• Clinical Research •

Layer-by-layer tear film measurement in patients with dry eye and meibomian gland dysfunction

Reiko Arita[1,2], Gaku Takeuchi[3], Kiyofumi Sasai[4], Masatsugu Nakamura[4], Masahiro Akiba[3]

[1]Department of Ophthalmology, Itoh Clinic, Saitama 337-0042, Japan
[2]Lid and Meibomian Gland Working Group, Saitama 337-0042, Japan
[3]Eyecare Business Division, Topcon Corporation, Tokyo 174-8580, Japan
[4]Product Development Division, Santen Pharmaceutical Co. Ltd., Osaka 530-8552, Japan
Correspondence to: Reiko Arita. Department of Ophthalmology, Itoh Clinic, 626-11 Minami-Nakano, Minumaku, Saitama 337-0042, Japan. ritoh@za2.so-net.ne.jp
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Abstract

● **AIM:** To evaluate parameters measured using the tear film imager (TFI) prototype, a new technology that enables to quantify the tear film thickness of lipid and mucoaqueous layers.

● **METHODS:** In this cross-sectional study, patients with dry eye, meibomian gland dysfunction (MGD), and non-dry eye/MGD from February 2020 to January 2021 were analyzed. Quantified TFI outputs included lipid layer thickness (LLT), mucoaqueous layer thickness (MALT), MALT rate of change (MALTR), and lipid breakup time. Two other interferometry devices, LipiView2 and DR-1α, were used for comparison. TFI outputs and other clinical parameters were analyzed using correlation coefficients. Each patient underwent one or several study visits. Baseline values of three device outputs, other clinical parameters, and their changes were examined.

● **RESULTS:** This study involved 28 patients (8 patients with dry eyes, 13 with MGD, and 7 with non-dry eye/MGD). Baseline TFI, LipiView2, and DR-1α values were associated with various clinical parameters. The LLT values estimated using TFI had a correlation with the plugging score in the upper eyelid (r=−0.42). Several TFI values have correlated better than LipiView2 and DR-1α, particularly with questionnaire scores. MALTR by TFI revealed a correlation between standardized patient evaluation on eye dryness (SPEED) and dry eye-related quality of life score (DEQS) scores (r=0.59, 0.43), respectively.

● **CONCLUSION:** TFI enabled to quantify the LLT and MALT separately over time and shows the moderate correlations between TFI measurements and clinical parameters, which yields the potential for TFI to serve as a complementary tool for assessing dry eye and MGD.

● **KEYWORDS:** dry eye; lipid layer thickness; meibomian gland dysfunction; mucoaqueous layer thickness; tear film imager

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INTRODUCTION

The corneal tear film is very thin, but it plays a key role in maintaining homeostasis on the ocular surface. The new definition of dry eye disease, proposed by TFOS DEWS II and other recent studies[1-2], emphasizes tear film instability as a central concept. This shift from focusing solely on tear volume to the stability and functionality of the tear film highlights the multifactorial nature of dry eye disease. By adopting this perspective, clinicians can better diagnose and treat dry eye by considering both structural and dynamic properties of the tear film. Several dry eye treatments make up for the tear film component deficiency, and the tear film-oriented disease or therapy concept asserts that understanding the condition of each tear film layer is critical for suitable diagnosis and treatment selection[3-4]. Assessing the tear film condition is critical because the tear film plays a fundamental role in maintaining ocular surface homeostasis and visual function[5-6]. Tear film instability is a hallmark of dry eye disease and a key factor influencing symptoms and disease progression. Accurate assessment of tear film condition, including its lipid and aqueous layers, provides insight into the underlying pathophysiology of dry eye and meibomian gland dysfunction (MGD). Moreover, understanding the tear film's structural and dynamic characteristics enables clinicians to tailor treatments effectively. For example, evaluating lipid layer thickness (LLT) and mucoaqueous layer thickness (MALT) can help determine the specific tear film deficiency, guiding the choice

of lipid-based treatments, aqueous supplementation, or other therapeutic strategies.

Numerous clinical tests and medical devices are available to determine tear film instability and measure tear volume. The interferometry approach is one of the major imaging technologies that is employed in LipiView2, DR-1α, and tear film imager (TFI)[7-8]. These devices can be used to quantify the LLT or assess the tear film stability. The lipid layer condition has been used to measure the efficacy of treatments in patients with dry eye and MGD[6,9]. These imaging technologies of tear film are effective for clinical trials and are often used to assess the efficacy of drugs and devices in patients with dry eyes or MGD[10-11], but their utilization as biomarkers remains insufficient. Further automation, quantification, and standardization are needed to fully use them as biomarkers[12-13]. Clarifying the correlation between tear film imaging parameters and existing clinical examinations is necessary to verify their validity as a biomarker. Several studies have been performed on lipid layer imaging using the interferometry technique; however, the association between lipid layer imaging parameters and traditional ophthalmic tests for dry eye or MGD is not always consistent[14]. Tear film imaging methods that cannot cover the wide field of view (FOV) of the cornea may have challenges in assessing tear film stability because of the tear dynamic characteristics of the cornea. The lipid layer extends from the bottom of the cornea to the top and stabilizes in a short time, and then tear film breakup starts more likely at the center of the cornea[15]. However, there are various reports and some uncertainty regarding the location of first tear film breakup in patients with dry eye and MGD. Kim *et al*[16] reported that there were regional differences in the first tear film breakup depending on the dry eye subtype, with the involvement of central breakup being higher in the aqueous-deficient dry eye (ADDE) and ADDE/MGD subtypes. Therefore, it is believed to be highly significant that tear film stability in the central cornea can be observed. For instance, LipiView2 measures only the inferior corneal area, which may be the cause of destabilization and challenges in interpreting the LipiView2 measurement results[8]. However, some imaging techniques cover the wide FOV of the cornea but have low objectivity and quantitative parameters. Noninvasive tear film breakup time (NIBUT), which is a tear stability indicator, uses the entire cornea as the assessment area, but its measurements may be difficult to interpret. A previous study found that NIBUT was not associated with conventional ophthalmic tests or LLT[17], because NIBUT shows large variations in measured values and is inappropriate for treatment monitoring[18]. The tear film lipid and aqueous layers may compensate for each other's functions; therefore, simultaneously measuring both layers are preferable[19]. Measuring and objectively quantifying the wider FOV, including the center of the cornea, are desirable for employing tear film thickness as a biomarker. TFI is a device that overcomes these difficulties by simultaneously quantifying the lipid and aqueous layers at the center of the cornea and measuring the LLT on the wide FOV of the cornea[20].

TFI is designed to objectively quantify tear film parameters, such as LLT, MALT, and their temporal changes. These parameters directly reflect tear film stability, and the ability to measure these parameters with nanometer-scale resolution supports a more precise diagnosis and monitoring of dry eye and MGD, addressing gaps in previous diagnostic approaches. For this, it is essential to quantify the changes in each layer of tear film over the large field of the cornea, however, none of the currently available devices cover. Objectiveness and quantification are essential features of ideal imaging biomarkers. Therefore, the aim of this study is to provide a preliminary investigation of the following points for dry eye, MGD and non-dry eye/MGD patients. Correlation between baseline TFI measurement results and conventional ophthalmic test results. Comparison of lipid layer thickness values measured by TFI and existing interferometry devices. Correlation between TFI measurement results and conventional ophthalmic test results in terms of change from baseline.

PARTICIPANTS AND METHODS

Ethical Approval This cross-sectional observational study and a small-scale short-term longitudinal study were performed at the Itoh Clinic, Saitama, Japan. Ethics approval was obtained from the ethics committees at Itoh Clinic (approved #: IRIN-20200106) and Santen Pharmaceutical Co., Ltd., Osaka, Japan (approved #: RINRI-2019-24). We have confirmed with the Ministry of Health, Labor and Welfare Japan that this research does not correspond to a "Specified Clinical Research". Participants with dry eye, MGD, and non-dry eye/MGD who visited the outpatient Itoh Clinic from February 2020 to January 2021 were recruited. All participants provided written informed consent before the examination and had visited the clinic at least once for evaluation, and some participants had several study visits for the same evaluation.

Participants This study included adults with aqueous-deficient dry eye, MGD, and non-dry eye/MGD. ADDE was diagnosed as 1) a fluorescein tear film breakup time (TBUT) of ≤5s, 2) presence of dry eye symptoms, 3) unanesthetized Schirmer's test values of ≤5 mm for 5min. These parameters align with the TFOS DEWS II emphasis on the multifactorial nature of dry eye disease and its diagnostic methodology[1]. MGD was diagnosed based on the MGD diagnostic criteria in Japan[21], *i.e.*, 1) existence of any chronic ocular symptom, 2) presence of lid margin vascularity, 3) obstruction of meibomian glands as shown by the detection of plugging and decreased

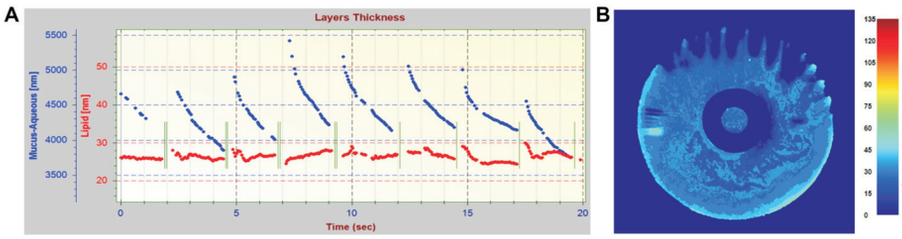

Figure 1 An example of the TFI output A: Example of lipid (red) and aqueous layer (blue) thickness over a 20-second time period. The vertical bar (green) represents the location where the blink is detected. B: Example of lipid layer 2D map at a certain time period. Color represents the thickness of the lipid layer in nanometer. Note that the center donut-shaped blue area is the location where the reflected light from tear was blocked due to the internal optical system. TFI: Tear film imager.

meibum expression in response to moderate digital pressure in at least one eye. Individuals who were not diagnosed with both dry eye and MGD were characterized as non-dry eye/MGD. Exclusion criteria included patients with ocular trauma, active ocular surface diseases, such as active corneal infection, those using lacrimal punctum plugs, and severe systemic diseases, such as autoimmune diseases including Sjögren's syndrome and rheumatoid arthritis, and pregnant or breastfeeding females. For each participant, the first visit was used for baseline value evaluations and the following visits were used for change evaluation from the first visit (baseline values).

Outcome Measures Each participant was requested to perform the following clinical examinations[22-23] on both eyes in the order listed: 1) standardized patient evaluation on eye dryness (SPEED) and dry eye-related quality of life score (DEQS) questionnaires for evaluating ocular symptoms. These are validated questionnaires and can assess the presence and severity of symptoms. These tools are widely accepted and provide a reliable measure of patient-reported outcomes, ensuring that symptom presence was objectively quantified; 2) measurements using TFI (prototype device, AdOM, Lod, Israel), LipiView2 (Johnson & Johnson, Stamford, CT, USA), and DR-1α (Kowa, Aichi, Japan); the measurement order of these devices was randomly predefined for each participant; 3) slit-lamp microscopy to assess upper and lower lid margin abnormalities, including plugging (scale of 0–3) and vascularity (scale of 0–3), irregularity (scale of 0–2), and thickening (scale of 0–2) were scored based on our previously reported technique; 4) slit-lamp microscopy to assess fluorescein-based TBUT, corneal and conjunctival fluorescein staining score (scale of 0–3 for each of nasal/temporal conjunctiva and cornea), and Marx line (mucocutaneous junction) score (scale of 0–3) after fluorescein dye instillation; 5) noncontact meibography system attached to a slit-lamp microscopy to determine the gland dropout (0–2 for each eyelid) and partial glands (0–3 for each eyelid); 6) unanesthetized Schirmer's test for 5min to evaluate the volume of tear fluid.

Tear Film Imager TFI is a newly developed white light interferometry-based device that measures the thickness of both lipid and aqueous sublayers of the tear film and monitors their trends over multiple blink cycles[20,24]. The reproducibility and other basic characteristics of TFI devices have previously been reported in a published paper evaluating the fundamental properties of these instruments[20]. For each measurement, the instrument is manually aligned to the corneal center, after which it records high-resolution spectroscopic data from the central cornea and interferometric images of the broader corneal field for up to 40s. Detailed operating principles are reported previously, explaining how direct thickness measurements of the lipid and aqueous sublayers are obtained *via* white light spectral analysis[25]. From these time-series data, TFI provides six key parameters: MALT, MALT rate of change (MALTR), LLT, lipid breakup time (LBUT), interblink interval (IBI), and lipid map uniformity (LMU). In addition, TFI can generate an LLT map across the wider corneal surface by leveraging image data. During examinations, subjects are instructed to blink naturally. Figure 1 depicts an example of the results evaluated using TFI.

LipiView2 LipiView2 is a tear thickness measurement device that noninvasively evaluates LLT based on the white light interferometry[22]. A 20-s video was recorded to illustrate the interference pattern of the tear film for each eye. The LLT derived from the image is determined as interferometric color units (ICUs), where 1 ICU reflects approximately 1 nm. The participants were instructed to naturally blink during the examination.

DR-1a DR-1α is a tear interferometer that can show interferometry images on the screen in real-time[22-23]. Investigators can assess NIBUT and score tear interferometric fringe patterns. The interferometric images were divided into three types: pearl-like appearance (monotonous gray interferometric fringe), Jupiter-like appearance (multicolored interferometric fringe), or crystal-like appearance (grayish amorphous interferometric fringe). Each type of interferometric image was converted to a numerical score for statistical analysis (pearl: 0, Jupiter: 1, and crystal: 2). Participants were instructed to blink twice naturally and then to keep both eyes open as long as feasible during the examination.

Table 1 Demographics of all enrolled subjects mean±SD

Parameters	Disease group			Total
	MGD	Dry eye	non-dry eye/MGD	
Patients (eyes)	13 (26)	8 (16)	7 (14)	28 (56)
Age (y)	50.8±14.7	60.3±7.7	68.3±9.5	57.9±13.6
Sex, male/female	6/7	0/8	4/3	10/18
History of contact lens usage, *n*	6	4	1	11
Eye allergy, *n*	6	4	2	12
History of eye operation, *n*	3	0	0	3
Vascularity[a]	1.7±0.87/1.2±0.75	0.9±0.89/0.6±0.51	0.5±0.52/0.4±0.50	1.2±0.96/0.8±0.72
Plugging[a]	2.3±0.88/1.4±0.99	0.9±0.85/0.8±0.75	0.6±0.65/0.3±0.47	1.5±1.13/1.0±0.93
Irregularity[a]	0.8±0.59/0.5±0.58	0.5±0.73/0.3±0.45	0.2±0.43/0.1±0.27	0.6±0.63/0.3±0.51
Thickening[a]	0.7±0.67/0.3±0.47	0.6±0.62/0.5±0.52	0.0±0.00/0.0±0.00	0.5±0.63/0.3±0.46
Mucocutaneous junction[a]	0.8±0.80/0.7±0.56	0.6±0.72/0.5±0.52	0.0±0.00/0.0±0.00	0.6±0.74/0.4±0.54
Dropout[a]	1.4±0.75/1.1±0.82	0.7±0.70/0.7±0.70	0.6±0.76/0.6±0.84	1.0±0.82/0.9±0.81
Partial glands[a]	2.3±0.88/2.2±0.94	1.9±1.02/1.6±1.09	1.2±1.19/1.2±1.31	1.9±1.08/1.8±1.14
BUT (s)	3.06±1.35	2.96±1.15	5.86±2.45	3.73±2.04
Fluorescein score				
Temporal/cornea	0.3±0.84/0.3±0.63	0.7±0.48/0.6±0.73	0.1±0.27/0.0±0.00	0.4±0.67/0.3±0.61
Nasal/conjunctiva	0.3±0.84/0.6±1.68	0.7±0.48/1.4±0.96	0.0±0.00/0.1±0.27	0.3±0.67/0.7±1.33
Schirmer's test (mm)	8.00±6.73	3.17±2.48	7.14±7.09	6.65±6.34

[a]Upper/lower eyelid, respectively. MGD: Meibomian gland dysfunction; SD: Standard deviation; BUT: Break-up time.

Statistical Analysis Statistical analysis was conducted using SAS version 9.4 (SAS, Cary, NC, USA). Descriptive statistics and frequency were determined for each item, and Pearson's or Spearman's correlation coefficients were estimated to analyze the associations between variables based on data types for baseline features. Wilcoxon signed-rank test was employed to assess the difference in the change in LLT values from baseline among groups. *P* values of <0.05 were considered statistically significant.

RESULTS

This study included 56 eyes from 28 participants (8 participants with dry eyes, 13 with MGD, and 7 with non-dry eye/MGD). Of the 28 participants, 9 participants who had visited the clinic several times were used to calculate the changes from baseline values. The demographics and basic parameters including objective findings of all enrolled participants are illustrated in Table 1. Table 2 shows the information including objective findings on participants who were observed at follow-up.

Table 3 depicts the correlation of baseline values among conventional ophthalmic test results and measurement values of TFI. The MALTR and LBUT correlated with Schirmer's test value when pick-up items revealed correlation coefficients of >0.4 (r=−0.46, 0.47). The LLT assessed using TFI demonstrated a correlation with the plugging score in the upper eyelid (r=−0.42). LMU correlated with BUT (r=0.40). LLT (TFI) and LLT (LipiView2) measured different areas, but their values were associated with each other (r=0.60), and LBUT was correlated with LLT (LipiView2) and NIBUT (r=0.48, 0.51).

TFI and LipiView2 can measure LLT on the cornea, but with different measurement areas. Each value was examined in each disease group. LLT (TFI) values were lower than LLT (LipiView2) values in all disease groups and were significantly lower only in the MGD group (Table 4).

The changes from baseline values of TFI measurements showed a high correlation with most ophthalmic test values (Table 5). Changes in MALTR and LBUT particularly correlated with most lid margin findings and symptoms. MALTR showed a very high correlation with plugging total score (r=−0.78), dropout lower and total scores (r=0.85, 0.87), and Schirmer's test value (r=−0.97). LBUT demonstrated a high correlation with irregularity total score (r=−0.94) and mucocutaneous junction score in upper and total score (r=−0.89, −0.78). MALTR revealed a correlation between SPEED and DEQS scores (r=0.59, 0.43). MALTR also showed a correlation with LLT (LipiView2), DR-1 score, and NIBUT. LLT (TFI) correlated with LLT (LipiView2), and LBUT correlated with LLT (LipiView2) and NIBUT.

DISCUSSION

TFI employed in this study can take an image of the lipid layer on the entire cornea objectively and can separately measure the lipid and aqueous layers at the center of the cornea with time. In addition, this study demonstrated that TFI values moderately correlated with Schirmer's test value, break-

Table 2 Demographics of follow-up subjects mean±SD

Parameters	Disease group		Total
	MGD	Dry eye	
Patients (eyes)	6 (12)	3 (6)	9 (18)
Age, y	54.2±13.3	54.0±5.0	54.1±11.2
Sex, male/female	3/3	0/3	3/6
History of contact lens usage, *n*	2	1	3
Eye allergy, *n*	3	2	5
History of eye operation, *n*	2	0	2
Vascularity[a]	2.1±0.51/1.7±0.65	1.2±0.98/0.5±0.55	1.8±0.81/1.3±0.83
Plugging[a]	2.3±0.97/1.6±0.79	1.0±0.63/0.8±0.98	1.8±1.04/1.3±0.91
Irregularity[a]	0.8±0.62/0.6±0.51	0.3±0.52/0.0±0.00	0.6±0.61/0.4±0.50
Thickening[a]	0.8±0.62/0.5±0.52	0.5±0.55/0.5±0.55	0.7±0.59/0.5±0.51
Mucocutaneous junction[a]	0.8±0.83/0.8±0.62	0.7±0.52/0.7±0.52	0.8±0.73/0.7±0.57
Dropout[a]	1.5±0.80/1.3±0.78	0.7±0.52/0.3±0.52	1.2±0.81/1.0±0.84
Partial glands[a]	2.3±0.89/2.2±1.03	1.7±0.82/1.0±0.63	2.1±0.90/1.8±1.06
BUT (s)	2.42±1.37	2.56±1.24	2.46±1.29
Fluorescein score			
Temporal/cornea	0.7±1.15/0.7±0.78	0.7±0.52/0.7±1.03	0.7±0.97/0.7±0.84
Nasal/conjunctiva	0.7±1.15/1.3±2.31	0.7±0.52/1.3±1.03	0.7±0.97/1.3±1.94
Schirmer's test (mm)	5.25±5.83	3.50±3.21	4.67±5.08

[a]Upper/lower eyelid, respectively. MGD: Meibomian gland dysfunction; SD: Standard deviation; BUT: Break-up time.

Table 3 Correlation coefficients between baseline values of TFI parameters and conventional ophthalmic test scores

Parameters	MALT	MALTR	LLT	LBUT	IBI	LMU
Objective signs						
Vascularity[a,b]	0.22/0.15/0.22	-0.15/-0.06/-0.14	-0.22/-0.21/-0.23	-0.14/0.01/-0.07	-0.18/-0.09/-0.13	-0.08/-0.11/-0.09
Plugging[a,b]	0.03/-0.01/0.02	0.33/0.26/0.33	-0.42[d]/-0.14/-0.32	0.07/-0.20/-0.06	-0.29/-0.17/-0.25	-0.25/-0.14/-0.20
Irregularity[a,b]	0.26/0.10/0.21	0.01/-0.10/-0.03	-0.38/-0.16/-0.33	-0.12/0.06/-0.05	-0.29/0.00/-0.20	-0.28/-0.33/-0.33
Thickening[a]	0.23/0.28/0.27	0.04/-0.09/0.00	-0.25/-0.05/-0.20	-0.14/-0.34/-0.22	-0.14/0.21/-0.03	-0.15/-0.02/-0.12
Mucocutaneous junction[a,b]	0.20/0.18/0.18	0.04/0.04/0.07	-0.30/-0.24/-0.28	-0.28/-0.27/-0.28	-0.12/-0.06/-0.10	-0.23/-0.34/-0.26
Dropout[a,b]	0.12/-0.09/0.01	-0.07/-0.16/-0.12	-0.02/-0.16/-0.11	0.10/-0.16/0.04	-0.27/-0.09/-0.21	-0.09/-0.17/-0.14
Partial glands[a,b]	0.04/0.03/0.03	0.13/-0.20/-0.05	-0.19/-0.22/-0.20	-0.06/-0.04/-0.04	0.00/-0.17/-0.08	-0.05/-0.05/-0.07
BUT[c]	-0.27	-0.27	0.17	0.30	-0.14	0.40[d]
Fluorescein scores[b]						
Temporal/cornea	-0.20/0.11	0.11/-0.10	0.33/0.16	-0.26/-0.08	0.03/0.05	0.03/0.03
Nasal/conjunctiva	-0.20/-0.20	0.11/0.11	0.33/0.33	-0.26/-0.26	0.03/0.03	0.03/0.03
Schirmer's test[c]	0.05	-0.46[d]	-0.13	0.47[d]	-0.30	-0.14
Subjective symptoms						
SPEED score[c]	0.24	0.17	-0.39	-0.10	0.04	-0.25
DEQS[c]	0.26	0.18	-0.34	0.06	0.16	-0.08
LipiView2						
LLT[c]	-0.37	0.09	0.60[d]	0.48[d]	-0.09	0.15
DR-1α						
DR-1α score[c]	0.17	-0.34	0.01	-0.21	0.11	-0.12
NIBUT[c]	0.00	-0.05	0.17	0.51[d]	-0.25	0.20

[a]Upper/lower/total eyelid, respectively; [b]Spearman's rank correlation coefficient; [c]Pearson's correlation coefficient; [d]Absolute correlation coefficient values of ≥0.4. TFI: Tear film imager; MALT: Muco-aqueous layer thickness; MALTR: MALT rate of change; LLT: Lipid layer thickness; LBUT: Lipid break-up time; IBI: Inter blink interval; LMU: Lipid map uniformity; NIBUT: Non-invasive break-up time; BUT: Break-up time; SPEED: Standard patient evaluation of eye dryness questionnaire; DEQS: Dry eye related quality of life score.

Table 4 Testing of differences between measuring devices for lipid layer thickness values for each disease group

Devices	LLT, nm (mean±SD)		
	MGD	Dry eye	Non-dry eye/MGD
TFI	43.5±21.7	54.0±25.2	77.3±35.8
LipiView2	58.9±23.8	66.0±32.0	100.1±27.7
[a]*P*	0.001	0.211	0.078

[a]Wilcoxon signed-rank test results between TFI and LipiView2 with significant level set at $P<0.05$. MGD: Meibomian gland dysfunction; LLT: Lipid layer thickness; SD: Standard deviation.

Table 5 Correlation coefficients between changes from baseline values of TFI parameters and those of conventional ophthalmic test scores

Parameters	MALT	MALTR	LLT	LBUT	IBI	LMU
Objective signs						
Vascularity[a,b]	-0.33/-0.39/-0.36	0.43/0.51/0.47	-0.05/0.10/-0.03	-0.50/-0.39/-0.63	-0.24/-0.41/-0.42	-0.02/0.15/0.05
Plugging[a,b]	-0.04/-0.31/-0.18	-0.11/-0.68/-0.78	0.55/0.16/0.41	-0.54/0.50/0.06	-0.52/0.21/-0.11	0.27/-0.19/0.06
Irregularity[a,b]	0.73/0.40/0.58	0.65/0.53/0.65	0.12/-0.03/0.07	-0.54/-0.54/-0.94	0.31/0.28/0.28	-0.22/-0.26/-0.27
Thickening[a]	0.37/0.27/0.36	0.65/0.53/0.65	0.17/-0.25/0.07	-0.54/NA/-0.54	0.50/0.73/0.59	0.04/NA/0.04
Mucocutaneous junction[a,b]	-0.23/-0.31/-0.27	0.54/-0.54/0.00	-0.40/-0.55/-0.49	-0.89/-0.50/-0.78	0.35/0.22/0.26	-0.61/-0.73/-0.71
Dropout[a,b]	0.61/0.38/0.61	0.67/0.85/0.87	0.65/0.16/0.59	0.54/0.54/0.54	0.00/-0.23/-0.23	0.27/0.00/0.13
Partial glands[a,b]	0.41/0.35/0.42	0.32/0.00/0.26	0.17/0.27/0.21	0.06/-0.24/0.06	-0.26/-0.13/-0.24	0.20/0.54/0.32
BUT[c]	0.07	0.51	-0.42	0.30	0.15	-0.38
Fluorescein scores[b]						
Temporal/cornea	-0.03/0.11	NA/NA	0.16/0.29	NA/NA	0.26/-0.04	-0.15/-0.33
Nasal/conjunctiva	-0.34/-0.28	-0.53/-0.53	0.24/0.23	NA/NA	-0.05/-0.01	-0.21/-0.23
Schirmer's test[c]	-0.08	-0.97	0.10	0.69	0.48	-0.05
Subjective symptoms						
SPEED score[c]	0.32	0.59	0.22	-0.50	0.32	0.15
DEQS[c]	0.40	0.43	0.35	0.31	0.38	0.14
LipiView2						
LLT[c]	0.05	0.66	0.48	-0.44	-0.27	0.09
DR-1α						
DR-1α score[c]	-0.34	-0.49	0.16	0.15	0.17	0.16
NIBUT[c]	-0.21	0.70	-0.24	0.72	0.16	-0.27

[a]Upper/lower/total eyelid, respectively; [b]Spearman's rank correlation coefficient; [c]Pearson's correlation coefficient. NA: There were no changes in objective signs and the correlation coefficient could not be determined. TFI: Tear film imager; MALT: Muco-aqueous layer thickness; MALTR: MALT rate of change; LLT: Lipid layer thickness; LBUT: Lipid break-up time; IBI: Inter blink interval; LMU: Lipid map uniformity; NIBUT: Non-invasive break-up time; BUT: Break-up time; SPEED: Standard patient evaluation of eye dryness questionnaire; DEQS: Dry eye related quality of life score.

up time, and plugging, which are typical objective signs of dry eye or MGD. MALTR and LBUT were also moderately correlated with changes in lid margin findings and symptoms. The rate of change in the aqueous layer thickness on the cornea or lipid layer stability may be used as an objective indicator as alternative to subjective symptoms such as SPEED score and DEQS. However, since the number of cases in this study was small and the correlation coefficient was only moderate, further research is needed to determine whether it can be used as a substitute for subjective symptoms. In addition, recently Mangwani-Mordani *et al*[26] also reported to investigate the tear film dynamics in dry eye patients using the TFI. Although their findings are still preliminary and have some limitations, they discuss the clinical usefulness of TFI and its correlation with various dry eye parameters. Furthermore, because dry eye is a multifactorial disease and the cornea is a tissue with hypersensitive sensory nerves, it may not be possible to simply evaluate the relationship between objective parameters and subjective symptoms of dry eye, as has been reported in various previous studies[12,27]. Therefore, MALTR in particular showed a correlation with the two subjective symptom scores and may become an objective surrogate marker of subjective symptoms in the future. In this study, we enrolled a wide range of patients with dry eye and MGD in order to perform exploratory measurements on a limited number of cases. In the future, to confirm the usefulness of TFI as a diagnostic tool

for dry eye and MGD, more detailed analysis using a larger number of cases with more clearly defined cases is necessary.

In previous studies, no correlations were reported between LLT and MGD findings, such as the meibomian gland status[14,28]. In these studies, LLT was assessed using LipiView2 or other devices that measure the inferior area, which is different from the area measured by TFI. Our study showed that LLT (TFI) was well associated with plugging, which is one of the MGD landmarks. TFI measures LLT at the center of the cornea, suggesting that LLT at the center of cornea may be strongly associated with MGD findings of the eyelid margin secreting meibum. Although most of the correlation coefficients are in the range of 0.4-0.6, which is a moderate correlation.

LLT values measured using TFI and LipiView2 were significantly different in patients with MGD. LLT measurement is performed at one point in the center of the cornea for TFI and at a fixed area in the inferior cornea for LipiView2. Therefore, the LLT value of TFI tends to be low in all groups of MGD, dry eye, and non-dry eye/MGD. These results indicate the importance of understanding the pathology by measuring LLT at wider areas than single spots on the cornea. However, as this is still a preliminary study and the number of cases is small, further study is needed to determine whether it can be used as a monitoring tool.

Changes in the aqueous layer thickness at the central cornea measured using TFI well reflected changes in MGD and dry eye findings. Measuring the changes in the aqueous layer on the cornea is important for observing the tear film characteristics of patients with MGD and dry eye[5,29]. In this study, aqueous layer changes were used not only for understanding the pathophysiology but also for treatment monitoring. The rate of the aqueous layer thickness change on the cornea is affected by tear evaporation; however, the evaporation may be affected by various factors other than LLT, such as lipid composition [6,30]. In this study, measurement values related to tear stability, such as MALTR and LBUT, were more correlated with changes in clinical findings than LLT itself, suggesting the importance of tear stability monitoring for treatment monitoring. The association between tear stability and subjective symptoms and that between NIBUT and subjective symptoms has been reported[31]. In this study, MALTR was correlated with changes in subjective symptoms. The rate of aqueous layer thickness change on the cornea may be used as an objective indicator for monitoring subjective symptoms. In addition, at this stage, a standard database set has not yet been established, and the correlation coefficients obtained in this study are moderate or low.

The degree of change and the variation in the findings were small because the follow-up period was approximately 3mo, and the number of cases was limited. This may overestimate the association between changes in findings and TFI measurements. While changes from baseline were observed during the standard 3-month follow-up period, future research should focus on clarifying the clinical significance of individual TFI values by examining the relationship between changes in clinical findings and TFI measurements over extended timeframes in larger study populations. Furthermore, as Mangwani-Mordani *et al*[26] describe, TFI may be useful as a biomarker for the diagnosis and management of dry eye and other ocular diseases.

In conclusion, TFI helped to determine the clinical parameters that could not be determined by existing devices. TFI has the advantage of simultaneously being able to measure the thickness of the lipid and aqueous layers and may be used for treatment monitoring in patients with dry eye and MGD.

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