A proposal of new ocular items in Sjögren's syndrome classification criteria

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Abstract

Objective

To verify whether ocular surface tests other than those included in primary Sjögren's syndrome (SS-I) classification criteria (Schirmer I, Break up Time, vital dye staining) may contribute to SS I diagnosis.

Methods

Two hundred and sixty-two patients (78 SS-1, 91 non-SS autoimmune diseases, 93 Sicca syndrome) filled a validated questionnaire on symptoms and were evaluated by Schirmer test without (Schirmer I) and with (Jones test) topical anaesthesia, Break Up Time (BUT), corneal aesthesiometry, tear clearance rate, vital dye (lissamine green) staining, impression conjunctival cytology, concentration of tear lysozyme and lactoferrin. Thresholds were selected from Receiver Operating Curves; sensitivity, specificity, likelihood ratio (LR+), predictive values were calculated for each test. A logistic regression model was constructed representing the best diagnostic index for SS.

Results

Data showed a poor diagnostic performance of Schirmer test I (LR+ 1.38) and BUT (LR+ 1.05); results from lissamine green staining may be unreliable due to incorporation bias. Tear lactoferrin (LR+ 4.52), Jones test (LR+ 6.24), tear lysozyme (LR+ 8.0), symptom questionnaire (LR+ 8.62), tear clearance rate (LR+ 18.73) and corneal aesthesiometry (LR+ 20.96) exhibited high diagnostic performance also taken together in the regression model.

Conclusions

Because many of the tests we have screened in this study can be carried out by a trained ophthalmologist in any clinical setting, we recommend that ocular surface impairment is studied with the combination of tests proved to be helpful for the SS I diagnosis.

Key words

Sjögren's syndrome, keratoconjunctivitis sicca, Schirmer tests, clearance rate, corneal aesthesiometry, tear chemistry, likelihood functions, predictive value of tests, regression analysis, ROC curve.

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Introduction

Ocular scleritis, episcleritis, uveitis and, most frequently, dry eye are often recognized in patients suffering from a variety of rheumatic diseases, but still it is questionable whether this is a consequence or an early manifestation of a rheumatologic disorder (1).

The so called "dry eye syndrome" is usually referred as a qualitative or quantitative alteration of tears and patients report mostly subjective symptoms of foreign body sensation, itching, burning, redness and photophobia. The ocular surface status has been included in almost all the diagnostic criteria for SS-I, in items separate for subjective symptoms and clinical evaluations, these including the Schirmer test I and a surface staining with Bengal Rose or Lissamine green dyes, in some also the Break-up Time (2).

Actually the ocular surface is considered as a functional unit where all components (tear film, cornea, limbus, conjunctiva, main and secondary lachrymal glands and Meibomian glands) work together (3) and assessment of ocular impairment consequently needs an integrated analysis (4).

The purpose of the present study was to establish the potential diagnostic value in SS-I diagnosis of a combination of clinical and laboratory tests related to the ocular surface involvement, other than those usually included in the classification criteria.

Patients

The study enrolled 262 patients, examined by the day hospital and the laboratory of the Ophthalmology Unit of the University of Bologna, during the period June 2002-August 2005. They were grouped as follows:

- Primary Sjögren's syndrome (SS-I) patients (78 subjects; 72 females, medium age 67.6 ± 9.8 yrs; 6 males, medium age 56.1 ± 10.2 yrs), diagnosed according to the American-European Consensus Group (5)
- Patients with other autoimmune diseases (91 subjects; 78 females, medium age 63.1 ± 10.2 yrs; 13 males, medium age 55.5 ± 10.4 yrs), classified as free from secondary Sjögren's Syndrome when the patients complained of ocular or oral symptoms in the absence of anti Ro (SSA) or anti La (SSB) and with salivary gland biopsy negative. Patients suffered from rheumatoid arthritis (n = 36; 32 females, 4 males), systemic lupus erythematosus (n = 16; 15 females, 1 male), connective tissue disorders (n = 23; 19 females; 4 males), sclerodermia (n = 16; 12 females, 4 males).
- Sicca syndrome (Sicca) patients (93 subjects; 87 females, medium age 61.1 ± 8.5 yrs; 6 males, medium age 53.4 ± 8.2 yrs) reporting subjective symptoms of dry eye and discomfort, slight dry mouth without serological positivity for any autoimmune disease, non contact lens wearers. These control patients were classified as free from secondary Sjögren's syndrome according to the American-European Consensus Group guidelines (5)

The research complies with the Declaration of Helsinki of the World Medical Association, and informed consent was obtained from each subject after a full explanation of the procedures.

Methods

All the tests were performed for each single patient in the same day and in the same time interval from 9 am to 12 am, in a dim light room controlled for temperature and humidity, following the sequence of tests elsewhere described (6)

Patients were requested to fill in the Ocular Surface Disease Index (OSDI) questionnaire on subjective symptoms, the score ranging from 0-12 (no disability), to 13-22 (light dry eye), to 23-32 (moderate dry eye), to 33-100 (severe dry eye) (7).

The Schirmer tests were carried out as described in (4) by using sterile Schirmer strips (ContaCare Ophthalmic) placed at the outer lateral inferior canthus with open eyes without (Schirmer test I, performed twice) or with (Jones test) anaesthesia (oxybuprocain 0.5%). Pathological value was regarded as ≤ 5 mm/wetting after 5 minutes for Schirmer I (4) and ≤ 3mm/wetting after 1 minute for Jones test (8).

Tear Break up Time (BUT) was performed as described elsewhere (4)

(pathological value < 10 sec). Lissamine green staining was performed only in patients without corneal epithelial defects as already reported (9) and scored (4) (pathological value > 9/18 in 6 conjunctival areas measured).
Corneal sensitivity was evaluated with a Cochet-Bonnet aesthesiometer (Luneau, Chartres, France) and scored according to (10) (pathological values were regarded as applied pressures higher than 0.57 gr/mm^2). Tear clearance rate was carried out as in (11), graded onto the colour of the fluorescein dye fading compared to a control staining scale dilution (pathological values ≤ 1/8 dilution). Conjunctival Impression cytology was performed, processed and scored as reported in (12) (pathological grade > 1).
Five μl tears were collected at the outer cantus, centrifuged and stored at –80°C until determination. Qualitative and quantitative analyses of the major tear proteins were performed by SDS-PAGE (Bio-Rad MiniProtean III Cell); the gels were stained with Biosafe Coomassie Blue G250 and scanned with Fluor-S Multiimager System (Bio-Rad). The densitometric volumes (with background subtracted) of the major protein bands separated in the gels were determined using gel-imaging software (Quantity One, Bio-Rad). Purified human proteins (Sigma) were used to construct the respective standard curves (densitometric volumes against known concentration). The concentrations of the lysozyme and lactoferrin proteins in tear samples were determined with reference to the standard curves [pathological value for lysozyme <1.40 mg/ml (13); lactoferrin < 1.80 mg/ml (14)].

Statistical analysis

Data coming from each eye of all the patients included in the study were statistically elaborated using the SPSS 9.0 and the the MedCalc 5.0 software. Each of the test was analysed for sensitivity, specificity, Receiver-Operating Characteristics (ROC) curves, likelihood ratio, positive predictive values, calculated comparing SS-I vs. sicca patients. The prevalence of the SS-I was calculated having as a reference the population included in the present study. The independent sample t-test, the Mann-Whitney test for unpaired data, the logistic regression analysis stepwise forward by likelihood ratio for selected groups of tests were applied; values for p less than 0.05 were regarded as statistically significant.

Results

The medium and standard deviation of all the tests are summarized in Table I and described hereby. The dry eye symptoms were reported by all of the participant to the study with varying severity and with a statistically significant difference among the groups ($p < 0.0001$).
The mean values of total tear secretion were higher than the pathological threshold for the Schirmer I test (5 mm/5') with statistically significant differences between SS I and the other two groups ($p < 0.05$). Data from the Jones test showed a reduction of the basal tear secretion only in the SS I patients, with statistically significant vs the other two groups ($p < 0.0001$)
Tear film stability as measured by Break Up Time was reduced in all the groups without any statistically significant difference.
The conjunctival epithelium integrity as evaluated by lissamine green staining was found to be in the pathological score only in the SS-I group, with statistically significant differences vs the other two groups ($p < 0.0001$)
Corneal sensitivity as measured by aesthesiometry was reduced in all the groups, worse in the SS I and with statistically significant differences among all the groups ($p > 0.0001$)
Tear turnover as evaluated by the tear clearance rate was reduced in the SS I group with statistically significant differences among all the groups ($p < 0.0001$).
The conjunctival imprint cytology score demonstrated pathological values in all the three groups, more severe in the SS-I patients, and with statistically significant differences only between SS-I and sicca patients ($p < 0.0001$) and between non SS A.D. and sicca patients ($p < 0.05$) .
Concentration of tear lysozyme and lactoferrin were reduced only in the SS I group with statistically significant differences vs. the other two groups ($p < 0.0001$).
Table II summarizes the cut off points for a positive diagnosis of SS-I, selected from ROC curves of all the tests with emphasis to specificity; the related sensitivity, specificity, likelihood ratio, positive predictive values are also listed for each test.
Table II also indicates the main parameters of the ROC curves for all the tests, indicating the diagnostic accuracy for each test: in the present study the validated subjective symptom questionnaire, lissamine green staining, Jones test, corneal aesthesiometry, tear clearance rate, tear lysozyme and lactoferrin concentration exhibited the best diagnostic performance as taken alone.
Logistic regression analysis stepwise forward by likelihood ratio for different combinations of variables was carried. Combination 1 considered variables included in the American-European re-

Table I. Summary of the results (medium + S.D.).

Test variable	Measure	SS-I	non-SS A.D.	Sicca
Questionnaire	score	45.8 ± 10.5	31.5 ± 7.3	28.1 ± 7.9
Schirmer I	mm / 5'	7.6. ± 5.7	11.2 ± 6.7	11.1 ± 7.4
Jones Test	mm / 1'	2.9 ± 2.3	5.6 ± 2.9	6.6 ± 2.8
Break Up Time	sec	4.8 ± 3.7	6.2 ± 5.4	5.7 ± 3.4
Lissamine green staining	score	9.4 ± 3.4	6.9 ± 3.7	5.5 ± 3.9
Corneal aesthesiometry	gr/mm^3	0.79 ± 0.11	0.57 ± 0.09	0.50 ± 0.1
Tear clearance rate*	score	3.2 ± 1.2	2.3 ± 0.9	1.7 ± 0.9
Conjunctival imprint cytology	score	1.9 ± 0.8	1.7 ± 0.7	1.4 ± 0.6
Tear lysozyme	mg / ml	1.12 ± 0.42	1.63 ± 0.46	1.81 ± 0.47
Tear lactoferrin	mg / ml	1.65 ± 0.60	2.43 ± 0.92	2.37 ± 0.98

* for the statistical analysis the Tear clearance rate results were converted into numerical values: dilution 1: 2=5; 1: 4=4; 1: 8=3; 1: 16=2; 1: 32=1; 1: 64=0.

Table II. Cut-off points of test variables for a positive diagnosis of SS-I and parameters relating to ROC curve analysis performed for each test, listed by increasing value of the area under the curve.

Test variable	Cut-off points	Specificity	Sensitivity	LR+	PPV	ROC curve analysis parameters		
						Area under the curve	Standard error	95% confidence interval
Break Up Time	≤ 10 sec	12.1	91.9	1.05	5.2	0.584	0.061	0.484 – 0.678
Conjunctival imprint cytology	score > 2	93.9	30.6	5.04	21.0	0.664	0.054	0.566 – 0.754
Schirmer I	mm ≤ 5	66.7	45.9	1.38	6.8	0.667	0.059	0.569 – 0.755
Tear lactoferrin	≤ 1.10 mg / ml	90.6	28.6	4.52	13.8	0.667	0.059	0.569 – 0.755
Tear lysozyme	≤ 0.90 mg / ml	96.9	25.0	8.0	29.6	0.785	0.056	0.667 – 0.877
Lissamine green staining	Score > 9	75.0	85.1	3.41	15.2	0.823	0.041	0.735 – 0.892
Jones Test	mm ≤ 3	87.9	75.7	6.24	24.7	0.839	0.046	0.756 – 0.903
Tear clearance rate*	score ≥ 3	97.0	56.8	18.73	49.6	0.844	0.037	0.761 – 0.907
Corneal aesthesiometry	gr/mm^3 ≤ 0.72	63.5	97.0	20.96	52.5	0.923	0.033	0.855 – 0.966
Questionnaire	Score ≥ 45	100	45.9	8.62	95.1	0.926	0.024	0.859 – 0.968

* for the statistical analysis the Tear clearance rate results were converted into numerical values: dilution 1: 2=5; 1: 4=4; 1: 8=3; 1: 16=2; 1: 32=1; 1: 64=0.
LR, likelihood ratio; PPV, positive predictive value.

vised criteria (questionnaire, Schirmer test I, BUT, lissamine green staining) but did not reach a satisfying significance (B = 0.121, p = 0.182) . Combination 2 considered the clinical variables that had shown the best diagnostic performance in our study (questionnaire, Jones test, corneal aesthesiometry, tear clearance rate) and Combination 3 considered those variables of combination 2 plus tear lysozyme and lactoferrin. Combination 2 and, even more, combination 3, exhibited a diagnostic performance higher than combination 1 (respectively B = 1.634 and $p < 0.0001$; B= 2.425 and $p < 0.0001$), as also it was evidenced by the corresponding ROC curves (Fig. 1).

Discussion

The items included in many diagnostic criteria for SS-I comprise questions on subjective symptoms and a clinical evaluation of ocular surface, but the role of these tests have been recently discussed. In particular, the Schirmer test I was demonstrated an unreliable test for the diagnosis of SS I (15, 16), showing a scarce repeatability (17) and relation with subjective symptoms (18). Our study also found that diagnostic performance of the Schirmer test I is poor, but sensitivity and specificity for Schirmer test may suffer from an incorporation bias since the same cut-off had been used to support the diagnoses by which patients were grouped and caution is therefore requested to draw any conclusion. It has been unascertained, however, that the Schirmer test produces many false negative test results (about half of the SS-I patients in our study tested negative for Schirmer I) in agreement with others (16) and only a very low value of a Schirmer test I can be assumed as a reliable indication for aqueous deficiency. It is conceivable that a significant part of SS-I patients included in the present study had met the ocular sign item with the positivity of the Rose Bengal staining only, or that at the time of our observation Schirmer value was influenced by the duration of the disease and/or by the therapy (1).

The role of BUT in the SS I diagnosis was reviewed (19) and also our data showed an high sensitivity for the test but low specificity. The vital staining exhibited a good diagnostic performance in this study, in agreement with previous works indicating vital dye score the test of choice (20); as for Schirmer, the result may be a possible consequence of a bias in data collection, since vital dye staining was not applied to patients with disepithelialized corneas.

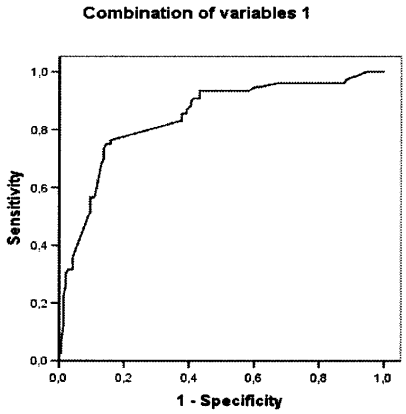
The updated scientific evidence suggests analysing the ocular surface also with other methods, specifically applied to investigate the different components of the unit (4, 21).

Conjunctival imprint cytology grades the ocular surface dryness analysing conjunctival or corneal epithelial cells collected atraumatically (22) and exhibits a good correlation with clinical severity (23). Our data indicates that dryness is a common finding in all the groups of patients and the best diagnostic performance is obtained at cut-off points of severe grade. The test can be carried out in specialized laboratories where specimens can be properly processed, and it appears not to be suitable for clinical routine.

The OSDI validated questionnaire, Jones test, corneal aesthesiometry, tear clearance rate, concentrations of tear lysozyme and lactoferrin showed the best diagnostic performance, either taken together and analysed in combination, as discussed hereinafter.

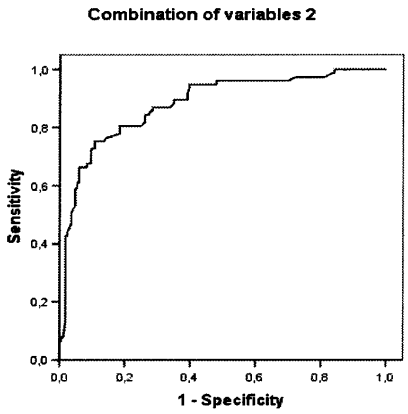
In the majority of the SS-I diagnostic criteria only a couple of simple questions on ocular subjective symptoms are indicated but, on the contrary, a scored symptom analysis has been proved to drive the clinician in the SS-I diagnosis (24). In the present study the OSDI validated questionnaire (7) exhibited the highest specificity and best diagnostic performance, strongly supporting the role of an exhaustive symptom analysis.

Tear secretion has been classically subdivided into a basal vs a reflex component, with different locations of production and neural controls (25). The Schirmer criterion have been widely used to discriminate and measure the different components of the secretion; our data showed a good diagnostic per-



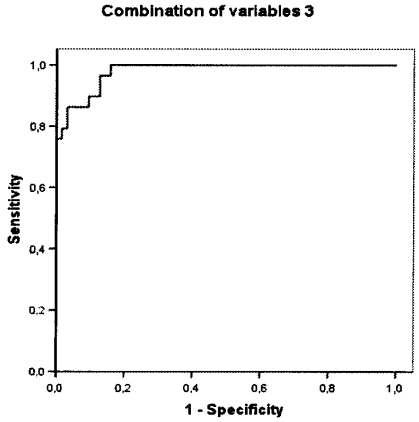
variables: Schirmer test I, Questionnaire
Lissamine Green staining

Area under the ROC curve = 0.801
Standard error = 0.281
95% Confidence interval = 0.791 to 0.834



variables: Questionnaire, Jones test, Corneal aesthesiometry, Tear clearance rate

Area under the ROC curve = 0.881
Standard error = 0.024
95% Confidence interval = 0.835 to 0.929



Variables: Questionnaire, Jones test
Corneal aesthesiometry,
Tear clearance rate, Tear lysozyme
Tear lactoferrin

Area under the ROC curve = 0.972
Standard error = 0.101
95% Confidence interval = 0.958 to 1.00

Fig. 1. Non-parametric ROC-curves of three combination of variables evaluated for their use in identifying SS in patients. Note the relative rounded curve (towards the upper left corner of the diagram) of all the three groups of tests, indicating good diagnostic performance; the best performance is showed by the combination of variables 3.

formance of the Schirmer test with topical anaesthesia (Jones test) in SS I classification, higher than that exhibited by test without anaesthesia: Jones test expresses the tear secretion without any stimulation from the ocular surface on the lachrymal gland and can be assumed a reference method to investigate the modification of lachrymal gland function.

Evaluation of corneal sensitivity, measured by aesthesiometry, and tear clearance rate is increasingly assuming a scientific role in the study of tear production. In fact, the more actual concepts on tear physiology (25, 26) indicate a tight neural control connecting all the components of the ocular surface unit (3) that would also control tear secretion. The ophthalmic branch of the trigeminal nerve, driven by corneal sensitivity, would act as afferent way of the neural arc while the facial nerve as efferent way and the final neuroanatomic integration of these two reflexes would be manifested in tear clearance rate (27).

In agreement with previous studies (28, 29) we demonstrated reduced tear clearance rate and corneal sensitivity in the SS I patients with statistically significant differences vs. the two other groups and a good diagnostic performance. The finding maybe related with an impaired parasympathetic control of lachrymal gland (30, 31) consequent to the presence of anti-M3 muscarinic (32) but not anti-SSA and anti-SSB (33) receptors.

Lysozyme is a protein secreted by both the acini and the tubular structures, while lactoferrin is only secreted in the acini of lachrymal gland; whether or not their concentration can be assumed as gland dysfunction index still is controverse (34, 35). However, a reduction of that both proteins in tears of SS-I patients has been documented (36-38) and although their analysis may be helpful in diagnosis, the test is rarely employed in the clinical practice outside a research setting, due to the technical difficulties in making determinations with a low amount of specimen.

Our data confirm a reduction of both proteins in tears, in agreement with others, but the best diagnostic performance is reached with cut-off points lower than those previously found (lysozyme < 0.90 mg/ml; lactoferrin < 1.1 mg/ml).

The logistic regression analysis of the symptom questionnaire, Jones test, aesthesiometry, tear clearance rate, taken together and corrected for their mutual influences demonstrated a diagnostic performance much higher than what found for the combination of tests usually considered in the diagnostic criteria and above discussed (Schirmer I, BUT, Lissamine Green staining). When the laboratory determination of tear lysozyme and lactoferrin was added to the combination the diagnostic performance reached higher levels (area under the curve in the ROC analysis: 0.972).

It has to be underlined, however, that in the clinical practice a lower performance of classical ophthalmological tests may be counterbalanced by the association with the other non-ophthalmological items. Because many of the tests we have screened in this study can be carried out inexpensively by a trained ophthalmologist in any clinical setting, we recommend that ocular surface impairment is studied at least with the combination of tests n. 2. The application of more expensive and time consuming laboratory tests of combination n.3 should be reserved to those patients in which a conclusive diagnosis cannot be defined by using the commonly used criteria.

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