Dry Eye Diagnosis

Santosh Khanal,[1] *Alan Tomlinson,*[1] *Angus McFadyen,*[2] *Charles Diaper,*[3] *and Kannu Ramaesh*[4]

PURPOSE. To determine the most effective objective tests, applied singly or in combination in the diagnosis of dry eye disease.

METHODS. Two groups of subjects— 41 with dry eye and 32 with no ocular surface disease— had symptoms, tear film quality, evaporation, tear turnover rate (TTR), volume and osmolarity, and meibomian gland dropout score assessed.

RESULTS. The subjects with dry eye had TTR, tear evaporation, and osmolarity significantly different from that of healthy normal subjects. Cutoff values between the groups were determined from distribution curves for each aspect of tear physiology, and the effectiveness of the cutoff was determined from receiver operator characteristic (ROC) curves. Values of 12%/min for TTR, 33 g/m^{-2}/h for evaporation, and 317 mOsmol/L for osmolarity were found to give sensitivities, specificities, and overall accuracies of 80%, 72%, and 77%; 51%, 96%, and 67%; and 78, 78%, and 79%, respectively when applied singly as diagnostic criteria in dry eye. In combination, they yielded sensitivities, specificities, and overall accuracy of 100%, 66%, and 86% (in parallel) and 38%, 100%, and 63% (in series), respectively. Discriminant function analysis incorporating these three factors in an equation allowed diagnosis with a sensitivity of 93%, specificity of 88%, and overall accuracy of 89%.

CONCLUSIONS. Tear osmolarity is the best single test for the diagnosis of dry eye, whereas a battery of tests employing a weighted comparison of TTR, evaporation, and osmolarity measurements derived from discriminant function analysis is the most effective. (*Invest Ophthalmol Vis Sci.* 2008;49: 1407–1414) DOI:10.1167/iovs.07-0635

Attempts have been made to define the condition of dry eye,[1] but the methods for its diagnosis vary widely, undermining attempts at definition by the use of differing diagnostic criteria and creating difficulties in comparisons of prevalence and efficacy of treatment regimens. Diagnosis of dry eye disease is made difficult by its multifactorial etiology, by the need for a comprehensive definition, and by the use of tests that are limited and variable in their assessment of the tears and the ocular surface.

Some studies have used reports of symptoms as the criterion for the diagnosis of dry eye[2-8] (see Table 1). It has been suggested that it is appropriate to diagnose dry eye from symptoms alone, as the condition rarely progresses to the stage of causing ocular discomfort without symptoms.[9] However, symptoms alone are inadequate for differential diagnosis of dry eye, because the same symptoms can be experienced with a range of ocular surface conditions and tear film disorders.[1,10]

The most common objective diagnostic test for dry eye, the Schirmer test, which has been in use for more than 100 years,[11] lacks standardization,[12] is inaccurate and unrepeatable because of the reflex secretion produced by its invasive nature,[13] and measures tear production only,[14] so that the evaporative aspects of dry eye are overlooked.[12] However, the low cost of strips, their ease of application, and the lack of availability of a more acceptable diagnostic test has led to the Schirmer test's being the most commonly applied clinical test for lacrimal secretary function in dry eye.[15]

Tear break-up time (TBUT) measurement with fluorescein is another widely used technique for dry eye diagnosis by clinicians. This test is considered to be more reliable than the Schirmer test, as it is repeatable[16] and minimally invasive; however, the instillation of fluorescein can destabilize the tear film.[17,18] The measurement of break up time in the absence of fluorescein (NIBUT) can overcome this problem and give a more accurate assessment of tear stability. But all forms of tear break-up measurement fail to give direct information on tear evaporation.[12]

Ocular surface staining with vital dyes such as rose bengal, lissamine green, and fluorescein have also been used to diagnose dry eye.[19-21] The disadvantage of these tests is that dry eye is measured by the extent of ocular surface damage, and therefore do not necessarily detect early dry eye or differentiate dry eye from other conditions causing ocular surface staining.[22]

Tear physiology tests can also be used to diagnose dry eye. Normal tear film dynamics require adequate production, retention on the ocular surface, and balanced elimination.[23] The quantitative aspects of tear dynamics include distribution, turnover (and drainage), evaporation, and absorption of the tears. An imbalance in any of these components would disrupt the normal tear physiology and lead to dry eye. The proportion of ocular surface absorption of a dye in eyes with normal corneas has been shown to be minimal at only 0.24% ± 0.13%.[24] Therefore, the contribution of tear absorption to the overall tear elimination can be said to be negligible. Tear osmolarity represents the end product of changes in tear dynamics[25] and is thought to be an attractive index for dry eye diagnosis.[26] Direct measurements of tear production, stability, evaporation and osmolarity have the potential to be accurate and sensitive tests for dry eye, and these are the tests assessed in this study.

It has been shown that there is poor correlation between symptoms and signs of dry eye. Only 57% of the symptomatic patients have been shown to have objective signs of dry eye.[9,10,27-29] This finding has been attributed to the symptoms preceding the signs, or the differing etiology and pathophysiology of dry eye.[30] Also, a single objective test for dry eye is of limited value without a report of symptoms.[9]

A large number of tests have been used singly, or in combination, to diagnose the condition with variable success,[13,17,23,31-45] (Table 1), because of the inherent variability

TABLE 1. Test Effectiveness in the Diagnosis of Dry Eye

Test	Author, Reference	Cutoff	Sens %	Spec %	False–ves %	PPV %	NPV %	OA %
Single Tests								
Symptoms	McMonnies[32]	Any	98	97	2	85	99.6	97
Symptoms score TERTC-DEQ	Narayanan et al.[31]	>32.29	75	100	25	100	96	96
Phenol red thread	Patel et al.[33]	≤10 mm	86	83	14	47	97	83
Rose bengal	Goren and Goren[34]	Any	25	90	75	31	87	80
Schirmer I	Lucca et al.[13]	<5 mm/5 min	25	90	75	31	87	80
Schirmer I	Farris[35]	<3 mm/5 min	10	100	90	100	86	87
Schirmer I	van Bijsterveld[45]	<5.5 mm/5 min	85	83	15	47	97	83
Schirmer I	Vitali et al.[36]	<10 mm/5 min	83	68	17	31	96	70
F BUT	Vitali et al.[36]	<10 s	72	62	28	25	93	64
N I BUT	Mengher et al.[17]	<10 s	83	85	17	49	97	85
TMS-BUT	Goto et al.[37]	<5 s	98	63	2	32	99	68
Meniscus height	Mainstone et al.[46]	≤0.35 mm	93	67	7	33	98	71
Meniscus radius	Yokoi and Kumoro[38]	≤0.25 mm	89	78	11	42	98	81
Tear film index	Xu and Tsubota[47]	≤95	67	60	33	23	91	61
Osmolarity	Gilbard et al.[40]	>312 mOsmol/L	95	94	55	73	99	90
Osmolarity	Tomlinson et al.[23]	>316 mOsmol/L	69	92	31	60	94	89
Osmolarity	Tomlinson et al.[23]	>316 mOsmol/L	59	94	41	63	93	89
Osmolarity	Tomlinson et al.[23]	>312 mOsmol/L	66	84	34	42	93	81
Osmolarity	Tomlinson et al.[23]	>322 mOsmol/L	48	99	52	89	92	91
Osmolarity	Sullivan[41]	>318 mOsmol/L	94	95	6	77	99	95
Lysozyme assay	van Bijsterveld[45]	Dia <21.5 mm	99	99	1	95	99.8	99
Ferning	Norn[42]	Area <0.06 mm²/μL	94	75	6	40	99	78
Lactoferrin	Lucca et al.[13]	<90 μg/dL	35	70	65	17	86	65
Combined Tests (Parallel)								
Schirmer or rose bengal	Farris[43]	<1 mm/min or any	77	49	23	21	92	53
Schirmer or TBUT	Farris[43]	<1mm/min or <10 sec	78	56	22	24	94	59
Schirmer or TBUT or rose bengal	Farris[43]	<1 mm/min or <10 sec or any	80	49	20	22	93	54
Combined tests (series)								
Schirmer and osmolarity	Farris[43]	<1 mm/min and >312 mOsmol/L	25	100	75	100	88	89
Lactoplate and osmolarity	Farris[43]	>90 and >312 mOsmol/L	35	100	65	100	90	90
Discriminant function								
Osmolarity, evaporation, and lipid	Craig[44]	<0.4	96	87	4	56	99	88

Sensitivities (Sens), specificities (Spec), false-negatives (False–ves), positive predictive values (PPV), negative predictive values (NPV), and overall accuracies (OA) of different tests, singly and in combination, and discriminant function are shown. TERTC-DEQ, Texas Eye Research and Technology Center Dry Eye Questionnaire; TBUT, tear break-up time; FBUT, fluorescein break-up time; NIBUT, noninvasive tear break-up time; TMS-BUT, topographic modeling system tear break-up.

of most tests and their inability to measure specifically the change in tear physiology in the multifactorial condition of dry eye.[10]

The purpose of this study was to assess as directly as possible tear physiology and meibomian gland function in a range of dry eye conditions in an attempt to optimize the diagnosis by determining the most suitable single test or battery of diagnostic tests. The study complied with the guidelines in the Declaration of Helsinki for research involving human subjects. All participants provided signed informed consent.

METHODS

Subjects

Two groups of subjects were recruited for the study, including those with dry eye and normal subjects with no symptoms or signs of anterior eye disease. Patients with dry eye were referred from two Glasgow hospitals to the Tear Physiology Unit in Glasgow Caledonian University for tear evaluation. Clinical diagnosis of dry eye was made by the referring ophthalmologists (KR, CD) on the basis of symptoms, clinical observations, Schirmer I test, tear break-up time, and autoantibody tests (in the case of Sjögren's syndrome). All the patients had

positive dry eye symptoms. Patients treated with tear supplementation were instructed not to use any solutions for 48 hours before tear evaluation. The dry eye group was made up of patients defined by the ophthalmologists in the following subgroups: Sjögren's syndrome, graft-versus-host disease (GVHD), or the general category other dry eye. Patients with blepharitis were excluded from the study.

Normal subjects were recruited initially by a general e-mail notice within the university. The inclusion criteria for normal subjects were less than two symptoms on the McMonnies Dry Eye Questionnaire,[32] noninvasive tear break-up time (NIBUT)[48] of greater than 10 seconds, and a Schirmer I test score[11,13,14] of >5 mm in 5 minutes. Subjects who had worn contact lenses and those with external ocular diseases in the previous 6 months were excluded from the study. None of the normal subjects had ever used tear supplements before. Signed consent was obtained from all the subjects and patients before recruitment into the study.

Investigations

In addition to the initial dry eye assessment performed at the hospital with the use of conventional clinical tests, tear analysis was performed at the Tear Physiology Unit at Glasgow Caledonian University on all subjects. The specific measures included, tear quality,[49] evaporation,[50]

TABLE 2. Results of Tear and Gland Assessments in Normal and Dry Eye

	Normal	**Dry Eye**	*P*
TTR (%/min)	15.24 ± 5.66	7.75 ± 5.94	<0.001
Evaporation (g/m²/h)	20.97 ± 10.06	37.89 ± 26.48	0.001
Osmolarity (mOsmol/L)	308.39 ± 9.29	328.71 ± 13.73	<0.001
Tear volume (mL)	7.98 ± 3.54	7.08 ± 2.85	0.234
Meibomian gland dropout score	0.56 ± 0.57	0.78 ± 0.99	0.691
Tear film quality (% frequency)			0.717
Grade 1	6.25	9.76	
Grade 2	15.63	39.02	
Grade 3	56.25	12.19	
Grade 4	21.87	29.27	
Grade 5	0	9.76	

Data are the mean ± SD.

turnover,[51-53] volume,[54] and osmolarity,[40,55,56] as well as meibomian gland function.[57]

Analysis of Data

The results obtained for individual measurements of tear quality, evaporation, turnover, volume and osmolarity, and meibomian gland function were analyzed by comparisons of means in normal subjects and patients with dry eye. Cutoffs and referent values for dry eye were initially obtained from the intercept of the frequency distributions of each tear measurement for dry eye and normal groups. Receiver operating characteristics (ROC) curves were plotted for the cutoff value, to determine the sensitivity and specificity of the measurement in defining dry eye.[58] The results for individual tear measurements were combined in parallel and series[34,43] and by discriminant function analysis in an attempt to improve diagnostic efficacy.[59]

RESULTS

Forty-one patients with dry eye (17 men and 24 women; average age, 51.63 ± 13.81 years) of which 17 (2 men and 15

women; average age, 52.35 ± 11.81 years) had a diagnosis of Sjögren's syndrome by the referring ophthalmologist as, 12 (12 men, average age, 47.01 ± 13.09 years) of GVHD, 12 (3 men and 9 women; average age, 55.25 ± 16.77 years) as general dry eye (aqueous deficient and/or evaporative); and 32 normal subjects (12 males and 20 females, average age, 52.31 ± 14.38 years), were examined. The mean and standard deviations of values obtained in assessments of these groups are shown (Table 2). Patients with dry eye were also classified into different groups in a severity matrix (Table 3), based on the dry eye severity scale proposed by Behrens et al.[60] and adopted by the recent Dry Eye Workshop (DEWS) report.[61]

Normal and Dry Eyes

All the measured variables were tested for normality. Tear evaporation, turnover rate, osmolarity, and volume were normally distributed. A two-sampled *t*-test showed that there were statistically significant differences between normal subjects and patients with dry eye for tear turnover rate (TTR; $P < 0.001$), tear evaporation ($P = 0.001$), and osmolarity ($P < 0.001$), but not for tear volume ($P = 0.234$). The frequency distribution of tear film quality grade was determined by χ^2 test, and no significant difference was seen between the groups ($P = 0.717$). The distribution of meibomian gland dropout score was not normal, and so a nonparametric test (Mann-Whitney U test) was used to test for significant differences between the subject groups. No significant difference was seen in the mean meibomian gland dropout scores ($P = 0.691$) between normal subjects and patients with dry eye. Abnormal meibomian gland function (meibomian gland dropout score ≥ 2), was present in 14% of normal subjects and 20.4% of patients with dry eye.

As significant differences had been demonstrated for tear turnover, evaporation, and osmolarity between normal subjects and those with and dry eye, the use of these aspects of tear physiology were assessed for their effectiveness in diagnosis.

TABLE 3. Patients with Dry Eye Grouped in the Four Severity Levels

Dry Eye Severity Level	1	2	3	4	Total Patients
Discomfort, severity and frequency	8 (Mild, episodic, with stress)	11 (Moderate, episodic or chronic)	13 (Severe, frequent, or constant)	9 (Severe, disabling, and constant)	41
Visual symptoms	27 (None or episodic mild fatigue)	3 (Annoying or activity-limiting episodic)	1 (Annoying, chronic and/or constant limiting activity)	3 (Constant and/or possibly disabling)	34
Conjunctival injection	— (None to mild)	— (None to mild)	— (+/−)	— (+/++)	—
Conjunctival staining	— (None to mild)	— (Variable)	— (Moderate to marked)	— (Marked)	—
Corneal staining (severity/location)	4 (None to mild)	7 (Variable)	16 (Marked central)	—	27
Corneal/tear signs	9 (None to mild)	10 (Mild debris, ↓ meniscus)	6 (Filamentary keratitis, mucus clumping, ↑ tear debris)	8 (Filamentary keratitis, mucus clumping, ↑ tear debris, ulceration)	33
Lid/meibomian glands	25 (MGD variably present)	9 (MGD variably present)	6 (Frequent)	1 (Trichiasis, keratinization, symblepharon)	41
TFBUT (s)	5 (Variable)	13 (≤10)	8 (≤5)	4 (Immediate)	30
Schirmer (mm/5 min)	5 (Variable)	9 (≤10)	14 (≤5)	13 (≤2)	41

Numerical data are the number of subjects at each level. Groups were determined by results of different clinical tests based on the severity matrix proposed by Behrens et al.[60] Not all of these investigations were performed on each patient to establish a positive diagnosis. The tests were conducted only as required at the discretion of the ophthalmologist. It can be seen that the number of patients falling into different severity levels varied widely with the tests. MGD, meibomian gland dysfunction; TFBUT, tear fluorescein break-up time.
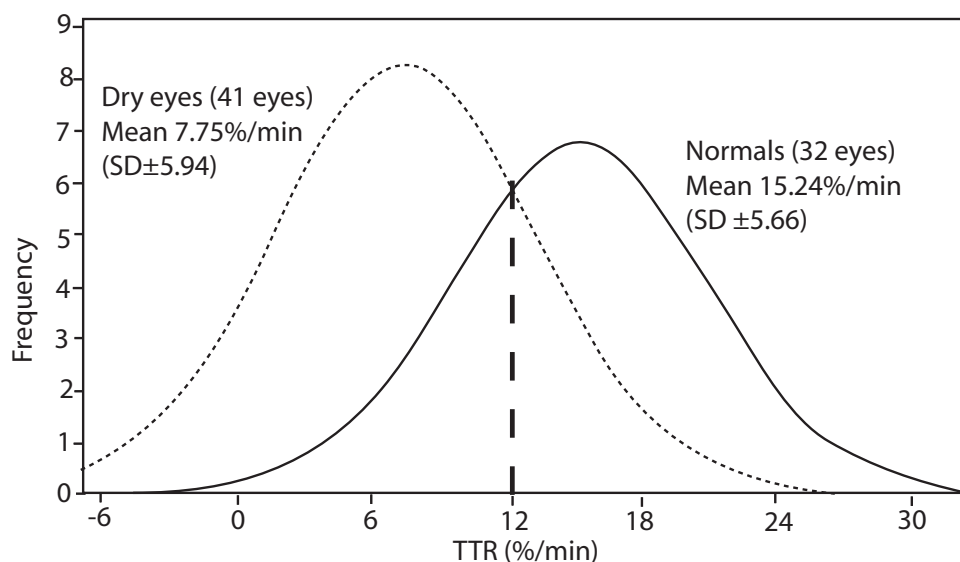
FIGURE 1. Distribution curves of TTR in normal patients and those with dry eye. *Dashed curve*: patients with dry eye; *solid line*: normal subjects: **bold dashed line**: intercept between the normal subjects and patients with dry eye (12%/min).

Distribution curves of TTR, evaporation, and osmolarity were plotted for normal subjects and patients with dry eye. As an example, the values for TTR are shown in Figure 1.
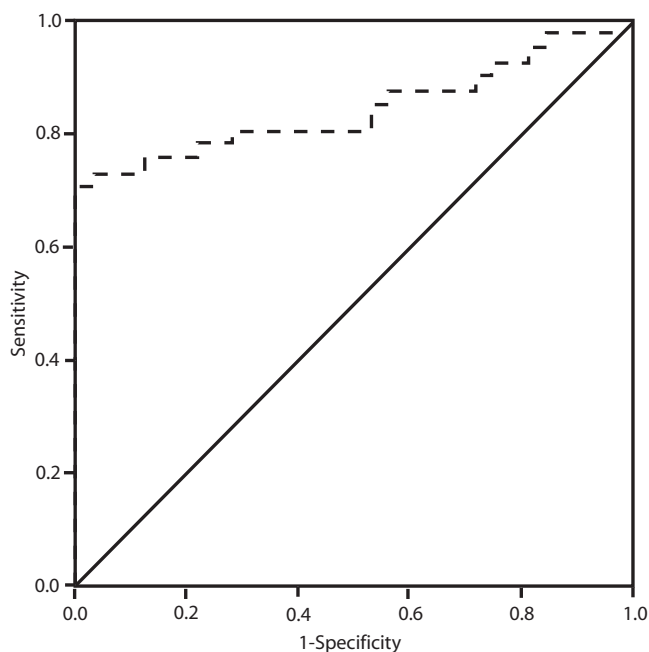
Single Test Results in the Diagnosis of Dry Eye

Figure 1 shows that TTR was lower in patients with dry eye than normal subjects. The intercept of the distribution curves for normal subjects and patients with dry eye was found to be at 12%/min. To evaluate the effectiveness of TTR in determining whether a subject has dry eye or is normal, an ROC curve was plotted from the TTR values of these two groups of subjects (Fig. 2). The area under the curve was found to be 0.84 (95% CI: 0.75–0.94). The sensitivity and specificity of a cutoff value of 12%/min as derived from the ROC curves was found to be 0.80 and 0.72, respectively, and the positive (PPVs)

and negative (NPVs) predictive values were 0.79 and 0.74, respectively.[62] The overall accuracy of this cutoff in discriminating between normal subjects and patients with dry eye was found to be 76.71%

A similar process was applied to tear evaporation and osmolarity, with the intercepts determined from the distribution curves. ROC curves were plotted, and the effectiveness of single tear measures in differentiating normal subjects from dry eye determined. These results are shown in Table 4.

Single tests may be used to diagnose dry eye but combinations in parallel or series may improve the efficacy of diagnosis.[34,43] This approach has the merit that there are distinct differences in diagnostic test results in different subgroups of patients with dry eye, which can aid clinicians in differential diagnosis of tear film disorders.[10] The use of a panel or battery of tests has been said to have the potential to improve the effectiveness of diagnosis.[10]

Combination Test Results in the Diagnosis of Dry Eye

The ability to differentiate persons with dry eye from normal subjects by combining, in parallel and series, objective measures of tear physiology was determined. Tests combined in parallel required a positive result on one test for diagnosis of dry eye; in series, all tests had to be positive.[43] The results are also shown in Table 4. As expected, parallel combinations improved the sensitivity and series combinations, the specificity of diagnosis.

Discriminant function analysis was also performed, to improve the efficacy of diagnosis and to identify the most suitable combination of tests. A forward linear step-wise model was used to exclude the parameters that offered little or no contribution to the diagnosis. All the measured parameters including TTR, tear evaporation, osmolarity, volume, film quality, and meibomian gland dropout scores were included in the analysis. The step-wise model removed tear film quality and meibomian gland dropout scores from the canonical function, leaving TTR, tear evaporation, and osmolarity as significant contributing factors in the diagnosis of dry eye (Table 5). A discriminant function incorporating three variables is acceptable.[63]

The most significant, single contributory measure to this function was osmolarity (with a standardized canonical discriminant function coefficient of 0.628), followed by TTR (coefficient, −0.528) and evaporation (coefficient, 0.343).[59]

FIGURE 2. ROC curves for prediction of dry eye from TTR. *Dashed line*: plot of the sensitivity of using TTR as a diagnostic tool against 1 − specificity.

TABLE 4. Test Effectiveness of Measures Used in the Diagnosis of Dry Eye

Test	Cutoff	Sens %	Spec %	False–ves %	PPV %	NPV %	OA%
Single Tests							
TTR	<12%/min	80	72	20	79	74	76.7
Evap	>33 g/m²/h	51	96	49	84	41	67.1
Osm	>317 mOsmol/L	78	78	22	86	73	79
Combined tests (parallel)							
TTR or Evap	<12%/min or >33 g/m²/h	95	62.5	5	76.5	86	80
TTR or Osm	<12%/min or 317 mOsmol/L	93	59	7	74.5	86	78
TTR or Evap or Osm	<12%/min or >33 g/m²/h or >317 mOsmol/L	100	66	0	81	100	86
Combined tests (series)							
TTR and Evap	<12%/min and >33 g/m²/h	36.5	100	63.5	100	55	64
TTR and Osm	<12%/min and 317 mOsmol/L	63	90	37	90	66	75
TTR and Evap and Osm	<12%/min and >33 g/m²/h and >317 mOsmol/L	38	100	62	100	52	63
Discriminant function (F)*							
TTR, Evap and Osm	>0.4	93	88	7	58	99	89

Specificity (Spec), sensitivity (Sens), false negatives (False–ves), positive predictive values (PPV), negative predictive values (NPV) and overall accuracies (OA) of different tests including tear turnover rate (TTR), evaporation (Evap) and osmolarity (Osm), singly and in combination, used in the diagnosis of dry eye. The intercepts found in the distribution curves between normal subjects and patients with dry eye for these tests were considered to be the cut-off value for dry eye.

* DE discriminant function: $F = -0.09$ TTR $+ 0.016$ Evap $+ 0.052$ Osm $- 16.25$.

The group to which the patient was allocated by the analysis was determined by inputting the values of TTR, evaporation (Evap), and osmolarity (Osm) into the following nonstandardized canonical equation derived from the discriminant function analysis:

Discriminant score (F_1)

$$= -0.09 \text{ TTR} + 0.016 \text{ Evap} + 0.052 \text{ Osm} - 16.25$$

The weighted midpoint of the group centroids of normal subjects and dry eye was found to be -0.4 on the study sample. This value was used as a cutoff for the function with discriminant score of more than -0.4 indicative of dry eye. This function classified 86.3% of the cases correctly. On cross-validation (i.e., when each case is classified by the functions derived from all other cases except that particular case), it was seen that 83.6% of the cases were classified correctly[64] (Table 6). This is a high percentage and indicates effectiveness of the discriminant function.[59]

The function was calculated for all the subjects in both groups. An ROC curve was plotted from the functions of normal subjects and patients with dry eye (Fig. 3). The area under the curve was 0.95 (95% CI: 0.90–0.99), indicating that the function was a reliable diagnostic tool for dry eye with a cutoff value of -0.4 (values > -0.4 indicating dry eye). This cutoff gave a sensitivity of 0.93 and a specificity of 0.88 (Table 4).

Dry Eye Subtypes

The discriminant function derived for differentiating all forms of dry eyes from normal eyes was applied to classification of

TABLE 5. Nonstandardized Canonical Discriminant Function Coefficients of the Measured Tear Parameters for the Separation of Normal and Dry Eye Groups

	Function
TTR	−0.090
Tear evaporation	0.016
Tear osmolarity	0.052
Constant	−16.25

Data determined by linear step-wise regression model of discriminant function analysis. Meibomian gland dropout scores and tear film quality were removed from the equation.

the subtypes of dry eye (Sjögren's syndrome, GVHD, and a general classification including aqueous deficiency and/or evaporative dry eye). For the various subtypes this discriminant function produced high sensitivity but low specificity and overall accuracy (reaching only 44% accuracy for subtyping of Sjögren's syndrome for instance). As a single measure, TTR offered the best differentiating tool for Sjögren's syndrome and GVHD from other types of dry eye, with fair sensitivities (75%–82%) and overall accuracies (68%–74%). This is not surprising, as both Sjögren's syndrome and GVHD disrupt lacrimal production causing significant aqueous deficiency.[65–67] However, the test was not effective in differentially diagnosing the two subtypes giving only low sensitivity, specificity, and overall accuracy.

DISCUSSION

The clinical and laboratory tests used for assessing the physiological characteristics of tears in this study have been shown

TABLE 6. Classification Results of the Original and Predicted Group Memberships of Normal Subjects and Those with Dry Eye[59]

		Predicted Group Membership		
	Group	Normal	Dry Eye	Total
Original				
Count	Normal	8	4	32
	Dry eye	6	35	41
%	Normal	87.5	12.5	100.0
	Dry eye	14.6	85.4	100.0
Cross-validated (a)				
Count	Normal	28	4	32
	Dry eye	8	33	41
%	Normal	87.5	12.5	100.0
	Dry eye	19.5	80.5	100.0

The derived discriminant function from discriminant analysis was applied to all subjects, and the subjects were reclassified as having normal or dry eyes in predicted group membership. In cross-validation, the function derived from all the subjects other than the tested subject was applied to the data from the tested subject, and the predicted group membership was determined. The discriminant function correctly classified 86.3% of the subjects and the cross-validation, 83.6%.
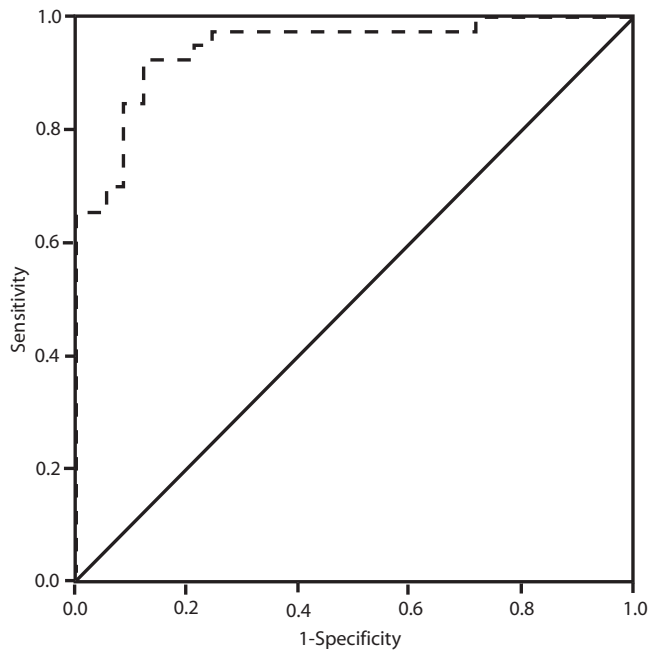
FIGURE 3. ROC curves for prediction of dry eye from the discriminant function. *Dashed line*: plot of the sensitivity of using the discriminant function as a diagnostic tool against 1 – specificity.

to be repeatable.[44,68,69] However, as all tests evaluate different characteristics of the tear film, it is unlikely that a single test can be a complete measure of dry eye. The results of the present study suggest that the best single tests for diagnosis are tear osmolarity and TTR. It has been suggested previously that hyperosmolarity can be used as an indicator of dry eye.[23,43,55,70,71] Tear evaporation was found to be less effective than osmolarity if used singly for the diagnosis of dry eye. But, it should be noted that the equipment requirements for the tests used in this study are expensive and not widely available in clinical practice. However, several more clinically applicable equivalents of these tests have been, or are being, developed. Fluorescein clearance measures have been reported to give good agreement with fluorophotometry,[72] new clinical osmometers are in development,[41] and measurements of evaporation are easy to apply and not time consuming.[73]

Discriminant function analysis has been used in diagnosis of several diseases, including coronary artery disease,[74,75] HIV infections,[76] liver disease,[77] Alzheimer's,[78,79] and alcohol dependency.[80] It is of use when several test results are combined into a diagnostic algorithm on the basis of their contribution to the discrimination between two groups of subjects. The technique was found to be superior to Bayesian analysis in predicting severe coronary disease.[75] Despite its obvious potential in a multifactorial condition such as dry eye requiring multiple test results in diagnosis, discriminant function analysis has rarely been applied in diagnosing this disease. One of the attractions of discriminant functions in the differential diagnosis of dry eye is that they can be updated and improved when more data are available from larger patient groups.[74] This refinement may allow identification of subtypes of dry eye. The use of combined tests derived from discriminant function analysis indicated that misdiagnosis of dry eye can be reduced significantly if assessments of tear turnover (production), evaporation, and osmolarity are included in a diagnostic battery of tests. Also, tear physiology tests are repeatable and reliable, unlike the currently used diagnostic tests.[16] It has been shown previously that there is a low site-to-site and day-to-day intra-individual variation in the results of evaporimetry.[81] Fluoro-

photometry[53] and its simpler alternative, the fluorescein clearance test,[72] are considered to be the gold standard for measuring TTR. It is therefore reasonable to argue that tear physiology tests can advance our ability to diagnose dry eye disease.

The determination of the effectiveness of any diagnostic test is made by considering the sensitivity and specificity of the test, which are characteristics of the test itself, and the predictive value of the test, which is influenced by the prevalence of the disease.[82] In the case of dry eye disease which has a prevalence of ~15% in general populations of Caucasians,[4,6,9] these measures of effectiveness apply to an extent. As illustrated in Tables 1 and 3, the choice of a test and its cutoff value for diagnosis of dry eye changes the relative values of sensitivity and specificity. Physiological factors and the seriousness of the condition must be taken into account when choosing a test and its cutoff value.[82] In diagnostic tests for dry eye, it is preferable to optimize overall accuracy and combine this with high sensitivity (and NPV).

On the basis of the results of this study (Table 4), the most appropriate single test for dry eye diagnosis would be osmolarity (giving overall accuracy of 79%, sensitivity of 78%, and NPV of 73%) and in parallel a combination of TTR, evaporation, and osmolarity (giving 86% overall accuracy, a 100% sensitivity, with 100% NPV). For a screening test for dry eye, the same single and combined tests would be chosen, with the combined test having improved sensitivity over the single test (100% vs. 80%). The choice of a weighted combination derived from discriminant function analysis would give comparable sensitivity, NPV, and (slightly) improved overall accuracy to the parallel combination of tests for use in diagnosis but would be less effective as a screening test because of its reduced PPV.

The test results in the present study compare very favorably with the effectiveness of other objective tests, as reported in the literature. From Tables 1 and 4, it can be seen that tear physiology tests are more effective in diagnosing dry eye than the commonly used tests, such as Schirmer and TBUT. The best diagnostic test in the literature would appear to be the lysozyme assay, yielding the highest reported overall accuracy combined with high sensitivity and NPVs.[45] However, this study involved patients with severe dry eye[43] and was probably influenced by significant spectrum bias, leading to overestimation of the test's sensitivity.[83] In the present study, patients classified as having general dry eye had borderline dry eye and those with Sjögren's syndrome and GVHD had moderate to severe dry eye, according to the clinical criteria, making the dry eye sample more inclusive of the general dry eye population. Therefore, the recommended cutoff values of the tear physiology tests in this study are suitable for diagnosing less severe forms of dry eye. However, it should be noted that several severe cases of aqueous-deficiency dry eye were included in our sample, and this may have led to the higher weighting of TTR in the discriminant function in comparison to tear evaporation. Also, the sample was from a secondary/tertiary eye care center. Correct diagnosis of moderate to severe dry eye is more essential in such settings, as misdiagnosis can have a huge impact on QOL and productivity of a patient.[84] Further diagnostic mechanisms are essential for diagnosing a milder form of the disease within primary eye care.

It must be remembered that the results of diagnostic efficacy in this and previous studies (Table 1) are generally affected by selection bias, because efficacy was assessed on the data from the patient samples on which the cutoff values for diagnosis were derived and not from an independent sample of new patients.[23] However, as this applied to all the single and combinations of tests compared, the findings of relative effectiveness in this study are valid.[23] Another difficulty with studies of diagnostic efficacy in diseases with no gold standard is that the classification of patients by any new test is compared with

a potentially flawed standard. In this case the comparison is with the ophthalmological diagnosis based on conventional clinical tests (Schirmer, TBUT, vital staining and auto antibodies). This problem will remain in the diagnosis of dry eye, as in many other diseases, until a gold standard is defined.

Blepharitis, a condition mainly caused by bacterial infections and/or dysfunction of the meibomian gland,[85,86] has been found to be associated with dry eye. However, as blepharitis is an independent clinical entity that shares only some of the signs and symptoms of dry eye,[87] patients with this disease were excluded from the study.

Another problem of diagnosis in dry eye is the issue of subtyping. The function derived by discriminant analysis for separating dry eye from normal was found to be more accurate than any of the individual tests or parallel or series combinations of tests, as it had higher sensitivity, specificity, and overall predictive values. Its effectiveness is similar to that previously reported by Craig et al. (*IOVS* 1995:36:ARVO Abstract 4823) in discriminating between normal subjects and patients with dry eye associated with rheumatoid arthritis. The previous study showed the difficulty of diagnosing subtypes of dry eye, even with discriminant function analysis. A similar problem has been noted with tests for hyperosmolarity in dry eye[23] and was again the case in the present study. None of the tests, either singly or in combination, or the discriminant function analyses was found to be adequate for the differential diagnosis of dry eye subtypes. However TTR was found to be effective in differentiating aqueous deficiency dry eye subtypes from other subtypes, and tear evaporation can be used to determine evaporative dry eye. Therefore, measuring TTR and evaporation can be used in combination to differentiate broadly between the two main subtypes of dry eye. This test, in itself, would be most beneficial in the treatment of dry eye, as the tear supplements currently available are directed toward one of these two types of dry eye.

References

1. Lemp MA. Report of the national eye Institute/Industry workshop on clinical trials in dry eyes. *CLAO J*. 1995;21:221–232.
2. Doughty MJ, Fonn D, Richter D, Simpson T, Caffery B, Gordon K. A patient questionnaire approach to estimating the prevalence of dry eye symptoms in patients presenting to optometric practices across canada. *Optom Vis Sci*. 1997;74:624–631.
3. Hikichi T, Yoshida A, Fukui Y, et al. Prevalence of dry eye in Japanese eye centers. *Graefes Arch Clin Exp Ophthalmol*. 1995;233:555–558.
4. Moss SE, Klein R, Klein BE. Prevalence of and risk factors for dry eye syndrome. *Arch Ophthalmol*. 2000;118:1264–1268.
5. Lee AJ, Lee J, Saw SM, et al. Prevalence and risk factors associated with dry eye symptoms: a population based study in Indonesia. *Br J Ophthalmol*. 2002;86:1347–1351.
6. Chia EM, Mitchell P, Rochtchina E, Lee AJ, Maroun R, Wang JJ. Prevalence and associations of dry eye syndrome in an older population: the blue mountains eye study. *Clin Exp Ophthalmol*. 2003;31:229–232.
7. Begley CG, Caffery B, Nichols K, Mitchell GL, Chalmers R, DREI study group: results of a dry eye questionnaire from optometric practices in North America. *Adv Exp Med Biol*. 2002;506:1009–1016.
8. Caffery BE, Richter D, Simpson T, Fonn D, Doughty M, Gordon K. CANDEES: the Canadian dry eye epidemiology study. *Adv Exp Med Biol*. 1998;438:805–806.
9. Schein OD, Munoz B, Tielsch JM, Bandeen-Roche K, West S. Prevalence of dry eye among the elderly. *Am J Ophthalmol*. 1997;124:723–728.
10. Pflugfelder SC, Tseng SC, Sanabria O, et al. Evaluation of subjective assessments and objective diagnostic tests for diagnosing tear-film disorders known to cause ocular irritation. *Cornea*. 1998;17:38–56.
11. Wright JC, Meger GE. A review of the Schirmer test for tear production. *Arch Ophthalmol*. 1962;67:564–565.
12. Cedarstaff TH, Tomlinson A. Human tear volume, quality and evaporation: a comparison of Schirmer, tear break-up time and resistance hygrometry techniques. *Ophthalmic Physiol Opt*. 1983;3:239–245.
13. Lucca JA, Nunez JN, Farris RL. A comparison of diagnostic tests for keratoconjunctivitis sicca: lactoplate, Schirmer, and tear osmolarity. *CLAO J*. 1990;16:109–112.
14. Cho P, Yap M. Schirmer test, I: a review. *Optom Vis Sci*. 1993;70:152–156.
15. Lamberts DW. Keratoconjunctivitis sicca. In: Smolin G, Thoft RA, eds. *The Cornea: Scientific Foundations and Clinical Practice*. Boston: Little Brown; 1987;387–405.
16. Nichols KK, Mitchell GL, Zadnik K. The repeatability of clinical measurements of dry eye. *Cornea*. 2004;23:272–285.
17. Mengher LS, Bron AJ, Tonge SR, Gilbert DJ. A non-invasive instrument for clinical assessment of the pre-corneal tear film stability. *Curr Eye Res*. 1985;4:1–7.
18. Patel S, Murray D, McKenzie A, Shearer DS, McGrath BD. Effects of fluorescein on tear breakup time and on tear thinning time. *Am J Optom Physiol Opt*. 1985;62:188–190.
19. Bron AJ, Evans VE, Smith JA. Grading of corneal and conjunctival staining in the context of other dry eye tests. *Cornea*. 2003;22:640–650.
20. Kim J. The use of vital dyes in corneal disease. *Curr Opin Ophthalmol*. 2000;11:241–247.
21. Khan-Lim D, Berry M. Still confused about rose bengal? *Curr Eye Res*. 2004;29:311–317.
22. Caffery BE, Josephson JE. Corneal staining after sequential instillations of fluorescein over 30 days. *Optom Vis Sci*. 1991;68:467–469.
23. Tomlinson A, Khanal S, Ramaesh K, Diaper C, McFadyen A. Tear film osmolarity: determination of a referent for dry eye diagnosis. *Invest Ophthalmol Vis Sci*. 2006;47:4309–4315.
24. Miller KL. Tear-film considerations with soft contact lenses (Abstract). *Oxyopia*. Berkeley, CA: University of California, Berkeley, Vision Science Program; 2002:29.
25. Mathers WD. Why the eye becomes dry: a cornea and lacrimal gland feedback model. *CLAO J*. 2000;26:159–165.
26. Tomlinson A, Khanal S. Assessment of tear film dynamics: a quantitative approach. *Ocul Surf*. 2005;3:81–95.
27. Hay EM, Thomas E, Pal B, Hajeer A, Chambers H, Silman AJ. Weak association between subjective symptoms or and objective testing for dry eyes and dry mouth: results from a population based study. *Ann Rheum Dis*. 1998;57:20–24.
28. Tomlinson A, Pearce EI, Simmons PA, Blades K. Effect of oral contraceptives on tear physiology. *Ophthalmic Physiol Opt*. 2001;21:9–16.
29. Versura P, Cellini M, Torreggiani A, Profazio V, Bernabini B, Caramazza R. Dryness symptoms, diagnostic protocol and therapeutic management: a report on 1,200 patients. *Ophthalmic Res*. 2001;33:221–227.
30. McCarty CA, Bansal AK, Livingston PM, Stanislavsky YL, Taylor HR. The epidemiology of dry eye in Melbourne, Australia. *Ophthalmology*. 1998;105:1114–1119.
31. Narayanan S, Miller WL, Prager TC, et al. The diagnosis and characteristics of moderate dry eye in non-contact lens wearers. *Eye Contact Lens*. 2005;31:96–104.
32. McMonnies CW. Key questions in a dry eye history. *J Am Optom Assoc*. 1986;57:512–517.
33. Patel S, Farrell J, Blades KJ, Grierson DJ. The value of a phenol red impregnated thread for differentiating between the aqueous and non-aqueous deficient dry eye. *Ophthalmic Physiol Opt*. 1998;18:471–476.
34. Goren MB, Goren SB. Diagnostic tests in patients with symptoms of keratoconjunctivitis sicca. *Am J Ophthalmol*. 1988;106:570–574.
35. Farris RL. Tear analysis in contact lens wearers. *Trans Am Ophthalmol Soc*. 1985;83:501–545.
36. Vitali C, Moutsopoulos HM, Bombardieri S. The European community study group on diagnostic criteria for Sjogren's syndrome:

sensitivity and specificity of tests for ocular and oral involvement in Sjogren's syndrome. *Ann Rheum Dis*. 1994;53:637–647.

37. Goto T, Zheng X, Okamoto S, Ohashi Y. Tear film stability analysis system: Introducing a new application for videokeratography. *Cornea*. 2004;23:S65–S70.

38. Yokoi N, Komuro A. Non-invasive methods of assessing the tear film. *Exp Eye Res*. 2004;78:399–407.

39. Xu KP, Yagi Y, Toda I, Tsubota K. Tear function index: a new measure of dry eye. *Arch Ophthalmol*. 1995;113:84–88.

40. Gilbard JP, Gray KL, Rossi SR. Improved technique for storage of tear microvolumes. *Invest Ophthalmol Vis Sci*. 1987;28:401–403.

41. Sullivan B. Clinical results of a first generation lab-on-chip nanolitre tear film osmometer (abstract). *The Ocular Surface*. 2005;3:S31.

42. Norn M. Quantitative tear ferning. Clinical investigations. *Acta Ophthalmol (Copenh)*. 1994;72:369–372.

43. Farris RL. Tear osmolarity: a new gold standard? *Adv Exp Med Biol*. 1994;350:495–503.

44. Craig JP. Tear physiology in the normal and dry eye. 1995.

45. van Bijsterveld OP. Diagnostic tests in the sicca syndrome. *Arch Ophthalmol*. 1969;82:10–14.

46. Mainstone JC, Bruce AS, Golding TR. Tear meniscus measurement in the diagnosis of dry eye. *Curr Eye Res*. 1996;15:653–661.

47. Xu KP, Tsubota K. Correlation of tear clearance rate and fluorophotometric assessment of tear turnover. *Br J Ophthalmol*. 1995;79:1042–1045.

48. Hirji N, Patel S, Callander M. Human tear film pre-rupture phase time (TP-RPT): a non-invasive technique for evaluating the precorneal tear film using a novel keratometer mire. *Ophthalmic Physiol Opt*. 1989;9:139–142.

49. Doane MG, Lee ME. Tear film interferometry as a diagnostic tool for evaluating normal and dry-eye tear film. *Adv Exp Med Biol*. 1998;438:297–303.

50. Trees GR, Tomlinson A. Effect of artificial tear solutions and saline on tear film evaporation. *Optom Vis Sci*. 1990;67:886–890.

51. Kok JH, Boets EP, van Best JA, Kijlstra A. Fluorophotometric assessment of tear turnover under rigid contact lenses. *Cornea*. 1992;11:515–517.

52. Occhipinti JR, Mosier MA, LaMotte J, Monji GT. Fluorophotometric measurement of human tear turnover rate. *Curr Eye Res*. 1988;7:995–1000.

53. Pearce EI, Keenan BP, McRory C. An improved fluorophotometric method for tear turnover assessment. *Optom Vis Sci*. 2001;78:30–36.

54. Kuppens EV, Stolwijk TR, de Keizer RJ, van Best JA. Basal tear turnover and topical timolol in glaucoma patients and healthy controls by fluorophotometry. *Invest Ophthalmol Vis Sci*. 1992;33:3442–3448.

55. Gilbard JP, Farris RL, Santamaria J 2nd. Osmolarity of tear microvolumes in keratoconjunctivitis sicca. *Arch Ophthalmol*. 1978;96:677–681.

56. Craig JP, Simmons PA, Patel S, Tomlinson A. Refractive index and osmolality of human tears. *Optom Vis Sci*. 1995;72:718–724.

57. Mathers WD, Shields WJ, Sachdev MS, Petroll WM, Jester JV. Meibomian gland dysfunction in chronic blepharitis. *Cornea*. 1991;10:277–285.

58. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. *Clin Chem*. 1993;39:561–577.

59. Hair JF, Anderson RE, Tatham RL, Black WC. *Multivariate Data Analysis with Readings*. Englewood Cliffs: NJ: Prentice-Hall International; 1995.

60. Behrens A, Doyle JJ, Stern L, et al. Dysfunctional tear syndrome: a delphi approach to treatment recommendations. *Cornea*. 2006;25:900–907.

61. Lemp MA, Baudouin C, Baum J, et al. The definition and classification of dry eye disease: report of the definition and classification subcommittee of the international dry eye WorkShop. *Ocul Surf*. 2007;5:75–92.

62. Altman DG, Bland JM. Diagnostic tests 2: Predictive values. *BMJ*. 1994;309:102.

63. Lachenbruch PA. Some misuses of discriminant analysis. *Methods Inf Med*. 1977;16:255–258.

64. Duda R, Hart P, Stork D. *Pattern Classification*. New York: Wiley; 2001.

65. Baudouin C. The pathology of dry eye. *Surv Ophthalmol*. 2001; 45(suppl 2):S211–S220.

66. Penn EA, Soong HK. Cataract surgery in allogeneic bone marrow transplant recipients with graft-versus-host disease (1). *J Cataract Refract Surg*. 2002;28:417–420.

67. Zoukhri D. Effect of inflammation on lacrimal gland function. *Exp Eye Res*. 2006;82:885–898.

68. Craig JP, Tomlinson A. Importance of the lipid layer in human tear film stability and evaporation. *Optom Vis Sci*. 1997;74:8–13.

69. Mathers WD, Lane JA, Sutphin JE, Zimmerman MB. Model for ocular tear film function. *Cornea*. 1996;15:110–119.

70. Bron AJ. Diagnosis of dry eye. *Surv Ophthalmol*. 2001;45(suppl 2):S221–S226.

71. Gilbard JP, Farris RL. Tear osmolarity and ocular surface disease in keratoconjunctivitis sicca. *Arch Ophthalmol*. 1979;97:1642–1646.

72. Macri A, Rolando M, Pflugfelder S. A standardized visual scale for evaluation of tear fluorescein clearance. *Ophthalmology*. 2000; 107:1338–1343.

73. Goto E, Endo K, Suzuki A, Fujikura Y, Matsumoto Y, Tsubota K. Tear evaporation dynamics in normal subjects and subjects with obstructive meibomian gland dysfunction. *Invest Ophthalmol Vis Sci*. 2003;44:533–539.

74. Douglass KH, Links JM, Chen DC, Wong DF, Wagner HN Jr. Linear discriminant analysis of regional ejection fractions in the diagnosis of coronary artery disease. *Eur J Nucl Med*. 1987;12:602–604.

75. Detrano R, Leatherman J, Salcedo EE, Yiannikas J, Williams G. Bayesian analysis versus discriminant function analysis: their relative utility in the diagnosis of coronary disease. *Circulation*. 1986; 73:970–977.

76. Lansky A, Fleming PL, Byers RH Jr, Karon JM, Wortley PM. A method for classification of HIV exposure category for women without HIV risk information. *MMWR Recomm Rep*. 2001;50:31–40.

77. Ellis G, Goldberg DM. Discriminant function analysis applied to laboratory tests in patients with hepatobiliary disease. *Comput Biomed Res*. 1979;12:483–501.

78. Visser PJ, Verhey FR, Ponds RW, Jolles J. Diagnosis of preclinical Alzheimer's disease in a clinical setting. *Int Psychogeriatr*. 2001; 13:411–423.

79. Sayeed A, Petrou M, Spyrou N, Kadyrov A, Spinks T. Diagnostic features of Alzheimer's disease extracted from PET sinograms. *Phys Med Biol*. 2002;47:137–148.

80. Vaswani M, Rao RV. Biochemical measures in the diagnosis of alcohol dependence using discriminant analysis. *Indian J Med Sci*. 2005;59:423–430.

81. Pinnagoda J, Tupker RA, Smit JA, Coenraads PJ, Nater JP. The intra- and inter-individual variability and reliability of transepidermal water loss measurements. *Contact Dermatitis*. 1989;21:255–259.

82. Wassertheil-Smoller S. *Biostatistics and Epidemiology*. New York: Springer-Verlag; 1990:66–74.

83. Knottnerus JA, van Weel C, Muris JW. Evaluation of diagnostic procedures. *BMJ*. 2002;324:477–480.

84. Goto E, Yagi Y, Matsumoto Y, Tsubota K. Impaired functional visual acuity of patients with dry eye. *Am J Ophthalmol*. 2002; 133:181–186.

85. Smith RE, Flowers CW Jr. Chronic blepharitis: a review. *CLAO J*. 1995;21(3):200–207.

86. McCulley JP, Dougherty JM, Deneau DG. Classification of chronic blepharitis. *Ophthalmology*. 1982;89(10):1173–1180.

87. Bowman RW, Dougherty JM, McCulley JP. Chronic blepharitis and dry eyes. *Int Ophthalmol Clin*. 1987;27(1):27–35.