Examining Tear Film Dynamics Using the Novel Tear Film Imager

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Purpose: The purpose of this study was to examine Tear Film Imager (TFI, AdOM, Israel) generated parameters across controls and dry eye (DE) subgroups and examine the changes in TFI parameters with contact lens (CL) placement.

Methods: The retrospective study (n = 48) was conducted at the Miami Veterans Hospital. Symptoms were assessed through validated questionnaires and signs of tear function by tear break-up time and Schirmer scores. Participants were grouped as 1) healthy, 2) evaporative, 3) aqueous deficient, and 4) mixed DE based on tear function. Seventeen individuals had a baseline scan and a repeat scan following CL placement. Descriptives were compared across groups and over time.

Results: The median age was 27 years, 74% self-identified as White, 45% as male, and 51% as Hispanic. Subjects in the aqueous deficiency category had lower muco-aqueous layer thickness (MALT) (2672 vs. 3084 nm) but higher lipid layer thickness (47.5 vs. 38.3 nm), lipid break-up time (4.4 vs. 2 seconds), and interblink interval (13.9 vs. 5.4 seconds) compared with the evaporative group. Subjects in the evaporative group had the highest MALT values (3084 vs. 2988, 2672, 3053 nm) compared with healthy, aqueous-deficient, and mixed groups. Symptoms were not significantly correlated with TFI parameters. CL placement significantly decreased MALT values (2869 → 2175 nm, $P = 0.001$).

Conclusions: The TFI provides unique information regarding the dynamic function of the tear film not captured by clinical examination. TFI generated metrics demonstrate a thinner aqueous layer in individuals with aqueous deficiency but highlight a thicker aqueous layer in those with evaporative DE.

Key Words: aqueous deficiency, evaporative dry eye, mixed dry eye, tear break up, tear film

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Dry eye (DE) is an umbrella term that encompasses a wide range of pain symptoms including "dryness," "grittiness," "burning," and "aching," among others, and visual disturbances.[1] Ocular signs of DE are likewise varied and can include tear instability, low tear production, ocular surface inflammation, and corneal and conjunctival staining.[2] Several diagnostic tests have been developed that examine various aspects of ocular surface health, such as fluorescein-assisted tear break-up time (TBUT) to evaluate tear stability, Schirmer strips to evaluate tear production, and vital dyes to highlight epithelial irregularity. Imaging technologies can provide further information on the status of the ocular surface. For example, the Keratograph 5M (Oculus, Arlington, WA) can quantify TBUT noninvasively and provides images that can be used to measure tear meniscus height (TMH) and detect meibomian gland (MG) dropout.[3] The LipiView II (TearScience, Morrisville, NC) can measure blink rate, central lipid layer thickness (LLT), and image MGs.[4] An important gap in these technologies is that they provide mostly static metrics and do not visualize and document changes in tear film dynamics over time.

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This is important as the human tear film is a dynamic system comprised 2 layers that overlap each other and interchange constantly to form a semiviscous gel: the outermost lipid layer and the innermost muco-aqueous layer.[5] The lipid layer is secreted by the MGs within the upper and lower eyelids and is thought to protect the ocular surface by modulating tear film evaporation.[5–7] The aqueous layer, produced by the main and accessory lacrimal glands, and the mucin layer, produced by the goblet cells within the conjunctival surface, further protect, lubricate, and nourish the ocular surface.[5]

The Tear Film Imager (TFI), developed by AdOM (Israel), is a novel device with nanometer resolution designed to capture aqueous and lipid layer dynamics over time.[8] TFI generated parameters include mucous-aqueous layer thickness (MALT) calculated as the average interblink thickness of the muco-aqueous layer, mucous-aqueous thinning rate (MALTR) defined as the change in thickness of the muco-aqueous layer in the time period from blink relaxation to initiation of next blink, LLT as the average thickness of the lipid layer, lipid break-up time (LBUT) determined from significant (>10%) drop in LLT between blinks, lipid map uniformity (LMU) calculated as the average LLT variation over the lower hemisphere, and interblink interval (IBI) defined as the time in seconds measured between natural blinking rates.[8,9]

Studies using the TFI have previously reported correlations between DE signs and quantitative TFI metrics. Specifically, one study within the Israel population categorized 49 individuals (69% female, 58.8 ± 15.9 years) into DE (n = 37) and control (n = 12) groups based on TBUT (≤7 seconds), Schirmer (≤10 seconds), and corneal staining (>1 on a 0–3 scale) scores.[9] Patients with DE were further subclassified into mild–moderate (n = 22) and severe (n = 15) groups based on these DE signs.[9] TFI generated LBUT values were found to positively correlate with clinically assessed TBUT (r = 0.73, $P < 0.001$).[9] LBUT was also noted to be significantly higher in the control group (11.2 seconds) as compared with the mild–moderate (3.3 seconds) and severe (2.9 seconds) DE groups, $P = 0.01$.[9] Differences in LLT maps were also noted between groups with controls having a more homogenous thickness across the 6.5-mm map compared with cases that had a more variable map.[9] Finally, TFI generated MALT values positively correlated with Schirmer scores (r = 0.31; $P = 0.04$).[9] As compared with the control group (3757 nm), MALT measurements tended to be thinner in the mild–moderate (2983 nm) and severe (3043 nm) DE groups.[9] These data highlight the potential utility of using the TFI to examine tear film dynamics in healthy individuals and those with ocular surface disorders.

While promising, the utility of using TFI to examine tear film metrics and quantify biomarkers for assessing DE requires more data from diverse populations. As such, the purpose of this study was to examine differences in TFI metrics in healthy persons, in individuals with signs of evaporative, aqueous-deficient, and mixed DE, and to examine changes in TFI parameters following contact lens (CL) placement.

METHODS AND MATERIALS

Study Population

A retrospective study of adult participants (older than 18 years) who underwent TFI imaging during a comprehensive exam and/or while being evaluated for DE was conducted at the Miami Veterans Affairs from May 2022 to May 2023. This study was approved by the Miami Veterans Affairs Institutional Review Board and conducted in accordance with the principles of the Declaration of Helsinki. Exclusions included a history of autoimmune disease, an active external ocular process, the use of any topical medications beyond artificial tears, history of any eye surgery, presence of nystagmus, and history of keratoconus or other forms of corneal pathology.

Questionnaires

As part of our standardized work up, all participants completed questionnaires regarding demographics (age, sex, race, ethnicity) and medical history, including ocular conditions and current medications (topical and systemic). Subjects completed validated questionnaires for DE, including the 5 Item DE Questionnaire (DEQ-5)[10] and the ocular surface disease index (OSDI).[11] Overall intensity of ocular surface pain was collected with a Numerical Rating Scale by grading the average pain over the previous 7-day period on a scale of 0 being "no pain" and 10 "the worst eye pain imaginable."

Ocular Surface Examination

Following TFI imaging, participants underwent a standardized ocular surface assessment which included grading of 1) anterior blepharitis 0 = none, 1 = mild, 2 = moderate, 3 = severe; 2) lower eyelid telangiectasias 0 = none, 1 = mild vessel engorgement, 2 = moderate vessel engorgement, 3 = severe vessel engorgement; 3) inferior MG plugging 0 = none, 1 = less than 1/3, 2 = between 1/3 and 2/3, 3 = greater than 2/3 lid involvement; 4) tear evaporation measured through TBUT (5 μL fluorescein instilled in the superior conjunctivae, seconds measured until the first black spot appeared in the tear film, 3 measurements taken with 5 seconds blink interval between measurements and averaged); 5) corneal epithelial cell disruption measured through corneal staining (National Eye Institute scale,[2] 5 areas of cornea assessed; score 0–3 in each, total 15); and 6) Schirmer score with anesthesia measured as mm of wetting at 5 minutes.

TFI Protocol

Subjects were instructed to refrain from drop instillation in OU an hour before TFI assessment. A certified TFI user insured individuals were in a dark room and were correctly positioned in the machine. During the coarse alignment, participants kept their eyes closed while the user adjusted the infrared light in the center of the right eyelid until a crisp circle ~10 to 15 mm was seen. Next, fine centering was conducted as the user asked the individual to open their right

eye and focus on the green light-emitting diode installed to enable fixation. Once fine centering was achieved in compliance with the alignment metrics required by the TFI, the scan was initiated automatically. Scans ranged from ~40 to 60 seconds, and participants were told to focus on the light-emitting diode but to avoid keeping their eye forcefully open as to allow normal blinking. In all individuals, scans were taken of the right eye, approximately 5 to 15 minutes apart.

As data processing occurred remotely, our access to processed outputs was not instantaneous. Consequently, our scans were reliant on intrinsic TFI quality control parameters, such as test duration, signal score, position score, and a color-coded signal bar (red, yellow, or green). Successful scans required adherence to specified score ranges (provided by the manufacturers) and the presence of a green signal bar. To obtain successful scans, we repeated scans at least twice in every participant and in some cases, up to 6 times. Following the sixth attempt, if a successful scan was not achieved, we concluded the scanning session. Participants who did not achieve a successful scan were excluded from statistical analysis. Participants who wore CLs underwent scans using the same protocol, once without and once following CL placement.

Determination of High-Quality TFI Scans

Quantitative results of the TFI parameters included MALT, MALTR, LLT, LBUT, LMU, and IBI. After receiving the postprocessed data, we examined ~100 scans to establish criteria for high-quality scans. We based our selection criteria on the graphical representation provided in the output. Specifically, high-quality scans were considered ones with 1) a lipid layer that had at least 1 continuous pattern graphed for ≥5 seconds and 2) a mucous-aqueous layer had at least 1 continuous downtrend pattern or trough graphed for ≥3 seconds (high-quality and low-quality scans demonstrated in Supplementary Figure 1a-b, Supplemental Digital Content 1, http://links.lww.com/ICO/B654). We included all available data for each patient (some of which had more than 1 high-quality scan) and found that even with multiple scans, 44% of individuals did not have a full set of metrics. The TFI metrics with the most missing data was LBUT (missing in 42%), followed by MALTR (9%) and MALT (6%). On the other hand, LMU and IBI were captured in at least 1 scan in 98% of subjects.

Groupings by Tear Status

Participants were grouped based on tear function as 1) healthy (TBUT > 10 s, Schirmer > 10 mm), 2) evaporative (TBUT < 10 s, Schirmer > 10 mm), 3) aqueous deficient (TBUT > 10 s, Schirmer < 10 mm), and 4) mixed (TBUT < 10 s, Sch < 10 mm). We recognize that these grouping are not as rigid as prior studies[2] but elected to use higher cut-off for tear dysfunction to examine tear dynamics in a wide range of otherwise healthy individuals.

Statistical Analysis

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) Statistics Software version 25.0 (IBM Corp Armonk, NY). Descriptive statistics were used to summarize participant demographics, ocular symptoms, and DE signs. Normality of the distributions of variables of interest was assessed using the Shapiro–Wilk test. Given that some measures were not normally distributed, Mann–Whitney *U* tests were run to assess the differences in TFI metrics regarding demographics. Spearman correlation coefficients were calculated to evaluate the relationship within TFI parameters and between ocular symptoms, DE signs, and TFI metrics. The Wilcoxon test was used to compare TFI parameters from scans taken during the same visit for the same participant. A *P*-value < 0.05 was considered statistically significant for all tests. We opted to give information on all variables being compared as opposed to correcting the *P*-value (eg, Bonferroni) since the latter methodology has its own limitations.[12]

RESULTS

Healthy, DE Subgroups and CL Users

Study Population

Our study population included 48 individuals with a median age and interquartile range (IQR) of 27 (6) years, 74% self-identified as White, 45% as male, and 51% as Hispanic. Individuals were overall healthy with no comorbidities noted including hypertension, hyperlipidemia, or diabetes. None of the individuals reported oral or topical medication use.

Individuals also had a low burden of DE symptoms, with 67% having a DEQ-5 ≤6 [median, IQR: 5, (6)] and 71% having an OSDI ≤13 [median, (IQR): 4.2, (12.5)]. Despite having a wide range of tear findings, all individuals had some eyelid abnormalities including mild or greater anterior blepharitis (61%), telangiectasias (61%), and MG plugging (53%). Subjects were divided into 4 groups based on tear stability (TBUT) and production (Schirmer) (Table 1). Overall, the groups were balanced with respect to demographic characteristics.

Correlations Within TFI Parameters

TFI parameters were extracted from high-quality scans, and median and ranges were calculated. Spearman correlations were first performed to examine relationships within TFI parameters. Significant positive relationships were seen between MALTR and LLT (rho = 0.44, $P = 0.004$) suggesting less rapid evaporation in individuals with increased lipid thickness. In addition, higher MALTR values significantly correlated with higher IBI (rho = 0.37, $P = 0.02$) suggesting less frequent blinking in individuals with greater tear film stability (less rapid evaporation) (see Supplemental Table 1, Supplemental Digital Content 1, http://links.lww.com/ICO/B656, and Supplemental Figure 2, Supplemental Digital Content 1, http://links.lww.com/ICO/B655). Increased LLT was also significantly correlated with higher LMU (rho = 0.55, $P < 0.0005$) suggesting that individuals with a thicker lipid layer also had a less uniform lipid layer (see Supplemental Table 1, Supplemental Digital Content 1, http://links.lww.com/ICO/B656, and Supplemental Figure 2, Supplemental Digital Content 1, http://links.lww.com/ICO/B655).

TABLE 1. Demographics, DE Symptoms, and Signs for Subgroups Created Based on Tear Function

Dry Eye Subgroups	Healthy (n = 18)	Evaporative (n = 10)	Aqueous Deficiency (n = 11)	Mixed (n = 9)
Demographics				
Age, yr, median (IQR), [range]	26 (4) [17–31]	28 (21) [22–54]	27 (12) [25–50]	31 (15) [24–42]
Sex, male, % (n)	44% (8)	40% (4)	55% (6)	44% (4)
Race, White, % (n)	83% (15)	80% (8)	64% (7)	56% (5)
Ethnicity, Hispanic, % (n)	41% (7)	50% (5)	55% (6)	67% (6)
Dry eye symptoms, median (IQR)				
DEQ-5 (0–22)	4 (9)	5 (7)	4 (5)	6 (8)
OSDI (0–100)	8.3 (17.7)	10.2 (17.2)	4.2 (4.2)	6.3 (10.4)
Dry eye signs, % (n)				
Anterior blepharitis, ≥1, (0–3)	61% (11)	70% (7)	73% (8)	33% (3)
Telangiectasias, ≥1 (0–3)	67% (12)	40% (4)	82% (9)	56% (5)
MG plugging, ≥1, (0–3)	50% (9)	30% (3)	73% (8)	56% (5)
Ocular surface disease parameters				
TBUT, s, median (IQR)	14 (3.2)	5.5 (2.9)	15 (2.7)	5.7 (2.8)
Corneal staining, ≥1 (0–15)	11% (2)	20% (2)	9% (1)	56% (5)
Schirmer, mm, median (IQR)	22 (23)	19.5 (16)	5 (4)	7 (4)

DEQ-5, 5 Item Dry Eye Questionnaire; IQR, interquartile range; OSDI, Ocular Surface Disease Index; TBUT, tear-break up time.

Quantified TFI Parameters for Healthy Subjects and DE Subgroups

Subjects in the aqueous deficiency category were found to have a lower median MALT (2672 vs. 3084 nm) and higher LLT (47.5 vs. 38.3 nm), LBUT (4.4 vs. 2 seconds), and IBI (13.9 vs. 5.4 seconds) compared with the evaporative group, highlighting that individuals clinically diagnosed with aqueous tear deficiency (ATD) had the lowest MALT values (although heterogeneity was noted within all groups with respect to all parameters) (Table 2). Interestingly, subjects in the evaporative DE group had the highest MALT values compared with the healthy, aqueous-deficient, and mixed groups (3084 vs. 2988, 2672, 3053 nm), suggesting a possible compensatory response to decreased LLT and tear stability (Table 2). Evaporation rate was most rapid in individuals in the mixed group compared with the healthy, evaporative, and aqueous deficient groups (−178 vs. −36, −30, and −31 nm/s), suggesting that tear stability was most impacted in individuals with both aqueous and lipid deficiency.

TFI Parameters with Repeated Measurements

Out of 31 non-CL wearers, 15 individuals had 2 good quality scans from which values could be compared for stability. Overall, Wilcoxon tests showed no significant differences in median TFI values between scans taken during the same visit (see Supplemental Table 2, Supplemental Digital Content 1, http://links.lww.com/ICO/B657), suggesting stability of measurements acutely.

TABLE 2. Median and IQR Ranges for TFI Parameters From First High-Quality Scan per Participant

Group	Mucous-Aqueous Layer		Lipid Layer			
Median, Min–Max, IQR	MALT (nm)	MALTR (nm/s)	LLT (nm)	LBUT (s)	LMU (nm²)	IBI (s)
Healthy n = 18	2988	−36	51.6	2.5	29	9
	1624–4817	−415 to 83	11.1–137.5	1.3–19.9	3.3–988	1–41
	1177 (n = 16)	83 (n = 16)	22.8 (n = 16)	5.4 (n = 12)	36 (n = 17)	15.9 (n = 18)
Evaporative n = 10	3084	−30	38.3	2	23.5	5.4
	2404–5156	−239 to 45	15.5–117.1	0.7–3.7	2.8–246	2.1–39
	1982 (n = 9)	145 (n = 9)	26.8 (n = 10)	2.4 (n = 4)	41 (n = 10)	4.1 (n = 10)
Aqueous deficiency n = 11	2672	−31	47.5	4.4	8	13.9
	1976–4855	−115 to −13	26.3–127.2	0.65–41	3.3–105	4.2–64
	1511 (n = 11)	33 (n = 10)	34.1 (n = 9)	10.9 (n = 8)	18.4 (n = 11)	32.1 (n = 10)
Mixed n = 9	3053	−178	34.9	4.2	15	11
	1561–4263	−456 to −15	11.9–76.8	2–9.3	2.7–47	3.2–44
	1162 (n = 9)	293 (n = 9)	25.8 (n = 9)	5.5 (n = 4)	32.6 (n = 9)	26.1 (n = 5)

IBI, inter-blink interval; IQR, inter-quartile range; LLT, lipid layer thickness; LBUT, lipid break up time; LMU, lipid map uniformity; MALT, mucous-aqueous layer thickness; MALTR, mucous-aqueous thinning rate; TFI, tear film imager.

TABLE 3. Median and IQR Ranges for TFI Parameters Before and After CL Placement

Group	MALT (nm)	MALTR (nm/s)	LLT (nm)	LBUT (s)	LMU (nm²)	IBI (s)
No-CL	2869	−60	39.3	3.1	20	4.5
[median, min–max, IQR, n = 17]	1981–3882, 1019 (n = 17)	−436 to −83, 123.5 (n = 16)	23–52, 19.6 (n = 15)	0.7–19.9, 8 (n = 7)	2.1–78, 26.5 (n = 16)	3–11, 3 (n = 16)
CL	2175	−151	38.4	2.9	21	6.2
[median, min–max, IQR, n = 17]	793–3358, 1106 (n = 16)	−252 to 37, 162.3 (n = 16)	12.5–111.5, 13.9 (n = 16)	0.6–5.5, 2.7 (n = 13)	3–128, 26.5 (n = 16)	2.3–21, 3.8 (n = 16)
P values	**0.001**	0.27	0.71	0.18	0.98	0.07

CL, contact lens; IBI, inter-blink interval; IQR, inter-quartile range; LBUT, lipid break up time; LLT, lipid layer thickness; LMU, lipid map uniformity; MALT, mucous-aqueous layer thickness; MALTR, mucous-aqueous thinning rate; TFI, tear film imager.

Changes in Quantified TFI Parameters Before and After CL Placement

Out of 17 CL wearers, all had 2 scans from which values could be compared for change with intervention (pre-CL vs. post-CL placement). At baseline (no CL), individuals had a significantly thicker muco-aqueous layer compared with the same participants with a CL in place (2869 vs. 2175 nm, $P = 0.001$) (Table 3). No significant changes in other values were noted with CL placement.

Relationship Between TFI Parameters and DE Symptoms and Signs

Spearman correlations were performed to examine relationships between TFI parameters and DE symptoms and signs. Symptoms were not significantly related to any of the quantitative TFI parameters (Table 4 and Fig. 1). The strongest correlations with respect to signs were between TBUT, MALTR, and LLT (rho: 0.30, $P = 0.05$ and rho: 0.44, $P = 0.002$, respectively). The remaining clinical signs did not show a significant correlation with TFI parameters (Table 4 and Fig. 1).

Relationship Between TFI Parameters and DE Symptoms and Signs in Participant Subgroups

Next, Spearman correlations between TFI parameters, DE symptoms, and signs were performed within each group (healthy, evaporative, aqueous-deficient, and mixed). For healthy participants (n = 18), positive correlations were noted between TBUT and TFI generated LLT and LMU (rho: 0.78, $P = 0.00$ and rho: 0.56, $P = 0.02$, respectively). In the evaporative group (n = 10), DE symptoms (ie, DEQ-5) positively correlated with LMU (rho: 0.69, $P = 0.03$) and MG plugging negatively with MALTR (rho: −0.73, $P = 0.03$). In the ATD group, Schirmer negatively correlated with LLT (rho: −0.66, $P = 0.04$). In the mixed group, plugging positively correlated with MALTR (rho: 0.68, $P = 0.04$) and LLT (rho: 0.78, $P = 0.01$).

DISCUSSION

DE is a highly prevalent condition with significant life burden, yet current diagnostic approaches are limited and poorly translated into improved patient outcomes. In this analysis, we investigated the dynamic changes in specific

TABLE 4. Spearman Correlations Between TFI Parameters and DE Symptoms and Signs

	MALT (nm)	MALTR (nm/s)	LLT (nm)	LBUT (s)	LMU (nm²)	IBI (s)
	Rho	Rho	Rho	Rho	Rho	Rho
Symptoms						
DEQ-5	0.23	0.15	0.14	0.06	0.09	0.09
OSDI	0.04	−0.03	0.11	−0.03	0.17	−0.13
Signs*						
Anterior blepharitis	0.24	0.23	−0.07	0.05	0.01	−0.10
Eyelid telangiectasias	−0.22	0.20	0.09	−0.10	−0.01	0.10
MG plugging	−0.01	0.07	0.15	0.15	−0.16	0.19
Ocular surface disease parameters						
TBUT	−0.13	**0.30**†	**0.44**‡	−0.03	0.08	0.24
Corneal staining	0.01	−0.08	−0.11	0.01	−0.01	−0.04
Schirmer	0.24	0.09	0.09	−0.24	0.09	−0.20

*Values from the right eye.
†$P < 0.05$.
‡$P < 0.01$.
DEQ-5, 5 Item Dry Eye Questionnaire; IBI, inter-blink interval; LBUT, lipid break up time; LLT, lipid layer thickness; LMU, lipid map uniformity; MALT, mucous-aqueous layer thickness; MALTR, mucous-aqueous thinning rate; OSDI, Ocular Surface Disease Index; TBUT, tear-break up time; TFI, tear film imager.

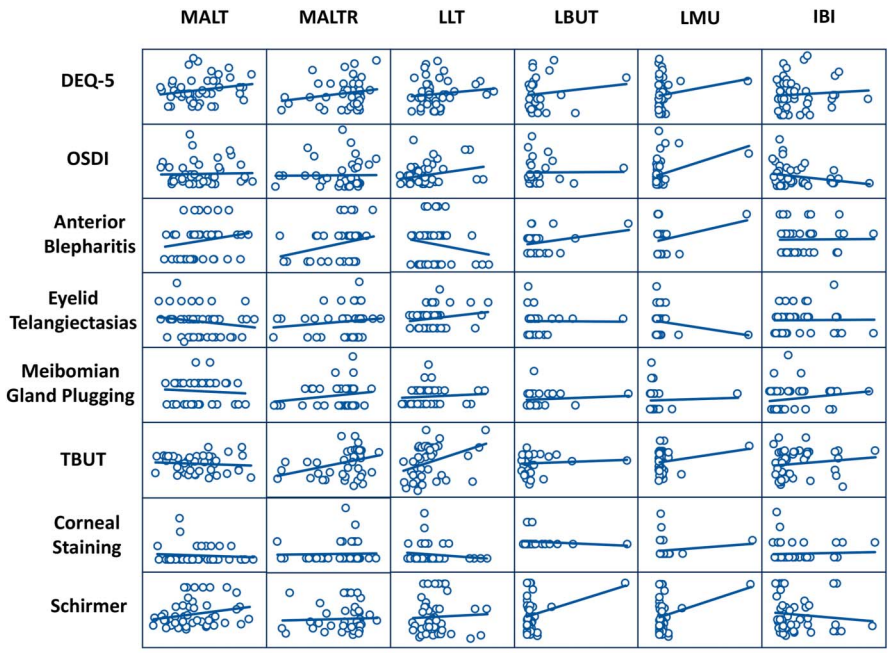

FIGURE 1. Matrix scatter plot showing Spearman correlations between TFI parameters and DE symptoms and signs. (The full color version of this figure is available at www.corneajrnl.com.)

TFI: *Tear Film Imager;* ***DEQ-5****: 5 Item Dry Eye Questionnaire;* ***OSDI****: Ocular Surface Disease Index;* ***TBUT:*** *Tear-Break Up Time;* ***MALT:*** *mucous-aqueous layer thickness;* ***MALTR:*** *mucous-aqueous thinning rate;* ***LLT:*** *lipid layer thickness;* ***LBUT:*** *lipid break up time;* ***LMU:*** *lipid map uniformity;* ***IBI:*** *inter-blink interval (IBI).*

layers tear of the human tear film over time and those occurring following CL placement. Overall, we found several significant correlations between TFI parameters and DE and identified specific tear film changes over time and following the administration of CLs. In our analysis, individuals with thicker lipid layers experienced slower tear evaporation as evidenced by higher MALTR (less negative) and LLT values. In addition, our data indicate that individuals with more stable tear film (higher MALTR values) blinked less frequently (IBI). Furthermore, those with thicker lipid layer (LLT) had less uniform lipid distribution (higher LMU).

When examining TFI parameters within the 4 groups (control, evaporative, aqueous-deficient, mixed DE), heterogeneity was noted with regards to all TFI parameters. However, across groups, individuals with a clinical diagnosis of ATD had a thinner MALT but increased LLT and increased tear stability (higher LBUT) compared with the evaporative DE group. On the other hand, the evaporative DE had the highest MALT when compared with the other groups, as a possible compensatory response to thinner lipid layer and decreased tear stability.[13] Those with both aqueous and lipid deficiency experienced the greatest impact on tear stability as seen by the most rapid evaporation rate when compared with the other groups. Over the short term, TFI generated tear measurements were found to be stable. CL wear most strongly impacted MALT values, with a decrease noted while wearing versus not wearing CL. Not surprisingly, TFI measurements did not relate to DE symptoms, highlighting the disconnect between symptoms and signs of disease.[14] TFI metrics (MALTR and LLT) most closely related to TBUT. Generally, TFI values gave information regarding tear film dynamics that was not otherwise captured on clinical testing.

Prior studies have examined tear parameters within DE subtypes using different imaging devices including the Keratograph 5M. A Korean study examined noninvasive TBUT (NIBUT) in individuals with ATD (n = 23, OSDI ≥ 12, Schirmer ≤ 5 mm, TBUT ≤ 5 seconds, corneal staining ≥ 3), meibomian gland dysfunction (MGD) (n = 95, OSDI ≥ 12, eyelid blood vessel engorgement and/or MG plugging, MG expressibility ≥ 1, or poor meibum quality ≥ 3), mixed DE (n = 132, both ATD and MGD criteria fulfilled), and controls (n = 55). NIBUT was significantly lower in the ATD and mixed DE subgroups compared with controls (ATD: 8.11 ± 4.12 seconds; MGD: 10.62 ± 4.39 seconds; mixed DE: 7.76 ± 3.86 vs. controls 10.56 ± 3.41 seconds). Similar to our findings, individuals with mixed DE were found to have the least stable tear film as indicated by the shortest NIBUT.[15] Other aspects of the tear film, such as TMH, have also been evaluated with the Keratograph 5M across DE subtypes. For example, in a Japanese study, TMH was lower in 23 patients with ATD (Schirmer ≤ 5 mm, TBUT ≤ 5 seconds, corneal staining ≥ 3) compared with 23 controls (0.14 ± 0.03 mm vs. 0.20 ± 0.05 mm, $P < 0.001$).[16] This is similar to our finding of low MALT values in the ATD

compared with the other groups. While it is encouraging that similar trends have been noted across studies using different devices, further studies are needed to elucidate relationships between machine generated tear film parameters and clinically defined DE subgroups.

LipiView II generated metrics have also been examined across DE subgroups. In a South Korean study of 326 individuals with DE (OSDI $\geq$ 12, Schirmer $<$ 5 mm or TBUT $\leq$ 5 seconds, corneal staining $\geq$1 with or without MGD), LLT values were higher compared with 64 controls [median (range): 84 nm (20–100) vs. 67 nm (33–100)].[17] However, other studies using LipiView have noted lower LLT in DE. In an American study of 137 individuals subgrouped by symptoms as mild–moderate DE (n = 61, Standard Patient Evaluation of Eye Dryness score 1–9); severe DE (n = 58, SPEED $\geq$ 10) and asymptomatic (n = 18, Standard Patient Evaluation of Eye Dryness = 0), the frequency of LLT $\leq$ 60 nm [61% (n = 37) in mild–moderate group; 74% (n = 43) in severe], while 72% of asymptomatic participants had LLT $\geq$ 75 nm.[18] The latter findings most closely align with our TFI data, as we found higher LLT controls compared with the other DE groups. However, it is important to note that differences in disease definition, along with population characteristics and instrument design, make comparisons across studies challenging.

Beyond subgroups, correlations between machine and clinically generated values have also been examined across studies, with disparate results. Some studies have noted moderate correlations, as seen in a Chinese study of 44 individuals with DE (conjunctival staining $\geq$1, corneal staining $\geq$2, TBUT $\leq$ 10 seconds, or Schirmer test $\leq$ 10 mm) where correlation coefficients were $>$0.3 (NIBUT and TBUT:rho = 0.55; NIBUT and Schirmer:rho = 0.41).[19] Other studies, on the other hand, have noted weak correlations, as was seen in a US study of 288 individuals with moderate-to-severe dry eye disease (OSDI $\geq$ 23 and $\geq$2: conjunctival staining $\geq$1, corneal fluorescein staining $\geq$4, TBUT $\leq$ 7 seconds, or Schirmer test 1–7 mm/5 minutes) where correlation coefficients were $<$3 (NIBUT and TBUT:rho = 0.18; TMH and Schirmer:rho = 0.15).[20] While the reason for these discrepancies is unclear, patient and disease-related factors may impact findings. Interestingly, our own studies found similar discrepancies using the TFI. In a prior Israeli study that focused on individuals with mild–moderate (n = 22, Schirmer 2–10 mm/5 minutes, TBUT 1–7 seconds, or corneal staining 1–2) and severe DE (n = 15, Schirmer $\leq$ 2 mm/5 minutes, TBUT $\leq$ 1 second, or corneal staining = 3), TFI generated LBUT strongly correlated with TBUT (r = 0.73, $P < 0.001$) and MALT moderately correlated with Schirmer (r = 0.31; $P = 0.04$).[9] In this study, which enrolled healthy controls and individuals with less severe disease, weaker correlations were noted between TFI tear metrics (ie, MALTR and LLT) and TBUT as a group but stronger correlations were noted when considering relationships within subgroups. In agreement across studies, weak correlations have been noted between machine generated parameters and patient-reported symptoms.[20] These studies highlight the complexity of DE, a disease that encompasses various symptoms and signs that often do not relate to one another.

The impact of CL wear on tear film properties has also been examined in prior studies. A Japanese study of 10 individuals (20 eyes) used swept source anterior segment optical coherence tomography (Tomey Corp, Nagoya, Japan) to measure TMH without and with placement of high (69.4%) and low (24.0%) water content (WC)-CL (1 CL randomly placed in each eye). Baseline TMH measurements (without CL) were taken and repeated 20 minutes later with CL wear. TMH values decreased with CL wear (244 $\pm$ 53 to 161 $\pm$ 38 μm, $P < 0.001$ in the high WC-CL group; 257 $\pm$ 44 to 198 $\pm$ 26 μm, $P < 0.001$, in the low WC-CL group).[21] Moreover, other studies have also shown the impact of CL use on tear stability (measured via NIBUT). In an Australian study (n = 11), baseline NIBUT (measured with a custom-made tearscope) was 21.3 $\pm$ 5.7 seconds which decreased with CL wear measured 6 hours later to 13.7 $\pm$ 4.3 seconds, $P = 0.003$.[22] These studies show quantitative changes in the tear film and decreased tear stability after CL placement. Similar to prior studies,[21,22] we found that MALT values decreased, with a similar trend noted for LBUT, with CL use. Further studies are required to further validate our findings.

Our study of novel tear film dynamics has several limitations to acknowledge including a small sample size, acute duration of assessment, and geographically restricted population. Furthermore, as we chose not to utilize the Bonferroni method (due to its own limitations), there is an increased risk of falsely rejecting the null hypothesis in our experiment (Type I error). Despite these limitations, our results suggest that TFI imaging offers quantification of tear film layer thicknesses and dynamics that are not able to be assessed with other clinical testing. Our preliminary findings, including correlations between TFI parameters, DE symptoms, and signs as a group and within subgroups, highlight the complex nature of the tear film with wide SD noted for our TFI metrics within and across groups and with variability with regards to correlations between TFI metrics and various clinical measures within subgroups. Encouragingly, we found that MALT decreased with CL, a finding noted in prior studies using different methodologies, providing validity for our preliminary observations. Noninvasive imaging modalities including TFI provides objective metrics and may help to improve DE classifications and eventually assist in development of precision-based treatment algorithms. However, data presented within are an initial interpretation of TFI metrics, and larger, longitudinal studies are needed that examine TFI metrics over time, with treatment, and in relationship to biomarkers generated by other imaging devices. Ultimately, the goal is to determine how tear film biomarkers can aid in the diagnosis and management of DE and other ophthalmic disease.

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