

Tear Film Osmolarity: Determination of a Referent for Dry Eye Diagnosis

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PURPOSE. To determine new referents, or cutoff levels for tear film hyperosmolarity in the diagnosis of keratoconjunctivitis sicca (KCS) and to assess their effectiveness in independent patient groups.

METHOD. A meta-analysis was performed on published data for tear osmolarity in samples of normal eyes and various subtypes of dry eye, and pooled estimates of the mean and standard deviations for normal and (all) dry eye subjects were determined. Diagnostic referents were derived from the intercept between the distributions of osmolarity in the two samples and from receiver operator characteristic (ROC) curves. This referent was tested for effectiveness of diagnosis in independent groups with normal and dry eyes.

RESULTS. An osmolarity referent of 315.6 mOsmol/L was derived from the intercept of the distribution curves, and 316 mOsmol/L from the ROC curve. When applied to independent groups of normal and dry eye subjects a value of 316 mOsmol/L was found to yield sensitivity of 59%, specificity of 94%, and an overall predictive accuracy of 89% for the diagnosis of dry eye syndrome.

CONCLUSIONS. Tear hyperosmolarity, defined by a referent of 316 mOsmol/L, was superior in overall accuracy to any other single test for dry eye diagnosis (Lactoplate, Schirmer test, and Rose Bengal staining), even when the other test measures were applied to a diagnosis within the sample groups from which they were derived. For overall accuracy in the diagnosis of dry eye, the osmolarity test was found to be comparable with the results of combined (in parallel or series) tests. (*Invest Ophthalmol Vis Sci.* 2006;47:4309–4315) DOI:10.1167/iovs.05-1504

The ocular surface requires a complete tear film to maintain its health and function; adequate production, retention, and balanced elimination of tears is necessary for this process. Any imbalance of these components can lead to the condition of dry eye.¹ A single biophysical measurement that captures the balance of inputs and outputs from the tear film dynamics is tear osmolarity. Osmolarity is the end product of variations in tear dynamics. Normal homeostasis requires regulated tear flow, the primary driver of which is osmolarity.²

It has been suggested that tear hyperosmolarity is the primary cause of discomfort, ocular surface damage, and inflammation in dry eye.^{3–7} In studies of rabbit eyes, tear osmolarity has been found to be a function of tear flow rate and evaporation.⁸ Gilbard⁹ has shown, in rabbit conjunctival cell cultures, that hyperosmolarity decreases the density of goblet cells, and Nelson and Wright¹⁰ report a 17% decrease in goblet cells for subjects with keratoconjunctivitis sicca (KCS). Armitage and Mazur¹¹ found that granulocyte survival is significantly decreased with increases in solute concentration.¹¹ Rabbit cells cultured in hyperosmolar states, above 330 mOsmol/L, show significant morphologic changes¹² similar to those seen in subjects with KCS^{13–15} Hyperosmolarity-induced changes in surface cells in KCS can be correlated with the amount and distribution of Rose Bengal staining.^{9,16}

The benefits of measuring tear osmolarity in the diagnosis of dry eye disease have been undermined by the difficulties of its measurement. These difficulties have hindered its acceptance and general application in clinical practice. The most commonly applied technique for measurement of tear osmolarity is through observation of the change in the freezing point of tear samples.^{3,5,16–21} The major benefit of this technique is that it requires only microliter samples of tears (~0.2 μ L). The instrument most frequently used is that by Clifton Technical Physics (Hartford, NY) and is the osmometer used in the seminal work of Gilbard and Farris.^{3,5,6–9,12,16,22} The disadvantages of the technique include the need for specialist expertise, constant maintenance of equipment, the length of the procedure, a large laboratory setup, and potential errors due to evaporation of the test sample or reference standards.^{21,23}

Ogasawara et al.²⁴ determined osmolarity indirectly through measurement of the electrical conductivity of tear samples. The benefit of this technique is that tear film osmolarity is measured in real time, and in situ, so that no errors occur during transfer or dilution of the tear fluid. The disadvantage, however, is that the sample size (up to 0.96 μ L) is dictated by the capacity of the sensor area. Also the placing of a sensor on the ocular surface is invasive and can precipitate reflex tearing. However, the development of new osmometers (i.e., OcuSense, Los Angeles, CA, which requires 0.2- μ L samples of tears placed into a disposable microelectrode array for semiautomatic reading of osmolarity),²⁵ may mean that the use of electrical conductivity as a means to obtain tear film osmolarity will gain greater acceptance.

The development of new instruments for measuring human tear film osmolarity with potential for clinical application make it appropriate to reconsider the utility of osmolarity as a differential diagnostic feature of KCS.²⁵ The suggestion of Farris²⁶ that tear osmolarity measurement offers a possible gold standard for the diagnosis of the disease needs re-examining in the light of these developments. This study was undertaken to determine new referent, or cutoff, for tear film hyperosmolarity in the diagnosis of KCS, by applying a meta-analysis to previously published measurements of tear osmolarity. These new referents were then assessed in an independent patient group.

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TABLE 1. Human Tear Osmolarity Recorded in Studies Reported in the Literature between 1978 and 2004

Study	Test	Normal Subjects (n)	KCS (Sum of Subtypes)
Mishima et al. ³²	FPD	304 ± 1.5 (33)	329 ± 4.7 (6)
Gilbard et al. ⁵	FPD	304 ± 10.4 (33)	343 ± 32.3 (30)
Terry and Hill ²⁷	VP	310 ± 5.7 (6)	—
Gilbard and Farris ¹⁶	FPD	—	365 ± 77 (20)
Farris et al. ³⁴	FPD	304.4 ± 7.2 (219)	329.6 ± 17 (123)
Benjamin and Hill ¹⁷	FPD	318 ± 31 (6)	—
Farris et al. ³	FPD	304 ± 8 (180)	326 ± 20 (111)
Farris ²²	FPD	302 ± 5 (51)	324 ± 11 (51)
Gilbard et al. ³¹	FPD	—	317 ± 2.4 (29)
Gilbard ³³	FPD	304 ± 0.3 (23)	314 ± 1.0 (31)
Craig and Tomlinson ¹⁷	FPD	303.5 ± 13 (100)	—
Mathers et al. ²⁰	FPD	303 ± 10 (72)	313.7 ± 13 (146)
Ogasawara et al. ²⁴	Con	296.4 ± 30 (17)	324 ± 41 (16)
Iester et al. ²⁸	FPD	—	349.5 ± 19 (58)
Isekei et al. ²⁹	FPD	283.3 ± 11.3 (56)	—
Thai et al. ³⁰	FPD	302.1 ± 11.9 (19)	—
Average tear osmolarity	All	302 ± 9.7 (815)	326.9 ± 22.1 (621)

Data were recorded in freezing-point depression (FPD) and vapor pressure (VP) osmolarity tests in normal subjects and those with all types of keratoconjunctivitis sicca (KCS).

METHODS

Meta-analysis of Human Tear Film Osmolarity

Over the past 27 years, several studies have measured tear film osmolarity in normal eyes and eyes with KCS^{3,5,16,17,19,20,22,24,27-34}, most of the studies used the freezing-point depression measurement technique. Data from these studies, collated in Table 1, were subjected to a meta-analysis.³⁵ Studies were included in Table 1 in which the original available information (mean, standard deviation, and sample size) facilitated combining or pooling of the data. The data were combined to obtain an overall mean and standard deviation for tear osmolarity in normal subjects and in those with KCS.

The estimates of the 95% confidence interval (CI) of the mean for the pooled samples of normal subjects and all subjects with KCS (Table 1) are shown in Figure 1. As two of the studies in the meta-analysis^{20,33} report values for osmolarity in subtypes of KCS (aqueous tear deficiency, meibomian gland dysfunction [MGD]; shown in Table 2), the 95% CI for these subtypes are also shown in Figure 1.

Deriving a Referent for Tear Osmolarity in the Diagnosis of KCS from the Meta-analysis

Normal distribution curves were derived (Fig. 2) for the pooled samples in the meta-analysis of normal subjects and those with KCS

described in Table 1, and the cutoff value of the intercept between the curves was found.³⁶ In addition the receiver operator characteristic (ROC) curves for this meta-analysis data set were derived (Fig. 3).³⁷ This ROC curve gave a second referent, or cutoff, that maximized sensitivity and specificity in differentiating persons with KCS from normal subjects.

Testing the Effectiveness of Tear Hyperosmolarity in the Diagnosis of KCS

The osmolarity referent derived by the intercept method and from the ROC curve was then applied to a second independent sample of normal subjects and those with KCS, referred for clinical evaluation to the dry eye physiology laboratory at Glasgow Caledonian University Eye Clinic, to determine the sensitivity and specificity in this sample. This sample comprised 25 normal subjects and 47 arthritic subjects with aqueous-deficiency dry eye (17 subjects) and primary Sjögren's syndrome (30 subjects), the data for which have been reported previously (Craig JP, et al. *IOVS* 1995;36:ARVO Abstract 4823). To this group were added a further 30 subjects with dry eye in whom tear physiology, including osmolarity, had been measured as part of their clinical management (Table 2). This additional group included 7 with MGD, 16 with aqueous-deficiency dry eye, and 7 with primary Sjögren's syndrome. Ophthalmic diagnosis (by two of the authors: KR

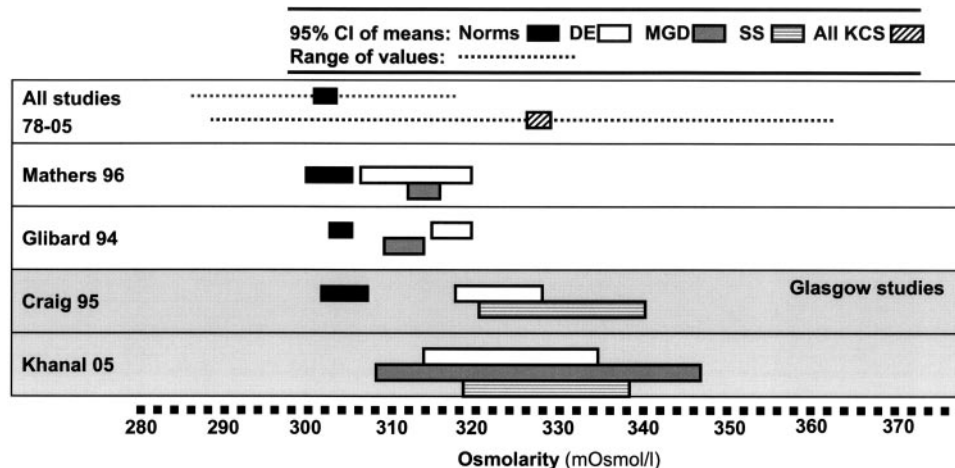


FIGURE 1. The spread of data obtained in studies of human tear osmolarity between 1978 and 2005 in normal subjects and patients with KCS. Data are shown for the range of osmolarities and the estimates of the 95% CI of the means for normal subjects and all groups of patients with KCS. Also shown are CIs for the estimates of the means for patients with KCS in studies in which these are reported. DE, aqueous deficiency dry eye; MGD, meibomian gland dysfunction; SS, Sjögren's syndrome. Data shown are from Mathers et al.,²⁰ Gilbard,³³ and Craig et al. (*IOVS* 1995;36:ARVO Abstract 4823), together with original data from the present study.

TABLE 2. Human Tear Osmolarity Recorded by Freezing-Point Depression Osmolarity Tests in Studies in Which Osmolarity in Subtypes of KCS Were Measured

Study	Normal Subjects (n)	KCS (Subtypes)
Gilbard ³³	304 ± 0.3 (23)	LGD: 316.2 ± 0.9 (19) L/MGD: 317.2 ± 1.1 (3) MGD: 311.5 ± 1.1 (9)
Craig and Tomlinson*	305 ± 7 (25)	DE (RA): 323 ± 15 (30) SS (RA): 331 ± 21 (17)
Mathers et al. ²⁰	303 ± 10 (72)	DE: 313 ± 9 (37) MGD: 314 ± 14 (109)
Tomlinson and Khanal ¹	—	DE: 324.1 ± 20.8 (16) SS: 327.8 ± 14 (7) MGD: 327.1 ± 25.1 (7)

LGD, lacrimal gland dysfunction; MGD, meibomian gland dysfunction; DE, aqueous-deficiency dry eye; SS, Sjögren's syndrome; RA, rheumatoid arthritis.

* IOVS 1995;36:ARVO Abstract 4823.

and CP) was on the basis of an ordered series of assessments including reports of dry eye symptoms (usually, dry, burning, or sticky eyes) recorded together with the time of occurrence, tear break-up time, and tear quality by slit lamp examination, Schirmer II test over 5 minutes with open eye test and proxymethacaine anesthesia and ocular surface staining with a single, mixed drop (fluorescein with proxymethacaine). Blood tests for autoantibodies were performed when necessary. The mean and SD of the tear osmolarities in the Glasgow samples of normal and subtypes of dry eye can be seen in Table 2, the normal distribution curves are shown in Figure 4.

Further information, in addition to determining the sensitivity and specificity of a test, about the efficiency of any diagnostic criterion for disease is obtained by calculating the predictive ability and the accuracy of prediction of that criterion when applied to diagnosis.³⁸ The predictive ability is the combination of the predictive value of a positive test (the proportion of positive tests that identify a disease as present) and the predictive value of a negative test (the proportion of negative tests correctly identifying normal individuals). The accuracy of a test is the proportion of all tests that give correct classification.³⁸ These predictive values were calculated for a new referent which was then applied to the meta-analysis and Glasgow Study patient samples.

RESULTS

Efficacy of a Referent Tear Osmolarity in the Diagnosis of KCS

The cutoff of the intercept between the distribution curves of the normal and KCS subjects in the meta-analysis studies was found to be 315.6 mOsmol/L; the ROC curve gave a second and similar referent of 316 mOsmol/L. The sensitivity and specificity of a referent of 316 mOsmol/L was 69% and 92% in the sample from which it was derived. The referent applied to the independent sample of normal and KCS subjects referred to the Dry Eye Physiology Laboratory at Glasgow Caledonian University Eye Clinic, yielded sensitivity and specificity in the diagnosis of dry eye of 59% and 94%. As expected, the efficiency of diagnosis (in terms of sensitivity) in this independent sample was less than when the osmolarity referent was applied to the sample from which it was derived (the meta-analysis data in Table 1).

The predictive value of a positive test for the Glasgow sample was 63% and the predictive value of a negative test, 93%; giving an overall accuracy of 89% (Table 3) assuming a prevalence of 15% for dry eye.³⁹⁻⁴¹ The overall accuracy in the

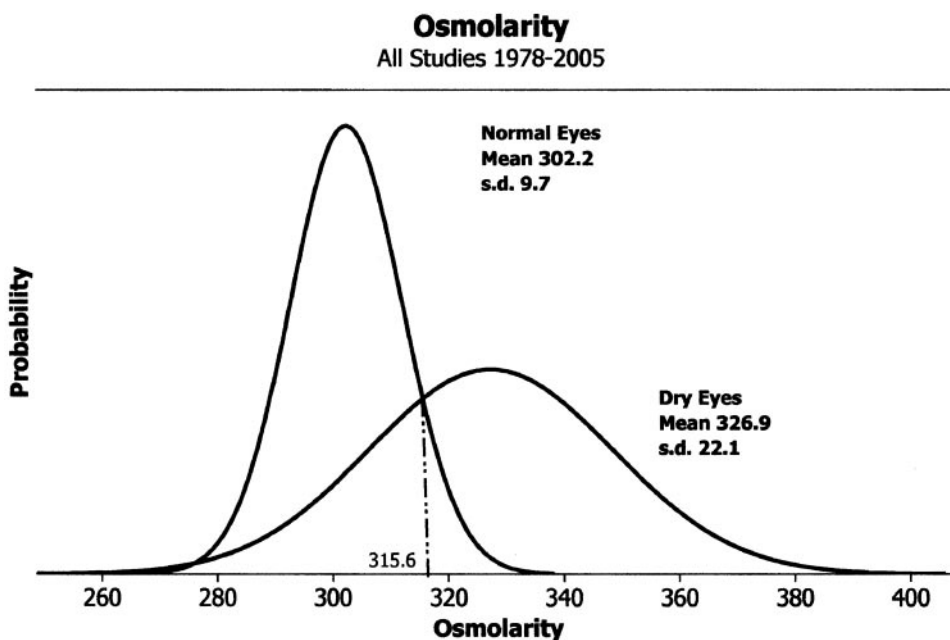


FIGURE 2. The normal distribution curves human tear osmolarity obtained in normal subjects and patients with KCS reported in the literature between 1978 and 2004. A referent or cutoff value for dry eye obtained from the intercept of these curves is at 315.6 mOsmol/L.

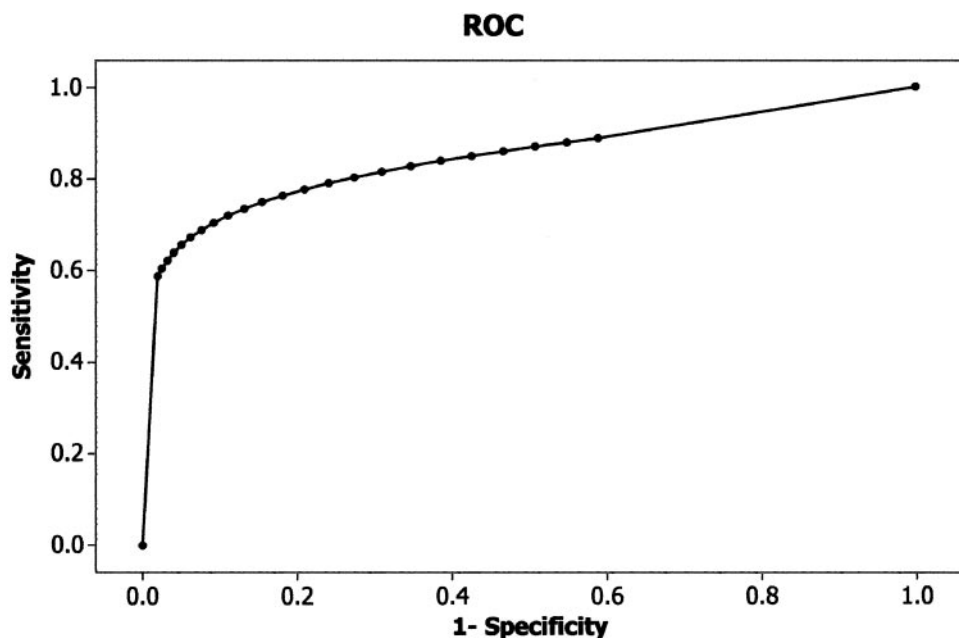


FIGURE 3. The ROC curves for the sensitivity and specificity of data obtained from the grouped analysis of studies of tear osmolarity between 1978 and 2004. The ROC curve is shown for osmolarities between 300 and 322 mOsmol/L.

independent sample was not changed from the accuracy of the referent when applied to the sample from which it was derived (meta-analysis studies; Table 1).

DISCUSSION

The difficulty of using a single parameter, tear osmolarity, to diagnose KCS lies in the overlap of levels of this parameter in normal and dry eyes (Fig. 1), which makes the choice of a referent, or a cutoff, difficult. Farris^{3,4} observed in his study that a significant overlap of values occurred between 293 and 320 mOsmol/L. This is reflected in the pooled analysis of all the data from Table 1 and is in spite of the highly significant difference between the sample means and the wide separation of the 95% CI for the estimates of these means (Fig. 1). Unfortunately, it is in the range of 290 to 320 mOsmol/L that the

determination must be made as to whether the osmolarity of a tear sample is abnormal. Gilbard et al.⁵ chose 312 mOsmol/L as a osmolarity referent for KCS on the basis of maximizing diagnosis and avoiding underdiagnosis. This value gave a sensitivity of 94.7% and a specificity of 93.7% in the determination of dry eye against diagnosis by a combination of dry eye tests.⁵ However, the original selection criteria for these subjects had included osmolarity. This introduced a selection bias into the study^{4,2} and contributed to the high sensitivity demonstrated by Gilbard et al.⁵ Also the sensitivity and specificity of the referent in the study by Gilbard et al. was not tested in an independent patient sample.

In the present study, we considered other approaches to determining an osmolarity referent for KCS and its effectiveness in the diagnosis of dry eye. A referent was derived from the intercept of the distribution curves for normal and dry

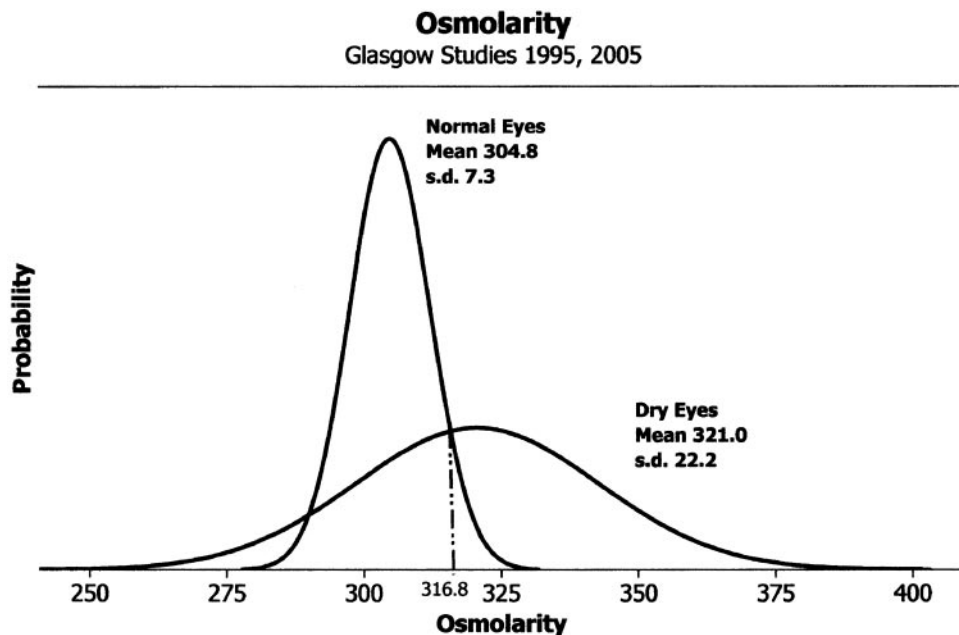


FIGURE 4. The normal distribution of human tear osmolarity measured in normal subjects and in those with KCS in two studies performed in our laboratory in Glasgow in 1995 and 2005. These curves are both normally distributed and have an intercept at 316.8 mOsmol/L.

TABLE 3. A Comparison of the Effectiveness of Various Individual and Combined Diagnostic Tests in the Differential Diagnosis of KCS

	Test	Cutoff	Sens. (%)	Spec (%)	PV+	PV-	Accuracy	False Positive	
Osmolarity									
	Meta-analysis	Osm	316	69	92	60	94	89	31
	Glasgow Studies	Osm	316	59*	94*	63*	93*	89*	41*
			312	66*	84*	42*	93*	81*	34*
			318	55*	97*	76*	92*	91*	45*
			322	48*	99*	89*	92*	91*	52*
	Gilbard/Farris ^{3,5,16,26,33,34}	Osm	312	94.7	93.7	73	99	90	6.3
Other Single KCS Tests									
	Lucca ⁴⁶	Lacto	<90	35	70	17	86	65	65
		Sch	<1 mm/min	25	90	31	87	80	75
	Goren, Goren ⁴⁴	RB	Any	25	90	31	87	80	75
	Van Bijsterveld ⁴⁵								
	Lysozyme diffusion test		<21.5 mm lysis	98.8	98.5	92	99.8	99	1.2
	Nelson Wright ⁴⁷		IB-GCD < 350/mm ²						
	Impression cytology	Epi area	>1000 μm; IP-GCD > 100/mm	100	87	58	100	89	0
Combined KCS Tests									
	Farris ⁶								
	Parallel: Osm or Sch		312; 1 mm/min	90	85	51	98	86	10
	Series: Osm + Lacto		312;<90	35	100	100	90	90	65
	Craig and Tomlinson†								
	Discr function osm, evap, lipid		>0.4	96	87	57	99	88	4

* The effectiveness of osmolarity referent values (Osm) in the diagnosis of KCS on independent samples of normal subjects and patients with dry eye. Comparisons are made with results from lactoplate (Lacto), Schirmer (Sch) and Rose Bengal (RB) tests and values of goblet cell diversity (GCD) from impression cytology performed on the epithelial (Epi), infrabulbar (IB), and infrapalpebral (IP) regions. evap, evaporation.

† IOVS 1995; 36:ARVO Abstract 4823.

eyes, and from the receiver operator characteristic (ROC) curves of the data. The derived referent of 316 mOsmol/L classified 41% of dry eyes as normal in the independent sample. An improvement in identification of dry eye in this sample of subjects can be achieved by decreasing the referent for hyperosmolarity (Table 3) to the 312 mOsmol/L suggested by Gilbard et al.⁵ and Farris et al.³ This change improves the sensitivity and reduces the false-negative rate, but the specificity and overall accuracy decline significantly. Increasing the referent's value maximizes accuracy (Craig JP, et al. IOVS 1995;36:ARVO Abstract 4823), but leads to misdiagnosis of almost half of KCS subjects as normal (Table 3). Therefore, the choice of different referents for diagnosis of KCS by osmolarity changes the relative of sensitivity and specificity (Table 3). The choice will also be influenced by physiological factors (the osmolarity at which cell damage occurs⁴³). In the case of potentially fatal conditions, high sensitivity is required in diagnosis, but for conditions such as dry eye with chronic, long-term morbidity, high specificity would be preferred, to avoid increasing burdens on healthcare delivery systems.

Another problem in considering the sensitivity, specificity, and predictive ability of any single test, or set of diagnostic criteria in dry eye diagnosis, is that these parameters are affected by the manner in which they are obtained and the population being diagnosed. The population effect is due to the multiple etiologies of the disease and the criteria used to define dry eye in the test sample. These effects are obviated when the samples are large, as in the meta-analysis in this study, but may have affected the diagnostic efficacy of a test or tests applied to the (smaller) number of patients in the Glasgow sample. In most reports (Craig JP, et al. IOVS 1995;36:ARVO Abstract 4823)^{5,26,44} the effectiveness of a test or tests is calculated by using the criteria on the population from which the referent was originally derived; thus overestimating effectiveness when applied to the diagnosis of future cases. This overestimate is compounded if the measurement is one of the criteria in the diagnosis of the disease.^{5,26,42} Ideally, in deter-

mining the utility of a measurement in the diagnosis of disease, a referent, or cutoff, for the disease should be developed on an investigational group of subjects and *then* tested successively on an independent sample of subjects. This gives a better estimate of its utility in diagnosis in a general population of subjects.

In Table 3 the effectiveness of osmolarity as a diagnostic test for KCS is compared with several other dry eye tests, both individually and in combination, as reported by other investigators.^{3,5,26,44-47} When comparing individual test results, the greatest accuracy appears to be for the lysozyme diffusion test of van Bijsterveld,⁴⁵ but it is probable that this test was administered to a severely affected population,²⁶ leading to spectrum bias and an overestimate of sensitivity in the results reported.⁴² This population would not be typical of a general population in which differential diagnosis is normally required. Nelson and Wright⁴⁷ report high sensitivity with impression cytology, and, although this is a test involving one technique, it requires a series of criteria to be met for diagnosis. Farris²⁶ has questioned the 100% sensitivity level reported for this test, as series tests are normally more specific than sensitive.

Osmolarity as a single test of tear physiology offers the ability to define and differentiate KCS and "normal" subjects with a relatively high degree of accuracy (90%) for referents in the range between 312 and 322 mOsmol/L (Table 3). A level of 316 mOsmol/L gives high sensitivity, specificity, and predictive accuracy, in the situation where it is applied to diagnosis of subjects in the population from which it was derived (Table 3, meta-analysis samples). It has overall accuracy comparable to the results reported by Farris et al.³ and Gilbard et al.⁵ but lower sensitivity, specificity, and predictive values of positive and negative tests. However, in the studies by Gilbard et al. and Farris et al.^{3,5} it must be remembered that osmolarity was an original criteria in patient selection for the osmolarity referent determination. In other comparisons of the effectiveness of diagnosis of single tests for KCS in the population from which referents were derived, the osmolarity of 316 mOsmol/L com-

pares very favorably in sensitivity, specificity, predictive ability, and overall accuracy (Table 3).

Farris²⁶ has taken the data reported by Goren and Goren⁴⁴ to compare the effectiveness of individual tests, as well as to describe their effect when used in combination, either in parallel or in series, in diagnosis (reproduced in Table 3). Tests in parallel, increase sensitivity at the expense of specificity and in series, the reverse applies.²⁶ The highest sensitivity and specificity are obtained for a combination, in parallel, of tear osmolarity with Schirmer strip wetting and in series, for osmolarity and the Lactoplate test (Table 3). As all these sensitivities and specificities are obtained with a test or tests applied to the patient samples from which the referents were derived, the single osmolarity test value of 316 mOsmol/L was assessed for diagnostic efficacy in the population from which it was derived. In this application, osmolarity alone gives a better overall accuracy than the best parallel combination tests, but the false negative rate of 31% with the single test was not as low as that of the parallel combination. This benefit is offset by the parallel combination offering only 51% predictive accuracy in a positive test.

Another statistical technique which may offer improved performance when using combination of tests in the diagnosis of dry eye disease is the discriminant function analysis.^{48,49} This approach has been adopted by Craig and Tomlinson (IOVS 1995;36:ARVO Abstract 4823) in dry eye diagnosis, giving a sensitivity of 96% and specificity of 87% when applied to the sample from which the discriminant function was derived. Discriminant function analysis incorporating multiple dry eye parameters also needs to be tested in independent patient samples.

The measurement of tear film osmolarity arguably offers the best means of capturing, in a single parameter, the balance of input and output of the lacrimal system. It is clear from the comparison of the diagnostic efficiency of various tests for KCS, used singly or in combination, that osmolarity provides a powerful tool in the diagnosis of KCS and has the potential to be accepted as a gold standard for the disease. The advent of new technology, making clinical testing of this feature of tear physiology simple, practical, and inexpensive could provide an impetus to its adoption in the diagnosis of KCS.

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