Diagnostic Tests in the Sicca Syndrome

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The lysozyme test is the most sensitive test for the diagnosis of the sicca syndrome. The agar diffusion method could be used as a routine diagnostic procedure. With a diameter limit of 21½ mm of lysis under the test conditions described, one out of every 100 diagnoses would be wrong. For the rose bengal test, using a limit of score of staining intensity of 3½, one out of every 20 to 25 times a diagnostic error could be expected. With the Schirmer's 1 test, using a limit of 5½ mm wetting the filter-paper strip, one out of every six persons would be misclassified. No difference in values between men and women was found at any age level in any test. Age effects were found with lysozyme and Schirmer's 1 tests, but not with the rose bengal test.

CONFLICTING information commonly results from the Schirmer's and the rose bengal tests in patients suspected of the sicca syndrome. This is particularly annoying since no factual information is available on the sensitivity of these tests.

Quantitative analysis of one of the constituents of the human tears, lysozyme, has been performed by a number of investigators using various techniques. They found this enzyme decreased in keratoconjunctivitis sicca. The significance of these findings has been greatly marred by the difficulty encountered with these techniques as routine clinical procedures.

The goals of this study were to introduce a quantitative test for lysozyme as a routine diagnostic procedure, to compare the discriminatory ability of the three tests, and to define limits for each test in terms of probability of misclassification.

The study was carried out on two groups, the first group of 550 normal individuals, the second of 43 patients with the sicca syndrome.

Submitted for publication Oct 16, 1968.

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The results indicated that decrease in the amount of lysozyme is the most sensitive indicator of the disease (Fig 1 to 3) and that the agar diffusion technique can be used as a routine clinical procedure.

Materials and Methods

In the first group only those persons were admitted who had no abnormal eye findings other than those attributable to refraction anomalies. The group consisted of 550 persons, ranging in age from 20 to 74 years. In each of five-year classes 25 men and an equal number of women were represented. The second group consisted of 43 patients, 11 men and 32 women.

All tests were performed in the unanesthetized eye. For the measurement of tearflow, the Schirmer's 1 test was carried out with sterile filter-paper strips in a routine fashion. Rose bengal in a 1% solution was used to study the degree of staining. The intensity of staining of both medial and lateral bulbar conjunctiva and of the cornea was scored, each section up to three points, so that a maximum score of nine could be obtained.

Tearfluid for the agar diffusion test was collected by placing a filter-paper disk (Whatman No. 3) with a diameter of 6 mm in the lower cul-de-sac, so that the disk was entirely covered by the lower lid to prevent evaporation of tearfluid and subsequent concentration of its

constituents in the disk. Only after the disk was entirely wet was it removed, excess tearfluid being drained by blotting it lightly between Whatman No. 3 filter-paper. The disks were then placed in a sterile screwcap bottle and kept at 4 C until processed, always within 24 hours. *M lysodeikticus* was used as a substrate for lysozyme determination. Freshly prepared and lightly dried meat infusion agar plates were flooded with a 24-hours broth culture, carrying an average of 5×10^7 viable organisms per milliliter, and excess fluid was removed. After the surface had been dried again for 20 minutes at room temperature, four disks were placed per dish on the medium. Fifteen milliliters of meat infusion agar was used per petri dish of 9-cm diameter, which allowed sufficiently wide zones of lysis. After 24 hours of incubation at 37 C, the diameter of lysis was measured with calipers on the reverse side of the dish to minimize parallax and recorded millimeters.

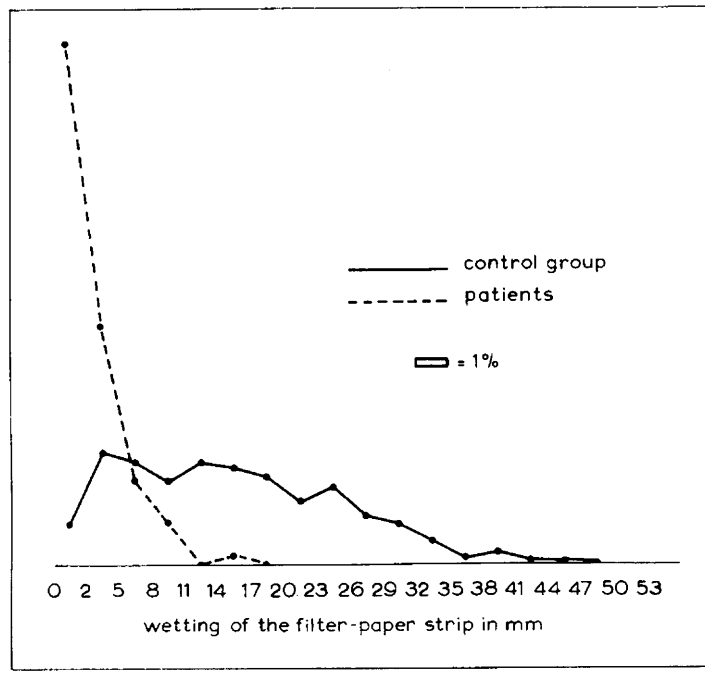


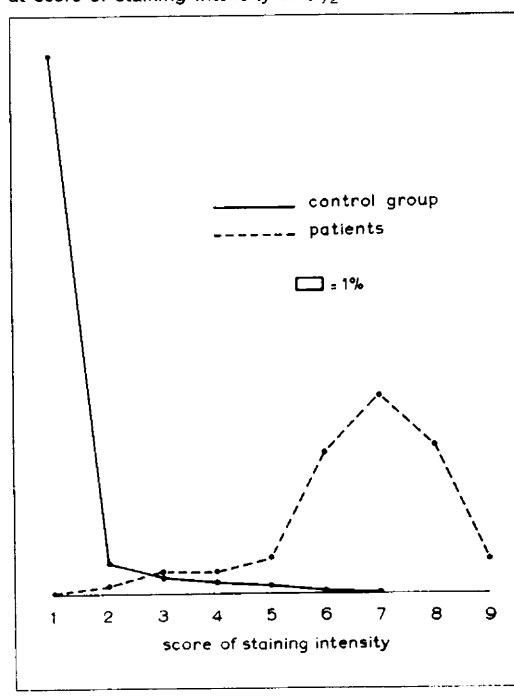
Fig 1.—Schirmer's test, with frequency polygons of data grouped in classes of 3 mm of control group and of patients and optimal limit at 5½ mm wetting of filter-paper strip.

Probabilities of misclassification were calculated for the control group and the patients, whereby the data of men and women were pooled.

As a check on the reproducibility of the technique of collecting tearfluid, 50 observations were carried out whereby two specimens of tearfluid were taken from each eye of the same person with an interval of 1½ hours and placed on one agar plate. The same test was carried out in another 50 paired observations whereby the filter-paper disks were placed on randomly selected agar plates, to measure the magnitude of the experimental error.

Age and sex effects were tested either with analysis of variance or the X^2 technique. Unequal distribution between the sexes and the small number of observations did not allow for analysis of these effects in the patient material.

Fig 2.—Rose bengal test, with frequency polygons of data of control group and of patients and optimal limit at score of staining intensity of 3½.



Results

Analysis.—*Schirmer's 1 Test.*—The frequency distribution of wetting of the filter-paper strips of the control and the patient group is shown in Table 1. If the probabili-

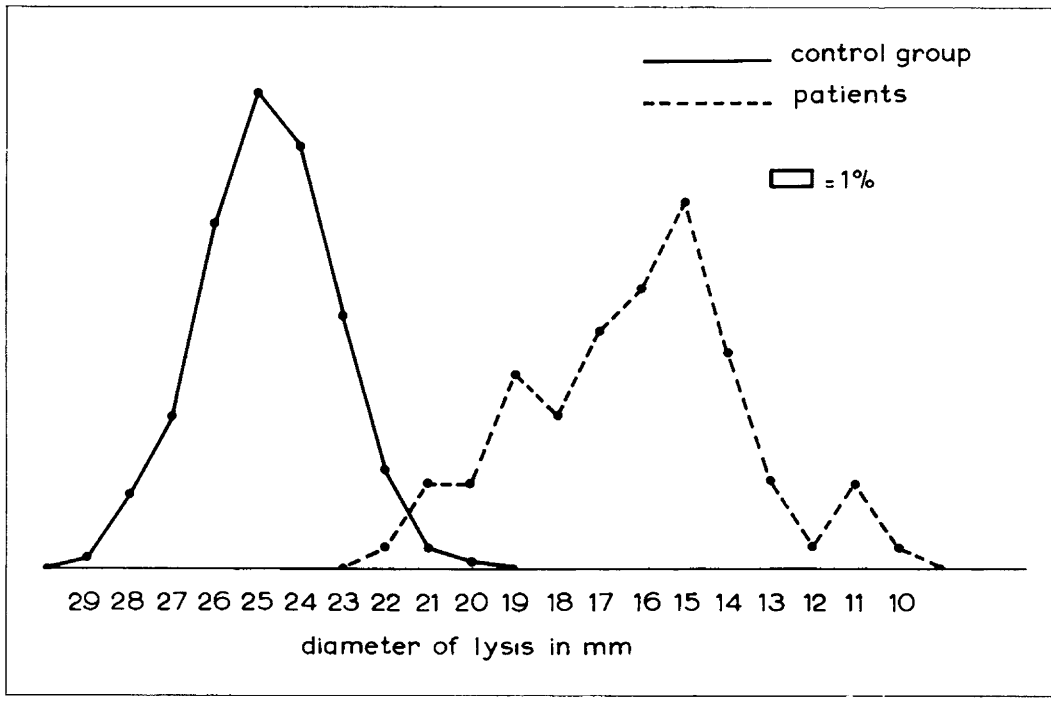


Fig 3.—Lysozyme test, with frequency polygons of data of control group and of patients and optimal limit at diameter of lysis of 21½ mm.

ties of misclassification are balanced, the best limit for the entire material was 5½-mm wetting of the filter-paper strip. With this limit the probability of misclassification of a person from the control group was 17% and of a patient 15%.

Rose Bengal Test.—The frequency distribution of the scores of the control group and patients is shown in Table 2. With a staining intensity score limit of 3½ the probability of misclassification of a person from the control group was 4% and of a patient 5%.

Lysozyme Lysis Test.—The frequency distribution of millimeters of diameter of lysozyme lysis in the control group and in patients is shown in Table 3. With a diameter limit of 21½ mm of lysis the probability of misclassification of a person from the control group and of a patient was 1%.

Using the entire material, the most sensitive indicator of the disease is the lysozyme lysis test, followed by the rose bengal test. The Schirmer's test was rather insensitive. From Table 1 to 3 the probabilities of misclassification for any given result of the three tests could be calculated.

Table 1.—*Schirmer's Test: Frequency Distribution of Wetting of Filter-Paper Strip in Control Group and Patients**

Control Group		Patients	
mm†	Frequency	mm	Frequency
0- 1	15	0	7
2- 3	83	1	25
4- 5	89	2	18
6- 7	82	3	8
8- 9	79	4	7
10-11	63	5	8
12-13	77	6	5
14-15	78	7	3
16-17	87	8	0
18-19	68	9	2
20-21	61	10	1
22-23	55	11	1
24-25	73	12	0
26-27	43	13	0
28-29	40	14	0
>30	107	15	1
Total	1,100	Total	86

* Data of men and women are pooled.
† Wetting of filter-paper strip in millimeters.

The combination of the Schirmer's test, the rose bengal test, and the lysozyme test did not give more information than the lysozyme test alone. The combination of the Schirmer's and the rose bengal test was not better than the rose bengal test alone.

Table 2.—*Rose Bengal Test: Frequency Distribution of Score of Staining Intensity in Control Group and in Patients*

Control Group		Patients	
S*	Frequency	S	Frequency
1	975	1	0
2	54	2	1
3	31	3	3
4	18	4	3
5	14	5	5
6	7	6	20
7	1	7	28
8	0	8	21
9	0	9	5
Total	1,100	Total	86

* Score of staining intensity.

Table 3.—*Frequency Distribution of Diameter of Lysis*

Control Group		Patients	
d*	Frequency	d	Frequency
29	6	22	1
28	44	21	4
27	91	20	4
26	204	19	9
25	282	18	7
24	250	17	11
23	150	16	13
22	57	15	17
21	12	14	10
20	4	13	4
Total	1,100	12	1
		11	4
		10	1
		Total	86

* Diameter of lysis in millimeters.

Table 4.—*Variance Components*

Variation	Variance
Between persons	1.4
Within persons	0.2
Between right and left eye (within persons)	0.2
Between plates	0.3
Experimental error	0.2

Reproducibility.—In both experiments no systematic differences could be found between first and second observation or between right and left eye. To give an impression of the order of magnitude of the different causes of variability we give in Table 4 a number of variance components. The variance "within persons" in this Table is, of course, the variance due to variability in 1½ hours of a certain person. It may be concluded that the lysozyme agar diffusion technique could be considered reliable, as the variance between persons is much higher than the other variances.

Age and Sex Effects.—When the data of men and women in the control group were compared in classes of five-year interval, no significant differences were found with the Schirmer's, the rose bengal, and the lysozyme test. Age effects were pronounced in the Schirmer's test. In the rose bengal test, where age effects could only be measured in two classes of 25-year interval, no effect was found. In the lysozyme test the age effect was significant, but the actual difference expressed in millimeters diameter of lysis was very small. No interaction between the age and sex effects could be found in the lysozyme test.

Patients Suspected of the Sicca Syndrome.—A group of 290 persons, 137 men and 163 women suspected of the sicca syndrome, was also tested. In regard to the Schirmer's and the lysozyme tests, the same limits and probabilities of misclassification were found as in the control group. In the rose bengal test a twofold increase in probability of misclassification was found with the optimal limit, as compared to the control group. Since the values of the tests in the persons of this group could not meet the criteria referred to earlier for the diagnosis of the sicca syndrome, the difference in probability of misclassification could be explained on the basis of unspecificity of the rose bengal test. Also, when the data of this group were compared with those of the control group, it was only with the rose bengal test in the age categories of 20 to 34 and 35 to 49 years that a significant difference in values was found. The age and sex effects followed the pattern of the control group.

Comment

Meyer[1] was the first to show that the tear lysozyme concentration as measured viscosimetrically was decreased in keratoconjunctivitis sicca. His observation was confirmed by Regan,[2] and McEwen and Kimura.[3] Thygeson and Kimura[4] showed that a decrease in concentration of lysozyme preceded all other symptoms of the sicca syndrome. In a recent study, Bonavida and Sapse[5] found the agar diffusion lysozyme test, in its design only slightly different from the type we used, reliable.

If several tests are compared on their discriminatory value for the diagnosis of a disease, the best test is the one that has the smallest area of overlap. These areas for the three tests used in our study are seen in the frequency polygons in Fig 1 to 3. Power analysis of the diagnostic tests in the sicca syndrome not only offered the possibility to compare the discriminatory ability of these tests, but also provided the opportunity to set limits for each of the three tests for normality and disease in terms of probability of misclassification. Meaningful limits have never been set for any one of these tests. The usual procedure in the past of taking the extreme values observed as limits is meaningless; consequently, radical opinions on the merits of these tests were not uncommon.

There remain several problems to be discussed. Undoubtedly the central question of this study is concerned with the selection of the groups. We investigated the discriminatory ability of the tests; but in one of the groups, that of the patients, the diagnosis was based to a great extent on those very tests. Bias was checked to a certain extent by accepting the diagnostic judgment of the various colleagues who cooperated with this study. The selection of the control group might also be a possible source of error in our analysis. From the number of persons rejected after middle age, it was obvious that the relative percentage of persons without any subjective complaints of the eye declined rather rapidly with advancing age, so that an entire absence of complaints might in fact not be representative for the older age groups. These possible errors in selection affected only the parameters of data in an absolute sense, but there was no reason to suspect that the results of the comparative study would have been affected, since all tests have been done on the same material. One must realize, however, that the probabilities of misclassification were dependent on the selection criteria.

In a general way, the distribution of values of the group of persons suspected of the sicca syndrome would either cover the distribution of the control group or the patients or would occupy a position somewhere between these distributions, dependent on the incidence of the disease and the specificity of the symptoms. Therefore, the values of this group could not be used for the analysis of misclassification. In regard to the incidence and the specificity of the symptoms of the sicca syndrome, a close relationship between the distributions of the group of persons suspected of the disease and the distribution of the control group could be expected.

Another issue is concerned with the type of agar diffusion technique used. Linearity could be found in a semilog dose-response plot, using eggwhite lysozyme in the dose range in which lysozyme is present in the human tear. Consequently, tear lysozyme could be assayed in the control group. The very nature of the disease, however, would not allow for a check on parallelism, nor, at best, full check on parallelism in material from patients. Rather than to settle for a low precision assay, we accepted a large margin of experimental error, but brought the test within reach of the clinician, with some degree of confidence based on the analysis of our material which indicated the experimental error considerably smaller than the biological variance.

Dr. G. J. Leppink directed and supervised statistical analysis for this investigation.

Key Words.—Lysozyme agar diffusion test; Schirmer's test; rose bengal test; sicca syndrome; probability of misclassification.

References

1. Meyer, K.: "Mucopolysaccharides and Mucoids of Ocular Tissues and Their Enzymatic Hydrolysis," in Sorsby, A. (ed.): *Modern Trends in Ophthalmology*, London: Butterworth & Co., Ltd., 1948, vol 2, pp 71-78.

2. Regan, E.F.: The Lysozyme Content of Tears, *Amer J Ophthal* **33**:600-605 (April) 1950.

3. McEwen, W.K., and Kimura, S.J.: Filter-Paper Electrophoresis of Tears, *Amer J Ophthal* **39**:200-202 (Feb) 1955.

4. Thygeson, P., and Kimura, S.J.: Chronic Conjunctivitis, *Trans Amer Acad Ophthal Otolaryng* **67**: 494-517 (July-Aug) 1963.

5. Bonavida, B., and Sapse, A.T.: Human Tear Lysozyme: II. Quantitative Determination With Standard Schirmer Strips, *Amer J Ophthal* **66**:70-76 (July) 1968.