Rose Bengal Staining of the Temporal Conjunctiva Differentiates Sjögren’s Syndrome from Keratoconjunctivitis Sicca

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PURPOSE. To compare the clinical presentation of 231 patients with primary Sjögren’s syndrome (pSS) with 89 patients with aqueous-deficient dry eye (keratoconjunctivitis sicca; KCS), to determine those procedures that best differentiate these groups in the eye care clinic.

METHODS. The records of all patients seen at the University Health Network Sjögren’s Syndrome Clinic from October 1992 to July 2006 were reviewed and documented. The diagnosis of pSS was based on the AECC (American European Consensus Criteria) of 2002. KCS control subjects were non-SS patients with symptoms of dry eye and Schirmer scores of ≤10 mm in 5 minutes in at least one eye. There were 90 variables used in the analysis of the total database. Recursive partitioning was used to generate tree diagrams that demonstrated which characteristics best distinguished pSS from KCS.

RESULTS. Recursive partitioning of the full database demonstrated that the serum immunoglobulin Ro and the status of the salivary gland biopsy were most important in distinguishing pSS and KCS. The presence of rose bengal staining of the temporal conjunctiva was the most important noninvasive ocular variable that separated the groups. Total rose bengal staining also improved sensitivity. When only noninvasive techniques were used, staining of the temporal conjunctiva and salivary gland biopsy were most important in distinguishing pSS from KCS.

CONCLUSIONS. Rose bengal staining of the ocular surface is an important observation in the detection of SS and the differentiation of pSS and KCS. (Invest Ophthalmol Vis Sci. 2010;51: 2381–2387) DOI:10.1167/iovs.09-4188

Sjögren’s syndrome (SS) is a systemic autoimmune disease that presents in eye care offices with symptoms of dry eye (DE). It is important to differentiate SS patients from those with other forms of DE disease, such as keratoconjunctivitis sicca (KCS), as they have systemic complications associated with the disease and up to a 40 times greater risk of MALT (mucosa-associated lymphatic tissue) lymphoma.1–3

The American European Consensus Criterion (AECC) for primary SS (pSS) has become the gold standard for the diagnosis of SS.4 The criteria include:

- Criterion I. Symptoms of DE
- Criterion II. Symptoms of dry mouth
- Criterion III. Signs of DE: ocular surface staining and/or Schirmer
- Criterion IV. Positive salivary gland biopsy
- Criterion V. Signs of dry mouth: salivary flow
- Criterion VI. The presence of anti-Ro and/or anti-La in the serum

This diagnostic standard requires that at least four of six of these criteria be present, one of which must be a positive antinuclear antibody test and/or a positive salivary gland biopsy result. These two critical diagnostic tests are both invasive and expensive and should be administered only to those most likely to have the disease.

In an effort to improve the eye care practitioner’s ability to make accurate referrals to rheumatology, we asked the question: Can SS-related DE be differentiated from aqueous deficient DE in the eye care clinic through noninvasive testing?

METHODS

Ethics approval was received from the University Health Network Research Ethics Board at the University of Toronto for this study. The protocol of the study adhered to the Declaration of Helsinki. A review of charts of patients seen at the University Health Network Sjögren’s Syndrome Clinic from its inception in October 1992 up to July 2006 was completed. pSS was diagnosed according to the American European consensus criteria (AECC) of 2002.4 KCS patients were those who did not meet the criteria for SS but did have symptoms of DE and Schirmer scores ≤10 mm.

Clinical Procedures of the SS Clinic

Patients were referred to the Sjögren’s Syndrome Clinic by rheumatologists and ophthalmologists who suspected or had confirmed that their patients had SS. Each one was examined on the same day by all practitioners and laboratories. During the day, patients attended optometry/opthalmology, rheumatology, x-ray, blood and urine collection, otolaryngology, and dentistry stations. The minor salivary gland biopsies, urine, and blood were analyzed within 1 month of the visit. The clinical testing regimen was determined by each discipline and adhered to the standard of clinical care for SS patients. All clinicians met within the month to review the chart and determine the diagnosis.
Patients completed a dryness questionnaire before the visit to the hospital and brought that information with them. The paper asked:

1. Have your eyes been dry for at least 3 months?  Yes  No
2. Do you use eye drops at least 3 times a day?  Yes  No

Please mark the scale to represent the level of eye dryness that you experience.

Not dry at all         1------2------3------4------5------6------7------8------9------10          extremely dry

The dry mouth questionnaire followed the same format:

1. Has your mouth been dry for at least 3 months?  Yes  No
2. Must you drink water when you eat dry foods?  Yes  No

Please mark the scale to represent the level of mouth dryness that you experience.

Not dry at all         1------2------3------4------5------6------7------8------9------10          extremely dry

Each patient proceeded through the various offices. The procedures and tests are listed in Table 1.

**Chart Review and Statistical Procedures**

The charts were reviewed by a trained optometry student and the data were entered into a database (Excel; Microsoft, Redmond, WA). When there were questions regarding the entries, such as unclear impressions or missing data, the rheumatologist was the final arbiter. A random sample of the charts was reviewed by one of the authors (BC) to ensure the quality of the data entered.

Statistical analysis included recursive partitioning, using the R statistical analysis language. The entire cohort (n = 320). Recursive partitioning (R package ‘rpart’) was used to make classification trees that demonstrated which characteristics best distinguished pSS from KCS.

### Table 1. The Clinical Procedures of the Sjögren’s Syndrome Clinic

<table>
<thead>
<tr>
<th>Ophthalmology</th>
<th>Rheumatology</th>
<th>Otolaryngology</th>
<th>Dentistry</th>
</tr>
</thead>
<tbody>
<tr>
<td>History: general ocular and dry eye</td>
<td>Musculoskeletal: myalgia, arthralgia</td>
<td>Inspection of ears, nose and throat</td>
<td>History: general oral and dry mouth</td>
</tr>
<tr>
<td>Schirmer I</td>
<td>Vascular: Raynaud’s phenomenon</td>
<td>Minor salivary gland biopsy</td>
<td>Inspection of mouth, tongue, and palate</td>
</tr>
<tr>
<td>General slit lamp examination with white light.</td>
<td>Cutaneous: dry skin, skin rash/vasculitis, alopecia, pruritus, photosensitivity</td>
<td></td>
<td>Unstimulated salivary flow</td>
</tr>
<tr>
<td>Fluorescein added from strips wetted with nonpreserved saline, using cobalt blue light.</td>
<td>Pulmonary manifestations</td>
<td></td>
<td>Stimulated salivary flow</td>
</tr>
<tr>
<td>Cornea graded 0–3, using five sections with cobalt blue light.</td>
<td>GI manifestations: dyspepsia, heartburn</td>
<td></td>
<td></td>
</tr>
<tr>
<td>One drop of topical anesthetic, followed by 1% liquid rose bengal. Staining of conjunctiva graded by van Bijsterveld scale.</td>
<td>Neurological manifestations</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lymphoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fatigue</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fibromyalgia</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Gynecology: vaginal dryness, dyspareunia</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Maternity: history of recurrent fetal loss</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Associated systemic diseases: hypothyroidism, diabetes, primary biliary cirrhosis, systemic lupus erythematosus, scleroderma, mixed connective tissue disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Renal manifestations: tubular acidosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>X-ray of hands, wrists, and chest evaluated for erosions, calcinosis, or interstitial fibrosis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In recursive partitioning, a tree-structured set of questions about the descriptor variables is used to recursively divide the data into groups that are as homogeneous as possible, and the data are presented as nodes of a tree. To identify the best split for a specific node, the algorithm considers all possible binary splits for each descriptor variable and chooses the optimal one. Thus, the ‘perfect’ variable would divide the patients in this study into 89 KCS and 231 pSS in the first splitting, providing 100% sensitivity and specificity.

For a categorical variable with only two levels, such as the presence of symptoms of dry mouth, there was only one split. For graded variables, such as rose bengal staining of the ocular surface, the split was defined by values less than or more than a chosen value. Several trees were created with different sets of variables.

**RESULTS**

A total of 420 charts were reviewed and 42 (10%) were excluded for unclear SS diagnosis, absence of SS and Schirmer scores higher than 10 mm, and absence of SS and no symptoms of DE. Also, the 58 (13.6%) patients with a diagnosis of secondary SS were excluded from this analysis. The study group included 89 KCS and 231 pSS patients and a total of 90 variables: 75 noninvasive and 15 invasive. The two cohorts are described in Table 2 and the variables are described in Table 3.

The recursive partitioning analysis produced the following trees (Figs. 1–3). The sensitivity and specificity of these trees are shown in Table 4.

**DISCUSSION**

The algorithms created by the recursive partitioning analysis of this large cohort of DE patients demonstrates the importance
of rose bengal staining of the conjunctiva in both identifying and differentiating pSS. In tree 1, when all 90 variables were used, only temporal staining of the conjunctiva contributed to the classification after serum Ro and biopsy findings were used. In tree 2, when only noninvasive variables except for salivary flow testing were used, temporal rose bengal staining was the most important variable for classification, followed by the severity of dry mouth. The remaining valuable noninvasive variables were the presence of eye signs as per the AECC, inferior corneal staining with fluorescein, and total rose bengal staining of greater than or equal to 2.25/9. Of note are the absence of other systemic factors and objective mouth variables in this tree. Tree 3 solidifies the surprising absence of impact of objective mouth variables in the differentiation of SS in this cohort, as the addition of salivary flow measurements did not improve sensitivity and specificity. The importance of noninvasive and inexpensive testing, such as rose bengal staining, to capture those patients with pSS is highlighted by comparing the first split in trees 1 and 2. The serum tests and biopsy tests captured 198 of 231 pSS patients whereas in tree 2, rose bengal staining and a dry mouth questionnaire captured 193 of 231 pSS patients. The difference in cost and invasiveness makes the simpler tests very appealing to the patient.

Although the common approach to comparing such cohorts is analysis of variance,7–10 cohorts of other rheumatic populations11,12 and SS13 have been studied with recursive partitioning. Our data lent itself to this type of analysis for many reasons. First, this form of analysis mimics the eye care practitioner's decision-making processes. Clinicians partition their patients daily into diagnostic categories, such as those with sight threatening or non–sight-threatening lesions or wet or dry AMD, for example. Also recursive partitioning is a good way of dealing with chart review data because, by its nature, it inevitably has both missing data points and changes in procedures over time. For example, in our data, the stimulated salivary flow test was used until 2004, when we added un-

### Table 3. Variables Measured in SS Clinic

<table>
<thead>
<tr>
<th>Classification of Variables</th>
<th>Noninvasive Variables</th>
<th>Invasive Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics</td>
<td>Age, sex</td>
<td>Biopsy, serum antibodies for Ro and/or La.</td>
</tr>
<tr>
<td>AECC criteria</td>
<td>Dry eye symptoms, dry mouth symptoms, dry eye signs (positive Schirmer or rose bengal), salivary flow positive</td>
<td>Biopsy focus score 0–4, Chisholm Mason biopsy score 3–4 (at least 1 focus in 4mm) is SS.</td>
</tr>
<tr>
<td>Variables associated with the AECC criteria</td>
<td>Severity of dry eye symptoms (0–10), years of dry eye, severity of dry mouth symptoms (0–10), years of dry mouth, rose bengal (RB) staining score of 4/9 or greater, RB value in worse eye 0–9, Schirmer failed (i.e., ≤5 mm, Schirmer value worse eye), unstimulated salivary flow score, stimulated salivary flow score.</td>
<td>Ro present, La present</td>
</tr>
<tr>
<td>Systemic autoimmune diagnoses</td>
<td>Presence of mixed connective tissue (CT) disease, CREST (calcinosis, Raynaud’s, esophageal, sclerodactyly, telangiectasia), RA, SLE, PBC.</td>
<td>IgG, IgM, IgA, M spike, WBC, ANA, RF, ATA. Anti-mic, TSH, AMA, SMA.</td>
</tr>
<tr>
<td>Other signs of autoimmune disease</td>
<td>Parotid swell, myalgia, arthralgia, fibromyalgia, lymphoma, X-ray positive.</td>
<td></td>
</tr>
<tr>
<td>Other systemic diseases</td>
<td>Blood work</td>
<td></td>
</tr>
<tr>
<td>Blood work</td>
<td>Diabetes, hypothyroid</td>
<td></td>
</tr>
<tr>
<td>Other systemic symptoms</td>
<td>Presence of: dysphagia, dyspepsia, vaginal dryness, dyspareunia, Raynaud’s, dry skin, pruritus, rash, alopecia, photosensitive skin.</td>
<td></td>
</tr>
<tr>
<td>Medications by category</td>
<td>Diuretics, depression, anticholinergics, anti-inflammatories</td>
<td></td>
</tr>
<tr>
<td>Other eye signs</td>
<td>Meibomian gland dysfunction, superior limbic keratoconjunctivitis (SLK), blepharitis</td>
<td></td>
</tr>
<tr>
<td>Rose bengal stain</td>
<td>Score 0–3: Worse eye (WE) temporal stain, WE corneal stain, WE nasal stain, each eye total of 3 areas.</td>
<td></td>
</tr>
<tr>
<td>Fluorescein stain</td>
<td>Presence of at least grade 1: WE temporal cornea, WE nasal cornea, WE superior cornea, WE inferior cornea, WE central cornea, each cornea by five quadrants</td>
<td></td>
</tr>
<tr>
<td>Dental information</td>
<td>Corneal stain of any kind</td>
<td></td>
</tr>
<tr>
<td>Dental information</td>
<td>Missing teeth 0–32, filled teeth, cervical cavities, D score 0–32, DMF (dentate, missing filled), candidiasis</td>
<td></td>
</tr>
</tbody>
</table>
stimulated salivary flow. Also, as with most data associated with complex diseases, our data are influenced by the complex and unbalanced relationship among the clinical variables. Classification trees are suitable for such analysis because they deal well with nonlinear relationships, high-order interactions, and missing values. These trees are descriptive, in that the model represents the systemic structure of the data in a simple manner. They are also predictive, in that a model accurately predicts unobserved data.

In fact, using this type of analysis, we have shown that SS can be identified in eye care offices with great sensitivity and can be differentiated from KCS to a moderate degree without invasive testing. Indeed, rose bengal staining of the temporal conjunctiva in the worse eye is a sensitive test for SS. Using

**Figure 2.** Tree 2: pSS and KCS. All noninvasive variables except salivary flow ($n = 74$). All numbers reflect KCS/pSS. x.w.e.rb.temp, temporal conjunctival staining with rose bengal of the worse eye; x.sev.dm, patient rating of severity of dry mouth on a scale of 10; x.eye.sign, the presence of rose bengal staining greater than or equal to $4/9$ in the worse eye and/or a Schirmer score $\leq 5$ mm in the worse eye; x.corn.stain.I, corneal staining with fluorescein in the inferior quadrant; and x.rb.val, the value of rose bengal staining in the worse eye on a scale of 9.

**Figure 3.** Tree 3: KCS and pSS. All noninvasive variables including salivary flow ($n = 75$). All numbers reflect KCS/pSS. x.w.e.rb.temp, temporal conjunctival rose bengal staining in the worse eye; x.sev.dm, patient rating of severity of dry mouth on a scale of 10; x.eye.sign, the presence of rose bengal staining greater than or equal to $4/9$ in the worse eye and/or a Schirmer score $\leq 5$ mm in the worse eye; x.s.sal.fl, amount of salivary flow per minute with stimulation; and x.corn.stain.I, corneal staining with fluorescein in inferior quadrant.
Rose Bengal Staining in Sjögren’s Syndrome and KCS

Table 4. Sensitivity and Specificity of the Classification Trees

<table>
<thead>
<tr>
<th>Tree</th>
<th>True</th>
<th>Predicted KCS</th>
<th>Predicted pSS</th>
<th>Sens.</th>
<th>Spec.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tree 1</td>
<td>All variables</td>
<td>KCS</td>
<td>75</td>
<td>14</td>
<td>99.57</td>
</tr>
<tr>
<td></td>
<td>pSS</td>
<td>1</td>
<td>230</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tree 2</td>
<td>Noninvasive without salivary flow</td>
<td>KCS</td>
<td>50</td>
<td>39</td>
<td>95.7</td>
</tr>
<tr>
<td></td>
<td>pSS</td>
<td>10</td>
<td>221</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tree 3</td>
<td>Noninvasive with salivary flow</td>
<td>KCS</td>
<td>50</td>
<td>39</td>
<td>96.1</td>
</tr>
<tr>
<td></td>
<td>pSS</td>
<td>9</td>
<td>222</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Rose bengal staining results combined with severity of dry mouth symptoms of 4.5/10 or greater and other staining signs, we were able to identify all but 3 of the 231 pSS patients. This noninvasive evaluation demonstrates a sensitivity of 96.1%. The specificity of 56.2% seems to be acceptable, given the importance of the SS diagnosis. Throughout this analysis, rose bengal staining by location and severity proved to be an important differentiation of SS disease.

There are some points that are noteworthy in this protocol. We used topical Alcaine (proparacaine hydrochloride 0.5%) before the instillation of 1% liquid rose bengal, and this may have influenced the results. The purpose of the anesthetic was to reduce the distinct discomfort that accompanies the instillation of rose bengal.15 There is some belief that topical anesthetics can contribute to staining.16,17 However, Forster found no such effect with tetracaine.18 Although the anesthetic was used on both groups, the effect may be different in SS patients and therefore may have caused some increase in staining. Nevertheless, even if there were something particular about the anesthetic effect, in combination with the dye, it was important diagnostically.

This result that emphasizes the importance of conjunctival staining, particularly of the temporal area, in the diagnosis of SS has not been described before, even though ocular surface staining has been used by eye care practitioners for many years to observe the effects of DE and Sjögren’s syndrome. Perhaps the frequency of the use of rose bengal in everyday practice is not high, as noted in a recent Delphi panel in which only 65% of DE specialists reported using rose bengal regularly, whereas 100% did use fluorescein.19 Both the lack of availability of and the patient’s sensitivity to liquid rose bengal may limit its use in clinical practice. The consistent use of 1% liquid rose bengal on a large cohort of DE patients in this protocol may have allowed this variable to become evident. Also, since ocular surface staining is usually added in DE diagnosis (i.e., corneal and conjunctival staining), we were able to identify all but 3 of the 231 pSS patients. This noninvasive evaluation demonstrates a sensitivity of 96.1%. The specificity of 56.2% seems to be acceptable, given the importance of the SS diagnosis. Throughout this analysis, rose bengal staining by location and severity proved to be an important differentiation of SS disease.

There may be many physiological factors that cause increased amounts of staining and perhaps progression to the temporal conjunctiva in SS. These include the unique inflammatory status of the lacrimal gland,31,32 lack of reflex tearing,33 tear film protein composition,34–36 inflammation of the ocular surface37–42 and alterations in mucin expression.43–44 It is the membrane-spanning mucins that have been most often linked to rose bengal staining.44–46 Alterations in the distribution of conjunctival epithelial MUC16 have been noted in non-SS DE by Danjo et al.45 and it was found to correlate significantly with staining scores of the temporal conjunctiva. Pfugfelder et al.44 also noted a correlation between membrane mucin absence and rose bengal staining. The concept that mucin changes relate to staining was furthered in two studies of a human corneal-limbal epithelial cell line (HCLE) that demonstrated that MUC16 surface protein protects against rose bengal invasion.46,47 None of these studies was specific to SS DE. In contrast to this line of reasoning, there is evidence that MUC16 is in fact overexpressed in SS. In a recent paper, Caffery et al.48 compared MUC16 expression in SS and KCS patients with that in normal subjects. MUC16 was found in 48% of SS patients as diagnosed by the AECC. They found that SS patients had significantly more nasal and temporal staining. Only 8.2% of non-SS DE subjects showed nasal staining versus 57.1% of the SS subjects, whereas temporal staining was present in 17% of the non-SS DE subjects compared with 48% of the SS patients.

Specific reference to temporal conjunctival fluorescein and rose bengal staining is rare in the literature but was noted to be one of the few DE observations that displayed moderate repeatability in a non-SS DE study by Nichols et al.28 However, such staining was found in only 16% of their subjects with mild to moderate DE. Tsubota et al.29 compared subjects with SS, DE with serum autoantibodies but not SS, and “simple” DE. Both fluorescein staining of the cornea and rose bengal staining of the conjunctiva were significantly worse in the SS group than in the other groups, which did not differ. Temporal staining was not differentiated in this study. Begley et al.,30 compared lissamine green staining of the nasal and temporal bulbar conjunctiva in normal subjects and non-SS DE and SS patients as diagnosed by the AECC. They found that SS patients had significantly more nasal and temporal staining. Only 8.2% of non-SS DE subjects showed temporal staining versus 57.1% of the SS subjects, whereas temporal staining was present in 17% of the non-SS DE subjects compared with 48% of the SS patients.

Why temporal staining was such a strong variable makes for interesting speculation. Very little is known about the progress of corneal and conjunctival staining in DE disease and how it changes with time of disease and severity. Some believe that conjunctival staining in DE begins in the nasal area and spreads to the temporal area with progression of disease.24,25 Therefore, temporal staining may be more prominent in SS because of the severity of DE in SS. The rationale is that the flow of tears begins from the lacrimal gland to the temporal side of the globe and then toward the nasal globe through the menisci. With reduced tear clearance and the natural flow of the tears, the blink moves tears toward the nasal puncta, where there is a loss of water by evaporation and thus a concentration of tears and inflammatory products at the nasal bulbar area that could be both hypersomotic and toxic.26,27
lium. Clearly, the relationship between mucins and rose bengal staining in DE disease should be investigated further.

CONCLUSIONS

In conclusion, the identification of Sjögren’s syndrome and its differentiation from other forms of DE disease can be improved in the eye care office by grading rose bengal staining of the conjunctiva, particularly the temporal area. Adding a dry mouth questionnaire and, when necessary, referring the patient for simple Ro and La blood analysis will improve both sensitivity and specificity of referrals to rheumatology.

References


