TEARLAB™ OSMOLARITY AS A BIOMARKER FOR DISEASE SEVERITY IN MILD TO MODERATE DRY EYE DISEASE. Gary N Foulks MD FACS[1], Michael A Lemp MD[2,3], Michael Berg[2], Rahul Bhola MD[1], Benjamin D Sullivan PhD[2]. [1]University of Louisville, [2]TearLab Corp., [3]Georgetown University

1. Introduction

Recently, lab-on-a-chip technology capable of simultaneous nanoliter volume specimen collection and analysis has been developed to address the barriers to *in vitro* diagnostic tear testing. The first in a series of tear fluid assays utilizing this methodology, is "intended to measure the osmolarity of human tears to aid in the diagnosis of dry eye disease in patients suspected of having dry eye disease, in conjunction with other methods of clinical evaluation" (TearLab™ Osmolarity System - FDA k083184). The TearLab™ uses electrical impedance of a 50 nL tear sample collected directly from the inferior lateral tear meniscus to calculate a patient's osmolarity in mOsms/L (Fig 1). The use of direct, unprocessed nanoliter tear samples allows the TearLab™ to resolve difficulties in both tear collection and specimen handling. Moreover, the TearLab™ measures osmolarity within a few seconds, as compared to 15 minutes required by other laboratory osmometers [Yildiz EH].

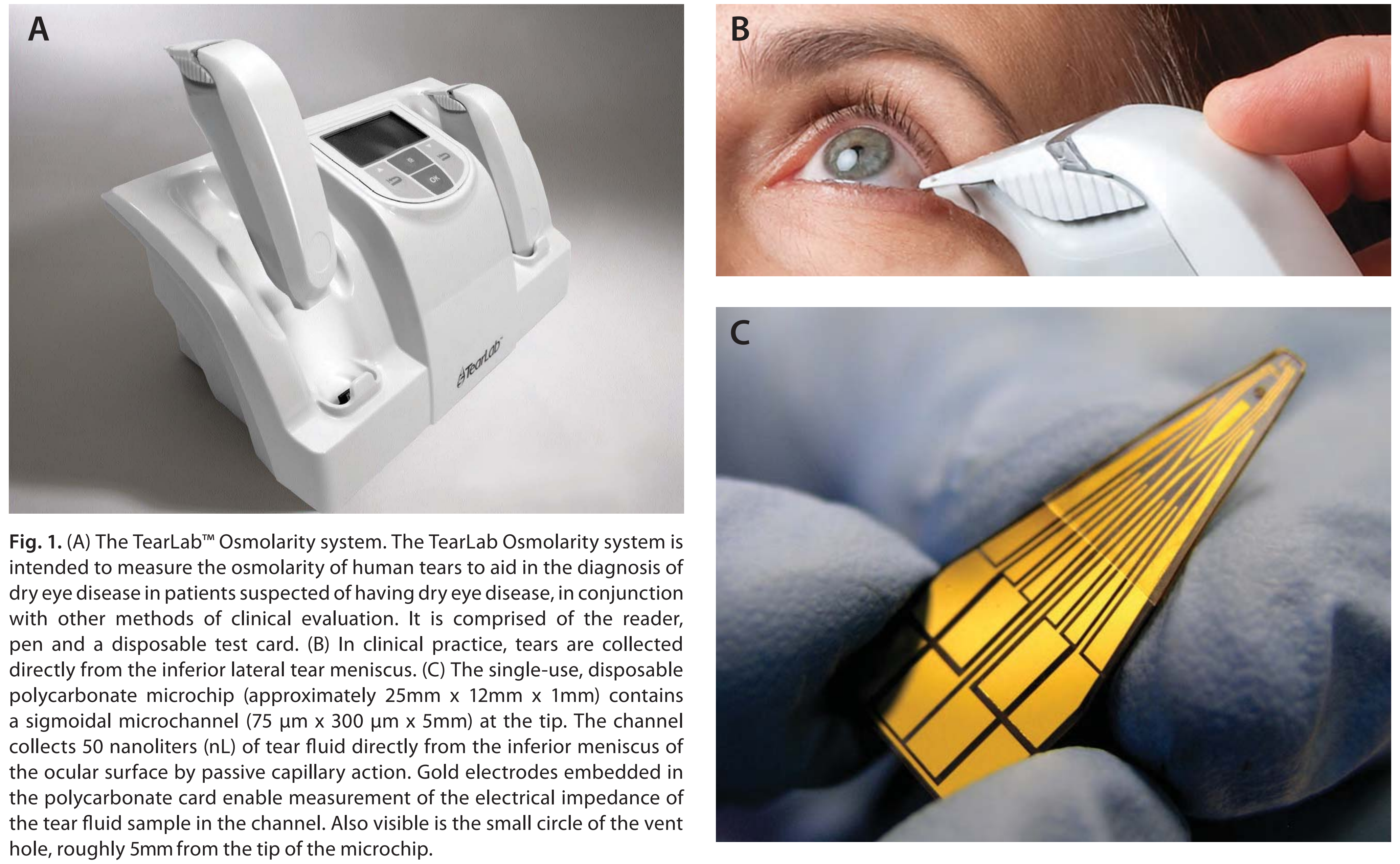

Fig. 1. (A) The TearLab™ Osmolarity system. The TearLab Osmolarity system is intended to measure the osmolarity of human tears to aid in the diagnosis of dry eye disease in patients suspected of having dry eye disease, in conjunction with other methods of clinical evaluation. It is comprised of the reader, pen and a disposable test card. (B) In clinical practice, tears are collected directly from the inferior lateral tear meniscus. (C) The single-use, disposable polycarbonate microchip (approximately 25mm x 12mm x 1mm) contains a sigmoidal microchannel (75 μm x 300 μm x 5mm) at the tip. The channel collects 50 nanoliters (nL) of tear fluid directly from the inferior meniscus of the ocular surface by passive capillary action. Gold electrodes embedded in the polycarbonate card enable measurement of the electrical impedance of the tear fluid sample in the channel. Also visible is the small circle of the vent hole, roughly 5mm from the tip of the microchip.

2. Purpose

PURPOSE: Dry eye disease is frequently characterized by conflicting signs, e.g., where patients with low tear production (Schirmer < 5 mm) may also present with a stable tear film (tear film breakup time (TBUT) > 20 s). Reconciling these conflicts is non-trivial, because 1) traditional signs carry significant analytical variation, 2) the relative importance of each sign is not clearly established [Dry Eye Workshop 2007], and 3) signs indicative of subtypes of the disease do not necessarily correlate with overall disease severity. Additionally, little data is available on the longitudinal or diurnal variation of existing clinical signs, which may play a critical role in the lack of correlation between these signs and symptoms of dry eye [Nichols K]. The current investigation attempts to resolve these conflicts by consolidating disparate types of clinical data into a single composite index of disease severity on {0,1}, with {0} representing no evidence of the disease and {1} representing the most severe form of dry eye. The index is derived from a 300 subject interim analysis of the TearLab Core Validation Study (CVS), a 13 site (7 EU, 5 US, 1 Japan) clinical trial, evaluating the ocular surface disease index (OSDI), Schirmer strips, corneal and conjunctival staining, meibomian gland scoring, TBUT, and osmolarity from the inferior tear margin. The present study investigates the relationship of individual signs to the overall disease severity index, in an attempt to better understand the relative importance of clinical signs in the diagnosis and management of dry eye disease, and specifically to determine if tear film osmolarity is a marker for severity, and in particular, for mild to moderate dry eye.

3. Methods

Continuous Mapping
An expert panel of physicians and optometrists, most of whom were DEWS authors, provided a progression of signs across a discrete severity scale, ranging from 1 (lowest) to 4 (highest) severity, as shown in Table 1. Linear or exponential functions were manually fit to normalized versions of these progressions as shown in Table 2. Generating a severity value on each basis is a straightforward substitution into the inverted curves. For instance, a Schirmer value of 4 mm would be converted as 1.4063 ln((**4**+1)/35)/-5 = 0.55/1.00, placing it between the DEWS severity grades of 2 & 3. Similarly, a corneal staining value of 16 would be calculated as **16**/16 = 1.00/1.00, representing the highest level of severity.

Construction of Basis Functions
In the most basic approach, each individual sign would occupy an orthonormal basis, contributing independent, equally important information to the state of the disease. Construction of properly rotated bases require removal of the overlap in information between the measurements to ensure that each clinical variable carries equivalent correlation risk against the overall index. For calculation of a Euclidean norm, this problem is reduced to dividing each clinical variable by an appropriate measure of redundant information. Jon Shlens' implementation of the infomax ICA algorithm was used to measure mutual information [Shlens J 2003, Bell AJ 1997]. Bases were scaled by the absolute value of the mean of the mixing matrix. Overall disease severity was calculated by the distance from the origin divided by the square root of the sum of the squared scaling factors for each sign. The highest severity mesaurement from each sign for each eye was used to construct the index.

Table 1. Modified DEWS Severity Scale.

Grade	**0**	**1**	**2**	**3**	**4**
Schirmer Test (mm)	35	7	5	2	0
TBUT (seconds)	45	7	5	3	0
Staining (NEI/Industry scale)	0	3	8	12	20
OSDI	0	15	30	45	100
Meibomian Grading Score	0	5	12	20	28
Osmolarity (mOsms/L)	275	308	324	364	400

Table 2. Results of Continuous Mapping to Clinical Breakpoints. Generating a severity value on each basis is a straightforward substitution into the inverted curves. For instance, a Schirmer value of 4 mm would be converted as 1.4063*ln((*4* +1)/35)/-5 = 0.55, placing it between the DEWS severity grades of 2 & 3. Similarly, a corneal staining value of 16 would be calculated as **16**/16 = 1.00, representing the highest level of severity in that dimension.

	Fitted Curve	**Inversion**
Schirmer Test (mm)	$35e^{-5x}$	$1.4063*\ln((y+1)/35)/-5$
TBUT (seconds)	$45e^{-5x}$	$1.3135*\ln((y+1)/45)/-5$
Staining (NEI/Industry scale)	$16x$	$y/16$
OSDI	$10e^{2.38x}-10$	$\ln((y+10)/10)/2.38$
Meibomian Grading Score	$27x$	$y/27$
Osmolarity (mOsms/L)	$125x+275$	$(y-275)/125$

4. Results

To define normal and dry eye subjects based on classical signs, the authors of the study protocol required that the subject be symptomatic with an with an OSDI ≥ 5. In addition, one eye had to exceed diagnostic thresholds on two out of the five classical signs, chosen from TBUT ≤ 7, Schirmer < 7, Corneal Staining > 0, Conjunctival Staining > 0, and Meibomian Scoring > 5. Typical conflicts within these categorizations are shown in Table 3, demonstrating that any one of the classical signs for dry eye are insufficient to properly diagnose the subjects.

Diagnostic performance of each indication, using the first quartile of the severity index as a threshold for normal and dry eye patients, is shown explicitly in Fig. 2, ROC curves in Fig. 3, and as text in Table 4. Quite a few of the indications exhibit poor correlation to overall severity, specifically Schirmer strips, TBUT, and OSDI.

To echo Dr. Tomlinson's findings as reported in IOVS in 2006, the measurement of tear film osmolarity arguably offers the best single parameter for characterization of the disease.

Of note, it was found that while the classical cut-off value for tear film osmolarity at 316 mOsms/L performs exceptionally well for separating normal and severe patients, the performance in the mild to moderate population is suspect. Shifting the cut-off value to 308 mOsms/L provided superior performance in the mild to moderate cohort, exhibiting the highest sensitivity of any of the indications.

Table 3. Examples of conflicts within patients defined by thresholds, i.e. "3 out of 6."

"Normal" Subjects	**Severity**	**Osm**	**TBUT**	**Sch**	**Cornea**	**Conj**	**Meib**	**OSDI**
A	0.43	337	1.7	5	2	3	4	0.0
B	0.38	326	3.3	29	0	0	4	56.3
C	0.38	326	2.0	9	1	4	4	4.2
D	0.37	337	7.0	29	0	6	0	25.0
"Dry Eye" Subjects								
E	0.22	315	15.0	20	2	2	2	6.3
F	0.26	307	21.7	12	0	1	9	33.3
G	0.26	321	21.7	25	0	2	6	18.2
H	0.27	294	3.7	30	2	0	1	20.8

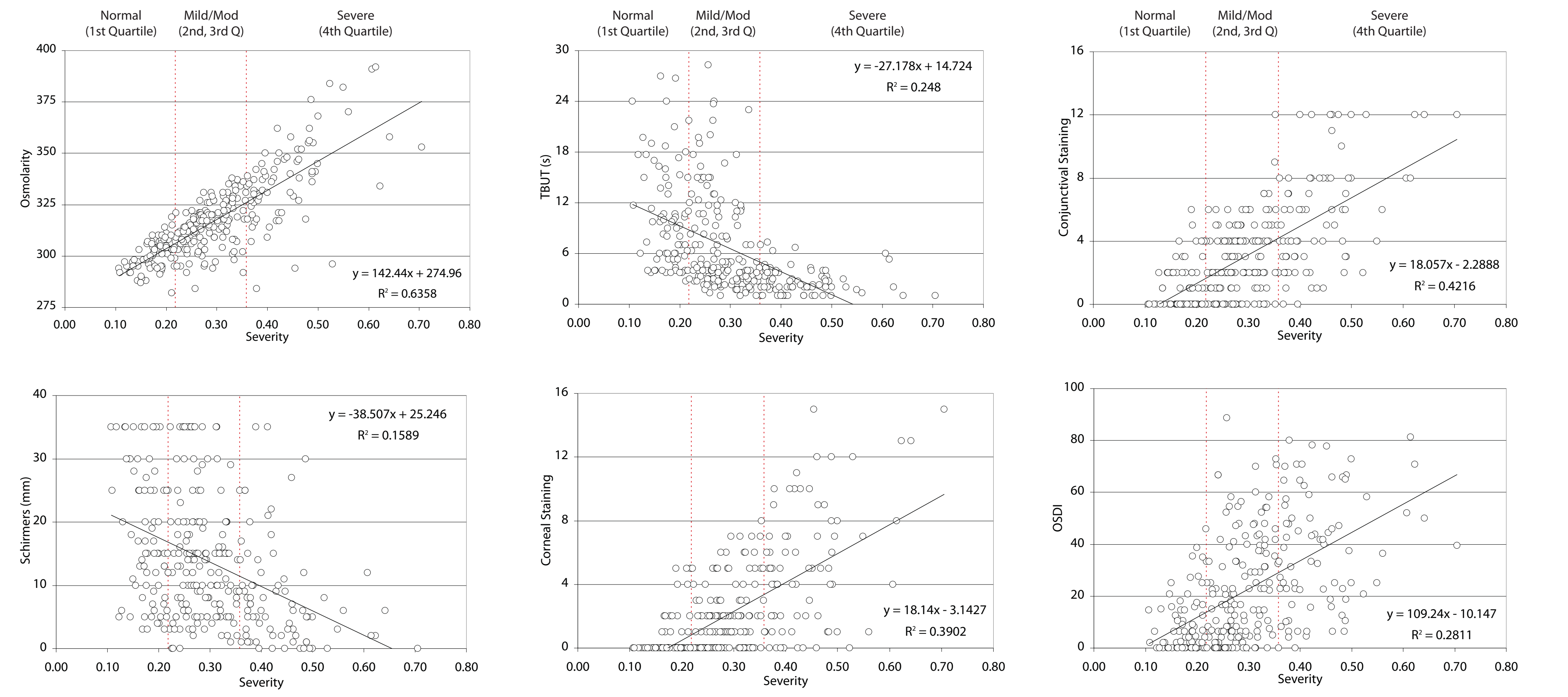

Fig. 2. (A) The three quartile-derived groups; normal, mild/moderate, and severe, are demarcated by the vertical dashed lines. Within the normal to moderate cohort, only osmolarity shows significant correlation to disease severity. OSDI shows good discrimination for the normal group, but poor discrimination across the remainder of the subjects. The other clinical signs perform well for the more severe patients, but poorly for normal through moderate quartiles.

Fig. 3

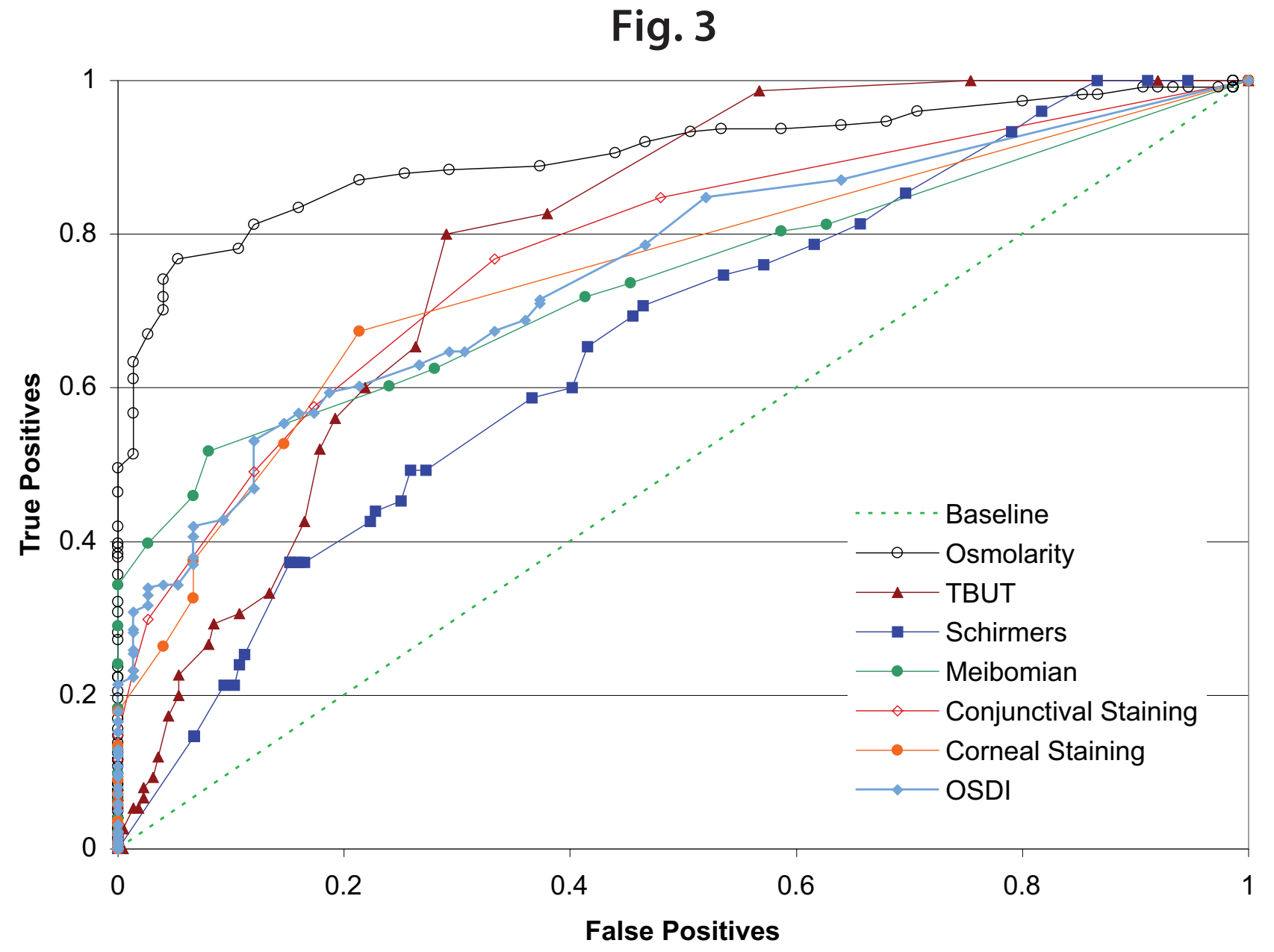

Table 4. Specificity and sensitivities of each clinical sign.

Osmolarity				**TBUT**			
308	Normal	Mild/Mod	Severe	**7**	Normal	Mild/Mod	Severe
Below	66	38	4	Above	45	48	1
Above	9	111	71	Below	30	101	74
	88.0%	**74.5%**	**94.7%**		**60.0%**	**67.8%**	**98.7%**
Schirmer Strips				**Corneal Staining**			
7	Normal	Mild/Mod	Severe	**1**	Normal	Mild/Mod	Severe
Above	59	108	30	Below	64	87	19
Below	16	41	45	Above	11	62	56
	78.7%	**27.5%**	**60.0%**		**85.3%**	**41.6%**	**74.7%**
Conjunctival Staining				**Meibomian Grading**			
1	Normal	Mild/Mod	Severe	**5**	Normal	Mild/Mod	Severe
Below	50	46	6	Below	57	75	14
Above	25	103	69	Above	18	74	61
	66.7%	**69.1%**	**92.0%**		**76.0%**	**49.7%**	**81.3%**
OSDI				**Osmolarity**			
15	Normal	Mild/Mod	Severe	**316**	Normal	Mild/Mod	Severe
Below	59	76	13	Below	74	79	8
Above	16	73	62	Above	1	70	67
	78.7%	**49.0%**	**82.7%**		**98.7%**	**47.0%**	**89.3%**

5. Conclusions

Osmolarity was found to have superior dynamic range and resolution as compared to the other signs, especically in the mild to moderate cohort. TearLab™ Osmolarity can be considered a surrogate biomarker for dry eye disease severity.

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8. References

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