# Prospective Study of the Diagnostic Accuracy of the In Vivo Laser Scanning Confocal Microscopy for Ocular Demodicosis

YU-JING WANG, MIN KE, AND XIAO-MIN CHEN

• PURPOSE: **We sought to determine the diagnostic accuracy of in vivo confocal microscopy (IVCM) for ocular demodicosis.**
• DESIGN: **Reliability and validity analysis.**
• METHODS: **This was a single-center study of consecutive patients presenting to Zhongnan Hospital of Wuhan University, Hubei, China, between February 2017 and February 2018 with blepharitis. After examination, the blepharitis was scanned by IVCM. The regrading of the shuffled image set was performed by grader 1 (experienced) and grader 2 (inexperienced). The regrading of the shuffled image set was performed by 2 graders 3 weeks later. Eyelash samples were collected for light microscopy. The main outcome measures were sensitivity, specificity, and positive and negative predictive values of IVCM compared with those of positive light microscopy under 2 definitions. Sensitivities and specificities for multiple graders were pooled and 95% confidence intervals (CIs) were calculated.**
• RESULTS: **Sensitivity of IVCM grader 1 and grader 2 based on the definition of "light microscopy–positive demodex" was 100% (95% CI 94.84–100%) and 98.8% (95% CI 93.02–99.94%), respectively. Sensitivity of IVCM grader 1, grader 2, and light microscopy to the definition of "definite diagnosis demodex" definition was 100% (95% CI 97.02–100%), 93.63% (95% CI 88.28–96.73%), and 56.69% (95% CI 48.55–64.49%).**
• CONCLUSIONS: **IVCM is better than traditional methods in detecting the number and sensitivity of demodex, and it is both highly sensitive and specific when performed by an experienced operator. (Am J Ophthalmol 2019;203:46–52. © 2019 Elsevier Inc. All rights reserved.)**

BLEPHARITIS IS A CHRONIC INFLAMMATORY PROCESS of the eyelid margin. It is a common eye disorder throughout the world and can affect any age group. Respondents to a 2009 survey of clinicians in the United States indicated that 37% to 47% of their adult patients had findings suggestive of blepharitis.[1] Clinical examination reveals the presence of scurf, telangiectatic vascular changes of the eyelid margin, inspissated meibomian glands, conjunctival hyperemia, punctuate keratopathy, cornea vascularization, and ulceration.[2]

The underlying causes of blepharitis and associated inflammation are not fully understood but likely involve several pathogenic mechanisms.[3] Chronic low-grade bacterial infection,[4] environmental factors,[5] and systemic diseases may contribute to the pathogenesis of blepharitis. A recent meta-analysis of the association between Demodex infestation and blepharitis based on 13 published case-control series (2098 patients with blepharitis and 2643 control subjects) suggesting that Demodex mites were related to chronic blepharitis.[6] Therefore, neglecting the etiologic origin of blepharitis can result in a protracted disease course.

The most common ectoparasites in humans are 2 Demodex species: *Demodex folliculorum* and *Demodex brevis*.[7] *D folliculorum* primarily inhabits the infundibular region of hair follicles, whereas *D brevis* thrives in the deeper sebaceous ductus and meibomian glands.[8] The prevalence of Demodex infestation increases with age, being observed in 84% of the population at 60 years of age.[9] Eyelash sampling and counting under light microscopy[10] are the key diagnostic methods. In vivo confocal microscopy (IVCM) has been used by dermatologists for the diagnosis of Demodex skin infection.[11] Recently, IVCM has come to wider clinical use as additional diagnostic modalities for ocular Demodex.[12] The purpose of this study was to compare the sensitivity, specificity, positive predictive value, and negative predictive value of confocal microscopy and light microscopy in the diagnosis of ocular Demodex.

## METHODS

THIS STUDY WAS APPROVED BY THE INSTITUTIONAL REVIEW board of Zhongnan Hospital of Wuhan University, Hubei, China and the Ethics Committee of the Zhongnan Hospital of Wuhan University, Hubei, China (ChiCTR1800016401). All patients provided written informed consent before enrollment in the study. This study adhered to the tenets of the Declaration of Helsinki and was conducted in accordance

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From the Zhongnan Hospital of Wuhan University, Hubei, China.
Inquiries to: Min Ke, Zhongnan Hospital of Wuhan University, No.169 Donghu Road, Wuchang District, Wuhan, Hubei, China; e-mail: 123631087@qq.com



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with the Standards for Reporting of Diagnostic Accuracy studies.[13]

• **STUDY PARTICIPANTS:** Consecutive patients presenting to the clinic between February 2017 and February 2018 in Zhongnan Hospital of Wuhan University, Hubei, China were assessed for eligibility and prospectively enrolled into the study if they were found eligible.

Male or female patients were eligible to participate in the study if they were ≥18 years of age on the date the informed consent form was signed and were able to understand study procedures and provide voluntary informed consent. The diagnoses of diseases used the *International Classification of Disease, 9th Revision, Clinical Modification*. We excluded any patients with acute and allergic ocular surface inflammation in the affected eyes; with a history of chemical, thermal, or radiation injury; keratoconus; a history of ocular surgery; or contact lens or drug use that would alter the ocular surface. One eye per patient was included as the study eye. In cases with bilateral blepharitis, the eye with the highest baseline blepharitis severity was the study eye; if severity was equal in both eyes, the right eye was designated as the study eye.

• **IVCM IMAGING:** IVCM was performed with the Heidelberg Retinal Tomograph/Rostock Corneal Module confocal microscope (Heidelberg Engineering, Dossenheim, Germany) using a standard operating procedure by an experienced ophthalmologist trained in performing IVCM and following a standard procedure. A sterile protective cap (TomoCap; Heidelberg Engineering) was mounted over the front of the microscope, and polyacrylic acid 0.2% (Viscotears; Novartis, Camberley, United Kingdom) was used as a coupling agent between the cap and the lens objective. Topical anesthetic (0.5% proxymetacaine hydrochloride; Bausch & Lomb, Kingston-upon-Thames, United Kingdom) was used to anesthetize the eye before performing microscopy. After an examiner asked the patient to look down, the cotton swab was used to flip the upper eyelid, and the center of the Tomo-Cap was applanated onto the upper eyelid edge vertically. The upper eyelid of all eyes was evaluated with IVCM (from temporal to nasal), allowing the examination of 9 lashes (3 lashes in the temporal, medial, and nasal eyelid) and their follicles. Nine sequences each containing 100 frames were taken in each eye. Focal distance was modified to evaluate the whole follicle and lash root, and every suspected image of mites was recorded.

• **LIGHT MICROSCOPY DIAGNOSIS:** Lash depilation was quickly performed after IVCM analysis as a standard technique. According to a modified method reported by Gao and associates,[10] 2 central lashes with cylindrical dandruff from each eyelid of the tested eye were removed and mounted with a coverslip. The number of mites was counted immediately, and the Demodex count was recorded as the mean mites per lash. The material was completed and counted by an independent operator.

• **IVCM GRADING:** Patient-identifying data were removed from all IVCM scans and images were arranged in a random order for each observer to assess. Our confocal graders applied all scans of all recruited patients and were graded for the presence or absence and the numbers of Demodex mites. The regrading of the shuffled image set was performed by 2 graders 3 weeks later. Eyelash samples were collected for microscopy. One grader had experience of performing IVCM and grading confocal images for Demodex for >2 years (grader 1) and the other had <1 year of experience (grader 2). All graders were masked to the light microscopy diagnosis and clinical appearance. Two graders were able to repeat the grading process ≥3 weeks after the first grading session.

• **CLASSIFICATION OF DIAGNOSTIC METHODS:** There is a lack of a criterion standard for the diagnosis of ocular Demodex; we have made 2 definitions of ocular Demodex diagnosis to act as a reference standard to allow for calculation of sensitivity and specificity. The first definition is "light microscopy–positive Demodex," in which Demodex is diagnosed on the basis of positive optical microscope results, and the mean mite count per lash is ≥1; the second definition is "definite Demodex," diagnosed on the basis of a positive result on IVCM and/or optical microscopy and a mean mite count per lash ≥1.

• **STATISTICAL METHODS:** In accordance with Hajian-Tilaki,[14] we used predetermined values of sensitivity and prevalence of disease as 60% and 80%, respectively. To ensure that the maximum marginal error of estimate did not exceed 7% with a 95% confidence interval (CI), the total required sample size was 155. Numeric data were reported as the mean ± standard deviation, and nonnumeric data were recorded as the presence (yes) or absence (no). Statistical significance of between-group differences in demographic or clinical features was assessed using the Kruskal–Wallis test and $\chi^2$ test for proportions. Sensitivity, specificity, positive predictive value, and negative predictive value were calculated. CIs for sensitivity and specificity are "exact" Clopper–Pearson CIs, and CIs for predictive values are standard logit CIs. We used the k score to calculate intragrader agreement (to assess reproducibility). The k scores were interpreted as in McHugh.[15] All statistical analyses were performed using SPSS software (version 17.0; SPSS, Inc., Chicago, IL) and reported as 2-tailed probabilities, with $P < .05$ considered statistically significant.

**TABLE 1.** Demographic Data and Clinical Features of Study Participants

| | Demodex Positive | Demodex Negative | *P* Value |
|---|---|---|---|
| Eyes, n (%) | 89 (52.3%) | 81 (47.6%) | |
| Age (y), mean ± SD | 52.9 ± 15.4 | 42.5 ± 14.1 | .000 |
| Male gender, n (%) | 41 (46.1%) | 38 (46.9%) | .517 |
| Duration of symptoms (d) ± SD | 37.8 ± 13.0 | 32.2 ± 14.8 | .009 |
| Clinical signs (%) | | | |
| Itching | 73 (82%) | 35 (43.2%) | .000 |
| Dryness | 53 (59.6%) | 61 (75.3%) | .021 |
| Irritation | 48 (53.9%) | 53 (65.4%) | .085 |
| Glare | 36 (37.1%) | 29 (35.8%) | .321 |
| Investigations | | | |
| IVCM, n (%) | 89 (100%) | 81 (100%) | |
| Light microscopy, n (%) | 89 (100%) | 81 (100%) | |

IVCM = in vivo confocal microscopy.

## RESULTS

• **STUDY PARTICIPANTS:** A total of 185 patients were assessed for study eligibility between February 2017 and February 2018 with blepharitis. 13 patients were excluded as Unable to tolerate pain in eyelash sampling, A few patients (n = 2) were unable to cooperate for the full IVCM imaging protocol. No adverse events were noted.

Descriptive demographic data and clinical features of study participants are presented in Table 1. The age of blepharitis patients with demodex is higher than that of patients without demodex. There was a statistically significant difference between the two groups in age ($P < 0.0001$). Compared with all others, demodex positive patients had a longer symptom duration ($P < 0.05$). Moreover, there are significant differences about the clinical signs of itching and dryness.

• **DETECTION OF DEMODEX BY IVCM:** Of patients with ocular demodex, IVCM images were considered positive and easily calculated by all observers showing typical highly reflective legs (Fig. 1). In some images, although experienced observer detected demodex elements, such elements were not recognized by inexperienced observer (Fig. 2).

• **ANALYSIS OF THE NUMBER OF MITES:** The mites' quantity information was observed by both examinations as shown in Figure 3, The mean mite count/lash tended to be higher in IVCM examinations when compared with the mite Counts in light microscopy, there were no

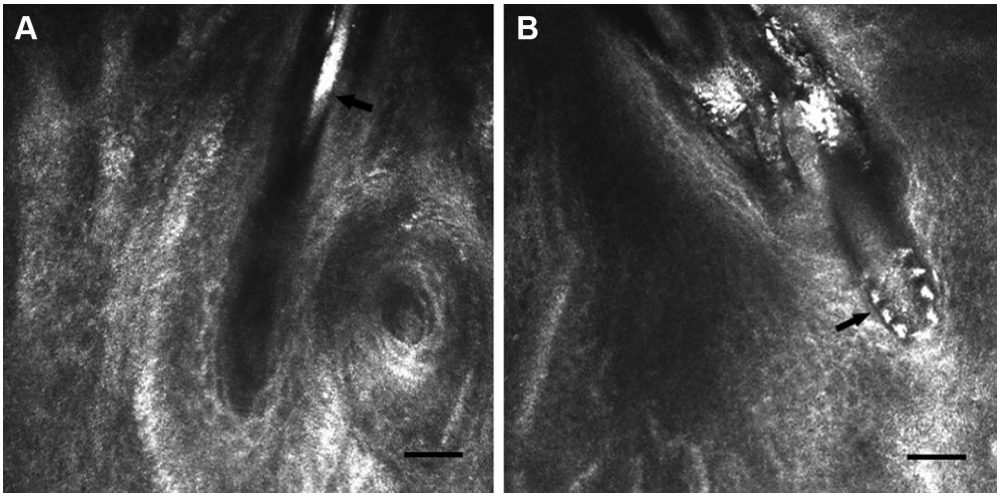


**FIGURE 1. In vivo confocal microscopy typical images of demodex. (A) Hair follicle without any demodex. Arrow marks the eyelash in the hair follicle. (B) Typical demodex in eyelash hair follicles with typical highly reflective legs. Arrow marks the demodex in the hair follicle. Scale bar = 50 μm.**

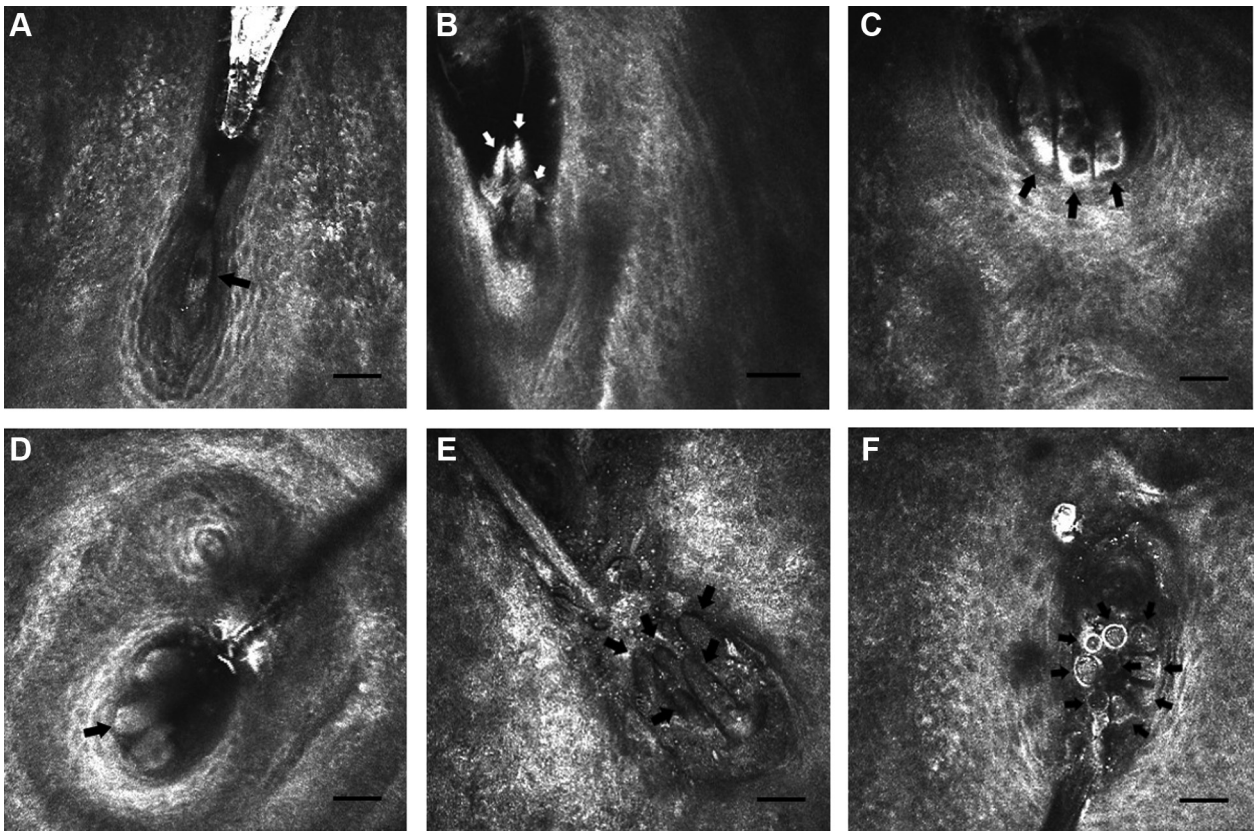


**FIGURE 2. In vivo confocal microscopy confusing images of demodex. (Row 1) A: Medium reflection structure of mite tail inside the follicle. B: Tails of 3 demodex with high reflection. C: Digestive particles of the abdomen inside 3 mites. (Row 2) D: Highly reflective mite front abdomen. E: Five mites attached to the opening of the lash. F: Cross section of 9 mites. Arrows mark the demodex in the hair follicle. Scale bar = 50μm.**

statistically significant differences in mean mite counts between the two graders groups (Table 2).

Summary statistics of the sensitivity, specificity, PPV and NPV of confocal microscopy, and light microscopy are shown in Tables 3 and 4. Sensitivity of IVCM grader 1 and grader 2 based on the definition of "light microscopy-positive demodex" was 100% (95% CI: 94.84%～100%), 98.8% (95% CI: 93.02%～99.94%), respectively. Sensitivity of IVCM grader 1, grader 2 and light microscopy to the definition of "definite diagnosis demodex" definition Was 100% (95% CI: 97.02%～100%), 93.63% (95% CI: 88.28%～96.73%), and 56.69% (95% CI: 48.55%～64.49%).

There was a perfect test reproducibility among the 2 graders' scores for IVCM grade, with a k score of 0.89 ($P < 0.001$).

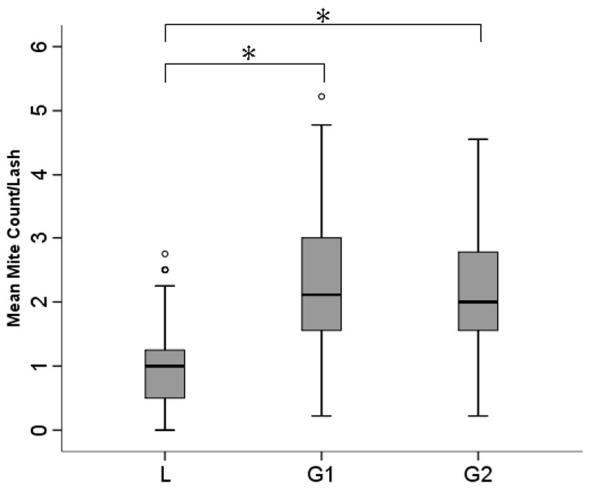


**FIGURE 3. Box plots showing the superior (Sup), inferior (Inf), and average (Avg) demodex number in the light microscopy group (L) and the in vivo confocal microscopy group (G1, G2). The boxes show the 75th percentile (top line), the median (middle line), and the 25th percentile (bottom line). The whiskers show the maximum (top bar) and minimum (bottom bar) values. Asterisks show the statistical difference between pairs of groups. *P < .05.**

## DISCUSSION

BLEPHARITIS IS A CHRONIC INFLAMMATORY DISEASE OF THE eyelids that is frequently encountered in clinical practice. The etiology of the disorder is complex and not fully

**TABLE 2.** Mean Mite Counts in Eye Lashes Assessed by Confocal and Light Microscopy

| | Light Microscopy | IVCM | |
|---|---|---|---|
| | | Grader 1 | Grader 2 |
| Mean mite count | 0.89 ± 0.56 | 2.27 ± 1.01 * | 2.12 ± 0.97 |

IVCM = in vivo confocal microscopy.
*$P < 0.05$ compared to control group.

understood. The first documented disorder associated with demodicosis was blepharitis, dated as early as 1899.[7] It has a high age-dependent prevalence and frequently is found in the eye with blepharitis,[8,16] ocular demodicosis was significantly more prevalent in patients with chalazia.[17] Because of the anatomic feature of the face, eyelids are not accessible to routine cleansing hygiene providing a favorable environment for Demodex mites to spread and flourish.

In our study, the age of blepharitis patients with demodex is higher than that of patients without demodex detected by confocal microscopy, consistent with previous optical microscope results. The clinical symptoms of demodex infected blepharitis patients last longer, probably because of demodex blepharitis is often overlooked. It has low specificity clinical manifestations in differential diagnosis of blepharitis and external diseases, leading to untimely or even wrong treatment.

Sampling and microscopic mite counting were performed, as previously established.[10,18] It allows superior evaluation of the mite species and identification of the life stage, that is ovum, larva, protonymph, nymph, or adult. However, the random error of the eyelash sampling method is very large, and it is greatly affected by factors such as baldness and age. Recently, IVCM has been used as a noninvasive method to diagnose demodex infestation. The current experimental data sample size is small,[12,19] therefore, we need to confirm the value of IVCM in diagnosing demodex infestation especially in giving reliable quantitative assessment in this study. In our experiments, patients who detected a large number of mites by IVCM did not show as many mites in the eyelash method, and even some patients had very few mites. This may be related to the tightness of the connection between the worm and the eyelashes. We found that some eyelashes roots with cylindrical dandruff did not detect more demodex through IVCM. This is inconsistent with the conclusion that the number of mites in the eyelash hair follicles of the previous eyelash sampling method that have a large number of cylindrical dandruff at the root.[18] Considering that confocal

**TABLE 3.** Diagnostic Validity and Reliability of Confocal Microscopy Compared With the Reference Standard of Positive Light Microscopy Demodex

| | Light Microscopy Diagnosis | | | | |
|---|---|---|---|---|---|
| | Positive | Negative | Total | | Value (95% CI) |
| IVCM grader 1 | | | | | |
| Positive | 89 | 68 | 157 | Sensitivity (%) | 100% (94.84% ~ 100%) |
| Negative | 0 | 13 | 13 | Specificity (%) | 52.35% (44.59% ~ 60.01%) |
| Total | 89 | 81 | 170 | PPV (%) | 56.69% (48.55% ~ 64.49%) |
| | | | | NPV (%) | 100% (N/A) |
| IVCM grader 2 | | | | | |
| Positive | 88 | 59 | 147 | Sensitivity (%) | 98.88% (93.02% ~ 99.94%) |
| Negative | 1 | 22 | 23 | Specificity (%) | 27.16% (18.15% ~ 38.37%) |
| Total | 89 | 81 | 170 | PPV (%) | 59.86% (51.44% ~ 67.76%) |
| | | | | NPV (%) | 95.65% (76.03% ~ 99.77%) |

CI = confidence interval; IVCM = in vivo confocal microscopy; N/A = not applicable; NPV = negative predictive value; PPV = positive predictive value.

**TABLE 4.** Diagnostic Validity and Reliability of Confocal Microscopy Compared and Light Microscopy Compared Against Definite Demodex Diagnosis

| | Definite Diagnosis | | | | |
|---|---|---|---|---|---|
| | Positive | Negative | Total | | Value (95% CI) |
| IVCM grader 1 | | | | | |
| Positive | 157 | 0 | 157 | Sensitivity (%) | 100% (97.02%～100%) |
| Negative | 0 | 13 | 13 | Specificity (%) | 100% (71.66%～100%) |
| Total | 157 | 13 | 170 | PPV (%) | 100% (N/A) |
| | | | | NPV (%) | 100% (N/A) |
| IVCM grader 2 | | | | | |
| Positive | 147 | 0 | 147 | Sensitivity (%) | 93.63% (88.28%～96.73%) |
| Negative | 10 | 13 | 23 | Specificity (%) | 100% (71.66%～100%) |
| Total | 157 | 13 | 170 | PPV (%) | 100% (N/A) |
| | | | | NPV (%) | 56.52% (34.87%～76.12%) |
| Light microscopy | | | | | |
| Positive | 89 | 0 | 89 | Sensitivity (%) | 56.69% (48.55%～64.49%) |
| Negative | 68 | 13 | 81 | Specificity (%) | 100% (71.66%～100%) |
| Total | 157 | 13 | 170 | PPV (%) | 100% (N/A) |
| | | | | NPV (%) | 16.05% (9.15%～26.25%) |

CI = confidence interval; IVCM = in vivo confocal microscopy; N/A = not applicable; NPV = negative predictive value; PPV = positive predictive value.

microscopy can show the number of aphids more intuitively, we will accurately explore the correlation between the number of aphids and clinical manifestations and signs, such as itchy and cylindrical dandruff.

Previous studies have shown that the incidence of mites in patients with blepharitis ranges from 28.8% to 90%,[8,20,21] the inconsistent diagnosis method and sampling method are the main reasons. At present, there is no uniform standard for the diagnostic threshold of demodex. Filho[22] proposed three eyelashes at random for each eyelid, more than one worm is considered to be positive for demodex. Wesolowska[23] thinks that one of the 10 eyelashes is positive. Improved sampling methods make infection rates higher.[10] Existing confocal studies show that the infection rate is significantly higher than the improved detection method.[12] Considering that demodex are also carried in the normal population, and it is ubiquitous in adult humans as suggested in one study.[24] this experiment defines the threshold of demodex infection as 1 or more per eyelash.

Our study shows that IVCM is both highly sensitive and specific in the diagnosis of demodex. These data support a role for IVCM to be used as an initial diagnostic test in evaluating suspected demodex. The sensitivity of IVCM ranged from 93.63% to 100% and a specificity of 27.16% to 100% depending on the definition of the reference standard. We found that experienced IVCM graders were able to detect demodex in 100% of light microscopy-positive participant. In our study, the sensitivity of experienced testers was slightly higher than that of less experienced testers, but the difference was not statistically significant. This shows that although the use of a skilled operator improved diagnostic accuracy, IVCM detection of demodex has a short learning period and high acceptability.

The main cause of IVCM false-negatives was the learning for the IVCM to capture any mite in the images, as well as the presence of a high degree of lipid metabolites and keratinized substances in hair follicles that could mask the presence of the mites. Some of these patients have excessive secretions and scales at the roots of the eyelashes due to severe blepharitis. We performed a simple cleaning to ensure that the images were clearly imaged, but whether the mild edge cleaning has an effect on the number of confocal detection of aphids will be verified in future experiments. In this experiment, the participants were able to tolerate IVCM imaging and showed a good cooperation due to the subject's upper eyelid was slightly externally rotated, the ocular surface was minimally exposed, and the discomfort was minimized during the operation.

Treatments for demodex blepharitis include physical and medical therapy. Physical therapy includes lid scrub with tea tree oil shampoo and lids massage daily.[25] However, there is currently no medical standard specification for the treatment of demodex. 2% metronidazole[26] and 5% tea tree oil are widely used in topical treatment clinically. Blepharitis patients with severe rosacea, seborrheic dermatitis, in addition to topical treatment, can be treated systematically by oral administration of ivermectin or metronidazole.[22, 27] At present, there are many different definitions of demodex infection, and only on the basis of perfecting the diagnosis of demodex blepharitis, can it be better treated. The most prominent advantage of IVCM

for the diagnosis of mites is that the number of aphids in each eyelash follicle can be accurately measured in real time. Perhaps the definition of demodex infection can be newly defined based on the number or distribution of the mites, which is the focus of our research in the next experiment.

Therefore, IVCM demonstrates good sensitivity, specificity and reproducibility in the diagnosis of demodex. Further studies are needed to validate the interpretation and utilization of these diagnostic tools to aid in prompt diagnosis and treatment, thereby improving prognosis of demodex.

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