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Meibomian Gland Structure in Participants with and Without Meibomian Gland Dysfunction

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Keywords: Meibomian gland structure; Meibomian gland dysfunction; Dry eye disease; Meibum secretion quality; Corneal staining; Tear film.

Abstract

Introduction: The purpose of this clinical study was to use rigorous clinical criteria to diagnose Meibomian Gland Dysfunction (MGD) and perform meibography to evaluate differences in meibomian gland structure compared with non-MGD participants.

Methods: A prospective, non-interventional, multi-center, clinical study included 3 cohorts (i.e. non-MGD, mild/moderate MGD, and severe MGD) classified using composite criteria. Differences between cohorts in meibomian gland structure, which included area of Meibomian Gland Loss (MGL) and whole/partial gland counts, were evaluated by meibography and a reading center provided standardized image grading. Independent of the cohort classification, the strength of associations between selected meibomian gland structure and function parameters, and Dry Eye Disease (DED) signs and symptoms, was also assessed.

Results: In the lower lid, there were statistically significant differences in area of MGL in most of the cohort comparisons on both days with the largest area of MGL observed in the severe MGD cohort, and lowest in non-MGD. In the upper lids, there were no significant differences in MGL between cohorts. Spearman's rank correlation coefficients showed a positive correlation of area of MGL with meibum secretion quality scores, non-expressible glands, and total DED symptoms.

Conclusions: MGD outcome variables related to meibomian gland structure of the lower lid were particularly suitable to discriminate between participants in our cohorts. It was previously reported that MGL reduces the volume of meibum secretion. The positive correlation between area of MGL and meibum secretion quality scores in our study suggested that the remaining glands produce reduced meibum secretion quality which could contribute to DED symptoms related to MGD. The positive correlation between non-expressible blocked glands with area of MGL poses a therapeutic challenge to salvage the remaining meibomian glands to improve meibum secretion quality and reduce patient DED symptoms.

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Introduction

Meibomian glands are modified sebaceous glands that secrete a lipid-rich meibum, which gives rise to the lipid layer of the tear film thereby reducing excessive evaporation of tear fluid [1]. Meibomian gland dysfunction (MGD) is commonly associated with Dry Eye Disease (DED), and now that MGD has been fairly well characterized as a clinical entity [2,3], several clinical studies have been performed to investigate potential therapies [4]. Techniques to image meibomian gland morphology have evolved and noninvasive meibography has developed using infrared light and an infrared-sensitive camera for imaging the meibomian glands in both the upper and lower lids [5,6]. Noninvasive meibography has been adopted into eye care practices and clinical studies have noted consistent anatomical features such as Meibomian Gland Loss (MGL) associated with increasing age in normal participants that were not associated with MGD [6]. Per the ICH standards for clinical trial investigations [7], especially those that support drug registration by the Food and Drug Administration (FDA), grading scales for disease quantification are required to measure change and establish efficacy endpoints [8]. Methods to quantify the area of MGL in both the upper and lower lids were developed and grading scales were established, including the meiboscore [9]. Some clinical studies equate higher meiboscores directly with the diagnosis of MGD without examination of meibum secretion quality [10,11]. As a result, cross-study comparisons of area of MGL with MGD diagnoses are challenging when different methods are used to diagnosis and define MGD, and varied number of grading scales are used to measure disease severity. Single arm studies using only participants with MGD, with no control group of participants with similar ages without MGD, make it difficult to interpret data and distinguish whether the MGL could be due to age alone, or to the MGD, or both [12].

The overall objective of this clinical study was to develop methods and procedures that can be used in drug registration studies for the treatment of MGD and DED that may lead to regulatory approvals. From this same clinical study, we have previously reported on many important aspects of clinical trial design in MGD patients that can inform on the design of large clinical trials such as the optimal signs and symptoms to be used as efficacy endpoints [13], patient-reported outcomes questionnaires [14], and meibum biochemical analysis [15]. Herein we report on the evaluation of meibomian gland structure using rigorous clinical criteria to diagnose MGD and categorize participants into cohorts of non-MGD, mild/moderate MGD, and severe MGD. Differences between cohorts in meibomian gland structure (i.e. MGL, whole and partial gland counts) were evaluated by meibography and an independent central reading center (CRC) provided standardized grading of the images thus reducing bias and variability in image grading that can occur across clinical sites [16]. Standardized grading scales were used by investigators for measuring outcomes for both meibum secretion quality and corneal staining. Independent of the cohort classification, the strength of associations between selected meibomian gland structure and function parameters, and DED signs and symptoms, was also assessed.

Methods

A prospective, non-interventional, multi-center, clinical study (NCT01979887) was conducted. In brief, for overall study entry, the key inclusion enrollment criteria included participants of either gender who were 40 years of age or older. Key exclusion criteria included those who had undergone any lid heating therapy or any therapeutic gland expression within 12 months; had worn a contact lens in either eye within 30 days prior; had performed lid hygiene within 48 hours; wore eye makeup within 8 hours prior; used eyelash growth-stimulating products within 30 days prior; used systemic or topical macrolides or tetracycline derivative drugs within 30 days; used any preserved topical artificial tear supplement within 30 days prior; used any non-preserved artificial tear supplement within 6 hours prior; used systemic anti-histamines within 30 days prior.

This study included 3 cohorts classified using composite criteria that followed the guidelines from the Tear Film and Ocular Surface Society (TFOS) International Workshop of MGD [4,17]: Non-MGD, mild/moderate MGD, and severe MGD. The composite criteria included meibum secretion quality, Schirmer tear testing, and total DED symptoms evaluation.

Cohort Criteria

Schirmer Tear Testing

To focus on participants with evaporative DED associated with MGD, for any participant to qualify for the study, they had to demonstrate that their DED was not due to aqueous deficiency. As a result, a Schirmer tear test score without anesthesia was performed on Day 1 to measure tear secretion rates and a score of ≥7 mm/5 min was required to be eligible for the study.

Meibum Secretion Quality

Meibum secretion quality was evaluated by expressing meibum from the central lower lid by applying the Meibomian Gland Evaluator (Johnson & Johnson Vision, Irvine, CA) just below the eyelash base. Meibum secretion quality was graded from 6 central meibomian glands of the lower lid using the Mathers' grading scale. Glands can be hyposecretory or obstructive subtypes of MGD, with the latter being more common [1], and for the purposes of this clinical study, they were combined as being 'non-expressible' glands, and these values were recorded. The Maximum Meibum Quality Score (MMQS) was defined as the maximum score among expressible glands as assessed by the investigator based on Mathers' meibum secretion quality grading scale: [18]

0 = clear excreta or clear with small particles (normal viscosity);

1 = opaque excreta with normal viscosity;

2 = opaque excreta with increased viscosity (gel-like);

3 = secretions retain shape, or secretions do not completely express but a toothpaste-like substance can be seen at the opening of the orifice; and

NE = non-expressible (i.e. nothing at the orifice).

Mean individual gland score was another measure of meibum secretion quality and was calculated by averaging the Mathers' grade values of the 6 central meibomian glands. For this calculation, the non-expressible glands were given a value of 3. Non-MGD required normal MMQS scores of 0 or 1, mild/moderate MGD = 2, and severe MGD = 3.

Ocular Symptom Questionnaire

The Ocular Symptom Questionnaire was performed on Day 1 and Day 22 and evaluated seven individual symptoms (i.e. blurred vision, burning, dryness, foreign body sensation, itching, light sensitivity, pain) and overall ocular discomfort using a

grading scale from 0 = none to 4 = very severe. Total symptom score was calculated by summing the scores for all seven individual symptoms and overall ocular discomfort for a maximum score of 32. The sum of scores of the worst 2 symptoms on the Ocular Symptom Questionnaire of 0 to 4 with neither symptom scored as >2 would qualify for the non-MGD or mild/moderate MGD, and ≥4 for severe MGD.

The Day 1 MMQS score, Schirmer tear test score, and total Ocular Symptom Questionnaire score were used for the assignment of participants into the non-MGD, mild/moderate MGD, and severe MGD cohorts as previously described in depth [13]. At least one eye was required to meet the specified criteria for each cohort, and this eye (or the right eye if both eyes met all the criteria) was designated as the study eye. The goal was to enroll enough participants to assign 25 participants to each study cohort (i.e. total of 75) who satisfied all the cohort criteria in at least one eye.

Meibography

Once participants met the enrollment criteria and were assigned to one of three available cohorts: non-MGD, mild/moderate MGD, or severe MGD), meibography using the Oculus Keratograph 5M Corneal Topographer (Oculus, Inc., Arlington, WA) was performed on both the upper and lower lids. Meibography measurements were performed on Day 1 and repeated on Day 22 for assessment of the degree of concordance between measurements. Images were sent to the University of Waterloo Central Reading Center (CRC) (Waterloo, Ontario, Canada) for an independent evaluation of the area of MGL. The CRC also counted the glands in both upper and lower lids and categorized each gland in a binary fashion as either 'whole' glands or 'partial' glands. Whole glands were considered normal anatomy; partial glands are truncated whole glands and were considered pathologic [19]. The upper lid has a larger, crescentic tarsal plate where differences can be as high as ~40% [20] between the center of the upper lid tarsal plate (i.e. largest vertical height) and ends of the tarsal plate. The lower lid tarsal plate is more rectangular and the height of the tarsal plate across the lid, and the length of the meibomian glands, is more uniform. Given the variability in meibomian gland lengths that occur across the upper lids, only partial gland counts from the lower lid were deemed reliable and recorded. For determining the area of MGL, the CRC employed a modified Arita grading scale [9] to determine the meiboscores. The original Arita grading scale to determine meiboscores was scored using the following for each eyelid: meiboscore 0, no loss of meibomian glands; meiboscore 1, area loss was less than one third of the total meibomian gland area; meiboscore 2, area loss was between one third and two thirds; meiboscore 3, area loss was more than two thirds. The CRC was confident that they were able to grade the area of missing glands with further granularity and adopted a modified grading scale where the area of missing glands of 0%, > 0 to 16%, 17 to 33%, 34 to 50.0%, 51 to 66%, 67 to 83%, and 84 to 100%, were assigned meiboscores of 0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0, respectively.

Whole gland counts were performed on both upper and lower lids on Day 1 and Day 22 and partial gland counts were performed on the lower lids on Day 1 and Day 22.

Independent of the cohort classification, the strength of correlations between selected meibomian gland structure parameters (i.e. area of MGL, whole and partial gland counts), functional parameters (i.e. number of non-expressible glands, MMQS, mean individual gland score), and DED signs and symptoms (i.e. total symptom score, corneal staining), was assessed.

To perform corneal staining grading, a fluorescein strip was moistened with 0.9% saline, and the strip was gently touched against the superior bulbar conjunctiva of the eye. Staining of the entire cornea was visualized 2 minutes later at the slit lamp using 10x magnification, a yellow barrier filter, and a cobalt blue filter for illumination. Corneal staining was graded using the Oxford Grading System [21].

Biostatistics

Cohort comparisons for meibography results (area of MGL, whole gland counts, and partial gland counts) were conducted using Cochran-Mantel-Haenzel (CMH) tests, stratified by site. The degree of concordance between Day 1 and Day 22 meibography results was assessed using Wilcoxon signed-rank tests, with corresponding nominal p-values provided. Spearman's rank correlation was used to measure the strength of associations between selected meibomian gland structure parameters, functional parameters, and DED signs and symptoms using average values of Day 1 and Day 22 assessments. The strength of the Spearman's rank correlation coefficients were graded as follows:[22]

Positive correlations: 0 to 0.19, very weak, 0.20 to 0.39, weak, 0.4 to 0.59, moderate, 0.6 to 0.79 as strong, and ≥ 0.80 as very strong.

Negative correlations: 0 to -0.19, very weak, -0.20 to -0.39, weak, -0.40 to -0.59, moderate, -0.6 to -0.79, strong, and -0.80 to -1, very strong.

Results

A total of 129 participants were enrolled into the study of which 75 participants (25 per cohort) qualified and were assigned to the non-MGD, mild/moderate MGD, and severe MGD cohorts. Most enrolled subjects were women (64.3%), and enrollment into the 3 cohorts was reasonably balanced with respect to gender (64%, 64%, and 72% women) and age (52.0(8.3), 52.8(6.3), and 58.8(11.9) years) in the non-MGD, mild/moderate MGD, and severe MGD cohorts, respectively. Table 1 summarizes the area of MGL in the lower and upper lids across all cohorts. There were no significant differences between day 1 and day 22 in area of MGL in both upper and lower lids, except in the lower lids in the severe MGD cohort, confirming reasonable consistency in grading between visits. In the lower lid, there were significant differences in most of the cohort comparisons on both days with the largest area of MGL being in the severe MGD cohort. In the upper lids, there were no significant differences between cohorts although numerically, the severe MGD cohort had a larger area of MGL.

In both the upper and lower lids, there were no significant differences (P >0.05) in the whole and partial gland counts between Day 1 and Day 22 (Table 2 and 3) except with the whole gland count in the upper lids in the severe MGD cohort only. With the lower lid whole gland counts, there were reductions that were significant (all P-values < 0.05 with an exception of p = 0.07 for Day 22 versus mild/mod MGD), on both days when comparing the severe cohorts with both the non-MGD and mild/mod MGD cohorts. The lower lid partial gland counts were increasing with MGD severity and there was significant difference between severe and non-MGD cohorts on Day 1 (P = 0.012).

With the upper lid whole gland counts, the numbers were similar across all the cohorts on both days and there were no significant differences except on Day 1 between the severe MGD vs mild/mod MGD cohorts (P = 0.04).

The number of expressible glands in the lower lid decreased with increasing severity of MGD across cohorts at both the Day 1 and Day 22. On Day 1, the proportion of participants with all 6 central glands expressible was 92.0% in the non-MGD cohort, 64.0% in the mild/moderate MGD cohort, and 44.0% in the severe MGD cohort. On Day 22, the proportion of participants with all 6 central glands expressible was similar to Day 1 in the non-MGD (88.0%) and mild/moderate MGD (62.5%) cohorts except for the severe MGD cohort that demonstrated a greater reduction in the severe MGD cohort to 29.2%.

Using averaged data from Days 1 and 22, the Spearman's rank correlation coefficients showed a very strong positive correlation between MMQS and the mean individual gland score (Table 4). Other notable correlations that have important clinical consequences (to be further discussed in the Discussion section) were moderate to strong positive correlations between the total symptom score with both the MMQS and the mean individual gland score. Moderate negative correlations were observed with area of MGL with whole gland counts and very strong positive correlations with partial gland counts. Non-expressible glands were moderately to very strongly correlated with MMQS, area of MGL and the mean individual gland scores. Area of MGL was moderately positively correlated with MMQS and the mean individual gland score and weakly positively correlated with total symptoms. Participant age was very weakly or weakly correlated with all the variables. In addition, corneal staining was also very weakly or weakly correlated with all the variables. Importantly, there was no positive correlation between total symptom score and corneal staining.

Table 1

Meibography of the Lower Lids: Area of Meibomian Gland Loss (Modified Arita Scale 0-3 Scale)						**Pairwise Comparisons: P-value (c)**		
		Non-MGD	Mild/Moderate MGD	Severe MGD	Total	Severe vs. Non-MGD	Mild/Mod vs. Non-MGD	Severe vs. Mild/Mod
Day 1	N	25	25	25	75	0.03	0.01	0.29
	Mean	0.42	0.72	1.28	0.81			
	SD	0.47	0.50	1.17	0.85			
Day 22	N	24	24	24	72	0.05	0.03	0.04
	Mean	0.35	0.73	1.63	0.90			
	SD	0.43	0.55	1.14	0.93			
Change (a)	N	24	24	24	72	0.73	0.92	0.20
	Mean	-0.08	0.04	0.40	0.12			
	SD	0.32	0.44	0.82	0.60			
	P-value (b)	0.34	0.83	0.04	0.21			
Meibography of the Upper Lids: Area of Meibomian Gland Loss (Modified Arita Scale 0-3 Scale)						**Pairwise Comparisons: P-value (c)**		
		Non-MGD	Mild/Moderate MGD	Severe MGD	Total	Severe vs. Non-MGD	Mild/Mod vs. Non-MGD	Severe vs. Mild/Mod
Day 1	N	25	25	22	72	0.08	0.37	0.22
	Mean	0.78	0.66	0.91	0.78			
	SD	0.87	0.67	0.90	0.81			
Day 22	N	25	23	21	69	0.17	0.89	0.07
	Mean	0.70	0.54	1.12	0.78			
	SD	0.84	0.67	1.02	0.87			
Change (a)	N	25	23	21	69	0.67	0.51	0.44
	Mean	-0.08	-0.11	0.19	-0.01			
	SD	0.31	0.37	0.68	0.48			
	P-value (b)	0.34	0.25	0.39	0.47			

(a) Change = Day 22 minus Day 1.
(b) P-values are based on Wilcoxon signed-rank test.
(c) P-values are based on Cochran-Mantel-Haenzel (CMH) method with modified ridit scores, stratified by site.

Table 2: Meibography of the Lower Lids: Whole Gland Count.

Meibography of the Lower Lids: Whole Gland Count						Pairwise Comparisons: P-value (c)		
		Non-MGD	Mild/Moderate MGD	Severe MGD	Total	Severe vs. Non-MGD	Mild/Mod vs. Non-MGD	Severe vs. Mild/Mod
Day 1	N	25	25	25	75	0.006	0.21	0.009
	Mean	14.9	14.2	10.6	13.2			
	SD	3.95	4.05	3.60	4.25			
Day 22	N	24	24	24	72	0.02	0.13	0.07
	Mean	14.5	13.9	11.3	13.3			
	SD	3.64	4.05	4.10	4.12			
Change (a)	N	24	24	24	72	0.12	0.77	0.15
	Mean	-0.5	-0.2	0.5	-0.1			
	SD	2.00	2.30	3.06	2.49			
	P-value (b)	0.31	0.98	0.48	0.99			
Meibography of the Upper Lids: Whole Gland Count						**Pairwise Comparisons: P-value (c)**		
		Non-MGD	Mild/Moderate MGD	Severe MGD	Total	Severe vs. Non-MGD	Mild/Mod vs. Non-MGD	Severe vs. Mild/Mod
Day 1	N	25	25	22	72	0.16	0.44	0.04
	Mean	17.5	18.7	15.7	17.4			
	SD	4.06	4.51	3.76	4.26			
Day 22	N	25	23	21	69	0.38	0.77	0.35
	Mean	18.0	18.8	18.1	18.3			
	SD	3.90	6.04	4.65	4.86			
Change (a)	N	25	23	21	69	0.67	0.51	0.44
	Mean	0.6	0.0	2.5	1.0			
	SD	3.08	3.54	3.40	3.45			
	P-value (b)	0.27	0.84	0.002	0.01			

(a) Change = Day 22 minus Day 1
(b) P-values are based on Wilcoxon signed-rank test
(c) P-values are based on Cochran-Mantel-Haenzel (CMH) method with modified ridit scores, stratified by site

Table 3: Meibography of the Lower Lids: Partial Gland Count.

Meibography of the Lower Lids: Partial Gland Count						Pairwise Comparisons: P-value (c)		
		Non-MGD	Mild/Moderate MGD	Severe MGD	Total	Severe vs. Non-MGD	Mild/Mod vs. Non-MGD	Severe vs. Mild/Mod
Day 1	N	22	24	20	66	0.012	0.07	0.98
	Mean	2.8	4.4	4.5	3.9			
	SD	4.53	4.09	3.66	4.13			
Day 22	N	20	22	24	66	0.16	0.08	0.92
	Mean	1.8	4.7	5.8	4.2			
	SD	2.53	4.67	5.08	4.57			
Change (a)	N	19	22	19	60	0.97	0.89	0.60
	Mean	-1.2	0.6	1.7	0.4			
	SD	4.34	3.07	4.92	4.23			
	P-value (b)	0.29	0.72	0.14	0.59			

(a) Change = Day 22 minus Day 1
(b) P-values are based on Wilcoxon signed-rank test
(c) P-values are based on Cochran-Mantel-Haenzel (CMH) method with modified ridit scores, stratified by site

Table 4: Spearman's Rank Correlation Coefficient Matrix (Lower Lid).

Variables	Area of MGL	Whole Gland Count Lower Lid	Partial Gland Count Lower Lid	MMQS	Non-Expressible Glands	Mean Individual Gland Score	Total Symptom Score	Corneal Staining	Age
Area of MGL	1.00	-0.46	0.82	0.47	0.51	0.50	0.39	0.19	0.25
Whole Gland Count		1.00	-0.15	-0.31	-0.16	-0.27	-0.19	-0.35	-0.02
Partial Gland Count			1.00	0.32	0.40	0.36	0.30	0.03	0.19
MMQS				1.00	0.59	0.89	0.60	0.12	0.28
Non-Expressible Glands					1.00	0.81	0.41	0.17	0.31
Mean Individual Gland Score						1.00	0.56	0.11	0.35
Total Symptom Score							1.00	0.07	0.21
Corneal Staining								1.00	0.15
Age									1.00

MGL: Meibomian Gland Loss.

Table 4: Spearman's Rank Correlation Coefficient Matrix (Lower Lid).

Correlation Coefficient	Heat Map Color	Strength of Correlation
≥ 0.80		very strong
0.6 to 0.79		strong
0.4 to 0.59		moderate
0.20 to 0.39		weak
0 to 0.19		very weak
0 to -0.19		very weak
-0.20 to -0.39		weak
-0.40 to -0.59		moderate

Discussion

Our clinical study demonstrated significant area of MGL and reduction in whole gland counts in the lower lids of participants in the MGD cohorts, compared to the non-MGD cohort, utilizing rigorous definitions of MGD and an independent CRC for analyzing meibography images. It appears that MGD outcome variables of the lower lid are particularly suitable to discriminate between participants in our cohorts, as differences were consistently found at both visits for each of these: whole gland count lower lid (non-MGD vs severe MGD) and area of MGL lower lid (non-MGD vs mild/moderate; non-MGD vs severe MGD). None of the variables measured for the upper lid (i.e. whole gland count or area of MGL) at both visits were found to be different between cohorts suggesting that the lower lid may be more suitable for detection of differences between participants, at least based on the cohort characteristics defined in this study. It is not known why the upper lids may be less prone to MGL compared with the lower lids. Hypothetically, forces that drive meibum into the tear film are the continuous secretion by the meibomian glands and gravity would aid in the passage of meibum through the ductal system more effectively from the upper lids. Furthermore, during a blinking event, the upper lid has greater muscular action compared with the lower lid with lid closure. The pretarsal orbicularis and the marginal muscle of Riolan, which encircles the meibomian gland near the lid margin, exerts a compressive action and milking of the glands that contributes to the secretion of meibum into the tear film [23,24]. Regardless of the differences in area of MGL between the upper and lower lids, the mechanism of developing MGL is the same. Stagnation of meibum in the glandular ducts, either from blockage at the orifice by hyperkeratinization or inspissated meibum, leads to a progressive increase in pressure in the ductal system extending into the secretory acini [3,24,25]. This leads to atrophic changes in the acini and full cornification of the epithelium of the ducts [26,27], which are visible clinically as glandular dropout on Meibography [18].

Other investigators have also noted that the lower lids have a higher propensity for MGL compared with the upper lids [28]. However, they did not compare with participants that did not have MGD. Since aging can also induce MGL without having MGD [29], this may be a potential confounder when assessing the area of MGL in participants with MGD [6]. For example, the sum of the meiboscores of the lower lid and upper lid area of MGL in our study was ~1 in the non-MGD cohort in our study in participants with an average age of ~55 years. This meiboscore is in the same range as previously reported in normal participants in the same age range without MGD [6]. A meiboscore in the 1 range translates to a 17 to 33% area of MGL that could occur based on age alone. Thus, it is important in clinical studies with MGD participants to also have age-matched controls since MGL attributed to MGD may be confounded by loss that occurs naturally in that age group.

Compared with the mean individual gland score, the MMQS is a simplified scoring technique when grading multiple gland secretions simultaneously during the manual meibum expression procedure [13]. The MMQS distills the overall meibum secretion quality of multiple glands down to one number. Not only is this an advantage in clinical studies to expedite the grading and recording process, MMQS is also an advantage when rapidly evaluating routine patients in an office setting to assist in diagnosing MGD and DED. Although the MMQS may not be as granular as grading the meibum secretion quality scores of multiple glands at once, the Spearman coefficient showed that MMQS demonstrated a very strong positive correlation and is a good proxy for judging overall meibum secretion quality for an individual eyelid.

The area of MGL was moderately negatively correlated with whole gland (i.e. normal glands) counts and very strongly positively correlated with partial gland (i.e. pathologic glands) counts. These results are intuitive since it would be expected that the greater the area of MGL, the greater the loss in whole glands. As whole glands gradually become truncated as they develop atrophic changes, partial glands start forming at a higher frequency. Notable is that the larger the area of MGL, proportionately, the more partial or damaged glands remain secreting meibum to replenish the tear film lipid layer. Importantly,

there was a positive correlation between partial gland counts and the MMQS & mean individual gland scores, meaning the higher the partial gland count, the lower the quality of meibum being secreted. The positive correlation between the meibum quality scores (i.e. MMQS and the mean individual gland score), and the total symptom score, suggested that not only do missing glands reduce the overall volume of meibum secretions, as has been previously reported [30], but in addition, the remaining glands produce reduced meibum secretion quality that may contribute to DED symptoms related to MGD. The quality of meibum secretions is important as it has been previously reported that altered lipid proportions in meibum secretions increase with MGD severity and this may impact tear film homeostasis and exacerbate patient symptoms [15].

In our clinical study, age was not well correlated with any of the variables that were studied. The majority of clinical studies that have found positive correlations between age and area of MGL were in normal healthy volunteers without a diagnosis of DED or MGD [6,29,31] or in DED participants that did not have a diagnosis of MGD [32]. Performing cross study comparisons of correlation results have to be interpreted with caution when they have disparate participant populations. For example, comparing correlation results in studies with a population of participants that are normal volunteers may yield different results when comparing correlation results in our study where 2/3's of the participants have a diagnosis of MGD. Having MGD may be a confounding factor in the correlation statistics where one of the variables, e.g. area of MGL, already exists across the majority of participants regardless of age.

Non-expressible glands were moderately to very strongly correlated with MMQS, the mean individual gland scores, and area of MGL, and this may have therapeutic implications. Non-expressible glands are frequently caused by terminal meibomian duct obstruction which leads to acini atrophy and glandular destruction [1]. There are several in-office procedural treatments available that may treat the inspissated meibomian gland orifices such as thermal pulsation devices, microblepharoexfoliation of the lid margin, or meibomian gland probing [4,33]. Careful examination of the lid margin to look for non-expressible glands caused by a blocked orifices, and performing meibography, can guide the eye care professional to customize the treatment regimen for a particular patient with MGD [34,35].

In our clinical study where two thirds were diagnosed with MGD, there was a weak positive correlation between area of MGL and corneal staining. In contrast, others have found a positive correlation of area of MGL and corneal staining [36], however, their clinical study population was normal volunteers from an academic community without a diagnosis of DED or MGD. Others have found a positive correlation between corneal staining and area of MGL in DED participants but did not specifically diagnosis and enroll participants with MGD [32]. A possible explanation for why our clinical study showed that area of MGL was only weakly correlated with corneal staining is from reports that MGD participants demonstrated increased Schirmer tear test scores with increasing area of MGL [37-39]. These compensatory mechanisms may be active in MGD participants to maintain tear film homeostasis [38,40] to reduce damage to the ocular surface and explain why we did not find a stronger correlation between area of MGL and corneal staining.

A positive correlation was lacking between total symptom score and corneal staining in our clinical study. The discordance between the signs and symptoms of DED has been observed in numerous clinical studies and this has been perplexing [3,41,8,42]. The FDA requires the demonstration of both signs and symptoms of a drug in at least 2 studies in order to gain approval for a broad label indication that includes treatment of both signs and symptoms of DED [43]. It is difficult to meet both a sign and symptom endpoint in the same clinical study [8]. Other health authorities, like the European Medicines Agency, only require an improvement in a DED sign or a symptom in clinical trials that potentially lowers the bar to gain drug approvals for DED in the EU [41].

With the exception of one clinical study [44], CRCs have not been used prospectively to analyze meibography images. This is surprising since eye-related clinical studies are conducive to using CRCs, especially in retina, where image acquisition and analyses are very common to determine eligibility and also to measure efficacy outcomes [45]. Other benefits for using CRCs is they provide inputs on the study design, provide photographer certification, and provide standardized grading of images [16]. The FDA has a guidance document that encourages sponsors to use centralized image interpretation especially in clinical study protocols using an image-based primary endpoint [46]. We anticipate that the traditional CRC approach using human graders will be augmented in the future using Artificial Intelligence (AI) and Deep Learning techniques to improve the accuracy and reproducibility in grading meibography images. These techniques have been successfully applied to reading retinal photographs and optical coherence tomography in detecting changes in diabetic retinopathy [47] and more recently to meibography images to better understand glandular morphology [48,49].

A limitation of our clinical study was that our defined participant population may not be applicable to all participants with MGD. It is common to have both aqueous deficient and evaporative dry eye from MGD coexist together [50] and the combination of both may have more severe DED [51]. However, our participant population with MGD excluded those with aqueous deficiency in part because there are a number of marketed products available in the United States that increase tear production in patients with aqueous tear deficiency [52]. Given the unmet needs, and the lack of approved drugs to specifically treat MGD, there is interest from sponsors to obtain drug approvals specifically for evaporative dry eye from MGD [53,54] thus the exclusion of participants with aqueous deficient dry eye in our study that could confound the outcome measures. Another limitation was grading of meibum secretion quality only included the lower lid. The Meibomian Gland Evaluator was developed specifically for the lower lid given the ease of access and visualization at the slit lamp of the lower lid compared with the upper lid. However, the meibomian gland anatomy of the upper lids appears to be better preserved with regards to whole gland numbers, even in our cohort of participants with severe MGD, and analyzing the meibum secretion quality of the upper lids would be of interest. Further research in this area is indicated to develop methods to accurately and reproducibly assess meibomian secretion quality from the upper lids. Lastly, clinical studies may lump the morphologic changes in glandular structure by meibography into categories of either 'whole' and 'partial' and measure the area of MGL on the tarsal plate [19]. Meibomian glands can also be distorted, tortuous, dilated, and lack well-defined acini [44,55] that may be pathologic but they may all be lumped into the category of 'whole' (i.e. normal) if the gland transcends the entire length of the tarsal plate. More research in glandular morphology is indicated to improve our understanding on how other characteristics of glandular mor-

phology can influence the quantity and quality of meibum secretions.

Conclusions

In this clinical study, MGD outcome variables related to meibomian gland structure of the lower lid were particularly suitable to discriminate between participants in our cohorts. A positive correlation between area of MGL and meibum secretion quality scores suggested that missing glands could reduce the overall volume of secretions but in addition, the remaining glands produce reduced meibum secretion quality which could contribute to DED symptoms related to MGD. The positive correlation between non-expressible glands with area of MGL and meibum secretion quality poses a therapeutic challenge to salvage the remaining meibomian glands to improve participants DED symptoms. The lack of a positive correlation between DED symptoms and the corneal staining sign reaffirms the paradoxical disconnect between the two and makes it more challenging from a regulatory viewpoint to bring innovative drugs to the market to treat patients with MGD and DED.

Data Sharing

AbbVie is committed to responsible data sharing regarding the clinical trials we sponsor. This includes access to anonymized, individual, and trial-level data (analysis data sets), as well as other information (e.g., protocols, clinical study reports, or analysis plans), as long as the trials are not part of an ongoing or planned regulatory submission. This includes requests for clinical trial data for unlicensed products and indications.

These clinical trial data can be requested by any qualified researchers who engage in rigorous, independent, scientific research, and will be provided following review and approval of a research proposal, Statistical Analysis Plan (SAP), and execution of a Data Sharing Agreement (DSA). Data requests can be submitted at any time after approval in the US and Europe and after acceptance of this manuscript for publication. The data will be accessible for 12 months, with possible extensions considered. For more information on the process or to submit a request, visit the following link: https://www.abbvie.com/our-science/clinical-trials/clinical-trials-data-and-information-sharing/data-and-information-sharing-with-qualified-researchers.html.

Disclosures

XX, **CZ** and **AN** are full-time employees of AbbVie and may hold AbbVie stock. **ITR** is an intern at AbbVie and has no additional conflicts.

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