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Imaging Biomarkers for Dry Eye Disease

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Abstract: The clinical, scientific, economic, and regulatory impact of validated biomarkers and surrogate endpoints has the potential to revolutionize the approach to ocular surface diseases. At present, there is a growing interest in developing biomarkers for dry eye disease, and other ocular surface disorders and imaging are of the most promising approaches to this issue. Among the several and constantly evolving imaging technologies, some tools that are aimed to assess tear film stability and volume, meibomian gland morphology and function, and ocular surface microanatomy are now supported by a good body of evidence. To date, clinical trials on ocular surface diseases have slowly started incorporating imaging biomarkers for disease diagnosis and stratification and as surrogate endpoints. Major efforts are still needed, mainly aimed to improve automatic acquisition and quantitative analysis, standardization (standard operating procedures, normative databases etc.), and validation of imaging biomarkers.

Key Words: Imaging—Biomarker—Clinical trial—Dry eye—Ocular surface.

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The definition of dry eye disease (DED) has been recently amended by the Tear Film and Ocular Surface Society Dry Eye Workshop II (TFOS DEWS II) to "Dry eye is 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."[1] This definition highlights the complexity of the disease, driven by five different etiologic mechanisms able to have an impact on several components of the ocular surface morphofunctional unit.

The proper assessment of DED patients is a major issue in both clinical practice and research.

As recently discussed in the TFOS DEWS II Diagnostic Methodology Report, "no single 'gold standard' sign or symptom that correlates perfectly with the DED state has been established."[2]

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Main problems are related to the lack of agreement between signs and symptoms, to their fluctuation over time, and to the significant overlap between normal and DED distributions of currently available metrics.[2]

Furthermore, the low success rate of recent clinical trials on DED, although due to several not yet fully understood concomitant factors, is pointing out the urgent need for the identification and validation of minimally invasive biomarkers to be used for patient selection and stratification and to monitor the response to the treatments.[3–5]

Biomarkers and Surrogate Endpoints: Conceptual Framework and Updated Definitions

Biomarkers and surrogate endpoints have significant potential for improving and accelerating the translation of scientific concepts into diagnostic and therapeutic approaches and technologies.[6] Proper validation and usage of these tools require, above all, common and shared language as well as definitions and conceptual framework.[6]

For this reason, the FDA-NIH Joint Leadership Council recently identified the harmonization of terms used in translational science and medical product development as a priority need and developed the BEST (Biomarkers, EndpointS, and other Tools) glossary.[7] This resource clarifies currently accepted definitions and describes some of the hierarchical relationships, connections, and dependencies among the terms it contains.[7]

A biomarker can be diagnostic, predictive, or prognostic and is defined as a "characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention, including therapeutic interventions. A biomarker is not an assessment of how an individual feels, functions, or survives."[6,7]

A surrogate endpoint is defined as "an endpoint that is used in clinical trials as a substitute for a direct measure of how a patient feels, functions, or survives. A surrogate endpoint does not measure the clinical benefit of primary interest in and of itself but rather is expected to predict that clinical benefit or harm based on epidemiologic, therapeutic, pathophysiologic, or other scientific evidence." A validated surrogate endpoint must be "supported by a clear mechanistic rationale and clinical data providing strong evidence that an effect on the surrogate endpoint predicts a clinical benefit."[6,7]

The Role of Imaging

Medical imaging is increasingly used for screening, diagnosis, prognosis, evaluating the natural history of disease, or monitoring therapeutic efficacy.[5]

The potential advantages of imaging include objective measurements, in vivo assessment, minimization of subjective bias, and, in

clinical research, the opportunity to have masked, standardized, and centralized evaluation of the images.[5]

Main limits of this approach are related to the availability of the technology and to the need for standardization of image acquisition, analysis, and interpretation.

Almost all medical imaging processes involve some aspects of standardization. However, in clinical practice, images are frequently handled with limited formal quantification, and a certain degree of variability among facilities and operators may have little or no impact on the ability to provide a diagnosis. Differently, imaging biomarkers for clinical trials require specific imaging process standards, extending beyond those typically performed in the medical care of a patient. Variability, indeed, may result in increased variability in endpoint measurements and may compromise the ability of the trial to achieve its objectives.[8]

In a recent analysis, we showed the growing use of imaging biomarkers in published clinical trials in ophthalmology, mainly for patient selection and stratification and as secondary surrogate endpoints.[5]

Imaging for Dry Eye Disease

At present, several technologies are available to assess the tear film and ocular surface in DED patients, and their usage is now reaching the clinical trials' design. Looking at trials recently registered on ClinicalTrials.gov (available at: https://clinicaltrials.gov/; search performed on Mar 28, 2019; study type: randomized clinical trials; status: recruiting; the first 10 results), we found that only 1/10 studies on "dry eye disease" included imaging biomarkers as inclusion criteria and surrogate endpoints, whereas 5/9 studies on "meibomian glands dysfunction" included imaging biomarkers, mainly as surrogate endpoints (Table 1). T1

The most widely used imaging approaches for DED patients and the most promising potential imaging biomarkers are aimed to assess tear film stability and volume, meibomian gland (MGs), and corneal and ocular surface microanatomy (Table 2). T2

Imaging to Assess Tear Film Stability

Tear film instability is one of the key elements of the DED vicious cycle,[1,9] and it is an important parameter to be considered for DED diagnosis.[2] Tear film break-up time is traditionally evaluated using sodium fluorescein to enhance the visibility of the tear film (F-break-up time [BUT]). F-BUT is a quick, inexpensive, and low-tech approach and has become one of the most widely used clinical tests for DED. Concerns related to the impact of sodium fluorescein on the tear film, to difficulties in standardizing the volume of instilled dye, and to the repeatability of the examination led to the development of imaging systems aimed to quantify the tear film stability without fluorescein instillation (noninvasive BUT [NI-BUT]).[10]

There are several commercially available NI-BUT systems, based on topographic or videokeratographic methods that analyze the interblink changes of reflected placido mires.[10–13]

Potential advantages of this approach include steady-state respect and good repeatability and reproducibility data in healthy subjects and DED patients.[12,14] Moreover, these systems allow for automatic qualitative and quantitative assessment of tear film stability and images and data storage. At present, main limits are the lack of validated diagnostic cutoff values and of agreement among the different systems, which cannot be used interchangeably,[15,16] the wide range of specificity for DED diagnosis (76%–94%),[2] and the conflicting data on the correlations between NI-BUT and other clinical parameters.[12,13,17,18]

Imaging to Assess Tear Film Volume

The quantitative assessment of tear film volume/secretion is an important component of DED patients' examination and a crucial element for DED subclassification.[2]

TABLE 1. *Imaging Biomarkers Usage in Current Clinical Trials on Dry Eye Disease and Meibomian Glands Dysfunction (https://clinicaltrials.gov/; Search Performed on Mar 28, 2019; Study Type: randomized Clinical Trials; Status: Recruiting; the First 10 Results)*

CT Number	Status	Imaging Biomarkers	Imaging Primary Surrogate Endpoint	Imaging Secondary Surrogate Endpoint
Clinical trials on "dry eye disease"				
NCT03204903	Recruiting	None	None	None
NCT02193490	Recruiting	None	None	None
NCT03785340	Recruiting	None	None	None
NCT03116776	Recruiting	None	None	None
NCT02767258	Recruiting	None	None	None
NCT03768115	Recruiting	None	None	None
NCT03888183	Recruiting	Surrogate endpoints. Inclusion criteria: TMH and NIBUT	TMH	5/8: TMH, lipid layer thickness, redness score, NIBUT
NCT03846453	Recruiting	None	None	None
NCT03676335	Recruiting	None	None	None
NCT03460548	Recruiting	None	None	None
Clinical trials on "meibomian glands dysfunction"				
NCT03434106	Recruiting	Surrogate endpoints	1/5: NIBUT	1/2: TMH
NCT03060005	Recruiting	Surrogate endpoints	2/6: NIBUT, TMH	None
NCT03652051	Recruiting	None	None	None
NCT03162497	Recruiting	Surrogate endpoints	OCT tear film thickness	2/13: lipid layer thickness, meibography
NCT03318874	Recruiting	Inclusion criteria: NIBUT	None	None
NCT03422146	Recruiting	None	None	None
NCT03708367	Recruiting	None	None	None
NCT03396913	Recruiting	Surrogate endpoint	None	1/12: meibography
NCT03492541	Recruiting	None	None	None

OCT, optical coherence tomography.

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TABLE 2. *Selection of the Currently Most Promising Potential Biomarkers for Dry Eye Disease*

Structure or Condition	Potential Imaging Biomarker
Tear film stability	Noninvasive break-up time
Tear film volume	Tear menisci height, area, radius, and depth
Meibomian gland	Infrared meibography quantitative and morphologic parameters
Ocular surface inflammation	Confocal assessment of corneal dendritic cells and other inflammatory cells
Corneal innervation	Confocal assessment of subbasal corneal nerves (quantitative and morphologic parameters)

For many decades, the Schirmer test has been the most popular test for tear film quantitative assessment, and it is still included among the preferred tests for DED diagnosis in the American Academy of Ophthalmology Dry Eye Preferred Practice Pattern.[19] However, major concerns related to its invasiveness and poor repeatability[20] led to major efforts aimed to validate more direct, noninvasive tests for tear film volume quantification.

The tear menisci, formed by the tears lying at the junctions of the bulbar conjunctiva and the margins of both the upper and lower eyelids, contain the majority of tear fluid, and the quantitative analysis of the tear menisci is, at present, the most direct approach to evaluate the tear film volume.[2] Different systems can perform meniscometry, but the largest amount of evidence currently supports the use of optical coherence tomography (OCT) assessment of the tear meniscus, extensively studied in the past 10 years.[2,21,22]

Upper and lower height, area, radius, and depth are, at present, the most studied parameters, and their OCT assessment showed good intra-rater and inter-rater repeatability, especially using spectral-domain OCT.[23,24]

The main advantages of OCT meniscometry are that this technology is a noninvasive and steady-state respectful technique, and image acquisition is easy and quick. On the contrary, image analysis may be complex, time-consuming, and operator-dependent, and the development of validated measurement software is needed.[2] Other major concerns are that measurements are instrument-dependent[2,24] and may be biased by conjunctivochalasis, disorders of lid margin congruity, and ocular surface–lid apposition.[21] Moreover, the combination of interfering factors related to head, eye, and eyelid movements suggests the potential clinical utility of developing systems for post-blink dynamic OCT meniscometry.[25]

There are conflicting data on the diagnostic accuracy of this technique and on its correlations with other dry eye tests.[2,10,21] An important issue is the heterogeneity of DED definitions and diagnostic algorithms adopted in the published studies. In light of the TFOS DEWS II report, additional efforts should be made to validate instrument-specific cutoff values, obtained starting from a standardized and widely accepted definition of DED and aimed to subclassify the disease by identifying aqueous-deficient forms.

Imaging to Assess Meibomian Glands

Meibomian glands are currently recognized as a key element of the ocular surface morphofunctional unit, and MGs' dysfunction (MGD), "a chronic, diffuse abnormality of the meibomian glands, commonly characterized by terminal duct obstruction and/or qualitative/quantitative changes in the glandular secretion," is known to be the leading cause of DED.[26]

Validated objective assessment of MGs would be essential for DED patients' subclassification and stratification and to monitor the response to some treatments.[27] Moreover, in vivo imaging studies have demonstrated MG changes associated with aging[28,29] and contributed to a better understanding of inflammatory/hyposecretive DED[30,31] and other ocular surface diseases.[32,33]

Technological evolution provided us several noninvasive (or nearly noninvasive) tools,[34] including infrared (IR) meibography,[35] in vivo confocal microscopy (IVCM),[36] and OCT meibography.[37]

In vivo confocal microscopy not only has a great potential allowing for in vivo study of the ocular surface tissues at a cellular level but also has major limitations mainly due to the small field of view and to the lack of validated and user-friendly software for image analysis.[27] Moreover, IVCM analysis is purely morphologic, without any tissue and cellular phenotyping, and recently published data seriously challenge the interpretation of confocal images of MGs acinar units.[38]

Optical coherence tomography meibography, performed by a long coherence swept laser source, might provide interesting and novel tridimensional information, but at present, we have only preliminary data obtained with customized systems, and there is need to standardize this approach and to obtain validated biomarkers by this technology.[37,39]

Instead, IR meibography has been extensively studied, and its use is supported by a large body of evidence.[35] This type of in vivo noninvasive examination can provide objective and repeatable, qualitative and quantitative, assessment of MGs morphology.[35] Moreover, the recent development of software for automated measurement of MGs area[40] is facilitating its increasing use in clinical practice and research. Published data on this technique have demonstrated its use for patients' stratification and to predict and to monitor the response to treatments,[41–44] strongly supporting its potentials as a useful biomarker and surrogate endpoint for clinical research on MGD and other ocular surface diseases.

However, working on the validation of potential IR meibography-based biomarkers, we have to consider that, at present, this approach has some important limits, including the poor diagnostic accuracy for MGD (when it is taken alone, without functional data),[45] the relatively low resolution, and the still subjective interpretation of the "dark drop-out areas."[27]

Imaging to Assess Cornea and Ocular Surface Microanatomy

In vivo confocal microscopy allows clinicians and scientists to perform a minimally invasive, high-resolution, steady-state respectful assessment of the ocular surface at the cellular level.[46,47]

Systems currently available on the market are a white-light confocal microscope (Confoscan 4.0; Nidek Co. Ltd., Gamagori, Japan) and a laser scanning confocal microscope (HRT-III/Rostock Cornea Module; Heidelberg Engineering GmbH, Heidelberg, Germany). Both the instruments have been used in several studies

on DED and the latter one able to go beyond the cornea, exploring the limbus, the bulbar, and tarsal conjunctiva and the eyelids margin, providing interesting images of several components of the ocular surface morphofunctional unit.[46,47]

Interestingly, IVCM is able to provide information on three of the five key etiologic elements included in the revised DED definition proposed by the TFOS DEWS II[1]: the ocular surface inflammation and damage and the neurosensory abnormalities.

About the inflammation, confocal studies described several types and patterns of inflammatory cells,[48] including dendritic cells,[49] activated keratocytes,[50] and conjunctival[51] and eyelid inflammatory cells.[30,52] At present, central corneal subbasal dendritic cells, interpreted as antigen-presenting cells, seem to be the most promising potential confocal biomarker of inflammation. These cells, in addition to showing increased density in DED patients (especially in the most "inflammatory types" of DED),[49] seem to be useful to monitor the response to treatments and even to predict the response to some therapies.[53]

About the epithelial damage, the literature reports conflicting confocal data on corneal epithelial density[47] and, more interestingly, preliminary data on conjunctival goblet cell density and squamous metaplasia (cell areas and nucleocytoplasmic ratios), with good correlations between confocal and impression cytology results.[54–56]

In vivo confocal microscopy allows for qualitative and quantitative evaluations of corneal nerves, mainly of the subbasal nerve plexus. Confocal studies showed DED-related decrease of nerves' density and increase of nerves' tortuosity.[50,57–60] Moreover, confocal data suggested correlations among nerves' changes, corneal sensitivity, symptoms, and inflammatory cells density.[57–60]

In summary, IVCM offers an exciting bridge between clinical and laboratory observations, enabling clinicians and scientists to gain insight into alterations of the ocular surface microstructure.

However, at present, despite the progress achieved in image quantitative analysis, main issues limiting the use of IVCM-based biomarkers in clinical trials on DED include the limited availability of this technology, the lack of validated software for fully automated image analysis, and the lack of validated cutoff values.

CONCLUSIONS

Recent rapid technological evolution is providing several instruments for imaging assessment of the ocular surface in health and disease. However, the full understanding of new image meaning and the development and validation of imaging biomarkers requires more time.

At present, a few imaging approaches have been extensively studied, obtaining growing evidence supporting their use as potential biomarkers of tear film volume and stability, MGs, and ocular surface microanatomy.

Tools for NI-BUT, OCT and other high-tech meniscometry, IR meibography, and IVCM, taken together, are able to in vivo investigate, in a steady-state respectful manner, four key etiologic elements of DED (tear film instability, ocular surface inflammation and damage, and neurosensory abnormalities)[1] and to provide information on tear film volume and MGs state, crucial elements for DED subclassification.[1]

These imaging tools, increasingly used in clinical practice, might play an important role also in clinical research, providing non-invasive biomarkers (for patients' diagnosis and stratification) and surrogate endpoints. Major efforts are still needed, mainly aimed to improve automated acquisition and quantitative analysis, standardization (standard operating procedures, normative databases etc.), and validation of imaging biomarkers.

REFERENCES

1. Craig JP, Nichols KK, Akpek EK, et al. TFOS DEWS II definition and classification report. *Ocul Surf* 2017;15:276–283.
2. Wolffsohn JS, Arita R, Chalmers R, et al. TFOS DEWS II diagnostic methodology report. *Ocul Surf* 2017;15:539–574.
3. Roy NS, Wei Y, Kuklinski E, et al. The growing need for validated biomarkers and endpoints for dry eye clinical research. *Invest Ophthalmol Vis Sci* 2017;58:BIO1–BIO19.
4. Novack GD. Why aren't there more pharmacotherapies for dry eye? *Ocul Surf* 2014;12:227–230.
5. Villani E, Massaro D, Scaramuzzi M, et al. Decade-long profile of imaging biomarker use in ophthalmic clinical trials. *Invest Ophthalmol Vis Sci* 2017; 58:BIO76–BIO81.
6. Villani E, Vujosevic S. Foreword: Biomarkers and surrogate endpoints in ophthalmic clinical research. *Invest Ophthalmol Vis Sci* 2017;58:BIOi–BIOii.
7. FDA-NIH Biomarker Working Group. *Best (Biomarkers, Endpoints, and other Tools) Resource*. Available at: http://www.ncbi.nlm.nih.gov/books/NBK326791/. AU2
8. U.S. Department of Health and Human Services. *Clinical Trial Imaging Endpoint Process Standards: Guidance for Industry*. Available at: https://www.fda.gov/files/drugs/published/Clinical-Trial-Imaging-Endpoint-Process-Standards-Guidance-for-Industry.pdf.
9. Bron AJ, de Paiva CS, Chauhan SK, et al. TFOS DEWS II pathophysiology report. *Ocul Surf* 2017;15:438–510.
10. Chan TCY, Wan KH, Shih KC, et al. Advances in dry eye imaging: The present and beyond. *Br J Ophthalmol* 2018;102:295–301.
11. Goto T, Zheng X, Okamoto S, et al. Tear film stability analysis system: Introducing a new application for videokeratography. *Cornea* 2004;23: S65–S70.
12. Hong J, Sun X, Wei A, et al. Assessment of tear film stability in dry eye with a newly developed keratograph. *Cornea* 2013;32:716–721.
13. Gumus K, Crockett CH, Rao K, et al. Noninvasive assessment of tear stability with the tear stability analysis system in tear dysfunction patients. *Invest Ophthalmol Vis Sci* 2011;52:456–461.
14. Tian L, Qu JH, Zhang XY, et al. Repeatability and reproducibility of noninvasive keratograph 5M measurements in patients with dry eye disease. *J Ophthalmol* 2016;2016:8013621.
15. Best N, Drury L, Wolffsohn JS. Clinical evaluation of the oculus keratograph. *Cont Lens Anterior Eye* 2012;35:171–174.
16. Lee R, Yeo S, Aung HT, et al. Agreement of noninvasive tear break-up time measurement between tomey RT-7000 auto refractor-keratometer and oculus keratograph 5M. *Clin Ophthalmol* 2016;10:1785–1790.
17. Abdelfattah NS, Dastiridou A, Sadda SR, et al. Noninvasive imaging of tear film dynamics in eyes with ocular surface disease. *Cornea* 2015;34(Suppl 10):S48e52.
18. Fuller DG, Potts K, Kim J. Noninvasive tear breakup times and ocular surface disease. *Optom Vis Sci* 2013;90:1086–1091.
19. Akpek EK, Amescua G, Farid M, et al. Dry eye syndrome preferred practice Pattern®. *Ophthalmology* 2019;126:P286–P334.
20. Nichols KK, Mitchell GL, Zadnik K. The repeatability of clinical measurements of dry eye. *Cornea* 2004;23:272e85.
21. Ibrahim OM, Dogru M, Takano Y, et al. Application of visante optical coherence tomography tear meniscus height measurement in the diagnosis of dry eye disease. *Ophthalmology* 2010;117:1923–1929.
22. Akiyama R, Usui T, Yamagami S. Diagnosis of dry eye by tear meniscus measurements using anterior segment swept source optical coherence tomography. *Cornea* 2015;34(Suppl 11):S115–S120.
23. Canan H, Altan-Yaycioglu R, Ulas B, et al. Interexaminer reproducibility of optical coherence tomography for measuring the tear film meniscus. *Curr Eye Res* 2014;39:1145–1150.
24. Chan HH, Zhao Y, Tun TA, et al. Repeatability of tear meniscus evaluation using spectral-domain Cirrus® HD-OCT and time-domain Visante® OCT. *Cont Lens Anterior Eye* 2015;38:368–372.

25. Bartuzel MM, Szczesna-Iskander DH, Iskander DR. Automatic dynamic tear meniscus measurement in optical coherence tomography. *Biomed Opt Express* 2014;5:2759–2768.
26. Nelson JD, Shimazaki J, Benitez-del-Castillo JM, et al. The international workshop on meibomian gland dysfunction: Report of the definition and classification subcommittee. *Invest Ophthalmol Vis Sci* 2011;52:1930–1937.
27. Villani E, Arita R. Imaging of meibomian glands: From bench to bedside and back. *Eye (Lond)* 2019;33:695–697.
28. Arita R, Itoh K, Inoue K, et al. Noncontact infrared meibography to document age-related changes of the meibomian glands in a normal population. *Ophthalmology* 2008;115:911–915.
29. Villani E, Canton V, Magnani F, et al. The aging meibomian gland: An in vivo confocal study. *Invest Ophthalmol Vis Sci* 2013;54:4735–4740.
30. Villani E, Beretta S, De Capitani M, et al. In vivo confocal microscopy of meibomian glands in Sjögren's syndrome. *Invest Ophthalmol Vis Sci* 2011; 52:933–939.
31. Chen X, Utheim ØA, Xiao J, et al. Meibomian gland features in a Norwegian cohort of patients with primary Sjögren's syndrome. *PLoS One* 2017; 12:e0184284.
32. Suzuki T, Morishige N, Arita R, et al. Morphological changes in the meibomian glands of patients with phlyctenular keratitis: A multicenter cross-sectional study. *BMC Ophthalmol* 2016;16:178.
33. Arita R, Itoh K, Maeda S, et al. Meibomian gland duct distortion in patients with perennial allergic conjunctivitis. *Cornea* 2010;29:858–860.
34. Geerling G, Baudouin C, Aragona P, et al. Emerging strategies for the diagnosis and treatment of meibomian gland dysfunction: Proceedings of the OCEAN group meeting. *Ocul Surf* 2017;15:179–192.
35. Arita R. Meibography: A Japanese perspective. *Invest Ophthalmol Vis Sci* 2018;59:DES48–55.
36. Ibrahim OM, Matsumoto Y, Dogru M, et al. The efficacy, sensitivity, and specificity of in vivo laser confocal microscopy in the diagnosis of meibomian gland dysfunction. *Ophthalmology* 2010;117:665–672.
37. Yoo YS, Na KS, Byun YS, et al. Examination of gland dropout detected on infrared meibography by using optical coherence tomography meibography. *Ocul Surf* 2017;15:130–138.e1.
38. Zhou S, Robertson DM. Wide-field in vivo confocal microscopy of meibomian gland acini and rete ridges in the eyelid margin. *Invest Ophthalmol Vis Sci* 2018;59:4249–4257.
39. Liang Q, Pan Z, Zhou M, et al. Evaluation of optical coherence tomography meibography in patients with obstructive meibomian gland dysfunction. *Cornea* 2015;34:1193–1199.
40. Arita R, Suehiro J, Haraguchi T, et al. Objective image analysis of the meibomian gland area. *Br J Ophthalmol* 2014;98:746–755.
41. Turnbull PRK, Misra SL, Craig JP. Comparison of treatment effect across varying severities of meibomian gland dropout. *Cont Lens Anterior Eye* 2018;41:88–92.
42. Finis D, König C, Hayajneh J, et al. Six-month effects of a thermodynamic treatment for MGD and implications of meibomian gland atrophy. *Cornea* 2014;33:1265–1270.
43. Arita R, Morishige N, Shirakawa R, et al. Effects of eyelid warming devices on tear film parameters in normal subjects and patients with meibomian gland dysfunction. *Ocul Surf* 2015;13:321–330.
44. Arita R, Suehiro J, Haraguchi T, et al. Topical diquafosol for patients with obstructive meibomian gland dysfunction. *Br J Ophthalmol* 2013;97: 725–729.
45. Finis D, Ackermann P, Pischel N, et al. Evaluation of meibomian gland dysfunction and local distribution of meibomian gland atrophy by non-contact infrared meibography. *Curr Eye Res* 2015;40:982–989.
46. Villani E, Baudouin C, Efron N, et al. In vivo confocal microscopy of the ocular surface: From bench to bedside. *Curr Eye Res* 2014;39:213–231.
47. Matsumoto Y, Ibrahim OMA. Application of in vivo confocal microscopy in dry eye disease. *Invest Ophthalmol Vis Sci* 2018;59:DES41–DES47.
48. Mantopoulos D, Cruzat A, Hamrah P. In vivo imaging of corneal inflammation: New tools for clinical practice and research. *Semin Ophthalmol* 2010;25:178–185.
49. Kheirkhah A, Rahimi Darabad R, Cruzat A, et al. Corneal epithelial immune dendritic cell alterations in subtypes of dry eye disease: A pilot in vivo confocal microscopic study. *Invest Ophthalmol Vis Sci* 2015;56: 7179–7185.
50. Villani E, Galimberti D, Viola F, et al. The cornea in Sjogren's syndrome: An in vivo confocal study. *Invest Ophthalmol Vis Sci* 2007;48: 2017–2022.
51. Wakamatsu TH, Sato EA, Matsumoto Y, et al. Conjunctival in vivo confocal scanning laser microscopy in patients with Sjögren syndrome. *Invest Ophthalmol Vis Sci* 2010;51:144–150.
52. Matsumoto Y, Shigeno Y, Sato EA, et al. The evaluation of the treatment response in obstructive meibomian gland disease by in vivo laser confocal microscopy. *Graefes Arch Clin Exp Ophthalmol* 2009;247:821–829.
53. Villani E, Garoli E, Termine V, et al. Corneal confocal microscopy in dry eye treated with corticosteroids. *Optom Vis Sci* 2015;92:e290–5.
54. Hong J, Zhu W, Zhuang H, et al. In vivo confocal microscopy of conjunctival goblet cells in patients with Sjögren's syndrome dry eye. *Br J Ophthalmol* 2010;94:1454–1458.
55. Villani E, Beretta S, Galimberti D, et al. In vivo confocal microscopy of conjunctival roundish bright objects: Young, older, and Sjögren subjects. *Invest Ophthalmol Vis Sci* 2011;52:4829–4832.
56. Kojima T, Matsumoto Y, Dogru M, et al. The application of in vivo laser scanning confocal microscopy as a tool of conjunctival in vivo cytology in the diagnosis of dry eye ocular surface disease. *Mol Vis* 2010;16: 2457–2464.
57. Benitez del Castillo JM, Wasfy MAS, Fernandez C, et al. An in vivo confocal masked study on corneal epithelium and subbasal nerves in patients with dry eye. *Invest Ophthalmol Vis Sci* 2004;45:3030–3035.
58. Benitez del Castillo JM, Acosta MC, et al. Relation between corneal innervation with confocal microscopy and corneal sensitivity with noncontact esthesiometry in patients with dry eye. *Invest Ophthalmol Vis Sci* 2007;48: 173–181.
59. Villani E, Galimberti D, Viola F, et al. Corneal involvement in rheumatoid arthritis: An in vivo confocal study. *Invest Ophthalmol Vis Sci* 2008;49: 560–564.
60. Villani E, Magnani F, Viola F, et al. In vivo confocal evaluation of the ocular surface morpho-functional unit in dry eye. *Optom Vis Sci* 2013;90: 576–586.