

TEAR OSMOLARITY - A NEW GOLD STANDARD?

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PROPOSAL

The purpose of this presentation is to propose tear osmolality measurement as a new gold standard for the diagnosis of keratoconjunctivitis sicca. Agreement on the diagnostic criteria is needed in order to permit meaningful comparisons of scientific results obtained from varying patient populations. The method employed is a review of previous studies of clinical symptoms, signs and diagnostic tests employed in making a diagnosis of keratoconjunctivitis sicca. Data from previous studies are reviewed and the results of individual and combinations tests are compared in regard to sensitivity, specificity and overall efficiency in establishing accurate diagnoses. The results are that the measurement of tear osmolality measurement provides the greatest sensitivity, specificity and overall efficiency of a single test. Adding either the Schirmer test without anesthetic or tear lactoferrin measured by the Lactoplate™ method in parallel to tear osmolality measurement did not increase the sensitivity of diagnostic testing beyond 90% which was obtained by using tear osmolality measurement alone. The specificity of such combination diagnostic testing was increased only from 95% to 100%. The simplicity of tear osmolality measurement and its established reliability supports the conclusion that this test is a reasonable candidate for a new international gold standard in the diagnosis of keratoconjunctivitis sicca.

INTRODUCTION

The Proceedings of the First International Seminar on Sjögren's syndrome was published in 1986 as a supplement to the Scandinavian Journal of Rheumatology. Prause,

Manthroe, Oxholm and Schiodt reported significant lack of agreement on the definitions and criteria used for Sjögren's syndrome.⁽¹⁾ Although ninety-six percent (96%) of the contributors agreed on the simultaneous presence of keratoconjunctivitis sicca (KCS) and xerostomia as the definition of primary Sjögren's syndrome, all required one or more abnormal objective tests for the definition of keratoconjunctivitis sicca. Only thirteen percent (13%) required subjective symptoms as well. The number of abstracts requiring differing number of tests were thirty (30) for at least two (2) tests, thirteen (13) for at least one (1) abnormal test, five (5) for at least three (3), and five (5) for at least four (4) abnormal objective tests.

Similarly, all contributors defined xerostomia by the presence of one or more abnormal objective tests, but only twenty-two percent (22%) required the presence of subjective symptoms as well. Twenty-nine percent (29%) required at least one abnormal objective tests, twenty-one (21) demanded at least two (2) abnormal objective tests while three (3) abstract contributors demanded at least three (3) abnormal tests.

Ninety-four percent (94%) of the participants agree upon the terminology of primary and secondary Sjögren's syndrome with all in agreement that secondary Sjögren's syndrome indicated along with, the keratoconjunctivitis sicca and/or xerostomia, another well defined chronic inflammatory connective tissue disease such as lupus, rheumatoid arthritis or dermato(poly)-myositis. The survey indicated that more criteria were required for a diagnosis of keratoconjunctivitis sicca than xerostomia.

From this conference, four (4) set of criteria emerged for the diagnosis of Sjögren's Syndrome.⁽²⁾ They were called the Copenhagen, Japanese, Greek and California criteria. The Copenhagen criteria were formulated in 1976 and 1977 and were based upon only objective tests.⁽³⁾ The objective tests used for the diagnosis of keratoconjunctivitis sicca and xerostomia required at least two (2) of three (3) tests which must be abnormal for a diagnosis of KCS or xerostomia. The Greek criteria required only one abnormal test, whereas the Japanese and California also required at least two (2) abnormal tests. The Schirmer I test and the van Bijsterveld Rose Bengal staining score were the most frequently used tests although the Copenhagen group also used the tear film break up time. The disadvantage seen for Rose Bengal staining and tear film break up time was the requirement for a slit lamp exam and an ophthalmologist. The Japanese and California criteria required abnormal fluorescein staining and/or van Bijsterveld Rose Bengal staining score as a separate point.

STATEMENT OF PURPOSE

As pointed out at this meeting, international discussions are needed to obtain agreement on the criteria for Sjögren's syndrome and its components so that meaningful comparisons of scientific results can be obtained from different patient populations.

The inability to compare results of studies becomes even more pronounced when in addition to the utilization of different tests, different cutoff or referent values are used for an abnormal test result. Populations for studies are selected in different settings which produces considerable variation in study results. For example, a rheumatology clinic and an ophthalmologist's office or a dental clinic may examine Sjögren's syndrome patients

presenting with a variety of chief complaints. In many cases the severity of symptoms of one organ system may mask or cause the patient to overlook mild symptoms from a disorder in another system. A study investigating the incidence of serum autoantibodies in Sjögren's syndrome reported a ten-fold disparity of serum autoantibodies was detected in the patients with keratoconjunctivitis sicca presenting in an ophthalmologist's office compared to those presenting in a rheumatology clinic.⁽⁴⁾

A "gold standard" for the diagnosis of the disorders of each organ system in Sjögren's syndrome is required which can be used by all researchers. The gold standard is the test or criteria used to unequivocally define the disease.⁽⁵⁾ At the present each investigator or geographic region seems to have their own criteria. I would like to review my efforts to determine if tear osmolarity could be a "gold standard" for keratoconjunctivitis sicca.

HISTORY OF THE TEAR OSMOLARITY TEST

Twenty-four years ago I was invited by Sai Mishima to join him and Zenichi Kubota to measure the osmolarity of microvolumes of tears in normals and dry eye patients in relation to their tear flow as measured by fluorescein dilution.⁽⁶⁾ We selected dry eye patients on the basis of history and clinical examination. Compared to a group of normals, the results indicated a distinct separation of the two groups into dry eye patients with elevated tear osmolarity and low flow and normals with only a slightly hypertonic tear film and greater tear flow (Figure 1).

Eight years later, a medical student, Jeff Gilbard, asked to do some summer research. We had a new instrument called a Nanoliter Tear Osmometer purchased from Clifton Technical Physics, Hartford, New York to replace an older, more cumbersome Ramsay-Brown micro-osmometer. We had not been able to standardize and use the instrument. Jeff made the instrument work and compared a group of dry eye patients collected on the basis of symptoms and at least one of the following signs: a deficient inferior marginal tear strip, debris in the tear film, or a viscous appearing tear film. A group of normal subjects and a group of patients with conjunctivitis were used as controls. Distinct separation of the dry eye population was evident and we adopted 312 mOsm/L as the cutoff or abnormal referent value. The tear osmolarity test in this initial study was found to be 94.7% sensitive and 93.7% specific (Figure 2).⁽⁷⁾

My next question was, "How does this compare with other diagnostic tests?" Having been stimulated to investigate tear tests because of the limitations of the Schirmer test, I wanted to compare its results with tear osmolarity, Rose Bengal staining and other clinical tests of tear function. In a group of 28 eyes with KCS diagnosed according to symptoms and at least one of three slit lamp findings, tear osmolarity was positive in all cases but the Schirmer test with anesthetic was positive in only 29% when using cutoff values of 312 Mosm/L for the tear osmolarity test and 5mm of wetting in five minutes for the Schirmer test with anesthetic.⁽⁸⁾ Similarly in a group of 23 eyes with KCS, tear osmolarity was positive in all cases but tear film break up time was positive in only 43% using referent values of 312 Mosm/L for the tear osmolarity test and less than ten seconds for the tear film break up time (BUT).⁽⁷⁾

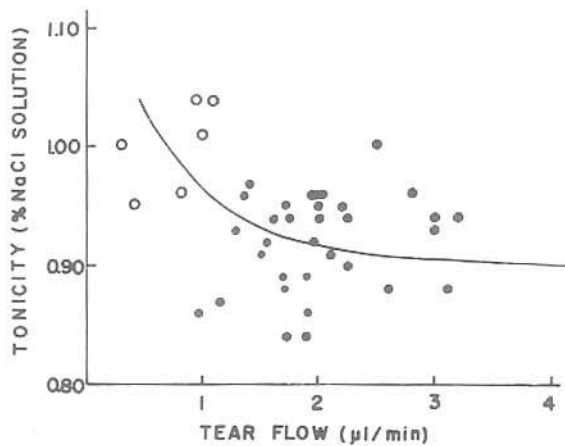


Figure 1. Osmotic pressure of the tears (equivalent of NaCl solution) and the rate of tear flow. Closed circles: Normal subjects. Open circles: cases of keratoconjunctivitis sicca. (From Mishima, Kubota and Farris, Excerpta Medical International Congress Series No. 222, pp 1801-1805. Copyright 1970 Elsevier North Holland. Reprinted by permission.)

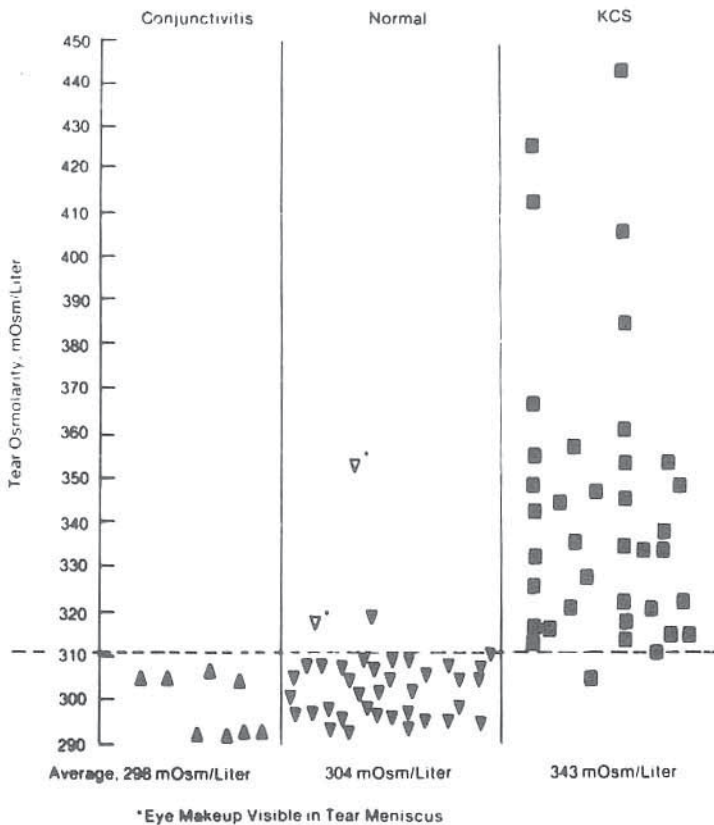


Figure 2. Tear osmolarity in conjunctivitis, normal eyes and KCS. (From Gilbard, Farris and Santamaria, Arch of Ophthalmol, 96:677-681. Copyright 1978 American Medical Association, Chicago, reprinted by permission.)

We then decided to go ahead and use tear osmolarity as a gold standard which would predict in a population of patients with symptoms of a dry eye the subsequent course of the disease and provide a more consistent association with the symptoms and clinical signs of KCS. We were aware that such a decision would subject us to the criticism that we were defining a new disease, but we understood that any new state of the art such as a new diagnostic test would require considerably more research before being accepted.⁽⁹⁾ We were fortunate to have studies completed about the same time by Rolando and Refojo who measured increased evaporation rates in external eye diseases.⁽¹⁰⁾ In addition, Jeff Gilbard and Ken Kenyon demonstrated that a hypertonic medium produced changes in epithelial cells growing in culture which resemble changes seen in the epithelial cells of dry eye patients.⁽¹¹⁾

We then collected a larger group of patients presenting only with complaints of eye discomfort and performed tear osmolarity tests as well as the tests of basal tear volume, Schirmer without anesthetic, Rose Bengal staining, and lysozyme and lactoferrin concentration in basal and reflex tears, as well as the percentage increase of the concentration of lysozyme and lactoferrin with reflex tearing.⁽¹²⁾ Tear osmolarity was 76% sensitive and 84% specific compared to the Schirmer test which was only 10% sensitive but 100% specific when 3mm or less of wetting in five minutes was considered abnormal.⁽¹⁰⁾ Rose Bengal staining using a cutoff value of 3.5 was only 58% sensitive but 100% specific. The lactoferrin concentration of reflex tears provided a specificity of 94% compared to only 67% specificity for the lysozyme concentration in tears. The percentage increase in lactoferrin was 95% sensitive when less than a 100% increase in lactoferrin with reflex tearing was considered abnormal.

OTHER STUDIES AND COMBINATION TESTING

How do these values compare with previous studies? van Bijsterveld was dismayed with the overlap of normal and abnormal values with the Schirmer test and Rose Bengal staining and found the lysozyme test measured by agar diffusion to be the most sensitive test.⁽¹³⁾ Analyzing his data, the sensitivity was 98.8% and the specificity was 98.5% using a diameter limit of 21.5mm of lysis as the cutoff. Rose Bengal staining was 95% sensitive and 96% specific with a referent value of 3.5. The Schirmer test was 58% sensitive and 83% specific with a referent value of 5.5mm of wetting in 5 minutes. The test population was selected on the basis of several practitioners' opinions and was most likely a more severely affected population than patients in our study who had only symptoms of eye discomfort as a criterion for entry.

Goren and Goren have published results of tear test in a more similar population which includes mildly affected as well as more severely affected KCS patients.⁽¹⁴⁾ Patients were selected only on the basis of symptoms. The patients with symptoms were divided into those with minimal symptoms, moderate to severe symptoms and both ocular and systemic symptoms. This study demonstrated the effect of combining tests into a battery of tests which is considered positive when any one of the tests within the group is positive. This is called combination testing by a parallel approach.⁽¹⁵⁾ A or B or both may be positive for the combination to be positive. Goren and Goren used combination testing in this manner i.e. a test batter was considered positive if any test within the group was positive. Even though a

"+" sign was used in their display of results, both tests were not required to be positive for the combination to be considered positive. Contrast this with a series approach in which the positive test from one test are retested with a second test. As a result, A and B must be positive for the combination to be considered positive. The data of Goren and Goren was used to recalculate test results after combining groups of patients with ocular symptoms ranging from mild to severe including those with or without systemic symptoms into one group in order to compare with our studies.⁽¹⁶⁾

TABLE 1 Patients with minimal to severe symptoms and combining those without and with systemic Involvement.

<u>TESTS</u>	<u>SENSITIVITY</u> (%)	<u>SPECIFICITY</u> (%)
BUT = A	52	72
Schir = B	66	77
LF = C	64	90
RB = D	25	90
A + B	78	56
A + C	71	64
A + D	57	62
B + C	79	70
B + D	77	49
C + D	70	54
A + B + C	84	51
A + B + D	80	49
A + C + D	74	54
B + C + D	83	62
A + B + C + D	87	44

Combining Tables 2, 3, and 4 Goren and Goren: Am J Ophthalmol 106:570,1988.
 LF - Lactoplate,TM But - Tear film breakup time, Schir-Schirmer without anesthetic,
 RB - Rose Bengal Staining. + = parallel combination testing, either test positive or
 both positive are a positive combination test.

The Schirmer test using less than 8mm of wetting as the cutoff for an abnormal value has the highest sensitivity, 66%, and the specificity is 60%.

Lactoferrin concentration in the tears was measured using the LactoplateTM and was 64% sensitive and 90% specific. Rose Bengal staining was only 25% sensitive and 90% specific using any staining as the cutoff for abnormal. Tear film break up time was 52% sensitive and 72% specific using less than 8 seconds as the cutoff for an abnormal test. Combining the tests so that parallel testing was done, that is one or more tests of the combination must be positive to consider the combination positive, a combination of all four tests was most sensitive at 87%. As would be expected with parallel testing, the combined sensitivity is greater than the individual sensitivities of the contributing tests. The specificity

of all four test using parallel testing was only 44%. Parallel testing results in the highest sensitivity but the lowest specificity. Series testing would have provided the opposite yielding lowest sensitivity but highest specificity but series combination testing was not reported. Of the combination of three tests, tear film break-up time, the Schirmer test and lactoferrin combination was the most sensitive at 84% with the lowest sensitivity of a three test parallel combination being tear film break-up, lactoferrin and Rose Bengal at 74%. The most sensitive of the two test parallel combination are Schirmer and lactoferrin at 79%, tear film break-up and Schirmer at 78% and Schirmer and Rose Bengal at 77%. The specificity of these parallel combinations are 69%, 56%, and 48% respectively. Thus overall efficiency appears best with the Schirmer and lactoferrin test combination.

TABLE 2 Combination tear testing: Tear Osmolarity, Lactoplate,TM and Schirmer

<u>TESTS</u>	<u>SENSITIVITY</u>	<u>SPECIFICITY</u>
	%	%
Tear Os = A	90	95
LF = B	35	70
Schir = C	25	90
Series:		
A and B	35	100
A and C	25	100
B and C	20	100
A and B and C	20	100
Parallel:		
A or B	90	65
A or C	90	85
B or C	40	60
A or B or C	90	55

Combining and calculating from Table I and II Lucca et al: CLAO Journal 16:109, 1990.

RECENT STUDIES

In our last study, we compared the performance of tear osmolarity, lactoplate and Schirmer tests in 20 keratoconjunctivitis sicca patients and 20 age matched controls.⁽¹⁶⁾ The diagnosis of keratoconjunctivitis sicca was made on the basis of history, symptoms, and clinical examination. Abnormal cutoff values for tear osmolarity, lactoferrin and Schirmer were 312 mOsm/L, 0.90 mg/dl, and less than 1mm/min of wetting.

The single test which provided the greatest sensitivity, specificity and overall efficiency was tear osmolarity. The next most sensitive test was the LactoplateTM test which was 35%

sensitive but 70% specific. The Schirmer test was 90% specific but only 25% sensitive. Adding any one of the other tests to tear osmolarity did not make the combination more sensitive with parallel testing, i.e. A or B positive. Specificity was 100% with series testing using any two of the tests in this fashion, i.e. A or B negative. Series testing maximizes specificity whereas parallel testing maximizes sensitivity.

Impression cytology is a more direct measure of the cellular damage produced by keratoconjunctivitis sicca than tear osmolarity.⁽¹⁷⁾ However, impression cytology reveals several variations of cell structure which may not be the result of a tear film deficiency but the result of ocular surface disease. Tear osmolarity measurement reflects only changes in the aqueous environment of the surface epithelium in a KCS patient with an excessively hypertonic tear film. Nelson⁽¹⁷⁾ has described the sensitivity and specificity of impression cytology as a diagnostic test for dry eye and has stated that impression cytology is superior to tear osmolarity with 100% sensitivity and 87% specificity. However, the referent value appears to require a series combination of goblet cell density less than 350 cell/mm², mean epithelial cell areas greater than 1000 square micron/cell on the interpalpebral bulbar ocular surface and goblet cell densities greater than 100 cells/mm² on the inferior palpebral ocular surface in the absence of inflammatory cells. The results of impression cytology in normal controls are not included in the paper. As demonstrated, series combinations are more specific but less sensitive than parallel combinations which leads us to question how the data produced a 100% sensitivity but only 87% specificity. The labor of cytologic examination and clinical judgement required to determine endpoints on each patient gathered from three impression samples seems to disqualify impression cytology when compared to the simplicity of tear osmolarity determination which provides one number from the thawing and disappearance of a final ice crystal through the microscope. I cannot explain why Nelson's studies yielded only 44% sensitivity for tear osmolarity and 75% specificity. It may be important here to explain that osmolarity is the term used more by the physiologist and osmolality is the term used by the chemist with both terms meaning the same for our purposes of tear tests.

CONCLUSION

In summary, tear osmolarity is a simple test to determine the freezing point of a microsample of basal tears. Studies have shown that it is a highly sensitive and specific test for keratoconjunctivitis sicca. Since the simplicity of the test and its reliability have become well established, it does appear to be a reasonable candidate for a new international gold standard in the diagnosis of keratoconjunctivitis sicca.

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