

# Ocular Surface Workup With Automated Noninvasive Measurements for the Diagnosis of Meibomian Gland Dysfunction

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**Purpose:** To analyze diagnostic performance of an ocular surface workup based on automated noninvasive measurements in the diagnosis of meibomian gland dysfunction (MGD).

**Methods:** Two hundred ninety-eight eyes of 149 patients with MGD and 54 eyes of 27 control patients were analyzed. Ocular Surface Disease Index (OSDI), noninvasive breakup time (BUT), lipid layer thickness, meibomian gland loss, and tear osmolarity were calculated. The correlations among variables in the MGD group were analyzed. The area under the curve (AUC) of receiver operating characteristic curves was calculated.

**Results:** OSDI, noninvasive BUT, and meibomian gland loss were significantly different between MGD and control groups (respectively,  $37.9 \pm 19.6$  vs.  $7.1 \pm 2.8$ ;  $8.8 \pm 3.6$  vs.  $11.0 \pm 3.0$ ;  $28.0 \pm 17.6$  vs.  $21.2 \pm 13.0$ ; always  $P < 0.05$ ). Positive correlations were found between lipid layer thickness and noninvasive BUT and between meibomian gland loss and OSDI (respectively,  $r = 0.169$ ,  $P = 0.004$ ;  $r = 0.187$ ,  $P = 0.004$ ). Noninvasive BUT had the highest diagnostic power as a single parameter, followed by meibomian gland loss (respectively AUC = 0.686, AUC = 0.598). When the diagnosis of MGD was made based on either noninvasive BUT or meibomian gland loss being abnormal, sensitivity was 86.2% and specificity 38.5%. When the diagnosis was made on both noninvasive BUT and meibomian gland loss being abnormal, sensitivity was 39.3% and specificity 85.6%.

**Conclusions:** This automated noninvasive ocular surface workup may represent a useful screening tool for the diagnosis of MGD. In case of positivity of either noninvasive BUT or meibomian gland loss, subsequent qualitative clinical tests should be performed to achieve a reliable diagnosis and more precise characterization of MGD.

**Key Words:** meibomian gland dysfunction, noninvasive breakup time, lipid layer thickness, meibography, tear osmolarity

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Dry eye disease was recently redefined as a “multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles.”<sup>1</sup> Meibomian gland dysfunction (MGD) represents the leading cause of evaporative dry eye, the most common subtype of dry eye.<sup>2,3</sup> MGD is characterized by hyperkeratinization of the meibomian gland ductal epithelium, leading to obstruction and plugging of the gland orifice. Moreover, quantitative and qualitative changes in the meibum lipid composition lead to increased viscosity and reduced gland outflow onto the tear film. The stasis of meibum inside the gland promotes proliferation of bacteria, producing lipases and esterases that increase the viscosity and melting temperature of the meibum, thus setting up a vicious spiral. Hyposecretion of meibomian lipids causes thinning of the tear film lipid layer, with consequent tear film instability, increased evaporation rate, and dry eye onset.<sup>4–6</sup>

There is currently no general consensus regarding the diagnostic workup for the diagnosis of MGD and monitoring of the treatment response. Routinely, the diagnosis is mainly based on slit-lamp examination of the lid margin and ocular surface epithelium, meibomian gland expressibility and secretion quality, and fluorescein tear breakup time (BUT).<sup>7</sup> However, these tests are invasive, requiring direct contact with the ocular surface, and subjective, which leads to the possibility of significant observer bias because of a low degree of standardization.<sup>8</sup> Recently, newer automated and noninvasive tests, including among others, noninvasive BUT, lipid layer thickness, tear osmolarity and noncontact meibography, have been developed to complement the diagnosis of MGD and dry eye disease. The purpose of this study was to analyze diagnostic performance of an ocular surface workup based on the above-mentioned automated noninvasive measurements in the diagnosis of MGD.

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## MATERIALS AND METHODS

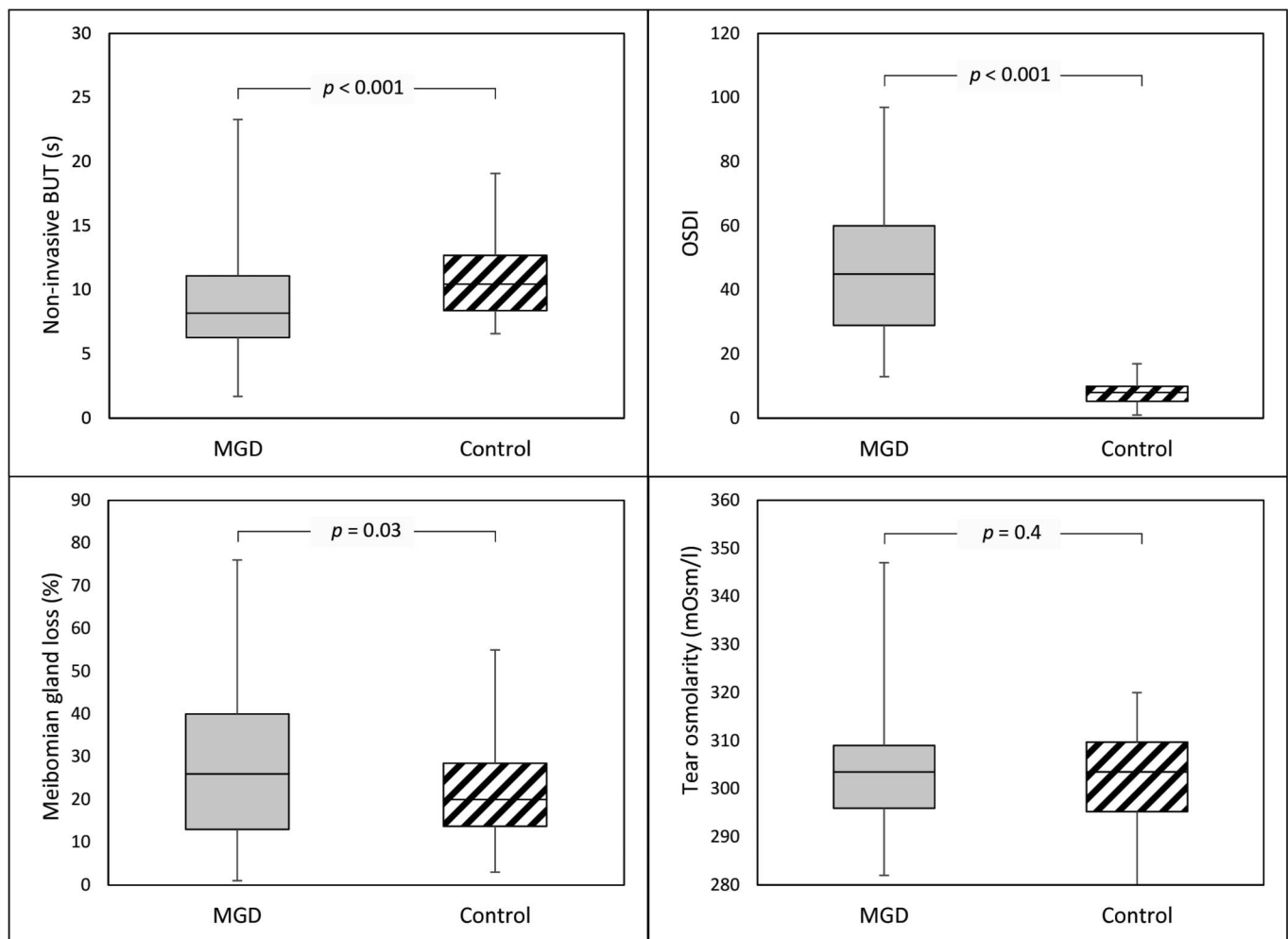
### Study Population

This cross-sectional study was conducted at Carones Ophthalmology Center (Milan, Italy) between September 2016 and July 2017. The study was performed in accordance with the principles of the Declaration of Helsinki and was approved by the local institutional review board. Written informed consent was obtained from all subjects before the examination. Patients with ocular discomfort symptoms (OSDI  $\geq 13$ ) and at least one clinical sign of MGD (namely terminal duct obstruction, plugging of the meibomian glands, turbid secretions, inflammation and swelling of the eyelid margin, or poor meibum expression) were enrolled in the MGD group. Healthy sex and age-matched patients acted as the control group. The exclusion criteria for both groups were age less than 18 years, concomitant presence of systemic uncontrolled disease, any active ocular surface disease other than MGD (including aqueous tear-deficient dry eye disease), and previous ocular trauma or surgery.

### Ocular Surface Workup

Before any examination, all patients completed the Ocular Surface Disease Index (OSDI) questionnaire to assess the severity of ocular surface symptoms. After slit-lamp examination, the following tests were performed in the following chronological order: noninvasive BUT, lipid layer thickness, tear osmolarity, and noncontact meibography of the lower eyelid.

The OSDI questionnaire consists of 12 questions regarding the presence and frequency of symptoms related to the ocular surface. The final scale ranges from 0 to 12 (no disability), to 13 to 22 (mild symptoms), to 23 to 32 (moderate symptoms), and to 33 to 100 (severe symptoms). The noninvasive BUT and lipid layer thickness were assessed by interferometry using the I.C.P. Tearscope (SBM Sistemi, Turin, Italy). Three measurements of the noninvasive BUT were recorded, and the median value was used for statistical analysis. The lipid layer thickness was graded from 0 to 5 based on the observed lipid layer patterns: absence of lipids (grade 0); open meshwork (grade 1); tight meshwork (grade 2); waves (grade 3); amorphous (grade 4); and color mixing



**FIGURE 1.** Boxplot analysis of noninvasive BUT, ocular surface disease index, meibomian gland loss, and tear osmolarity for both MGD and control groups.

**TABLE 1.** Correlation Factors Among Variables in the MGD Group

	OSDI	Noninvasive BUT	Meibomian Gland Loss	Lipid Layer Thickness	Tear Osmolarity
OSDI	1	0.100	<b>0.187</b>	0.110	-0.103
Noninvasive BUT		1	0.070	<b>0.169</b>	-0.057
Meibomian gland loss			1	-0.041	0.053
Lipid layer thickness				1	-0.083
Tear osmolarity					1

Bold are for  $P < 0.05$ .

(grade 5). Meibography was performed by capturing infrared images with the BG-4M noncontact meibography system (SBM Sistemi, Turin, Italy). Images were digitally analyzed using ImageJ software (National Institutes of Health; <http://imagej.nih.gov/ij>), and meibomian gland loss was defined as the percentage of gland loss in relation to the total tarsal area of the lower eyelid. Tear osmolarity was tested using the TearLab Osmolarity System (TearLab Corporation, San Diego, CA), collecting a 50-nL tear sample from the inferior lateral tear meniscus.<sup>9</sup>

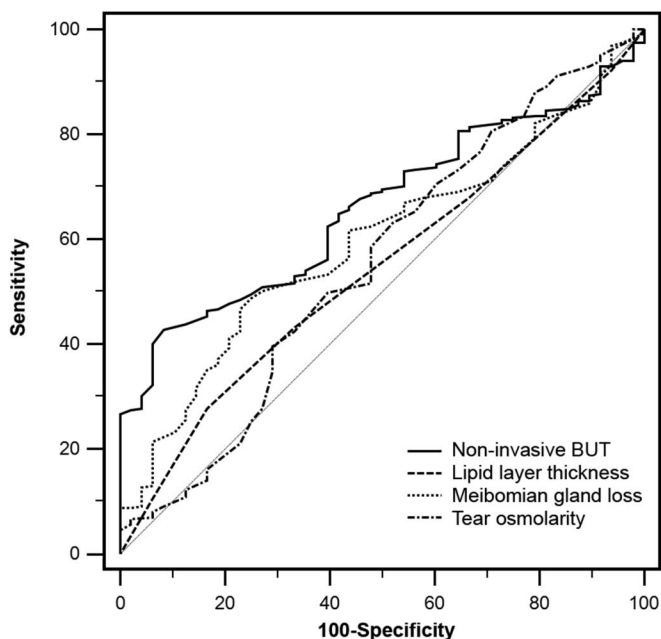
**Statistical Analysis**

Data analysis was performed using SPSS statistical software (SPSS Inc, Chicago, IL). Values were expressed as mean ± SD. The Mann–Whitney *U* test was used to compare OSDI, noninvasive BUT, lipid layer thickness grade, meibomian gland loss, and tear osmolarity between both groups. The correlations among the parameters in the MGD group were calculated using Spearman correlation analysis. Venn diagrams were used to show how the measured parameters might contribute to the diagnosis of MGD. Receiver operating

characteristic (ROC) curves with calculations of the area under the curve (AUC) were used to describe the accuracy of each parameter for differentiating patients with MGD from controls. Results were considered statistically significant for  $P < 0.05$ .

**RESULTS**

A total of 352 eyes of 176 patients were included in the study. Of these, 149 patients were affected by MGD (105 women and 44 men; mean age  $53.4 \pm 15.5$  years), whereas 27 healthy patients acted as a control group (22 women and 5 men; mean age  $52.9 \pm 15.2$  years). There was no significant difference in the distribution of both age and sex between both groups. The median values and variability of the OSDI, noninvasive BUT, meibomian gland loss, and tear osmolarity in both groups are shown in Figure 1. The mean OSDI was significantly higher in the MGD group than in controls (respectively  $37.9 \pm 19.6$  vs.  $7.1 \pm 2.8$ ;  $P < 0.001$ ). The noninvasive BUT was significantly shorter in the MGD group than in the control group ( $8.8 \pm 3.6$  vs.  $11.0 \pm 3.0$ ;  $P < 0.001$ ), whereas meibomian gland loss was significantly higher in the MGD group than in the control group ( $28.0 \pm 17.6$  vs.  $21.2 \pm 13.0$ ;  $P = 0.029$ ). There were no significant differences in both lipid layer thickness and tear osmolarity between the MGD group and control group (respectively,  $22.2 \pm 1.3$  vs.  $2.4 \pm 1.5$  and  $303.5 \pm 9.8$  vs.  $302.7 \pm 8.5$ ; always  $P > 0.05$ ).



**FIGURE 2.** ROC curves for noninvasive BUT, meibomian gland loss, lipid layer thickness, and tear osmolarity.

**TABLE 2.** Areas Under the ROC Curves (AUCs) With 95% Confidence Intervals (CIs), Sensitivity, and Specificity for the Calculated Cutoff Values\* of Each Parameter

Characteristics	AUC	95% CI	Cutoff	Sensitivity, %	Specificity, %
Noninvasive BUT	0.686	0.635–0.734	≤9.6 s	65.8	63.0
Meibomian gland loss	0.598	0.544–0.651	>20%	59.7	61.1
Lipid layer thickness	0.545	0.490–0.599	≤2	57.7	33.3
Tear osmolarity	0.555	0.500–0.609	>303 mOsm/L	49.3	53.7

\*Determined as the value whose corresponding point on the ROC curve was nearest to the coordinate (0,1).

The correlation analyses among the measured variables in the MGD group are summarized in Table 1. Positive correlations between lipid layer thickness and noninvasive BUT ( $r = 0.169$ ,  $P = 0.004$ ) and between meibomian gland loss and OSDI ( $r = 0.187$ ,  $P = 0.004$ ) were found.

The ROC curves of noninvasive BUT, lipid layer thickness, meibomian gland loss, and tear osmolarity are shown in Figure 2. The AUC values showed that noninvasive BUT had the highest diagnostic power as a single parameter, followed by meibomian gland loss, tear osmolarity, and lipid layer thickness (Table 2).

A Venn diagram analysis was performed to show how the 4 automated parameters might be able to differentiate patients with MGD from controls (Fig. 3). The cutoff value for each score was determined as the score whose corre-

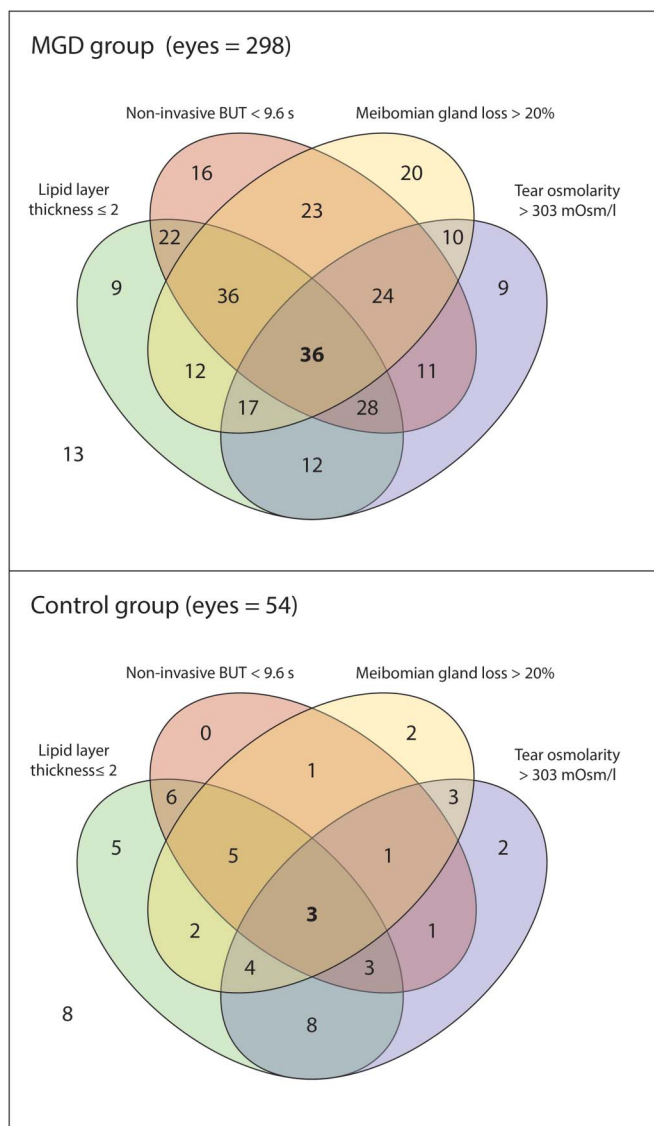
sponding point on the ROC curve was nearest to the coordinate (0,1). Cutoff values considered abnormal were noninvasive BUT  $\leq 9.6$  seconds, lipid layer thickness grade  $\leq 2$ , meibomian gland loss  $> 20\%$ , and tear osmolarity  $> 303$  mOsm/L. Noninvasive BUT showed a sensitivity of 65.8% and a specificity of 63.0%; meibomian gland loss showed a sensitivity of 59.7% and a specificity of 61.1%; the lipid layer thickness grade showed a sensitivity of 57.7% and a specificity of 33.3%; and tear osmolarity showed a sensitivity of 49.3% and a specificity of 53.7%.

The 2 parameters with the highest AUC (namely noninvasive BUT and meibomian gland loss) were combined in parallel, and the diagnostic accuracy was calculated. When the diagnosis of MGD was made based on either noninvasive BUT or meibomian gland loss being abnormal, the sensitivity was 86.2% and the specificity was 38.5%. When the diagnosis was made on both noninvasive BUT and meibomian gland loss being abnormal, the sensitivity was 39.3% and the specificity was 85.6%.

## DISCUSSION

The accurate diagnosis and classification of dry eye are complicated by the heterogeneous nature of the disease and the variability of signs and symptoms.<sup>10</sup> Various diagnostic assessments have been proposed to qualitatively and quantitatively characterize the entire ocular surface system. However, to date, no universally accepted diagnostic workup for the diagnosis of MGD has been established. Several tests used routinely in daily practice require direct contact with the eye and/or the use of eye drops. The resulting alteration of the tear film volume and composition may not only influence the measured variable itself but also have disruptive effects on the results of subsequent tests. In addition, some tests require the clinician's judgment to reach a score and, therefore, are open to significant observer bias. Furthermore, measurements obtained using traditional tests are often affected by low values of repeatability and reproducibility.<sup>11</sup>

Recently, new automated noninvasive quantitative tests have been developed to overcome these drawbacks. They include, among others, tear film interferometry, noncontact meibography, and tear osmolarity. In particular, interferometry is a technique that studies the surface reflection pattern and dynamics of the lipid layer of the tear film, thus allowing the measurement of the tear film stability and the thickness of the lipid layer.<sup>12</sup> The measurement of BUT with a noninvasive technique eliminates the disturbance on the tear film caused by instillation of fluorescein dye.<sup>13</sup> Meibography allows in vivo observation of the meibomian gland morphology; the gland structural changes may be graded with different scoring systems.<sup>14,15</sup> In addition, new digital software allows automated calculation of the total meibomian gland area in the lower and upper eyelids.<sup>16</sup> Tear film osmolarity has been reported as the single best metric to diagnose and grade severity of dry eye.<sup>17</sup> However, some authors questioned its clinical utility because of the high variability of measurements and the lack of correlation with dry eye signs and symptoms.<sup>18</sup>



**FIGURE 3.** Venn diagram analysis of both MGD and control groups. Cutoff values of noninvasive BUT, meibomian gland loss, lipid layer thickness, and tear osmolarity were determined from ROC curves.

In this study, we performed the diagnostic workup using automated noninvasive measurements of various ocular surface parameters in both patients with MGD and healthy controls. Noninvasive BUT was significantly shorter in the MGD group compared with the control group. This result is consistent with previous studies and confirms that MGD reduces the stability of the tear film.<sup>19,20</sup> The ROC analysis showed that the noninvasive BUT was the parameter with the highest AUC, indicating that it has the highest power to differentiate between MGD and control patients. No association between the noninvasive BUT and OSDI was found, in disagreement with other authors.<sup>18</sup> However, this finding is not surprising because it is well known that ocular surface symptoms have low and inconsistent correlations with clinical signs, including both fluorescein tear and noninvasive BUT.<sup>21</sup>

Meibomian gland loss was shown to be significantly higher in patients with MGD than in controls. Moreover, a significant correlation between meibomian gland loss and OSDI was found, in accordance with previous studies.<sup>10,20,22</sup> The ROC analysis indicated that the meibomian gland loss score was the second best parameter to differentiate patients with MGD from the normal population. Its high diagnostic accuracy and positive correlations with lid margin abnormalities and meibum quality and expressibility were previously demonstrated.<sup>19</sup>

Lipid layer thickness showed a significant correlation with noninvasive BUT but did not significantly differ between patients with MGD and controls. It should be also noted that lipid layer thickness showed inconsistent correlations with TBUT, ocular symptoms, and meibomian gland loss across different studies.<sup>10,23–26</sup> This finding may be related to several confounding factors potentially influencing its measure, such as the use of lipid-containing eye drops, the palpebral aperture, and the gaze position.<sup>26</sup>

Tear osmolarity did not significantly differ between patients with MGD and controls. Furthermore, it did not show any correlation with the other measured parameters. It was previously hypothesized that tear osmolarity may not be increased in patients with MGD because the disease alone may not be sufficient at overwhelming the homeostatic control in many patients.<sup>27</sup> However, previous studies showed that there was increased variability attributable to errors in repeated measurements in both patients with dry eye and those with MGD compared with control participants, thus making the clinical interpretation of its measurements unclear.<sup>18,28</sup>

In this study, the noninvasive BUT and meibomian gland loss resulted in the tests with the highest diagnostic power; by combining the 2 tests in parallel, MGD may be strongly suspected when 1 of these 2 tests is abnormal. However, it should be highlighted that these values are lower compared with the other traditional tests: for instance, Arita et al<sup>19</sup> reached the diagnosis with a sensitivity of 84.9% and a specificity of 96.7% using the combination of meibomian gland loss, lid margin abnormalities, and ocular symptoms. However, our automated ocular surface workup has several potential advantages: the techniques are noninvasive, not altering the volume or the properties of the tear film, and the results of subsequent tests; they are examiner independent and

may be easily performed as a screening tool by trained nonophthalmologist medical personnel, and they are more objective than clinician-derived estimates, thus minimizing the risk of observer bias. In addition, the diagnostic power of the proposed workup could be further improved in clinical practice by incorporating the OSDI score, which is also a noninvasive measure that can be administered by nonophthalmologists.

In conclusion, the automated noninvasive ocular surface diagnostic workup used in this study may represent a promising diagnostic tool for MGD diagnosis. Although no single test has proved able to reach the diagnosis with sufficient accuracy, MGD may be strongly suspected when one between noninvasive BUT and meibography combined in parallel is abnormal. In case of positivity of either noninvasive BUT or meibomian gland loss, subsequent qualitative clinical tests should be performed to achieve a reliable diagnosis and more precise characterization of MGD.

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