

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 severity of dry mouth symptoms were the major factors 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}

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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/ophthalmology, 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.

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

1-----2-----3-----4-----5-----6-----7-----8-----9-----10
 Not dry at all 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,⁵ using the R statistical analysis language,⁶ of 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 2. Description of the Two Groups

	KCS	pSS	P
Patients, <i>n</i>	89	231	
Age, y	55.47 ± 13.89	51.67 ± 13.94	0.065
% Female	87.6%	89.6%	0.76
Schirmer scores	3.67 ± 3.08	2.86 ± 3.12	0.079
DE severity (0-10)	5.70 ± 2.45	6.24 ± 2.87	0.52

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.⁵ This splitting is performed to its completion and then pruned back to an appropriate size.⁵ 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

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

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

TABLE 3. Variables Measured in SS Clinic

Classification of Variables	Noninvasive Variables	Invasive Variables
Demographics	Age, sex	
AECC criteria	Dry eye symptoms, dry mouth symptoms, dry eye signs (positive Schirmer or rose bengal), salivary flow positive	Biopsy, serum antibodies for Ro and/or La.
Variables associated with the AECC criteria	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	Biopsy focus score 0-4, Chisholm Mason biopsy score 3-4 (at least 1 focus in 4mm) is SS. Ro present, La present
Systemic autoimmune diagnoses	Presence of mixed connective tissue (CT) disease, CREST (calcinosis, Raynaud's, esophageal, sclerodactyly, telangiectasia), RA, SLE, PBC	
Other signs of autoimmune disease	Parotid swell, myalgia, arthralgia, fibromyalgia, lymphoma, X-ray positive	
Other systemic diseases	Diabetes, hypothyroid	
Blood work		IgG, IgM, IgA, M spike, WBC, ANA, RF, ATA, Anti-mic, TSH, AMA, SMA
Other systemic symptoms	Presence of: dysphagia, dyspepsia, vaginal dryness, dyspareunia, Raynaud's, dry skin, pruritus, rash, alopecia, photosensitive skin	
Medications by category	Diuretics, depression, anticholinergics, anti-inflammatories	
Other eye signs	Meibomian gland dysfunction, superior limbic keratoconjunctivitis (SLK), blepharitis	
Rose bengal stain	Score 0-3: Worse eye (WE) temporal stain, WE corneal stain, WE nasal stain, each eye total of 3 areas	
Fluorescein stain	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	
Dental information	Corneal stain of any kind Missing teeth 0-32, filled teeth, cervical cavities, D score 0-32, DMF (dentate, missing filled), candidiasis	

RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; PBC, primary biliary cirrhosis; M-spike, M-protein level; WBC, white blood cell count; ANA, antinuclear antibodies; RF, rheumatoid factor; ATA, anti-thyroglobulin antibody; Anti-mic, anti-microsomal antibodies; TSH, thyroid specific hormone; AMA, anti-mitochondria antibody; SMA, smooth muscle antibody.

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 populations^{11,12} 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-

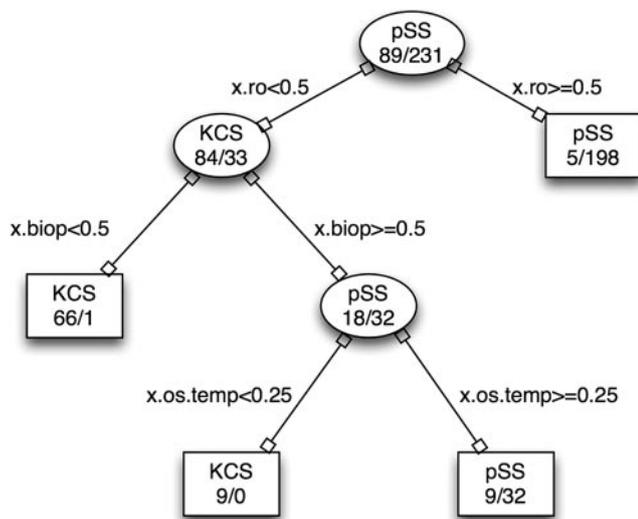


FIGURE 1. Tree 1. All numbers reflect KCS/pSS. x.ro, presence of anti-Ro immunoglobulin in serum; x.biop, biopsy score for SS; and x.os.temp, temporal conjunctival staining with rose bengal.

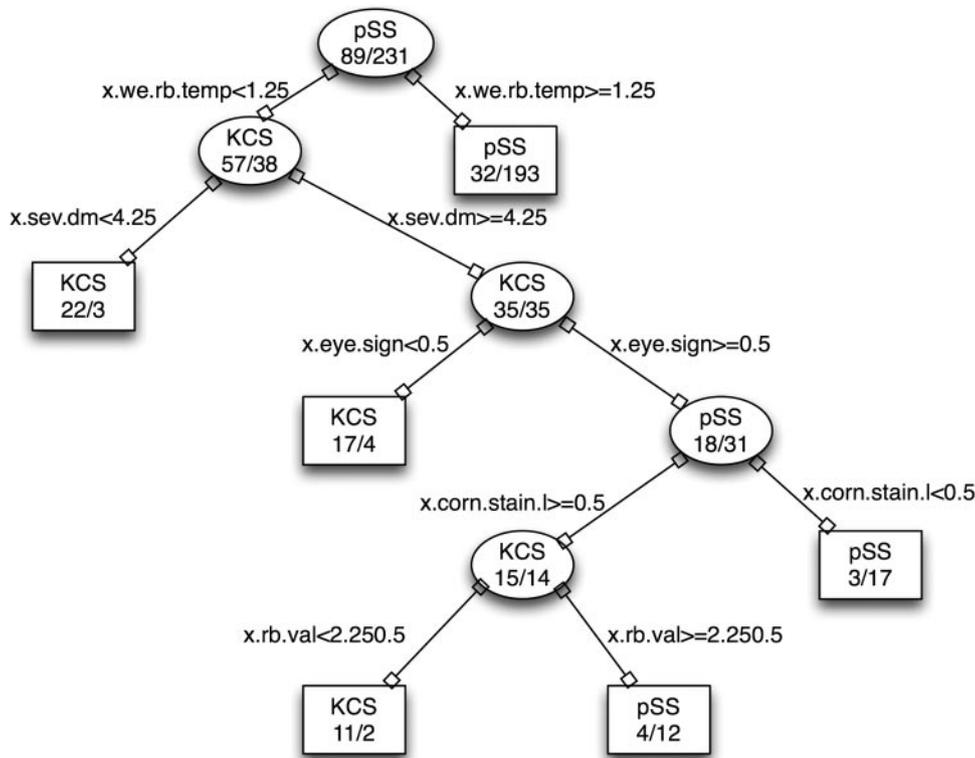


FIGURE 2. Tree 2: pSS and KCS. All noninvasive variables except salivary flow ($n = 74$). All numbers reflect KCS/pSS. $x.we.rb.temp$, temporal conjunctival staining with rose bengal of the worse eye; $x.sev.dm$, patient rating of severity of dry mouth on a scale of 10; $x.eye.sign$, the presence of rose bengal staining greater than or equal to 4/9 in the worse eye and/or a Schirmer score ≤ 5 mm in the worse eye; $x.corn.stain.l$, 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.

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.^{5,14} 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.⁵

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

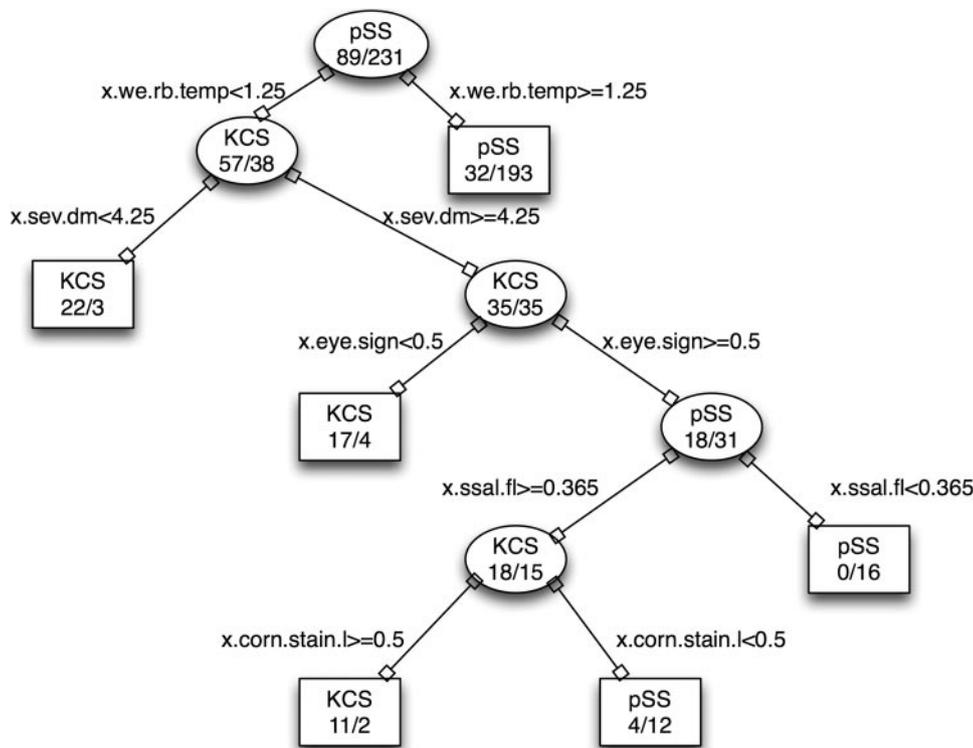


FIGURE 3. Tree 3: KCS and pSS. All noninvasive variables including salivary flow ($n = 75$). All numbers reflect KCS/pSS. $x.we.rb.temp$, temporal conjunctival rose bengal staining in the worse eye; $x.sev.dm$, patient rating of severity of dry mouth on a scale of 10; $x.eye.sign$, the presence of rose bengal staining greater than or equal to 4/9 in the worse eye and/or a Schirmer score of ≤ 5 mm in the worse eye; $x.ssal.fl$, amount of salivary flow per minute with stimulation; and $x.corn.stain.l$, corneal staining with fluorescein in inferior quadrant.

TABLE 4. Sensitivity and Specificity of the Classification Trees

Tree	True	Predicted KCS	Predicted pSS	Sens.	Spec.
Tree 1 All variables	KCS pSS	75 1	14 230	99.57	84.26
Tree 2 Noninvasive without salivary flow	KCS pSS	50 10	39 221	95.7	56.18
Tree 3 Noninvasive with salivary flow	KCS pSS	50 9	39 222	96.1	56.18

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) are combined as in criterion III for SS,⁴ clinicians may have missed the impact of conjunctival staining on its own in DE disease.

We believe that the more common clinical practice of using fluorescein alone is not sufficient for observation of the conjunctiva and for the differentiation of DE and SS. Although the AECC for SS diagnosis lists fluorescein, rose bengal, and lissamine green in the description of the staining procedures,⁴ rose bengal is considered the best dye for the observation of conjunctival staining, while fluorescein staining is very useful in observing the cornea.²⁰ Both Nichols et al.²¹ and McCarty et al.²² found a poor correlation between fluorescein and rose bengal conjunctival staining. Also, Yokoi and Kinoshita²³ found that fluorescein was not a useful tool in severe DE cases.

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}

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 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.

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,²⁹ tear film protein composition,³³⁻³⁶ inflammation of the ocular surface³⁷⁻⁴² and alterations in mucin expression.^{37,43,44}

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. Pflugfelder 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 higher concentration in tears in SS, and MUC16 mRNA was found in increased concentration in the conjunctival epithe-

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.

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