Parotid (ml/min/gland) | 0.17 (0.19) | 0.19 (0.15) | –0.09 to 0.05 |
| SM/SL (ml/min/glands) | 0.25 (0.31) | 0.42 (0.28) | –0.29 to –0.05* |
| Parotid lag phase (s) | 171 (202) | 52 (83) | 53 to 184* |

SM/SL, submandibular/sublingual.

**Table 3** Composition of stimulated glandular salivas (mean (SD)) of SS positive patients (SS) and SS negative patients (non-SS) in the "observation group". Statistical test used: independent sample *t* test. Significance marked with *. 95% Confidence interval of the difference (CI-diff) given. (Note: if zero is not included in the interval the difference is significant)

| | Parotid saliva | | | | | SM/SL saliva | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | SS | | Non-SS | | | SS | | non-SS | | |
| | Mean (SD) | No | Mean (SD) | No | CI-diff | Mean (SD) | No | Mean (SD) | No | CI-diff |
| Sodium (mmol/l) | 24 (14) | 48 | 4 (4) | 42 | 14 to 28* | 18 (14) | 43 | 6 (6) | 41 | 8 to 17* |
| Potassium (mmol/l) | 23 (7) | 48 | 30 (21) | 42 | –14 to –8* | 20 (16) | 43 | 20 (6) | 41 | –5 to 5 |
| Chloride (mmol/l) | 33 (21) | 28 | 18 (6) | 29 | 6 to 23* | 30 (24) | 21 | 16 (5) | 31 | 4 to 23* |
| Calcium (mmol/l) | 1.2 (0.7) | 42 | 1.3 (0.8) | 37 | –0.4 to 0.3 | 1.9 (0.7) | 33 | 2.2 (1.6) | 40 | –0.8 to 0.3 |
| Phosphate (mmol/l) | 4.4 (2.1) | 39 | 5.8 (2.9) | 35 | –2.5 to –0.2* | 2.4 (1.2) | 29 | 3.9 (1.7) | 41 | –2.2 to –0.9* |
| Urea (mmol/l) | 5.2 (2.2) | 41 | 6.1 (2.5) | 40 | –1.9 to 0.2 | 3.3 (2.1) | 31 | 4.0 (1.9) | 41 | –1.6 to 0.3 |
| Total protein (g/l) | 1.4 (0.9) | 37 | 1.2 (0.6) | 37 | –1.9 to 0.5 | 0.7 (0.4) | 33 | 0.7 (0.4) | 36 | –0.1 to 0.2 |
| Amylase ($10^3$U/l) | 566 (409) | 44 | 842 (486) | 38 | –435 to –36* | 139 (212) | 31 | 138 (121) | 36 | –86 to 88 |

SM/SL, submandibular/sublingual; No, number of cases included. Missing cases result from insufficient amount of saliva available for full sialochemical analysis owing to an extremely low secretion rate in these patients.

### Statistical analysis

Data were submitted for statistical analysis using MedCalc version 5.0 in order to calculate receiver operating characteristic (ROC) curves, and the statistical package for the social sciences (SPSS) version 9.0 was used for the remaining statistical procedures,[16] including independent sample *t* test and (multiple linear) logistic regression analysis. A significance level of 0.05 was predefined in all cases.

By selecting diagnostic indicators[13] and combining these into a model, two different diagnostic approaches were applied, one by univariate and one by multivariate analysis. In the univariate analysis cut off points from ROC curves of the relevant diagnostic indicators were selected and combined into a definition for a positive diagnosis of SS. In the multivariate analysis (in which the diagnosis of SS is descriptive of a set of jointly relevant diagnostic indicators) a logistic regression model, including variables stepwise backward by likelihood ratio, was constructed.[17] It predicts the true state (SS or non-SS) of a patient.

Diagnostic indicators and tests were evaluated by the ROC curve and likelihood ratio. The ROC curve provides an index of diagnostic accuracy of a test, whereas the likelihood ratio expresses its usefulness by measuring the change in certainty of diagnosis (post-test probability = likelihood ratio × pretest probability).

## RESULTS

### Variables of inflammation and immune activation in SS

The inflammatory nature of SS was reflected by significant changes in the following blood variables: erythrocyte sedimentation rate, levels of C reactive protein, and immunoglobulins (total, IgG, and IGA) (table 4). The level of serum IgG was the most discriminating inflammatory variable for SS, with raised values (>15 g/l) in 93% of the patients with SS and in 20% of the SS negative patients.

### Sialometrical and sialochemical variables: cut off points for SS

Cut off points for a positive diagnosis of SS were selected from ROC curves of the potentially relevant sialometrical and sialochemical variables (fig 1, table 5). The cut off points were selected with emphasis on specificity (up to 1.00) to compensate for the specificity loss which will inevitably occur when variables are combined as a test for SS.

### Diagnostic approach: combined cut off points as a test for SS

Parotid and SM/SL variables were combined as a test for SS, which increased the sensitivity up to 0.92, however, at the expense of specificity (table 6). When sialochemical variables only were used, 8% of the patients could not be diagnosed

**Table 4** Results (mean (SD)) of blood tests ("observation group") in patients with primary SS (pSS), patients with secondary SS (sSS), total number of patients with SS (SS), and SS negative patients (non-SS). Significant difference between "SS" and "non-SS" marked with *. Statistical test used: independent sample *t* test. 95% Confidence interval of the difference (CI-diff) given. (Note: if zero is not included in the interval the difference is significant)

| | pSS (n=33) | sSS (n=25) | SS (n=58) | Non-SS (n=42) | CI-diff (SS *v* non-SS) |
|---|---|---|---|---|---|
| Haemoglobin (mmol/l) (N: 7.5–9.9) | 8.2 (0.5) | 7.7 (0.8) | 8.0 (0.7) | 8.4 (0.7) | –0.7 to –0.2* |
| MCV (fl) (N: 80.0–96.0) | 87.7 (4.5) | 90.0 (4.8) | 88.8 (4.7) | 89.3 (4.3) | –2.6 to 1.5 |
| Leucocyte count ($10^9$/l) (N: 4.0–11.0) | 5.7 (1.3) | 6.8 (2.0) | 6.2 (1.7) | 8.0 (2.4) | –2.7 to –0.9* |
| Neutrophils (%) (N: 45–75) | 66 (8) | 65 (13) | 65 (10) | 66 (10) | –5 to 5 |
| Lymphocytes (%) (N: 25–50) | 24 (6) | 25 (10) | 25 (8) | 26 (9) | –6 to 3 |
| Thrombocyte count ($10^9$/l) (N: 150–300) | 235 (65) | 278 (110) | 254 (89) | 253 (74) | –35 to 36 |
| ESR (mm/1st h)† (N: 0–6) | 35 (28) (100%) | 44 (38) (78%) | 40 (33) (91%) | 15 (19) (35%) | 13 to 37* |
| CRP (mg/l)† (N: 0–5) | 9 (11) (65%) | 15 (24) (68%) | 12 (18) (66%) | 3 (6) (28%) | 3 to 15* |
| Immunoglobulins(g/l): | | | | | |
| Total† (N: –18) | 29.2 (7.8) (97%) | 30.0 (14.1) (90%) | 29.9 (10.6) (94%) | 18.0 (4.5) (33%) | 8.3 to 15.5* |
| IgG† (N: 8.5–15.0) | 22.5 (7.1) (95%) | 23.0 (9.5) (90%) | 22.8 (8.1) (93%) | 13.4 (3.5) (20%) | 6.7 to 12.1* |
| IgA† (N: 0.9–4.5) | 3.7 (2.6) (16%) | 4.5 (5.1) (33%) | 4.1 (3.7) (23%) | 2.7 (1.4) (8%) | 0.3 to 2.6* |
| IgM† (N: 0.6–2.6) | 3.0 (3.8) (32%) | 2.5 (2.5) (25%) | 2.8 (3.3) (29%) | 1.9 (0.8) (20%) | –0.2 to 2.0 |

†If group mean is above normal range (N), the prevalence (%) of raised values is given between brackets.

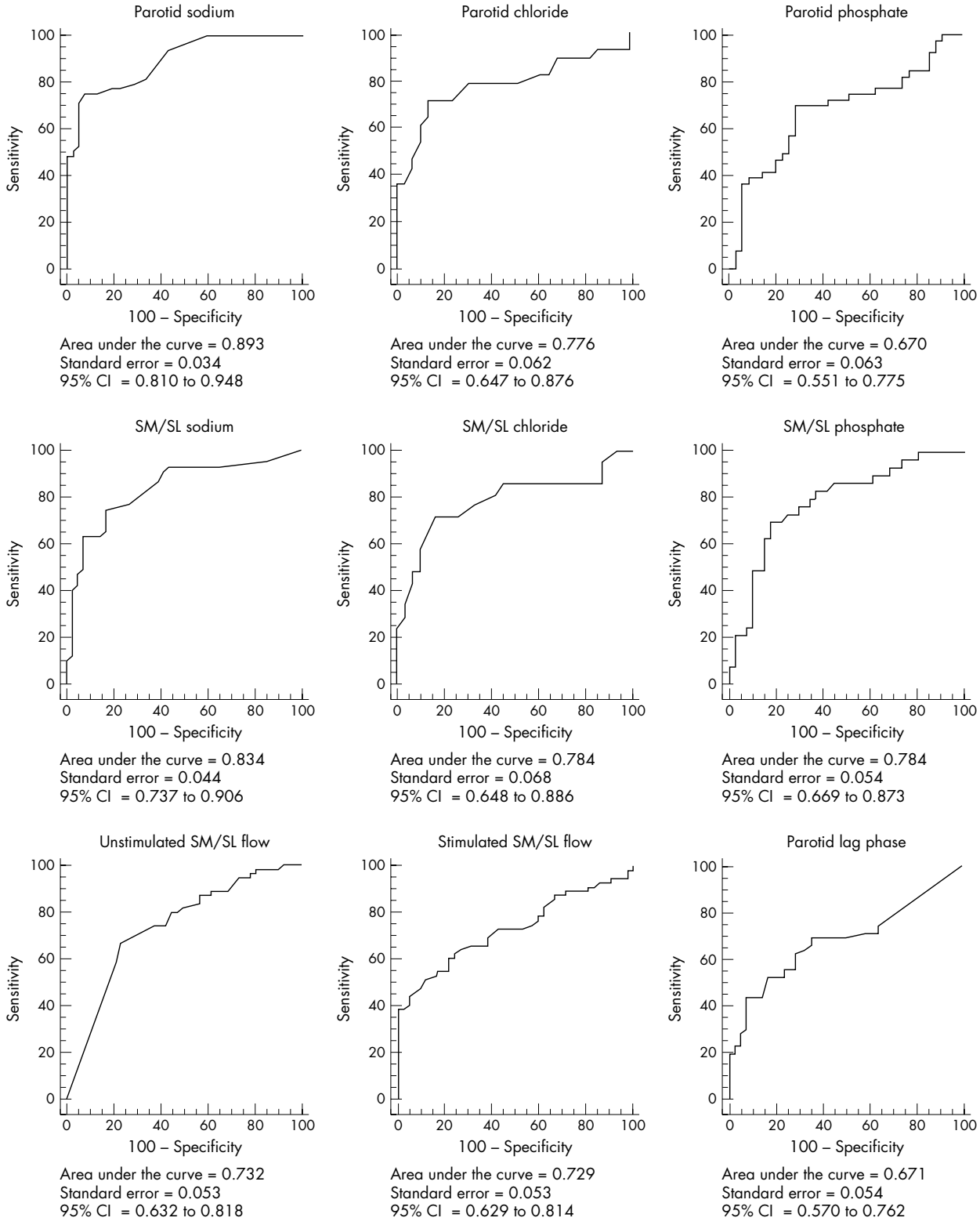


**Figure 1** Non-parametric ROC curves of sialochemical and sialometrical variables in identifying SS in patients referred for diagnosis. Fifty eight patients had SS; 42 did not.

owing to missing data (sufficient saliva could not be collected for full sialochemical analysis).

To improve the diagnostic potential of the tests, sialochemical variables were combined with sialometrical variables. This resulted in four tests with high specificity and moderate sensitivity (table 7). When sialometrical and sialochemical variables were combined, all patients could be classified (that is, no cases were lost owing to missing data).

### Diagnostic approach II: a logistic regression model as test for SS

Alternatively, logistic regression models were constructed representing a diagnostic index for SS.[17] Sialochemical variables

**Table 5** Cut off points of sialometrical and sialochemical variables for a positive diagnosis of SS. Variables are ranked by likelihood ratio

| Test variable | Cut off point | Specificity | Sensitivity | LR | PPV | NPV |
|---|---|---|---|---|---|---|
| Sialometry | | | | | | |
| Stimulated SM/SL flow* | <0.05 ml/min | 1.00 | 0.38 | ∞ | 1.00 | 0.55 |
| Parotid lag phase | >2.20 min | 0.93 | 0.42 | 6 | 0.89 | 0.54 |
| Unstimulated SM/SL flow | ≤0.01 ml/min | 0.76 | 0.67 | 3 | 0.78 | 0.64 |
| Stimulated SM/SL flow† | ≤0.20 ml/min | 0.76 | 0.62 | 3 | 0.77 | 0.60 |
| Sialochemistry (stimulated) | | | | | | |
| Parotid sodium* | ≥20 mmol/l | 1.00 | 0.48 | ∞ | 1.00 | 0.62 |
| Parotid sodium† | ≥10 mmol/l | 0.95 | 0.71 | 14 | 0.94 | 0.74 |
| Parotid chloride | ≥30 mmol/l | 0.93 | 0.46 | 7 | 0.87 | 0.64 |
| SM/SL chloride | >20 mmol/l | 0.90 | 0.57 | 6 | 0.80 | 0.76 |
| SM/SL sodium | >10 mmol/l | 0.85 | 0.63 | 4 | 0.82 | 0.69 |
| SM/SL phosphate | ≤2.50 mmol/l | 0.85 | 0.55 | 4 | 0.73 | 0.73 |
| Parotid phosphate | ≤4.75 mmol/l | 0.71 | 0.67 | 2 | 0.72 | 0.66 |

*Restricted cut off point, with highest specificity; †widened cut off point, with increased sensitivity and decreased specificity.
LR, likelihood ratio; PPV, positive predictive value; NPV, negative predictive value; SM/SL, submandibular/sublingual.

**Table 6** Sialochemical and sialometrical variables as tests for SS

| Criteria for classifying SS test (cut off point approach) | Specificity | Sensitivity | LR | No | PPV | NPV |
|---|---|---|---|---|---|---|
| **Parotid sialochemical variables** | | | | | | |
| 1. Parotid (stimulated) sodium ≥20 mmol/l or Parotid (stimulated) chloride ≥30 mmol/l | 0.95 | 0.56 | 11 | 90 | 0.93 | 0.66 |
| 2. Parotid (stimulated) sodium ≥20 mmol/l or Parotid (stimulated) chloride ≥30 mmol/l or Parotid (stimulated) phosphate ≤4.75 mmol/l | 0.69 | 0.81 | 3 | 90 | 0.75 | 0.76 |
| **SM/SL sialochemical variables** | | | | | | |
| 3. SM/SL (stimulated) sodium >10 mmol/l or SM/SL (stimulated) chloride >20 mmol/l or SM/SL (stimulated) phosphate ≤2.50 mmol/l | 0.71 | 0.81 | 3 | 84 | 0.74 | 0.78 |
| **Parotid and SM/SL sialochemical variables** | | | | | | |
| 4. Parotid (stimulated) sodium ≥20 mmol/l or Parotid (stimulated) chloride ≥30 mmol/l or Parotid (stimulated) phosphate ≤4.75 mmol/l or SM/SL (stimulated) sodium >10 mmol/l or SM/SL (stimulated) chloride >20 mmol/l or SM/SL (stimulated) phosphate ≤2.50 mmol/l | 0.62 | 0.92 | 2 | 92 | 0.74 | 0.87 |
| **Sialometrical variables** | | | | | | |
| 5. Stimulated SM/SL flow <0.05 ml/min or Parotid lag phase >2.20 min | 0.93 | 0.57 | 8 | 100 | 0.92 | 0.61 |

LR, likelihood ratio; No, number of cases included in the analysis out of total (observation group n=100). Cases were rejected when data was missing in all variables used in the criteria; PPV, positive predictive value; NPV, negative predictive value; SM/SL, submandibular/sublingual.

were included in a logistic regression model stepwise backward by likelihood ratio, which resulted in tests for SS with high specificity (average 0.93) and moderate sensitivity (average 0.76 )(tests 10–12, table 8). However, about 50% of the patients could not be classified owing to missing data (lack of saliva).

When both sialometrical and sialochemical variables were included in a logistic regression model, 10% of the patients could not be classified owing to missing data. However, no improvement of test accuracy (specificity, sensitivity) was seen.

### Salivary test enhancement by including serum IgG

By stratifying for raised serum IgG (normal ≤15 g/l) and accordingly widening the salivary cut off points (for the cut off point approach), or including serum IgG in the logistic model, the sensitivity of the sialometrical/sialochemical tests for SS increased by, on average, 15% (tables 9 and 10). The calculated likelihood ratio, however, remained unchanged.

### Evaluation

Both diagnostic approaches were evaluated by applying them in a separate group of patients, the test group. The outcomes for salivary flow, salivary composition, and blood tests in the test group were comparable with those in the SS and non-SS patients in the observation group (data not shown). The test definitions (cut off points) and test formulas (logistic regression) with the highest likelihood ratio, combined with the lowest number of rejected cases (owing to missing data), were considered as the most useful clinically and were, therefore, evaluated. Table 11 list the selected tests. These tests were also evaluated after including serum IgG.

The selected test definitions and test formulas had on average equal sensitivity and specificity in the test group compared with the observation group. By using the selected test formulas (logistic regression), 15% of the patients in the test group could not be diagnosed owing to missing data. However, by using the selected test definitions (cut off point), all patients could be classified.

## DISCUSSION

Many sialometrical and sialochemical variables can contribute to the diagnosis of SS. Some have greater diagnostic potential than others, as can be determined by the likelihood ratio as well as by the shape of an ROC curve. High potential is

**Table 7** Sialometrical and sialochemical variables combined as tests for SS

| Criteria for classifying SS test (cut off point approach) | Specificity | Sensitivity | LR | No | PPV | NPV |
|---|---|---|---|---|---|---|
| **Sialometrical and sialochemical variables** | | | | | | |
| 6. Stimulated SM/SL flow <0.05 ml/min or Parotid (stimulated) sodium ⩾20 mmol/l | 1.00 | 0.66 | ∞ | 100 | 1.00 | 0.68 |
| 7. Stimulated SM/SL flow <0.05 ml/min or Parotid (stimulated) sodium ⩾20 mmol/l or Parotid (stimulated) chloride ⩾30 mmol/l | 0.95 | 0.71 | 14 | 100 | 0.95 | 0.70 |
| 8. Stimulated SM/SL flow <0.05 ml/min or Parotid (stimulated) sodium ⩾20 mmol/l or Parotid lag phase >2.20 min | 0.93 | 0.74 | 11 | 100 | 0.93 | 0.72 |
| 9. Stimulated SM/SL flow <0.05 ml/min or Parotid (stimulated) sodium ⩾20 mmol/l or Parotid (stimulated) chloride ⩾30 mmol/l or Parotid lag phase >2.20 min | 0.90 | 0.78 | 8 | 100 | 0.92 | 0.75 |

LR, likelihood ratio; No, number of cases included in the analysis out of total (observation group n=100); PPV, positive predictive value; NPV, negative predictive value; SM/SL, submandibular/sublingual.

**Table 8** Logistic regression models (formulas) for sialometrical and sialochemical variables combined as tests for SS. The probability of a subject having SS is represented in logistic regression formula as "p". A p value <0.5 is considered positive for SS. For comparison with cut off point approach see tables 6 and 7

| Formulas for classifying SS test (logistic regression approach) | Specificity | Sensitivity | LR | No | PPV | NPV |
|---|---|---|---|---|---|---|
| **Parotid sialochemical variables** | | | | | | |
| 10. Parotid sodium ($X_1$) and parotid chloride ($X_2$) (parotid phosphate removed by analysis) Formula: LNR ($p$)=2.8603–0.2044$X_1$–0.0547$X_2$ | 0.93 | 0.75 | 11 | 57 | 0.91 | 0.79 |
| **SM/SL sialochemical variables** | | | | | | |
| 11. SM/SL chloride ($X_1$) and SM/SL phosphate ($X_2$) (SM/SL sodium removed by analysis) Formula: LNR ($p$)=0.9882–0.1411$X_1$+0.6928$X_2$ | 0.90 | 0.72 | 7 | 49 | 0.81 | 0.85 |
| **Parotid and SM/SL sialochemical variables** | | | | | | |
| 12. Parotid sodium ($X_1$) and parotid chloride ($X_2$) and SM/SL phosphate ($X_3$) (SM/SL sodium, SM/SL chloride, and parotid phosphate removed by analysis) Formula: LNR ($p$)=1.6479–0.2274$X_1$–0.1268$X_2$+1.3265$X_3$ | 0.96 | 0.80 | 20 | 43 | 0.92 | 0.90 |
| **Sialometrical variables** | | | | | | |
| 13. Stimulated SM/SL ($X_1$) flow and parotid lag phase ($X_2$) Formula: LNR ($p$)=–0.1546+1.1286$X_1$–0.0050$X_2$ | 0.60 | 0.67 | 2 | 100 | 0.68 | 0.58 |
| **Sialometrical and sialochemical variables** | | | | | | |
| 14. Parotid sodium ($X_1$) and stimulated SM/SL flow ($X_2$) (parotid chloride and lag phase removed by analysis) Formula: LNR ($p$)=0.6765–0.2353$X_1$+3.3929$X_2$ | 0.90 | 0.76 | 8 | 90 | 0.89 | 0.78 |

LR, likelihood ratio; No, number of cases included in the analysis out of total (observation group n=100). Cases were rejected when data were missing in any of the variables in the formula; PPV, positive predictive value; NPV, negative predictive value; LNR, $\log_e$; SM/SL, submandibular/sublingual.

indicated by a high likelihood ratio and by an ROC curve that approaches the upper left corner of the diagram.

Owing to the nature of the disease, it is not always possible to collect sufficient saliva for full sialochemical analysis, whereas the salivary flow rate can obviously be determined at any level of glandular dysfunction. On the other hand, when the disease is still incipient, sialometry may not show any loss of glandular function, whereas the salivary composition may already have changed significantly. Therefore, a combination of at least one sialometrical and one sialochemical variable is preferred for a diagnostic test to cover all stages of the disease. Although the use of a combination of variables has the advantage of an increased sensitivity, the number of variables to be combined is limited by the extent of loss of specificity.

Variables can be combined by applying their cut off points into a set of criteria for a diagnosis of SS, but also by using a logistic regression model that predicts the true state of a patient (SS or non-SS) based upon the selected variables. The univariate method—the cut off point approach—has the advantage that the sensitivity and specificity of the test can be adjusted to its purpose (for example, screening, diagnosis) by selecting the proper cut off points. The multivariate method—the logistic regression approach—has the advantage of using the full (joint) discriminative potency of the variables included and correcting for their mutual influences. This method has the limitation that if any variable is missing the test cannot be carried out, because all variables are required in the formula. This may frequently occur, as sialochemistry is often impaired in xerostomic patients by lack of saliva. However, this problem of having only small amounts of saliva available for sialochemical analysis may be less important if only a few variables are selected for assessment (only the variables required for the diagnostic test), compared with the wide selection of variables which we needed to assess in our study.

**Table 9** Diagnostic potential of sialometrical/sialochemical tests for SS, after stratifying for raised serum IgG (normal ≤15 g/l). Adjusted (widened) salivary cut off points are applied if serum IgG is raised in order to improve the sensitivity

| | Criteria for classifying SS test (cut off point approach) | Specificity | Sensitivity | LR | No | PPV | NPV |
|---|---|---|---|---|---|---|---|
| | **Sialometrical variables and IgG** | | | | | | |
| 15. | IgG ≤15: Stimulated SM/SL flow <0.05 ml/min<br>IgG >15: Stimulated SM/SL flow ≤0.20 ml/min | 0.95 | 0.53 | 11 | 100 | 0.94 | 0.61 |
| | **Sialochemical variables and IgG** | | | | | | |
| 16. | IgG ≤15: Parotid (stimulated) sodium ≥20 mmol/l<br>IgG >15: Parotid (stimulated) sodium ≥10 mmol/l | 1.00 | 0.69 | ∞ | 90 | 1.00 | 0.74 |
| | **Sialometrical/sialochemical variables and IgG** | | | | | | |
| 17. | IgG ≤15: Parotid (stimulated) sodium ≥20 mmol/l or Stimulated SM/SL flow <0.05 ml/min<br>IgG >15: Parotid (stimulated) sodium ≥10 mmol/l or Stimulated SM/SL flow ≤0.20 ml/min | 0.95 | 0.83 | 17 | 100 | 0.96 | 0.80 |
| 18. | IgG ≤15: Parotid (stimulated) sodium ≥20 mmol/l or Parotid (stimulated) chloride ≥30 mmol/l or Stimulated SM/SL flow <0.05 ml/min<br>IgG >15: Parotid (stimulated) sodium ≥10 mmol/l or Parotid (stimulated) chloride ≥30 mmol/l or Stimulated SM/SL flow ≤0.20 ml/min | 0.90 | 0.86 | 9 | 100 | 0.93 | 0.83 |
| 19. | IgG ≤15: Parotid (stimulated) sodium ≥20 mmol/l or Parotid (stimulated) chloride ≥30 mmol/l or Stimulated SM/SL flow <0.05 ml/min or Parotid lag phase >2.20 min<br>IgG >15: Parotid (stimulated) sodium ≥10 mmol/l or Parotid (stimulated) chloride ≥30 mmol/l or Stimulated SM/SL flow ≤0.20 ml/min or Parotid lag phase >2.20 min | 0.88 | 0.91 | 8 | 100 | 0.91 | 0.88 |

LR, likelihood ratio; No, number of cases included in the analysis out of total (observation group n=100). Cases were rejected when data were missing in all variables used in the criteria; PPV, positive predictive value; NPV, negative predictive value; SM/SL, submandibular/sublingual.

**Table 10** Logistic regression models (formulas) for sialometrical/sialochemical variables and serum IgG combined as tests for SS. The probability of a subject having SS is represented in logistic regression formula as "p". A p value <0.50 is considered positive for SS. For comparison with cut off point approach see table 9

| | Formulas for classifying SS test (logistic regression approach) | Specificity | Sensitivity | LR | No | PPV | NPV |
|---|---|---|---|---|---|---|---|
| | **Sialometrical variables and IgG** | | | | | | |
| 20. | Stimulated SM/SL flow ($X_1$) and serum IgG ($X_2$)<br>Formula: LNR ($p$)=$5.2645+3.3610X_1-0.3968X_2$ | 0.85 | 0.84 | 6 | 100 | 0.88 | 0.81 |
| | **Sialochemical variables and IgG** | | | | | | |
| 21. | Parotid sodium ($X_1$) and serum IgG ($X_2$)<br>Formula: LNR ($p$)=$6.5479-0.1596X_1-0.3193X_2$ | 0.90 | 0.82 | 8 | 90 | 0.90 | 0.82 |
| | **Sialometrical/sialochemical variables and IgG** | | | | | | |
| 22. | Stimulated SM/SL flow ($X_1$), parotid sodium ($X_2$), and serum IgG ($X_3$)<br>Formula: LNR ($p$)=$5.5999+5.3278X_1-0.2138X_2-0.3501X_3$ | 0.93 | 0.83 | 12 | 90 | 0.92 | 0.84 |
| 23. | Stimulated SM/SL flow ($X_1$), parotid sodium ($X_2$), Parotid chloride ($X_3$) and serum IgG ($X_4$) (parotid lag phase removed by analysis)<br>Formula: LNR ($p$)=$6.9853+5.7582X_1-0.2423X_2-0.0432X_3-0.3755X_4$ | 0.96 | 0.85 | 21 | 53 | 0.96 | 0.87 |

LR, likelihood ratio; No, number of cases included in the analysis out of total (observation group n=100). Cases were rejected when data were missing in any of the variables in the formula; PPV, positive predictive value; NPV, negative predictive value; LNR= $\log_e$; SM/SL, submandibular/sublingual.

The limitation as well as the strength of the logistic regression model is reflected by the results from this study. The diagnostic approach with a logistic regression model was frequently inapplicable (rejected cases varying from 10 to 50%), whereas the approach by combined cut off points was far more universally applicable (rejected cases varying from 0 to 10%). The impaired applicability of the logistic regression model was counterbalanced by a higher likelihood ratio (likelihood ratio of 21 *v* 17 of the cut off point approach). Both approaches (logistic regression and cut off point) were adequate for diagnosing SS using only two or three salivary variables. The logistic regression approach, having the highest likelihood ratio, is the best option for diagnosing individual patients, whereas the cut off point approach, being more universally applicable, may have greater value for diagnosing series of patients.

From both methods, the tests that combined the highest likelihood ratio with the lowest number of rejected cases were selected for clinical use (table 11: tests 7, 14, 17, and 22). The selected tests appeared to be equally accurate on a separate group of patients, indicating their general applicability. In clinical practice, only two salivary variables are required for diagnosing SS—that is, the sodium concentration in stimulated parotid saliva and the stimulated secretory flow rate of the SM/SL glands. With these variables, the logistic regression formula (table 8: test 14) accurately predicts the presence or

**Table 11** Evaluation of sialometrical/sialochemical tests for SS on a "test group". The probability of a subject having SS is represented in logistic regression formula as "p". A p value <0.50 is considered positive for SS. For comparison with test results in the "observation group" see tables 7–10

| | Criteria (cut off points)/test formulas test (logistic regression) for classifying SS | Specificity | Sensitivity | LR | No | PPV* | NPV* |
|---|---|---|---|---|---|---|---|
| | **Cut off points: sialometrical/sialochemical variables (for comparison see table 7)** | | | | | | |
| 7. | Stimulated SM/SL flow <0.05 ml/min or<br>Parotid (stimulated) sodium ⩾20 mmol/l or<br>Parotid (stimulated) chloride ⩾30 mmol/l | 0.86 | 0.83 | 6 | 20 | 0.71 | 0.92 |
| | **Logistic regression: sialometrical/sialochemical variables (for comparison see table 8)** | | | | | | |
| 14. | Parotid sodium ($X_1$) and stimulated SM/SL flow ($X_2$)<br>(parotid chloride and lag phase removed by analysis)<br>Formula: LNR ($p$)=0.6765–0.2353$X_1$+3.3929$X_2$ | 1.00 | 0.67 | ∞ | 17 | 1.00 | 0.85 |
| | **Cut off points: sialometrical/sialochemical variables and IgG (for comparison see table 9)** | | | | | | |
| 17. | IgG ⩽15: Parotid (stimulated) sodium ⩾20 mmol/l or<br>Stimulated SM/SL flow <0.05 ml/min<br>IgG >15: Parotid (stimulated) sodium ⩾10 mmol/l or<br>Stimulated SM/SL flow <0.20 ml/min | 0.86 | 0.83 | 6 | 20 | 0.71 | 0.92 |
| | **Logistic regression: sialometrical/sialochemical variables and IgG (for comparison see table 10)** | | | | | | |
| 22. | Stimulated SM/SL flow ($X_1$), parotid sodium ($X_2$), and serum IgG ($X_3$)<br>Formula: LNR ($p$)=5.5999+5.3278$X_1$–0.2138$X_2$–0.3501$X_3$ | 0.83 | 0.91 | 13 | 17 | 0.83 | 0.91 |

LR, likelihood ratio; No, number of cases included in the analysis out of total (test group n=20). Cases were rejected when data were missing; PPV, positive predictive value; NPV, negative predictive value; *prevalence of SS in test group 35%; LNR= $\log_e$; SM/SL, submandibular/sublingual.

absence of SS. When data are missing, the cut off point criteria (table 7: test 7) can be used as an alternative to diagnose the patient.

Because SS is a chronic disease with overactivation of the immune system, it is not surprising to find that serum IgG is the most discriminating immunological variable. This finding is in agreement with published reports.[18–21] By including this serological variable, the diagnostic approach of SS by sialometry and sialochemistry may be further improved, because the presence of raised serum IgG is accompanied by an increase of prior probability for SS. Because only the sensitivity of the test is optimised (no remarkable increase of likelihood ratio was observed), we conclude that adding serum IgG to the method of choice (tables 9, 10: tests 17 and 22) may be worthwhile in (patient) populations with low prevalence of SS, but not in general.

Until now, sialometry and sialochemistry have been useful methods that contribute to the differentiation of salivary gland diseases. By defining cut off points and constructing proper models, glandular sialometry and sialochemistry have become clinically applicable methods which, when combined, form a reliable diagnostic technique for SS. Because the collection of saliva takes only few minutes and is non-invasive, and the analysis requires no laboratory other than for routine blood testing, we feel that glandular sialometry and sialochemistry may eventually replace other, more invasive, techniques for diagnosing SS.

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