REDESIGNING THE MANAGEMENT AND TREATMENT ALGORITHM FOR DRY EYE CLINICIANS

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Doctor of Philosophy

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Thesis abstract

Dry eye disease multifactorial aetiologies and complexity makes a single treatment not functional enough to manage different subtypes. Instead, a tailored-managed-therapy plan is needed to lead to an effective treatment of signs and symptoms.
An organisation tool to manage dry eye disease was created in 2017 by Tear Film & Ocular Surface Society Dry Eye WorkShop II, yet no differentiation between disease subtype (evaporative dry eye or aqueous dry eye) or severity, was made as a cause of lack of level 1 studies.
This thesis sought to compile a series of experimental studies to provide; Additional scientific evidence of management strategies (Chapter 1:), by the means of; identifying therapeutic effects from all different treatment stages (Chapter 2:), assessing if further modifications of this guideline are needed (Chapter 3:) and to disseminate the current prescribing patterns of therapies across the globe (Chapter 4:).

Accordingly, this thesis has found that:

- Dry eye is being managed worldwide similarly to TFOS DEWS II recommendations, with exception of home-made facecloths.
  Products prescribed for evaporative dry eye and aqueous dry eye are different.
  North American and Asia/Middle Eastern regions tend to use a more pharmacological approach at lower levels of dry eye disease severity.

- Between liposomal drops, liposomal sprays and emulsion-drops, the decrease in symptomatology was similar, no statistically significant changes happened to homeostatic markers during a 2-week treatment.

- Only lipid-based artificial tear provides relief for patients with the evaporative subtype of dry eye, and both lipid-based and a non-lipid based artificial tears, show a 1-month symptomatology decrease for both dry eye subtypes, with signs taking 3-4 months to show a statistically significant improvement.

- The use of an automatic massaging-mask provides improvement in symptomatology (subjectively improving severity) after 2 weeks of mask-treatment twice a day.

- Eyelid warming therapy, improves Meibomian gland expression quality, regardless of its dropout extent. Debridement helps removing orifice obstruction in patients with Meibomian gland dropout, yet forcible expression provides nonadditional benefit on partial MGs.

Keywords: dry eye disease, algorithm, management, therapy, treatment, artificial tears, eye mask, meibomian gland dysfunction, cardiovascular health, eye care practitioners.

Dedication

Per la meva família, que sempre heu estat al meu costat acompanyant-me en cada moment, donant-me suport durant aquests 4 anys tant complicats.
L´ajuda incondicional a cada pas durant aquesta educació professional.
Per ensenyar-me aquesta energia, tranquil·litat, confiança, manera de ser, fer i estimar.
La vida no es perfecte, però vosaltres, la meva pinya, m´heu donat el millor possible.
Sense el vostre amor no seria aquí. Mil gràcies per aquesta vida.
"Oblida el que et va fer mal, però mai oblidis el que et va ensenyar".

To my family, for always being by my side, supporting me during these 4 very challenging years.
For the inconditional help in every step of this professional education.
For teaching me how to have this energy, comfort, confidence, way to be and way to love.
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Without your love I would not be where I am today. Thank you for this life.
"Forget what hurt you, but never forget the lessons learned".

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List of abbreviations

AAS - Autologous/allogeneic serum

ADDE – Aqueous dry eye disease

AM - Amniotic membranes

AT - Artificial tears

BCL – Bandage Contact Lens

BAK - Benzalkonium

CAM - Commercially available warm lid compress/face mask

CIE – Cascade of inflammatory events

CL - Contact lens

CLD – Contact lens discomfort

CONSORT - Consolidated Standards of Reporting Trials

CSA - Cyclosporine A

CT - Clinical trials

CLW - Contact lens wearers

Conj. – Conjunctival

DE – Dry eye

DEC – Dry eye clinic

DED – Dry eye disease

DEM – Dry eyes mist

DEQ-5 – Dry Eye Questionnaire - 5

DEWS – Dry eye WorkShop

ECP – Eye care practitioner

EDE – Evaporative dry eye disease

EES – Ectoin eye spray

EFA - Essential fatty acids

EUn - Emustil unidose

GVHD - Graft versus host disease

HK - Hong Kong

HPMC - Hydroxypropyl methylcellulose

HPO+ - Hyperosmolarity

HVPL - High viscosity enhancing preserved lubricants

HVUL - High viscosity enhancing unpreserved lubricants

HWC - Homemade warm lid compress

IP - Intraductal probing

IPr - Independent prescriber

IPL - Intense pulsed light

IR - Infrared light

IVCM - In-vivo corneal nerve microscopy

GVHD – Graft versus host disese

LcL - Lipid containing lubricants

LFA-1 - Lifitegrast

LG – Lissamine green

LIPCOF – Lid-parallel conjunctival folds

LLT – Lipid layer thickness

LMD - Lid margin debridement

LOLG - Liposic ophthalmic liquid gel

LVPL - Low viscosity enhancing preserved lubricants

LVUL - Low viscosity enhancing unpreserved lubricants

LWE – Lid wiper epitheliopathy

LwS - Lid wipes/scrubs

NaFL – Fluorescein

NSAID - Non-steroidal anti-inflammatory drugs

MCG - Moisture chamber spectacle/goggles

MGE - Therapeutic meibomian gland expression

MG – Meibomian gland

MGD – Meibomian gland dysfunction

NaFl – Flourescein soduim

NIBUT – Non-Invasive breakup time

NIKBUT - Non-Invasive keratometric breakup time (on oculus keratograph 5m)

OAb - Oral antibiotics

OAS - Optrex actimist spray

ODC - In office demodex lid control

OI - Ointment

OLH - In office lid hygiene

OP - Optive plus

OS - Oral secretagogues

OSA -Other surgical approaches

OSD – Ocular surface disease

OSDI – Ocular surface disease index

OSH - Ocuvers spray hyaluron

OTC - Over the counter

OTP - In office thermal pulsation of lids

PEG 400 - Polyethylene glycol 400

PI - Principal investigator

PO - Punctal occlusion

PRP - Autologous platelet rich plasma

QoL - Quality of Life

RCT - Randomised control trial

RF – Risk factors

RGP – Rigis gas permeable (Contact lens)

RET – Refresh eye therapy

ROAU - Refresh optive advance unidose

ROSU - Refresh optive sensitive unidose

ROM - Refresh optive advance multidose

ROAM - Refresh optive advance multidose

RU - Refresh ultra

SANDE - Symptom assessment questionnaire in dry eye

SAz - Oral azythromicin

SH - Sodium hyaluronate

SL – Slit lamp

SS - Sjögren syndrome

STH – Clinicas soothe

Sy – Systane

SB – Systane balance

SSS - Saline solution spray

TA – Tears again

TAb - Topical antibiotic

TAS - Tears again spray

TAz - Topical azythromicin

TBUT- Tear breakup time

TCl - Therapeutic contact lenses

TCo - Topical corticosteroids

TCy - Topical cyclosporine

TFBUT - Tear fluorescein breakup time

TFOS – Tear film and ocular surface

TLF - Topical lifitegrast

TLL – Tear lipid layer

TM – Tear mist

TMH – Tear meniscus height

TPGS – Polyethylene glycol succinate

TS - Topical secretagogues

TTa - Topical tacrolimus

TT – Thera tears

TTO - Tea tree oil

UNSW - University of New South Wales

VDU - Video display unit

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Chapter 1: General introduction. Focused on dry eye disease management.

1.1 Definition and classification

Dry eye disease (DED) is a common condition affecting the ocular surface and adnexa, it results in an increment of osmolarity which leads to a cascade of inflammatory events (CIE) in the ocular surface (1). Its prevalence ranges from approximately 5% to 50% of the population (studies with symptoms with or without signs), and up to 75% if based primarily on signs (2).

The prevalence, aetiology, management, and therapy of this condition has been a challenge for researchers and practitioners for more than two decades. Thanks to Tear Film and Ocular Surface (TFOS) Dry Eye WorkShop (DEWS) report in 2007 (3), the definition of dry eye started to become more specific regarding the pathophysiologic basis of the disease, including tear hyperosmolarity and ocular inflammation alongside the influence on visual function (3). Symptoms were considered as the central feature of the disease. It was the first time that dry eye condition was formally defined as a disease. Because of the ongoing research, a better understanding of the condition has been obtained. Tear Film and Ocular Surface (TFOS) Dry Eye WorkShop (DEWS) II team created a second report after 10 years of research, creating a state-of-the-art evidence of dry eye disease in different areas such as disease classification, epidemiology, tear film, pathophysiology, diagnosis, and treatment of the disease, and also on the role of hormones, pain and sensations. In this report DED has been defined as:

"A multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film, and accompanied by ocular symptoms (4-6), in which tear film instability (7-9) and hyperosmolarity (10-13) ocular surface inflammation and damage (14), and neurosensory abnormalities (15) play etiological roles."

In comparison to the first report, the 2017 TFOS DEWS II report, mentions neurosensory abnormalities, introduces the new term "loss of homeostasis" and clarifies that hyperosmolarity of the tear film and inflammation of the ocular surface play a causal etiological role, considering all aspects of tear imbalance (16).

DED can be differentiated from other ocular surface disease (OSD) with the use of triaging questions, which are part of a normal history and symptoms assessment, along with the determination of dry eye risk factors. However, eye care practitioners may not be the first port of call for patients with ocular symptoms, so the triaging questions

introduced for the first time in TFOS DEWS II can aid pharmacists and GPs to manage such patients more appropriately.

In the TFOS DEWS II report from 2017 a new algorithm scheme regarding clinical decisions has been made for the purpose of a clear classification. In the diagram (*Figure 1.1)* the left part of the oriented flow chart has been subdivided in to two major clinical differences: If the patient presents with symptoms or if the subject is asymptomatic. Starting with the assessment of the symptomatology and following with triaging questions and secondary tests to differentiate from OSD (17).

1.1.1 Symptomatic patient with signs

Further testing is needed to identify if there are other comorbidities mediating that could hinder the disease, for that triaging questions, risk factors, symptomatology questionnaires and specific homeostasis measurements need to be performed (17).

1.1.2 Symptomatic patient without demonstrable clinical signs

This type of patients could not fall into the DED group but might become sufferers in the future.

1.1.2.1 Early stage or pre-clinical DED

Even if signs are not present, patient's symptomatology being consistent with DED, could indicate an episode of DE or a pre-clinical state (16). In both cases, it should be monitored as the condition could further develop and signs could be visible with time. Eye care practitioners (ECPs) should education the patients on the disease progression and its prevention.

1.1.2.1.1 Neuropathic pain

Neuropathic sensation comes from a lesion within the somatosensory nervous system. It might cause associated pain, which only in about ~5% of the patients comes from the ocular surface. In most cases the sensation is not triggered by the ocular surface. In this status the pain disproportionally outweighs the signs (15), therefore a DE treatment could be ineffective, and general pain management referral is needed.

1.1.3 Asymptomatic patient with signs of OSD

This type of patients requires careful examination of the OS as some type of management might be required.

1.1.3.1 Predisposition to DED

In this prodromal stage, time and provocation could lead to the development of DED symptoms. In this case preventative management should be used.
An example could be treating blepharitis, which in a pre-operative anterior eye assessment of cataract or refractive surgery might prevent the patient of being at risk of developing DED symptoms. Having this type of DED can adversely affect refractive surgery outcomes, especially in patients with decreased corneal sensation where wound healing can be impaired. An increased risk of infection and complications with ocular surgery can be associated with ocular morbidity, especially in patients with connective tissue disorders, such as rheumatoid arthritis, lupus or secondary sjögren's syndrome (SS) (18).

1.1.3.2 Reduced corneal sensitivity

In longstanding ocular surface disease (OSD) cases, corneal sensitivity decreases, by which discomfort is masked and corneal nerve damage appears. This masked discomfort renders the patient asymptomatic (19). Other types of corneal disease, where sensitivity has decreased, could also lead to this dysfunctional sensation.
In these cases, the ECP should consider whether DE management needs to be considered or a referral to a corneal specialist is needed.

1.1.4 Asymptomatic without signs

In these cases, the patient does not require any treatment, and general advice on ocular health should be given as well as explanations on when to seek ECP's help.

1.1.5 Differentiation

In the right part of the chart (*Figure 1.1*) the aetiologies of DED; aqueous dry eye disease (ADDE) and evaporative dry eye disease (EDE) can be seen. Several researchers have reported that EDE is more common than ADDE (2, 20-22).

The issue in classification arises from the differentiation of the two main subtypes EDE and ADDE. DED prevalence was proven to be three times more likely to be subclassified as EDE than ADDE, and over 30% of the patients were diagnosed having a mixture of both types (20).
It has been reported that the most common cause of EDE is meibomian gland dysfunction (MGD), being defined as a;

"*Chronic*, *diffuse abnormality of the meibomian glands*, *commonly characterized by terminal duct obstruction and/or qualitative/quantitative changes in the glandular secretion. MGD may result in alteration of the TF*, *symptoms of eye irritation, clinically apparent inflammation*, *and OSD (23)"*

Many cases of DED are likely to be a mixture of both EDE and ADDE with overlapping signs. Conditions affecting the lid, such as; blink abnormalities and MG changes, tend to classify as EDE, and conditions affecting the ocular surface, mucin deficiency, contact lens wear or the lacrimal gland (Sjögren or non-Sjögren) tend to classify as ADDE (2, 16, 24). Thus, a diagnostic continuum was found, demonstrating a possibility in mixture of the two subtypes, as an overlapping of signs (25, 26). Specifically, as the disease progress, both components start to be clinically more apparent (16). Even Vitali et al., in 2002, whilst writing about the criteria for Sjögren syndrome classification, mentioned that particularly in early stages of the disease the previous classification criteria could lead to a misclassification (27).

A new classification created by TFOS DEWS II accommodates patients with conflict between signs & symptoms, including even a diagnosis of "normal" (16).

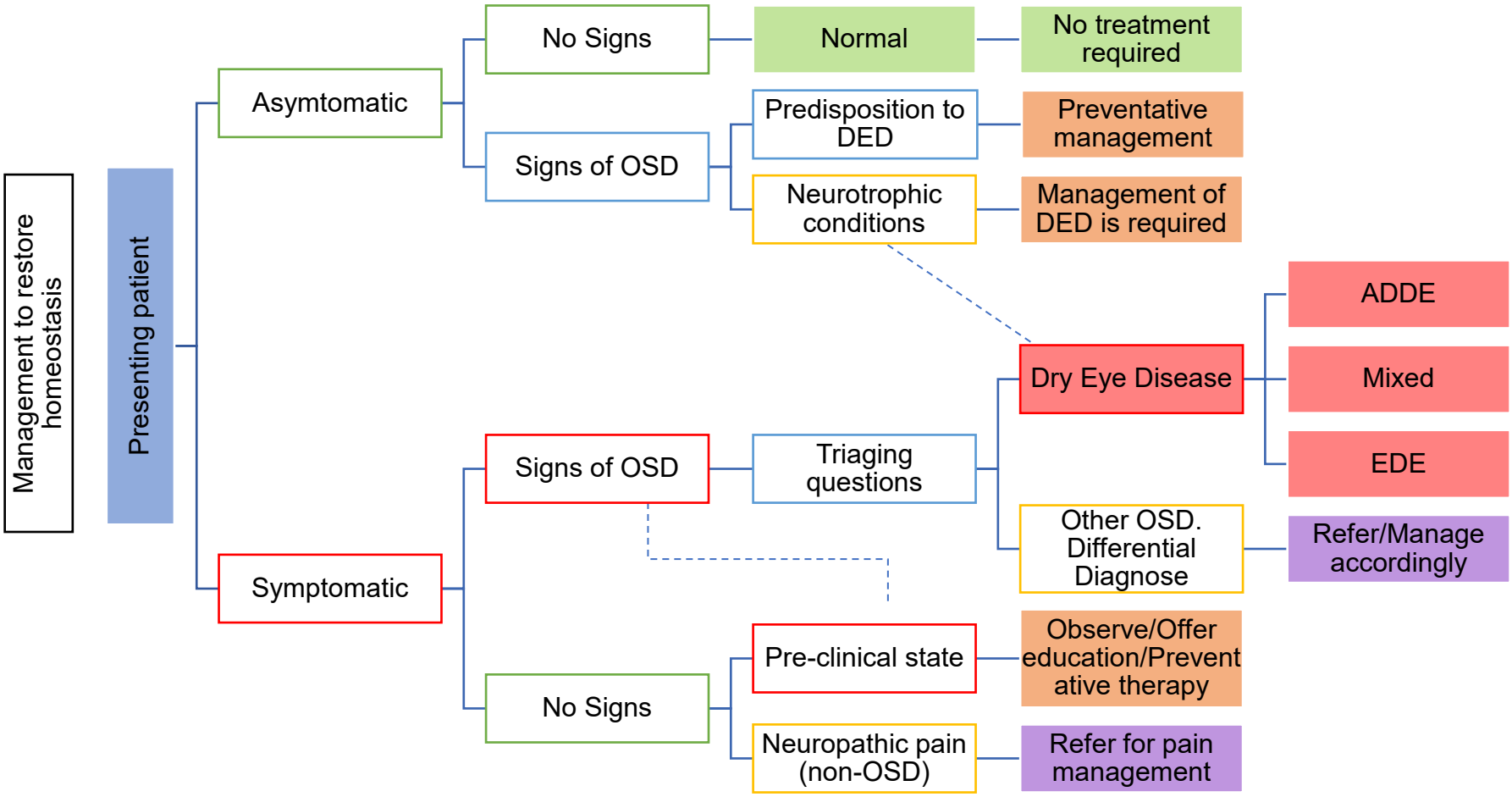

Figure 1.1. Illustration of the classification of dry eye disease (Adaptation from TFOS DEWS II)

1.2 Epidemiology

Epidemiology remains a challenge due to a lack of standardisation of a diagnosis. A decade and a half ago, the prevalence of DED was reported to be from 5% to 35% (24), on population aged 50 or older, but recently, in the latest epidemiology review, the range has been extended to a suggested 5-50% (2). In terms of OSD and with the presence of signs, the prevalence increases even more, reaching up to 75% (2).

1.3 Risk factors

For more than a decade now risk factors (RF) had been divided into Conclusive, Probable and Inconclusive.

In this report just the conclusive RF are stated, which have been divided into:

- Non-modifiable:
    - Increasing age has been tested to be the most consistent RF to increase the potential development of DED (28-34).
      A later result of a British female twins' meta-analysis study proved that apart from escalating with age the signs exhibit a greater gain per decade than the symptoms, peaking at 40-50 years old (34).
    - Female gender has also been verified to be a significant RF. A significantly higher percentage of women compared to men, have DED, especially in older age groups (2, 30-32, 35).
    - Asian ethnicity has been proved as well to be a conclusive RF with an increased risk of odds ratio of 1.5-2-2x, even though the reason remains unclear yet. In England Vehof et al in 2014 reported a 20.8-point score prevalence on signs & symptoms (34) whilst Tan et al from Singapore in 2015 reported a 30.1 (36).
    - Meibomian Gland Dysfunction (MGD); There is limited epidemiologic prevalence since TFOS MGD epidemiology report (37) found lack of agreement in classification and definition.
    - Sjögren syndrome ia an autoimmune chronic disorder, which affects the lacrimal glands in the eye inducing ADDE. From all the ADDE patients it is estimated that about 10% might be suffering from Sjögren syndrome (38).

- Modifiable:

    - Hormonal imbalance replacement, specifically after menopause and androgen deficiency during ageing which specifically increases the risk to develop DED (39). The lack of androgen promotes MGD as it affects the lipid content which leads to a more EDE (40-42).
    - For visual display users (VDU) the reduced amount of blinking rate (43) and/or blink incompletion together with the corneal exposure, has been hypothesised as a probable mechanism for dryness whilst using VDUs. When the blinking rate is reduced, the OS is exposed to the atmosphere increasing the amount of evaporation, leading to a more frequent TF instability and possible ocular damage and subjective symptomatology (44-46). The highest prevalence has been found in young adults (47-50).
    - According to population-based studies DED is 4 times more prevalent in contact lens wearers (CLW) (35, 36, 51-53) and a higher prevalence on severe DED symptomatology was associated to CLW (48). DED is also associated with CL intolerance and discontinuation of CL wear as it impacts on the OS homeostasis (54).
    - Environment factors such as pollutions, high altitude, wind, and low humidity are RFs for DED. A couple of studies proved that in metropolitan areas the amount of prevalence of DED is higher than in rural areas but there is still unknowns' of how these environmental factors specifically affects the DED (55-59).
    - Medications such as antihistamines, isotretinoin, anxiolytics, and antidepressants, reported a higher incidence of DED (19, 60). The possible approach to reduce/eliminate the side effects include 'changing the route of administration from oral to topical, discontinuation of the drugs, dose adjustments, switching to another medication or more aggressive management of the induced dry eye' (19).
    - The most common manifestation after haematopoietic stem cell transplantation (GVHD – graft attacks the host following transplantation) is a lacrimal gland destruction apart from severe MGD (61).

Other factors such as the effect of digital devices and impact of climate, specifically on young adults have not clearly been defined (2).

1.4 Core mechanisms of dry eye.

1.4.1 Tear Hyperosmolarity and tear film instability. The vicious cycle

In the most simplistic model, tear hyperosmolarity (HPO+) can be considered as the starting point of the disease, nevertheless, a patient can enter in the vicious cycle of DED at any point as ocular inflammation can be due to different disorders e.g. allergies, loss of goblet cells, xerophthalmia, altered mucin expression and topical preservative toxicity (62). (Figure *1.2*).

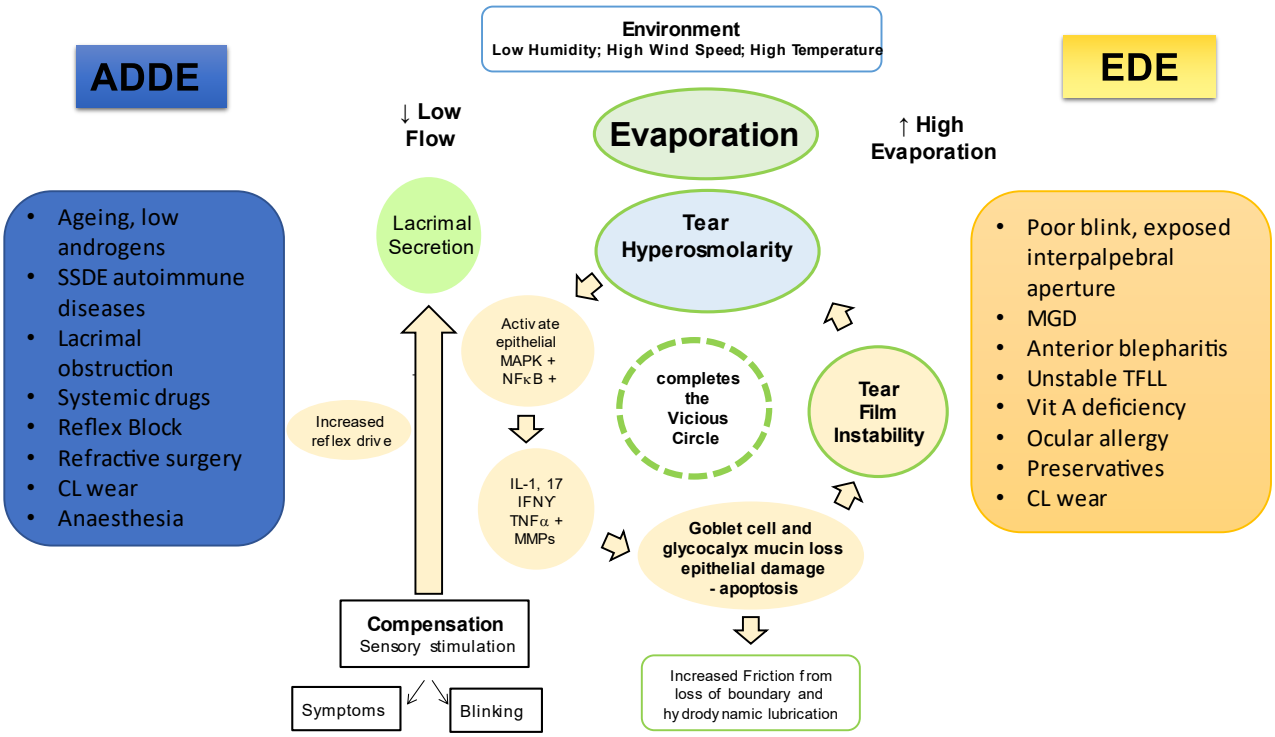

Figure 1.2. Vicious cycle of dry eye. Adaptation from Bron et al. TFOS DEWS II Pathophysiology Report

In both DE subtypes the main indicator of DED is TF HPO+ which arises from tear evaporation.

This HPO+ directly damages the ocular surface by starting a cascade of warning events in the surface epithelial cells and indirectly by initiating inflammation (1, 63), creating symptomatology (64). When inflammatory mediators and HPO+ of the tear film coexist, goblet cell loss (65, 66) and epithelial cell apoptosis occur (64, 67) by damaging the expression of glycocalyx mucins. Goblet cell loss is a feature of both forms of DED (65, 68). The inflammatory mediators gathered in the ocular surface reinforce the epithelial damage causing tear film instability. Once the TF stability is compromised, the friction between the lids and the globe increases after each blink, which causes the

characteristic punctate epitheliopathy, lid wiper epitheliopathy and further symptomatology. The reduction in tear volume and decrease in surface wettability, leads to an early tear breakup, which exacerbates tear hyperosmolarity, completing the cycle of vicious events causing ocular surface damage.
If the vicious cycle remains untreated, the condition stimulates corneal nerve endings, generating symptoms of discomfort, increased blinking rate and possibly a compensatory increased reflex drive in lacrimal tear film stimulation and secretion.

It has been proven that tear HPO+ can provide a clinical homeostasis marker for DED severity as it does correlates well (69, 70). Measurement of tear osmolarity is therefore a diagnostic test for clinical use that has potential to be used as an efficient, quantifiable biomarker for dry eye disease, in addition to the five commonly used signs and symptoms tests (NIBUT, corneal, conjunctival and lid margin staining and dry eye symptoms questionnaires) (17, 71).

Even though osmolarity has been chosen as a diagnostic test for hallmarking DED, there are still interstudy variations (72) for specific biomarkers which accentuates the need for a more detailed reporting of the methodology as information on subject characteristics, quality control and analysis methods to ameliorate non-subjective metrics.

Other areas for DED diagnostics are in development specifically on proteomics, inflammatory markers (73), and in-vivo corneal nerve imaging (IVCM) predictive biomarkers *(Figure 1.3)* (74, 75).

The development of homeostasis biomarkers can help in clinical research and eye care practitioners in moving forward in our profession with faster detection and control of therapeutic results.

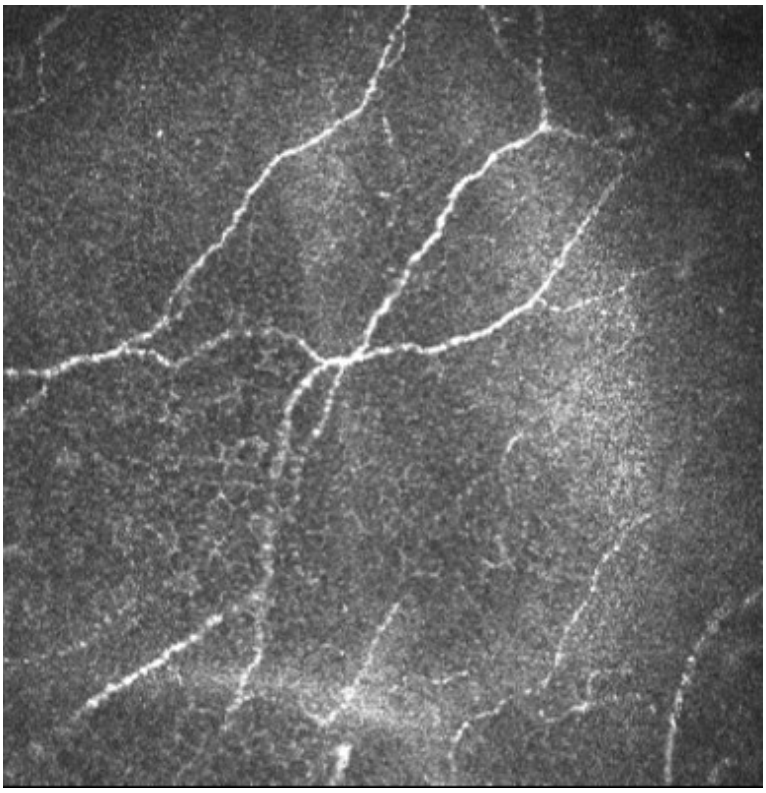

Figure 1.3. In vivo corneal microscopy. Image taken in Aston University dry eye clinic

1.5 Severity and grading

A serious clinical weakness is still tangible upon DED severity grading DED clinical features do not show a strong association with symptoms (4, 76-78). In general, there used to be a lack of uniformity in testing procedures, standardised dyes, protocols and consistency of recorded data and results, which has improved with the TFOS DEWS II guidelines (*Figure 1.4*), yet up to date there is no gold standard for grading the severity of the disease. Symptoms alone are not sufficient for a clinical diagnosis and a management plan for treating the disease (79, 80).

Even TFOS DEWS II – Clinical Trial design report mentioned the distinct difficulty in comparing results from clinical trials (CT) (81). “DE is a disease entity where there is a constellation of symptoms or even, a specific condition which might be associated with other conditions”. With such broad conceptual approach result comparison is much more challenging (81).

Should we ask ourselves if tackling separately signs in one study and symptoms in another would help us better understanding how patients feel instead of reporting the condition as a whole?

What was clear was the need for a consensus of clinical signs that would better reflect all aspects of the disease, and that is what TFOS DEWS II recommended.

For the purpose of selecting a treatment, the use of signs and symptoms, together with the subtype classification can help to tailor the treatment plan.

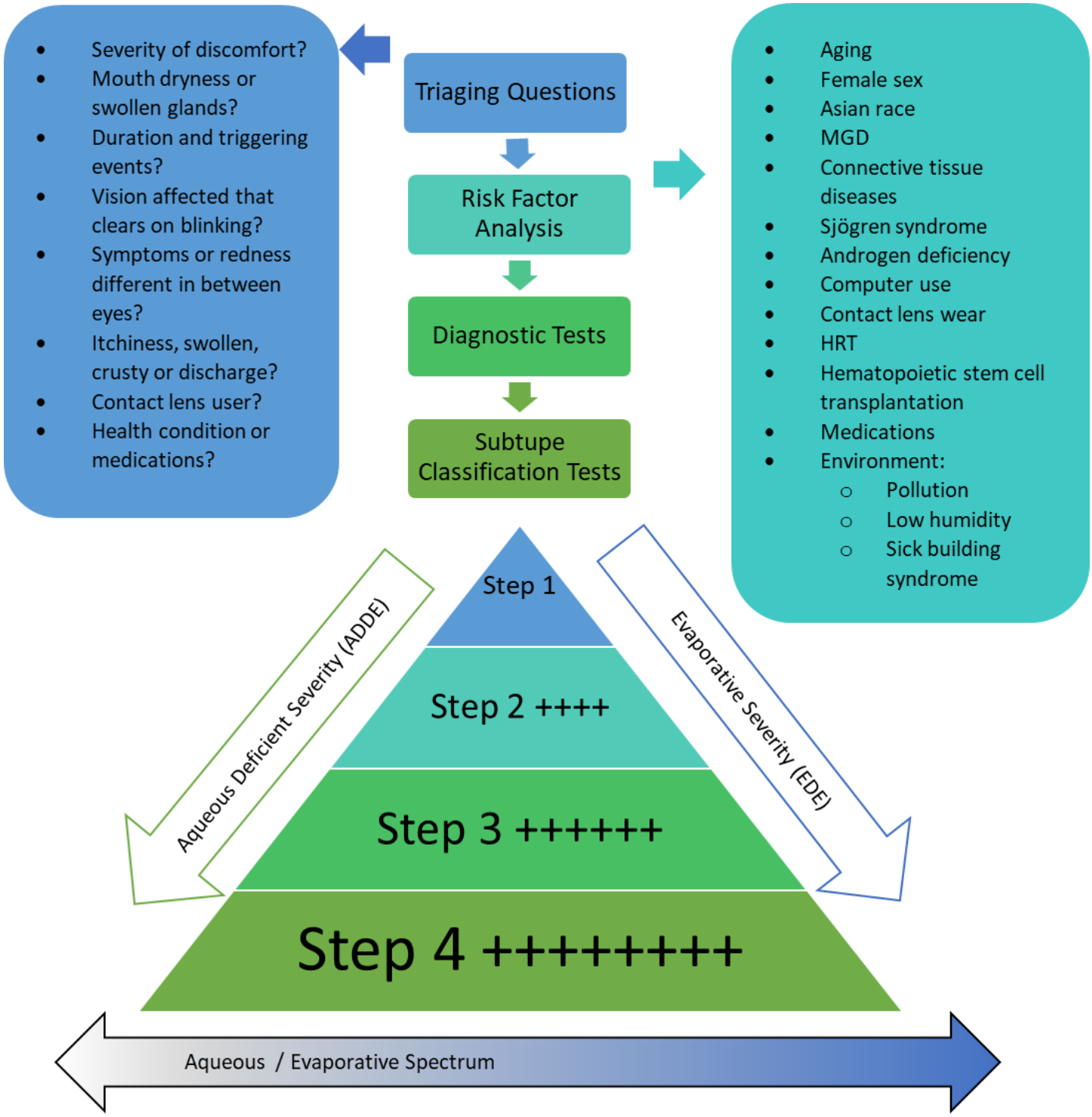

Figure 1.4. Diagrammatic representation of the process associated with assessing and step treating DED (Adaptation from TFOS DEWS II).

1.5.1 Subclassification

Before managing DED, a diagnosis and subtype classification must be made in order to understand which treatment can provide a greater benefit. Several epidemiological studies have reported that evaporative dry eye (EDE) is approximately three times more common than aqueous deficiency dry eye (ADDE), and over 30% of the patients are diagnosed with a mixture of both subtypes overlapping on the dry eye spectrum (20, 22). In fact, it has been observed in population-based studies that MGD (*Figure 1.7)* is the leading contributor cause to EDE (20, 82, 83).

Once DED has been diagnosed, determining the subtype is the following step to treatment success. Even though the diagnostic value of the following tests requires further study and has not been established in DED, TFOS DEWS II diagnostic report (17) mentions them due to its value in assisting to subtype classification:

For ADDE:

- Tear volume (meniscometry measurement – volume or height) by either slit lamp beam height compared to tear meniscus height (estimation) or more reliably by using anterior OCT calliper imaging (quantification). Normality ≥0.20mm (*Figure 1.5*) (17).

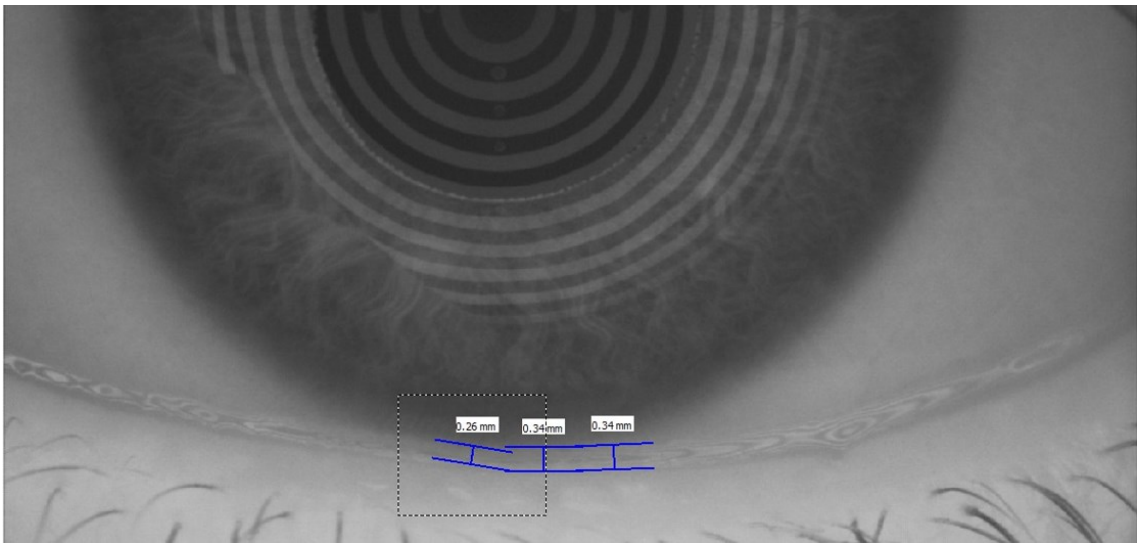

Figure 1.5. Tear meniscus height. Image taken in Aston University Dry Eye Clinic

- Schirmer test without anaesthetic by measuring the wetted length from the notch after 5 minutes. Normality ranges from ≤5 mm/5 min (84) to ≤10mm/5 min (85).

For EDE:

- Duct observation, meibum quantity, quality and expressibility reflect Meibomian gland function by the application of a known force through the eyelid and the evaluation of its lipid output. Slit lamp can be used and Meibomian gland expressor or digital force can be used to apply pressure. Normal lipid volume excreta should form an oil-dome, with a clear-fluid consistency upon expression (86-88) during 10-15 seconds (88).

- Assessment of blepharitis/demodex with slit lamp to discriminate Meibomian gland dysfunction (MGD) from blepharitis, as it is a contributor of DED symptomatology.

- Interferometry: performed with a broadband illumination that allows visualisation of the lipid layer kinetics, shows different fringe patterns depending on the lipid layer thickness (LLT). Useful instruments are LipiView *(Figure 1.7),* DR-1 system or Tearscope. Range of normality for LLT is approximately 20 to 60nm (89, 90) or a closed meshwork/wave pattern (number 2-3) on Keeler-Guillon scale (91, 92).

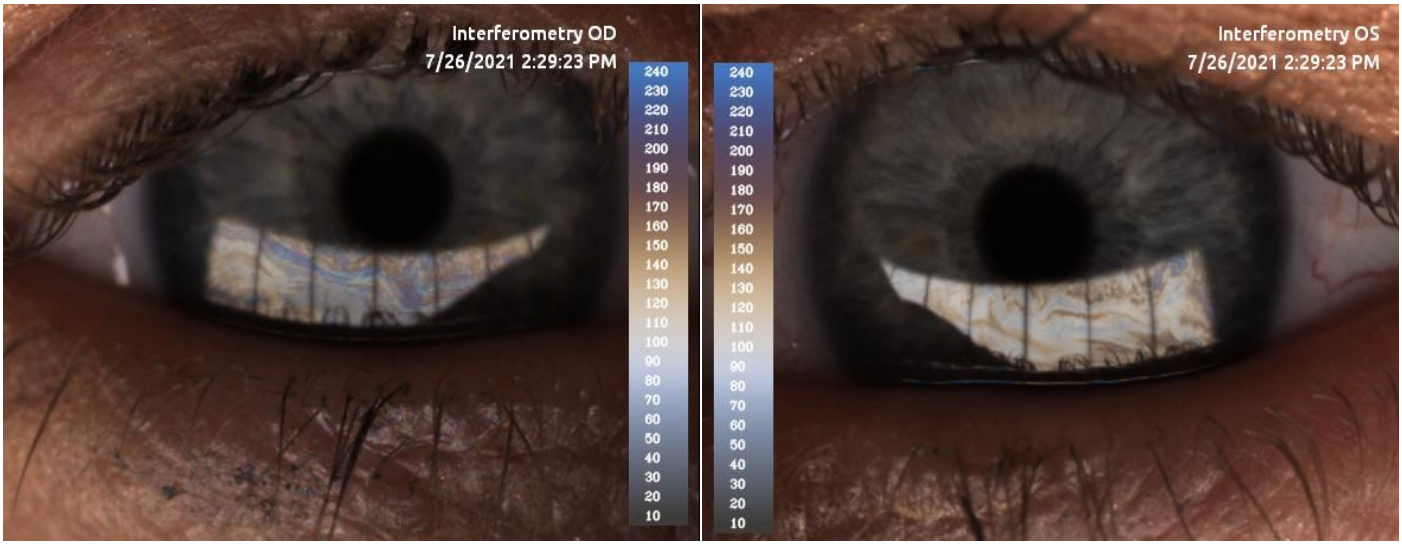

Figure 1.6. Interferometry example of a colorful fringe pattern (Keeler-Guillon grading scale) [5]. Thickness higher than average on both eyes. Image taken in Aston University with LipiView.

- Meibography (*Figure 1.7*) aids on understanding morphology and silhouette of the Meibomian glands (MG) under infrared light to determine shortening and/or gland dropout. Normality would be seeing hyper-illuminated regions which refer to the MGs and having no dropout or gland shortening (93).

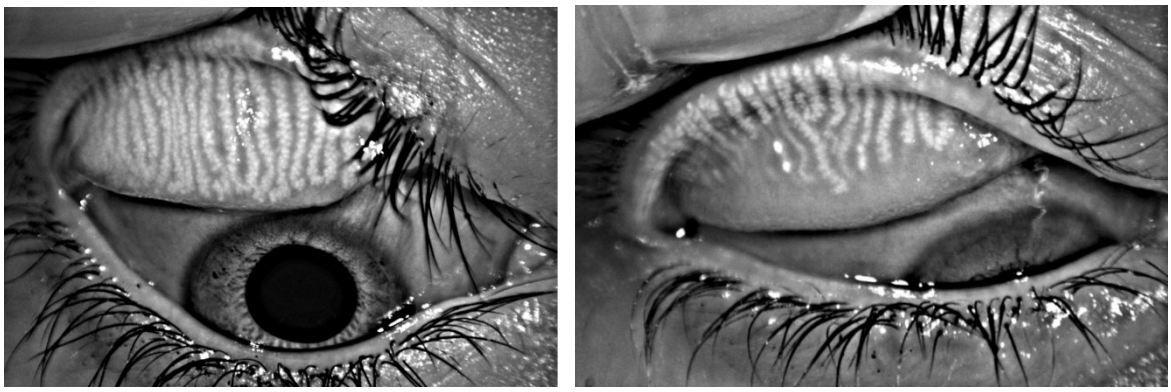

Figure 1.7. Meibomian glands under infrared light. Left picture: Full extent of Meibomian glands, Right picture: Shortening and dropout of Meibomian glands. Images taken on Aston University Dry Eye Clinic with Corneal Analyser 800 (Topcon).

- Blink analysis assessment can be observed with a slit lamp using fluorescein and perceiving a dark line in between two areas of fluorescein indicating the lower limit of the upper lid after a blink. It can also be assessed with a high-speed camera and even smart phones. In order to avoid blinking stimulation, infrared light (IR) should be used (17). Normality is reported between 10-15 complete blinks per minute (94, 95).

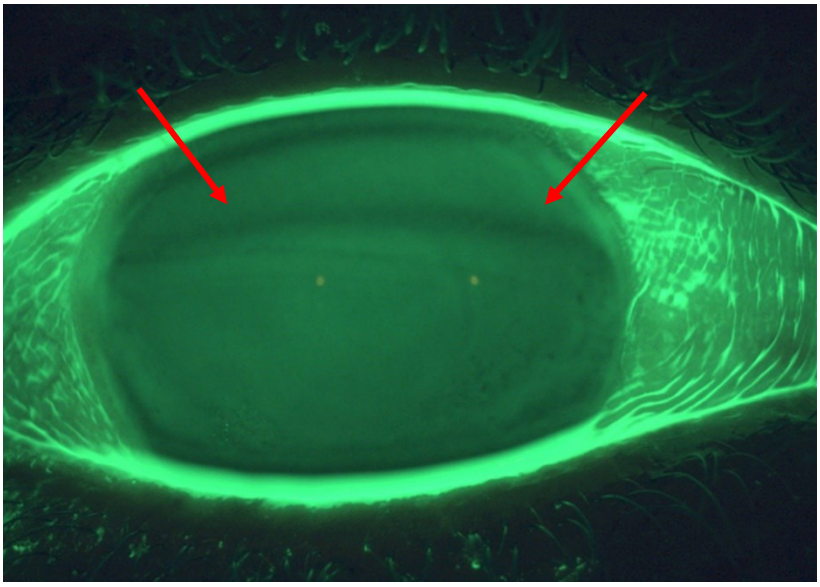

Figure 1.8. Blinking completion

1.5.2 Symptomatology

Use of validated questionnaires such as OSDI self-administrated or DEQ-5, with cut-off values of ≥6 (96) and ≥13 (97) respectively.

On one hand, OSDI is the main symptom questionnaire used for clinical trials due to its widespread use and range of questions, on the other hand DEQ-5 is more discriminative and asks for frequency and severity of discomfort and watery eyes. For research projects OSDI is the questionnaire to use due to its establishment and validity versus other questionnaires. For daily practice, DEQ-5 surveys more the different range of DED symptoms within longer periods of time, which could make the test more discriminative.

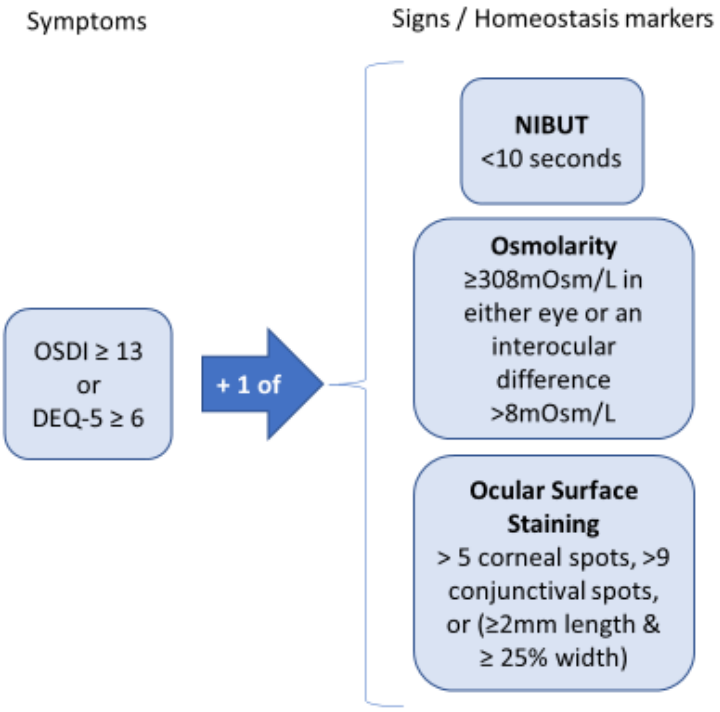

Figure 1.9. Patient investigation for DED diagnose. Adaptation from The Ocular Surface 2017 15, 539-574DOI: (10.1016/j.jtos.2017.05.001).

1.5.3 Clinical signs/Homeostasis markers

If any of the three homeostasis markers mentioned below is positive in conjunction with a sufficient symptomatology score, DED can be diagnosed.

1.5.3.1 Non-Invasive Break-Up Time (NIBUT)

Recording the mean of three measurements, or the moment of blink if the patient cannot withhold the eye open for a longer time (98). In case of using this test as a diagnostic measurement, the lowest time score should be considered for both eyes. Objective

methods are preferred, in case of using automated instruments a positive decision can be as low as 2.7s (99) and up to 10s (100) for subjective observation measurements.

1.5.3.2 Osmolarity:

Used with a temperature stabilised calibration device. An absolute measure as well as the intraocular difference eyes can be diagnostic (80, 101). A certain result is considered to be be ≥ 308 mOsm/L in either eye (70, 102) or an interocular difference >8 mOsm/L (80).

1.5.3.3 Ocular Surface Staining:

Considered to be a late sign of DED, it is considered a positive result when there is staining in either eye (17), as mentioned in (Figure 1.9).

1.5.3.3.1 Lissamine Green:

Mainly for evaluating lid margin and conjunctival damage. Lissamine Green (LG) should be instilled after saline solution has been in contact with the strip for 5s eluting the dye (17, 103, 104). Desired viewing time is after 1-4 minutes after instillation. A positive score would be >9 conjunctival spots for the conjunctiva (105) and ≥2 mm in length and ≥25% sagittal width (excluding Marx's line) for lid wiper epitheliopathy (LWE) (*Figure 1.10*) (17).

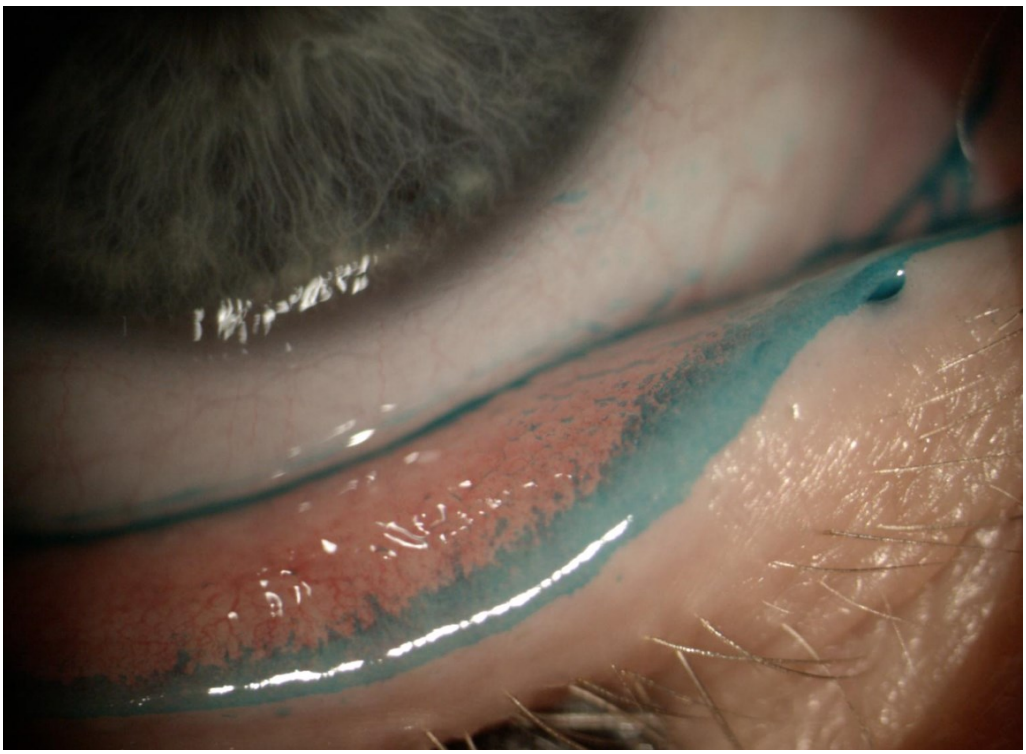

Figure 1.10. Lid wiper epitheliopathy and Marx's line stained with Lissamine Green

1.5.3.3.2 Fluorescein staining:

It is used to observe corneal and even lid damage. A positive score is <5 corneal spots (*Figure 1.11*) (17).

To observe ocular staining with Sodium Fluorescein (NaFl), viewing is advised 3 to 6 minutes after repeat instillation, using 2 separate wet strips with 2 saline drops (106).

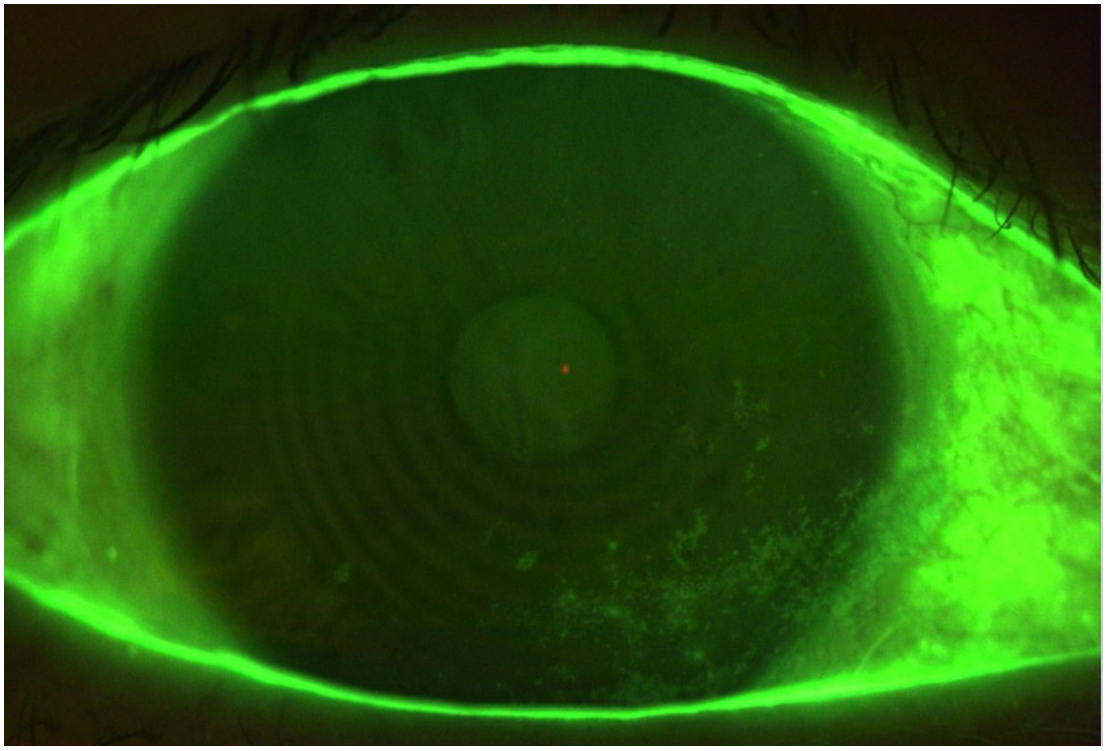

Figure 1.11. Corneal staining with NaFl)

1.6 Management and treatment

When managing or treating a patient, a comprehensive analysis of symptoms and signs must be done. As mentioned above, the continuum spectrum of EDE and ADDE, needs to be kept in mind when diagnosing and creating a treatment plan (***Error! Reference source not found.***). Consequently, the therapy approach must be done accordingly to the subtype classification and the possible masquerade ocular conditions. An accurate diagnose is necessary to assess the major cause of DED, as an incorrect subclassification would lead to patient dissatisfaction, as not all the required subclinical signs would be treated equally which would leave the patient with some subclinical symptoms.

In the original TFOS DEWS II diagram, ADDE and EDE are designed as a continuum, to demonstrate that they are not separate entities. Therefore, elements of each shall be

considered when managing and treating DED, as otherwise patients may continue having symptoms and may address dissatisfaction towards the treatment product/plan or even the ECP.

As mentioned before factors found to be influencing DED categorical distinctions for subclassification have been explored in several studies and reports (17, 25, 26, 107). The conflict between signs and symptoms remains in most of the patients (4, 78, 108) which puts the clinicians in the difficult position of having some aspects measured subjectively, which might indicate high severity, while other patients might suggest a lower severity (81). It was also identified that while there is lots of good evidence that many treatments work compared to a placebo helping reduce symptomatology, there are few evidence-based comparative studies that would inform when to change from one treatment type to another either with disease severity or sub-classification of the disease, only a couple of studies had compared the clinical effect of some of the products (21, 91, 109-115). A criterion indicating which treatment is best suited to alleviate a precise DE sign or symptom is still unavailable.

As ECPs, our aim is to restore the homeostasis by breaking the vicious cycle. According to the literature, consistent treatment use is linked to:

- Clinically significant DED symptomatology changes after 1 month.
- Clinically significant DED sign changes after 3-4 months of regular use (91).

In order to break this cycle, historically the primary course of action for DED treatments was the use of topical application of eyedrops, gels and sprays to stabilise and/or re-build the TF. The products principally differ in which element of the TF they are aiming to replace.

Over the past decade there has been a dramatic increase in products for treating DE, such as ‘artificial tears (AT), liposomal sprays, ophthalmic inserts, anti-inflammatory or immunosuppressant drops, antibiotics, dietary omega-3 essential fatty acids, autologous serum, intense-pulsed-light (IPL), punctual plugs, moisture-retaining eyeglasses, hydrophilic bandage contact lenses and secretagogues’ (116, 117) but particularly lipid-containing lubricants.

ECPs aim should be to restore the homeostasis by breaking the vicious cycle, with the idea of not returning to the vicious cycle if possible. According to the literature, patients

are supposed to continue with their treatment for at least 3 (118-120) or 4 months, after that, clinical changes are unlikely (91).
Therefore, the management guidelines (121) recommend a more generalised treatment from an early and mild stage disease with conventional therapies to more advanced forms of treatment (121, 122). Management and therapy were regarded as a 4 stages plan indicating what might be considered as first line and later treatments considering disease aetiology.

In this literature review the explanation of the algorithm approach (123) is more in regard to the patient's needs than the staging itself.

The primary course of treatment as stated by TFOS DEWS II can be found on (*Table 1.1*). Management and therapies were structured by TFOS DEWS II under 4-stages, starting from a more generalised treatment for disease of mixed aetiology, to more advanced therapies (121, 122). Despite the ongoing research on DED treatments, there is still an absence of level-1 studies that provides therapeutic evidence for treating each of the DED subtypes. Still, the proposed TFOS DEWS II guidelines provide an organisation tool for ECPs for when it might be best to initiate a treatment. ECPs should also select what might provide a better benefit for their patients according to their own experience, abilities, product availability and patient needs.

Table 1.1. Adapted staged management & treatment recommendations for dry eye disease Adapted from "TFOS DEWS II. Management and Therapy Report. (p.615) by Jones. L., et al., 2017. The Ocular Surface.

Step 1
Education of the condition
Modification of environment
- Desiccating conditions and environmental pollutants
- Blink rate / exercising
- Contact lens wear
- Identification of offending topical/systemic medications

Dietary modifications
- General hydration state
- Supplements (essential fatty acids - EFAs)

Ocular lubricants
Lid hygiene + Warm compress

Step 2
Preservative-free lubricants (aqueous, lipid or other supplementation)
Tear Conservation
- Punctal occlusion temporary/permanent
- Moisture chambers spectacles and humidifiers

Overnight treatments (ointment, moisture goggles)
In-office physical heating and expression of Meibomian glands
- Physical treatments
- Debridement
- Therapeutic expression
- LipiFlow

In-office intense pulsed light (IPL)
If demodex present → tea tree oil treatment
Pharmaceutical approach:
- Topical antibiotics, corticosteroid (limited duration), secreretagogues, non-glucocorticoid immunomodulatory drugs (Cyclosporine A), lymphocyte function-associated antigen 1 (LFA-1) (Lifitegrast)
- Oral macrolide therapy or tetracycline therapy antibiotics

Step 3
Oral secretagogues
Biologics
- Serum eye drops
  Autologous serum, allogeneic serum, umbilical cord serum, platelet preparations
- Therapeutic contact lens options (Soft BCLs, RGP sclerals)

Step 4
Topical corticosteroids → Longer duration
Biologics (Amniotic membranes/grafts)
Surgical approaches

Dry eye disease management Step 1

1.6.1.1 Education regarding the dry eye condition, prognosis, and treatment

Information and prognosis must be always mentioned to the patients. Advice on the condition's management should be given to the patient, if possible, both oral and written. A clear explanation makes patients feel more reassured, less concerned about their condition and is a good standard of care for all medical practitioners.

1.6.1.2 Dietary Modifications

Water consumption and whole-body hydration state has been examined stating its potential role in DED control, even though its therapeutic effect on the tear film is not clear, advice on water consumption as well as diet should be advised (121, 124).

Essential fatty acids (EFAs) specifically Omega-3 have been found to have an anti-inflammatory effect. Both Omega-3 and 6 modulate the overall inflammatory system acting as mediators (125). EFAs also have a direct impact on fatty acid saturation on cardiovascular system (126) and MG contents (127). The ideal intake ratio is thought to be a 4:1 ratio, Omega-3 and 6 respectively (128). Some studies mentioned a therapeutic benefit in DED using omega-3 (129), correlating it to improvement on tear osmolarity, break-up time, symptomatology scores, inflammatory mediators (130) and Schirmer results (131). The ingestion of EFAs has proven to be beneficial on the ocular surface, but there are still some conflicting results. On the DREAM study a group was kept on omega-3 whilst the other group was withdrawn of it by only using placebo tablets which contained refined olive oil, and after 12 months no statistically significant differences were found in between groups for signs and symptoms of DED (132, 133).

1.6.1.3 Modification of environment

1.6.1.3.1 Offending medications

1.6.1.3.1.1 Topical medications:

A considerable amount of literature has been published showing that chronic topical medication with preservatives could be associated with allergic, toxic, or inflammatory reactions. Specially, cases treated with glaucoma medication where preservatives such as Benzalkonium (BAK) irritate the ocular surface (134), suggesting that certain medications contribute to develop further DED (135-137). Therefore, some other preservative free (PF) alternatives were created such as; Polyquad®, demonstrating less

toxicity to the ocular surface, with similar therapeutic effects (138) and SofZia®, demonstrating an improvement in signs and symptoms in the ocular surface (139, 140).

1.6.1.3.1.2 Systemic medications:

Patients on 'antihistamines, beta-blockers, antidepressants, diuretics, anxiolytics, antipsychotics, anti-Parkinsonian drugs, isotretinoin, estrogenic therapy and systemic chemotherapy' (60, 141) have been reported to have a higher incidence of DED. Possible solutions would be to change the route of administration from oral to topical, discontinue the drugs if possible/dose adjustments or to switch to other medication less aggressive to the ocular surface eye (141).

1.6.1.4 Insufficient blinking

A lower and/or incomplete blink rate alters the tear film dynamics, (increases tear film evaporation, reduces tear volume, causes tear film destabilisation (142, 143) and is associated with MGD at early age (144, 145)). When the ocular surface is exposed to adverse conditions such as low humidity, air movement, increase/decrease air temperature or pollutants, the blinking rate decreases (146). Also, tasks that require a higher state of concentration such as reading or VDU have a significant impact on reduced blinking rate (147).

Relative temperature/humidity and air flow are environmental factors that ECPs can tell their patients to modify. A benefit of performing blinking exercises such as 10 seconds blink cycles every 20 minutes during the day has been proven to increase lipid quality grade and symptomatology scores (148). A voluntary forceful blink can help the motor memory to perform a more efficient blinking pattern and rate, which improves symptomatology and tear film quality (148-150).

1.6.1.5 Contact Lens Wear

Dry eye disease is a major factor of contact lens discomfort (CLD), DED and MGD. Most of the discomfort is relating to fit, design, material, replacement period and care system (151). Contact lens (CL) use is known to reduce meibum quality and meibomian gland morphology after prolonged lens exposure (152), decreasing also mucin and aqueous TF layer and protein concentrations (153). The destabilisation of the TF creates an increase of evaporation which in turn creates an increased friction during blinking (154). Yet, the driving pathophysiology is unknown (132, 155). The strategies to manage CLD and CL-induced DED are to improve environment (heaters - air humidifiers, screen time - blinking exercises, screen adjustment to reduce exposure), add tear supplements

dietary supplementation, adjusting CL replacement frequency, changing or eliminating care system, or lens design and the use of topical medication if needed (124).

1.6.1.6 Ocular lubricants

Even though artificial tears are largely palliative, they are the most popular treatment for DED (121); the subtype of dry eye needs to be clearly established to tailor which layers of the tear film needs replenishment (156-159). Few randomised control trials (RCT) compared the superiority of a particular over the counter (OTC) product over another one (77, 91, 160).

1.6.1.6.1 Aqueous supplementation

Aims to enhance lubrication and extend the retention time on the ocular surface. These products are specifically useful for ADDE and low tear production patients such as Sjögrens's sufferers.

1.6.1.6.2 Viscosity-enhancing agents:

Once in contact with the surface of the eye this lubricant increases tear film thickness, promotes tear retention, protects against desiccation, relieves DED symptomatology and improves goblet cell density (161). The higher the viscosity the longer the time the product remains on the ocular surface. High viscosity eyedrops are recommended mostly for overnight use due to a temporarily vision blur, to keep the eye lubricated in case of recurrent corneal erosions and for lid closure abnormalities.

1.6.1.6.3 Osmotic agents,

Aid in balancing the tear film osmolarity. Osmolarity balanced artificial tears protect cells under osmotic stress and have been shown to have anti-inflammatory properties (162), improving patient symptoms and signs (163). These artificial tears are appropriate for ADDE (111), but not many studies compared the use of hypo/hyperosmolar drops on tear osmolarity.

1.6.1.6.4 Lipid-containing-drops

Lipid-containing products are a newer and more attractive option for treating DED specifically EDE (164). These lipid-based drops/sprays are an alternative to hydrogel-based artificial tears and have been proven to work in several studies; with lipid-based drops (110, 113, 165-167) and liposomal sprays (109, 168, 169). They have been shown to ameliorate lid parallel conjunctival folds, tear break-up time, Schirmer test, lipid layer thickness, tear evaporation rates, tear stability and ocular symptoms (21, 91, 109, 168-

171). Consequently, it can be said that phospholipid-based products help in enhancing the lipid layer, improving subjective comfort, and tear film stability (172). Liposomal-based products can be recommended in every subtype of DED (21, 91, 111, 173), as they closely mimic the tear film composition, but they will be more effective on EDE sufferers, as it will be seen in *(Chapter 4:*).

1.6.1.7 Lid hygiene

New varieties of lid cleansing products such as wipes, gels, sprays, solutions, swabs, foams, and scrubs (Figure 1.10) are available nowadays. The rationale behind using them is to reduce the microbial overgrowth by reducing the bacteria associated with blepharitis and to improve ocular comfort (23, 174). A daily hygiene of lids and lashes is recommended due to the alleviation of symptoms of DED, MGD, blepharitis and mites infestation in case of demodex (175). Some therapies such as Blephex (Figure *1.12*) cannot be performed during COVID-19 due to the risk of spreading contaminated debris.

Anti-demodex wipes with tea tree oil (TTO) as an active ingredient, act by having an antimicrobial, anti-inflammatory, antifungal and antiviral mechanism (176), by which the population of those mites declines (177). Even though TTO is toxic for the eye, over the counter pre-formulated wipes use a safe concentration (25% whole TTO), otherwise 5% twice a day or 50% once weekly was found to be sufficient to eliminate Demodex infestation (178, 179). Other research has advocated the use of Manuka honey to reduce bacteria on the eyelid margin by having natural antibiotic and anti-inflammatory properties, but further research is warranted on this.

Extra care must be taken to show how to perform the lid cleansing, keeping the eyes closed due to its possible irritation. Explaining the life cycle of the mite (3-4 weeks) to the patient, can make them understand the importance of compliance.

A monthly follow-up is ideal to observe the reduction of Demodex infestation.

Some patients still use baby shampoo (*as seen in Results*), and as ECPs, our advice should be to not favour this behaviour as it has been proven that its use reduces goblet cell function, aggravating DED (180). Therefore, patient education remains a key aspect of the treatment.

Figure 1.12. Lid cleansing devices. Left: Blephex. Right: NuLids. Image taken in Aston University Dry Eye Clinic

The only issue found in the literature is that even though symptomatology decreases with lid hygiene, after 6 weeks of use, only 55% of the patients remain compliant (181). Lack of accepted guidelines for lid cleansing are noted in the literature as well; with some studies suggesting a daily regimen while others suggest its use twice a day with a proposed duration of the procedure ranging from 5-15 minutes in between studies.

1.6.1.8 Warm compresses

Many management and therapy options had been developed to warm the lids. Common in practice, we can find warm compresses such as eye bags, hot-eye masks and disposable eyelid warming devices. More novel heat treatments include steam-based devices, radiance heat-based masks, warm moist air devices and water-propelled massaging masks (*Figure 1.13)*. Studies regarding what the most optimal temperature to melt the meibum of the glands might be, suggest reasonable variability (182). Patients with MGD (183), need greater temperature to melt the meibum due to the increased viscosity (184, 185).

Devices that cannot keep the desired temperature for the full time of the treatment such as homemade face masks, might be insufficient for an adequate treatment of MGD (186). Massaging in general has many health benefits such as relaxation and the slowing of

pain transmission in chronic cases (187). Patient education on eyelid massaging after having used a thermal device, helps the glands release its contents. This treatment is also poorly standardised with most studies using this method once or twice a day from 5 to 10 minutes (188).

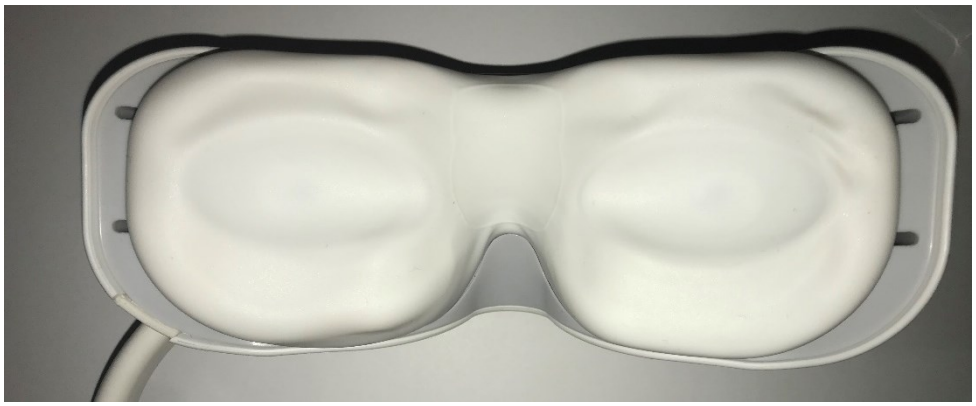

Figure 1.13. AURAI Eye Mask, automated water-propelled massager.
Image taken on Aston University Dry Eye Clinic.

1.6.2 Dry eye disease management Step 2

1.6.2.1 Preservative-free lubricants

Patients with chronic medications who are continuously exposed to preservatives, are more susceptible to toxicity and adverse changes to the ocular surface (189-193). Patients using artificial tears could also have more sensitivity reactions to preservatives, hence, a preservative free product could be used, decreasing inflammation, and increasing the antioxidant contents (194).

1.6.2.2 Tear Conservation approaches

Historically it was thought that DED was largely due to aqueous deficiency hence tear replacement artificial tears or punctal plugs (PP) were mostly used to maintain the tears protecting the ocular surface. Recently, treatments mimicking the tear film and even new tear stimulation methods have been developed.

1.6.2.2.1 Punctal Occlusion temporary/permanent

A few studies demonstrated a clear benefit of this intervention, with symptoms and clinical results improving after the plugs are in place. This technique is more successful when combined with other DED treatments (195). Yet its use is somehow controversial as the TF or any medication instilled remains for a longer period on the ocular surface.

This prolonged exposure to inflammatory cytokines could lead to toxicity reactions if the medication stays in the ocular surface for longer than expected. Most ECPs refer using this management approach for ADDE (*as seen in Results*), to retain the tear film and keep the ocular surface lubricated.

Surgical punctal occlusion or cauterisation (for a more severe condition), is exclusively for patients who cannot tolerate punctal plugs. Comparative case series demonstrated that permanent punctal closure improves symptoms and signs such as Schirmer scores, ocular surface staining and tear break-up time in patients with Graft-versus-host disease (GVHD) and Stevens-Johnson syndrome (196, 197).

1.6.2.2.2 Moisture chamber spectacles and humidifiers:

Specifically designed goggles seal the contour of the orbital rim creating a humid environment by slowing the evaporation of the tears. Its clinical efficacy has been demonstrated in several reports (198, 199), but not enough well-designed studies have investigated the therapeutic value of these devices.

Only one controlled study reported that the use of humidifiers provided an improvement in break-up time and subjective comfort (200).

1.6.2.3 In-office physical gland treatments

Three types of physical treatments are available for Meibomian glands and aim to improve the functionality of the MGs by clearing up any unwanted obstruction.

1.6.2.3.1 Heating devices:

1.6.2.3.1.1 Lipiflow®

Lipiflow pads are used on the palpebral conjunctiva to soften or liquify MGs contents *(Figure 2.1).* By means of an inflatable air bladder, pressure is applied from both the internal and external part of the lid, to excrete the glands contents.

This device heats up the glands up to 42.5ºC (therapeutic levels) for 12 minutes. It is a safe and effective treatment for MGD having a sustained effect (201).

Depending on MGs state before treatment, outcomes might vary and expectancies should be discussed prior treatment, as not all patients will have the same benefit.

Clinical reports and clinical trials reported that MGs yielding function is restored and DED symptomatology improves, with results lasting from 3 months (202) to 12 months (203) and up to 3 years (204), depending on the study.

Figure 1.14. LipiFlow treatment. Johnson & Johnson. “TearScience® LipiFlow® Thermal Pulsation System” (https://www.jnjvisionpro.com/products/eye-medical-devices/lipiflow-treatment).

1.6.2.3.1.2 Tixel

Is a thermo-mechanical treatment for fractional ablations. This treatment is mostly used for cosmetic skin rejuvenation, to reduce scars and stretch marks but it is also being tested for DED (Figure 1.15). The tip of the device penetrates 100–1000μm into the skin, and thermal energy is transferred onto the tissue (205). The initial temperature of the tip is 400°C and it is assumed that upon skin contact it evaporates the tissue’s water content and cools down to 150°C. A rapid increase on this type of lasers during the past years on the dermatological field is due to the few side effects and clinical improvements (206).

A paper presented at 2020 ASCRS annual meeting reported that signs and symptoms of DED improve after its use in 77 individuals having an increase on NIBUT and a decrease on symptomatology scores (188). This treatment was initially trialled with two sessions, requiring the use of analgesic creams (207), despite the use of anaesthetic creams in non-ocular trials, which still reported some discomfort with little to no pain (208). The main idea behind its efficacy is to coagulate the dermal tissue by desiccation (209), creating collagen remodelation and the increase of its formation. The pathophysiological benefit behind its efficacy on DED cases remains unclear, due to a lack of clinical trials on this therapy. One could argue that the creation of new collagen retightens the skin and as connective is in contact, muscle tightness occurs pressing on the MGs, yet this idea has not been proven. Despite the good results on Shah et al. study on 2020, further clinical studies are needed to prove its efficacy and potential as a DED treatment.

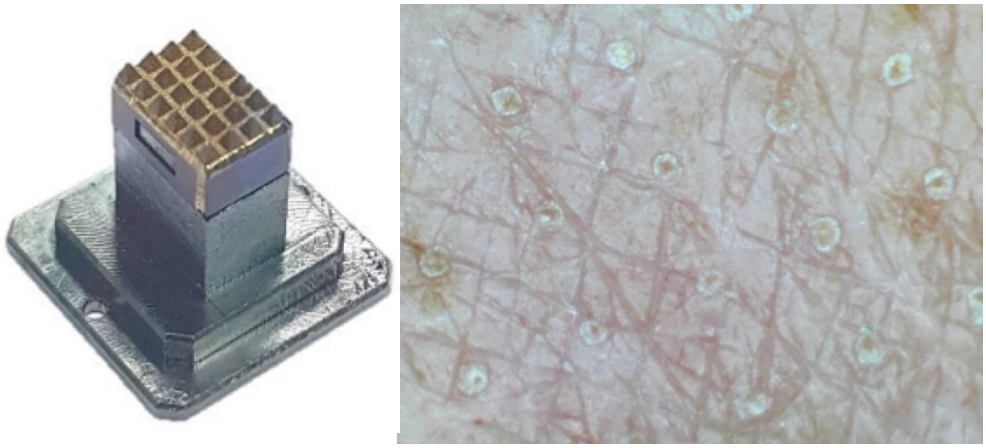

Figure 1.15. Tixel treatment. From left to right: Picture of device tip, and array of coagulation points on human skin after Tixel treatment. Novoxel. “Ablative coagulation sites”. (https://www.novoxel.com/index.php?todo=technology)

1.6.2.3.2 Physical treatments

Are used to forcefully express and/or clear up the contents or openings of the MGs.

1.6.2.3.2.1 Debridement

Is a procedure performed by a mechanically wiping force along the eyelid margin, applied with a stainless-steel golf club spud *(Figure 1.16*). It is used to remove any possible keratinised tissue that might be blocking the openings of the glands, preventing the meibum to flow onto the ocular surface, and to remove any debris. Positive results had

Figure 1.16. Golf club spud for lid debridement. Image taken in Aston University Dry Eye Clinic.

been reported with improvement in symptomatology, MG function and decreased ocular staining (210, 211), tear breakup time and reduced inflammation (212). Its use after warm compresses increases also the quality of expression (213). In addition; improvement in CL discomfort has been reported, allowing the patients to double the amount of wearing time (214).

1.6.2.3.2.2 Therapeutic Meibomian gland expression

This procedure is performed to empty the blockages from the MGs so that a better quality meibum can be produced *(Figure 1.17).* The eyelid to treat is isolated, and 2 forces from the internal tarsal conjunctiva and external lid come together below the lid margin (only where glands are present) and the MGs are compressed by the stationary paddles (*Figure 1.18)*. The use of anaesthetics is needed to reduce the blinking reflex and to decrease the sensation due to the force applied to express the MGs contents is on the maximum tolerable force (215), and the procedure can become quite uncomfortable.

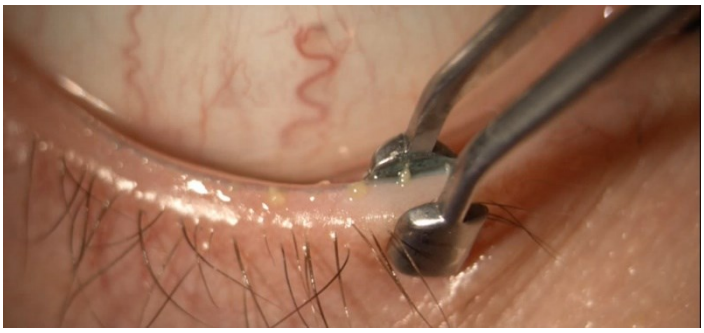

Figure 1.17. Therapeutic expression of the Meibomian Glands. Inspissated excretion. Image taken on Aston University Dry Eye Clinic.

Figure 1.18. Arita Meibomian gland compressor for therapeutic gland expression. Image taken in Aston University Dry Eye Clinic.

1.6.2.3.2.3 Meibomian gland intraductal probing

The procedure entails the insertion of a 2mm probe into the MGs to open/dilate them and remove any obstructive material. Even though it is a safe procedure, it is quite invasive in nature, and there is a possibility of tissue damage/haemorrhage. Nonetheless, patients reported a rapid relief of symptoms after the procedure, being symptom-free up to a year on one of the studies (216), an increase on meibum viscosity was also noted in a case report (217).

1.6.2.4 In-office Intense Pulsed Light (IPL)

Intense pulsed light delivers high intensity pulses of light from 500 to 1,200nm (*Figure 1.19).* At this wavelength, coagulation and ablation is induced, specifically on new vessels, such as telangiectasic vessels. This procedure has been tested providing very good results on lipid quality and quantity (218), improving symptoms and signs (219), reducing inflammatory markers and changing the lipid excreta of the Meibomian glands. Between studies differences are present such as the number of sessions ranging from 3-4, and the amount of time the outcomes last, extending to 11 weeks (220). The combination of IPL and manual MG expression seems to be a safe and efficient way to treat MGD, and a very promising modality of MGD treatment (220-223).

The underlying mechanism is poorly understood. It has recently been suggested that the absence of visible gland structure may not be due to absolute atrophy or loss of function, instead the loss of activity. Some case reports suggest that MGs can be improved upon treatment (both IPL and LipiFlow), indicating a possible gland reactivation (224), yet those are not to be always expected on clinical settings.

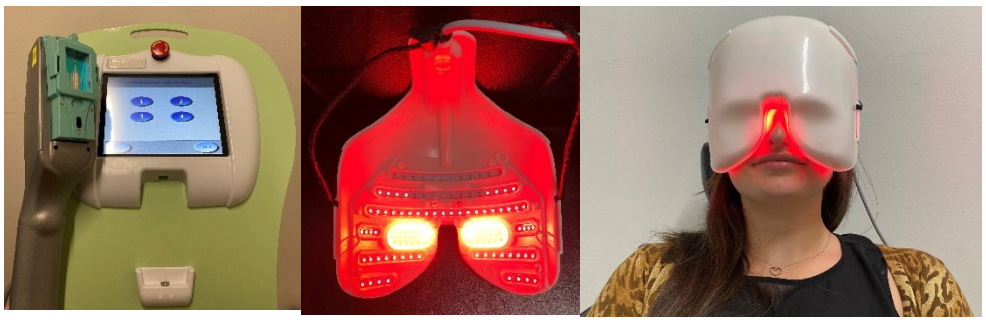

Figure 1.19. IPL (Low Level Light therapy + polychromatic light inducing thermal stimulation). Images taken on Aston University Dry Eye Clinic.

Depending on the causing mechanism of DED and its severity, a pharmaceutical approach can be used:

1.6.2.5 Systemic and topical antibiotics

These medications act by having a bactericidal effect keeping bacteria from reproducing. Tetracyclines and analogues are used to treat chronic blepharitis improving tear film stability, symptomatology (225), corneal staining and meibomian quality (226). Meibomian glands are also altered by these medications. A stimulatory effect with Azythromicin was reported (227) in which an increase of cholesterol esters, phospholipids and lysosomes was present which proved beneficial on EDE cases.

1.6.2.6 Topical corticosteroid with a limited duration

Because of the inflammatory nature of DED, corticosteroid treatment acts by reducing inflammation, pain and signs and symptoms of DED. Side effects need to be taken into account with prolonged therapy, including secondary glaucoma, delayed wound healing, susceptibility to infection and cataract formation (228).

1.6.2.7 Topical secretagogues

Are pharmacologic agents used to stimulate the secretion of a desired substance.

1.6.2.7.1 Aqueous/Mucin

The secretion of both aqueous and mucin layer comes from a direct pharmacological stimulation of conjunctival epithelial cells and goblet cells.

- Aqueous pharmacological stimulation, improves TF stabilisation which can be noted on NIBUT (159, 229, 230). One of the approved secretagogues used is Lacritin Aqueous.
- Stimulation of mucin-like glycoproteins has an impact on gel forming mucins. This mucins are synthezised by the lacrimal glands and corneal epithelia, helping to maintain and create epithelial barrier, lubrication, hydration and prevent pathogen binding to the ocular surface (231). Some of used secretagogues are; Diquafosol tetrasodium - Diquas® (non-FDA approved) -. Mucin secretagogues: Rebamipide - Mucosta® (non-FDA approved) - Galectin-3, Eupatilin.

1.6.2.7.2 Lipid

The pharmacological action for lipid stimulation has an effect on MGs (232), acting by either;

- Upregulating gene expression on lipid metabolic pathways, with Insulin-like growth factor 1 (IGF-1) – studies are still underway.

- Downregulating gene expression for keratinisation, with androgens.

1.6.2.8 Topical non-glucocorticoid immunomodulatory drugs

These remedies have anti-inflammatory and immunosuppressive effects, but their clinical efficacy is compromised by the metabolic effects of long-term treatment. Some of the most used are presented below:

1.6.2.8.1 Cyclosporine A (CSA)

Is the most commonly used therapy for ADDE 0.05 % (233), especially for moderate to severe DED (160). For CSA to be effective an extended period of use is needed. Studies with CSA reported an improvement of tear production by reducing many inflammation markers, tear osmolarity and recovering density of goblet cell density (121).

1.6.2.8.2 Tacrolimus

Is a class of macrolide that acts by inhibiting protein synthesis in bacteria, stimulating the lacrimal glands, and preventing further destruction of the tissue. It has been proved to increase tear stability and overall ocular surface status, especially on patients that are intolerant to CSA (234).

1.6.2.8.3 Non-steroidal anti-inflammatory drugs (NSAID)

These aids are used to relieve pain, inflammation and swelling specially after surgery. A fast reduction of symptomatology has been reported for Diclofenac on Sjögren patients, yet caution should be taken as it can reduce corneal sensitivity, therefore no more than 1 month use is advised (235). Examples of NSAIDs are pranoprofen, diclofenac, ketorolac and indomethacain.

1.6.2.8.4 Biologic drugs:

These products act by different means depending on the defect to treat, by either, reducing friction, improving nerve regeneration and goblet cell density, inhibiting inflammatory reactions, and regulating immune cell trafficking (121). Most of these therapies have been investigated in animal models therefore, its use requires further exploration.

1.6.2.9 Topical lymphocyte function-associated antigen 1 (LFA-1) (Lifitegrast)

This medicine acts by preventing an “immunologic synapse” which is a critical step in the pathway toward T-cell activation and cytokine release (236). This anti-inflammatory

therapy used twice daily for almost a year proved to be well tolerated providing an improvement in symptomatology and in inferior corneal staining.

1.6.3 Dry eye disease management Step 3

1.6.3.1 Oral secretagogues

Are used for a longer period than on Step 2 (*1.6.2.7*), but careful follow-ups should be performed.

1.6.3.2 Biologic serum eye drops:

1.6.3.2.1 Autologous serum:

Its function has been tested to enhance corneal wound healing, reepithelisation (237-240) by inhibiting the release of cytokines as well as increasing the number of goblet cells and mucin expression in the conjunctiva (241, 242). Overall tear break-up time, ocular staining and conjunctival impression improved, as well as the symptoms, showing a 60-80% positive response.

1.6.3.2.2 Allogeneic serum

It has been proven to work in severe patients having graft-versus-host-disease. An improvement in symptoms, ocular staining, break-up time, goblet cell density and tear osmolarity was found (243); it can also be used if autologous serum is not available. No significant complications had been associated with this product.

1.6.3.2.3 Umbilical cord serum

This serum has similar characteristics to allogenic serum, it contains transforming growth factors (244). Umbilical cord serum has been proved to be efficient in patients resistant to conventional treatments (245) and with graft versus host disease (GVHD) (246), improving in break-up time, ocular surface staining and impression cytology.

1.6.3.2.4 Autologous platelet rich plasma (PRP)

This product is a blood derivative with a high concentration of platelets which has very similar biochemical characteristics to those of the human tears. PRP provides successful treatments in moderate to severe dry eye cases by enhancing corneal epithelial wound healing, inhibition of inflammatory cytokines, increasing the number of goblet cells and mucin expression in the conjunctiva (247). Certain advantages are seen over autologous serum which is quite poor on growth factors, and inconsistencies in the methods of preparation and percentages are reported on the literature (248).

PRP has been proven to be an effective alternative with little differences between subtypes of DED, it was also proven beneficial for Sjögren patients (249).

Even though these products have had very good results on clinical trials, its use in practice is narrowed to ophthalmologists and specialist ECP's as its production is quite expensive and time-consuming due to the material needed, its extraction and processing procedures,

1.6.3.3 Therapeutic contact lens options

Evidence on epithelial healing improvement and pain reduction on persistent epithelial defects (250) has been noted on soft contact lenses (CL), particularly on silicone hydrogel lenses.

Therapeutic CL are used for ocular discomfort, corneal support during healing (251) and as protection from environmental and friction stress (252).

1.6.3.3.1 Soft CL

Current literature suggests a good long-term tolerance of soft CL use (252). A study with soft CL on irregular corneas, reported that 84% of all participants were wearing them comfortably and with good visual performance even after 12 months (253).
When soft CL are used with a medical aim to protect the ocular surface, it means that it is already somehow compromised, therefore, it can be more vulnerable and prone to possible infections (254) (Figure 1.20). Studies reported a risk on infections due to CL wear to be 14.4 per 10,000 lens wearers (255).

1.6.3.3.2 Rigid scleral lenses

Scleral lenses were the first lenses used for therapeutic purposes (252) due to its sealed tear reservoir they provides constant lubrication, exposure protection and visual improvements (Figure 1.20) (254, 256).

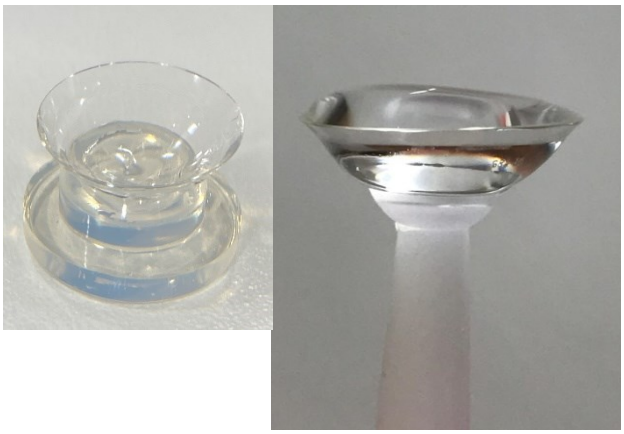

Figure 1.20. Left: Soft bandage contact lens. Right: Scleral lens. Image taken in Aston University Dry Eye Clinic.

1.6.4 Dry eye disease management Step 4

1.6.4.1 Topical corticosteroids

Longer duration of corticosteroids must be used carefully and followed-up due to the possible complications associated (infections, ocular hypertension, and cataracts), that is why repeated-short term therapies are an alternative approach (257, 258).

1.6.4.2 Biological substitutes

Substitutes such as amniotic membranes are used to heal ocular surface conditions, mostly used by ophthalmologists and specialist ECPs. The tissue is placed on the eye and held in position by a vehicle/prosthetic device such as bandage contact lens, surgical glue, or sutured in. Its biological activity typically dissolves in 3-7 days (Figure 1.21Figure 1.21*).* This device claims to expedite restoration of corneal epithelial health and epithelialization by reducing inflammation, scarring, pain and promote healing, having a sustained symptom improvement for 4 months (259-262).

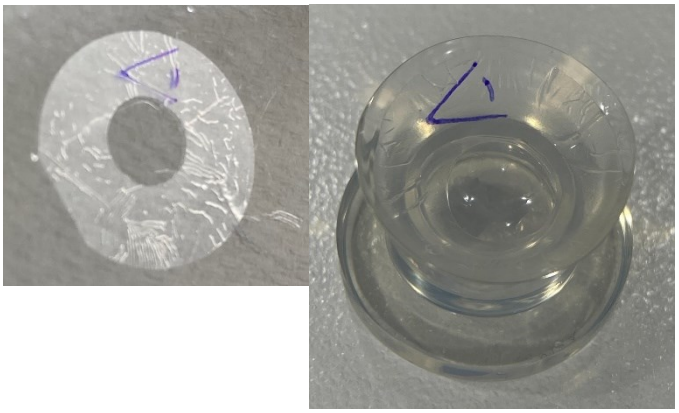

Figure 1.21. Left: NuVision amniotic membrane, Right: Amniotic membrane loaded onto a bandage contact lens. Image taken in Aston University Dry Eye Clinic.

1.6.4.3 Surgical approaches

Normally performed in cases where protection is needed due to corneal exposure if continuous lubrication is needed. Some of the procedures are:

1.6.4.3.1 Tarsorraphy

Either a temporary or permanent, partial, or total closure of the lid by either sutures, surgical glue, adhesive tape, or botulin toxin injection. Used as a last resort on severe dry eye cases and persistent epithelial defects (263).

If the conjunctiva is altered:

1.6.4.3.2 Correction of conjunctivochalasis

A recession of excessive tissue - by either thermal cauterization, conjunctivoplasty or fixation to the sclera is surgically performed (264).

1.6.4.3.3 Conjunctival reconstruction

Reconstructions are mostly used for conditions where the tissue becomes scarred or needs transplanting such as pingueculae, pterygiums, Stevens-Johnson syndrome.

1.6.4.3.4 Amniotic membrane grafts

These biological membranes are used for persistent corneal scarring/ulceration, epithelial defects, and Stevens-Johnson syndrome (265, 266).

If lubrication is needed continuously:

1.6.4.3.5 Mechanical dacryoreservoirs

Lubricant reservoirs act by delivering drops from a reservoir to a catheter, in severe dry eye cases.

1.6.4.3.6 Minor and major salivary gland transplantation

Transplantation acts by providing a functioning tissue to the eye in cases of congenital lacrimal secretory disorders or surgical damage, severe aqueous deficiency secondary to cicatricial conjunctivitis, and Stevens-Johnson syndrome (267).

In summary, there are a lot of available therapies to manage dry eye and guidelines are available on how to treat this disease. Even if some more advanced therapies are not within average clinical reach, efforts should be made to advise on disease prognosis, environmental modifications, iatrogenic risk factors and lid hygiene to start with. Depending on the disease subtype appropriate management should be taken, and if the condition is more severe, therapeutic management in case the ECP is independent prescriber (IP) trained, or referral would be the next step on the treatment strategy.

Although major advancements have been made in new therapies, a lot more research is needed to understand the temporal-therapeutic profile of each treatment, to understand when a specific therapy is more beneficial and to optimise compliance and effective application.

1.7 Aims and objectives of the thesis

The latest guidelines created by TFOS DEWS II on 2017 (121) is an organisation tool to treat DED. This evidence-based algorithm focuses on the best therapy to start a patient on. Moving from a more simplistic approach to a more advanced-specific treatment.
Its creation also shed light on the much-needed RCTs and level 1 studies on different treatment efficacies and the lack of evidence of treatments for each specific DED subtype and severity.

Despite this condition being able to be managed with multiple therapies treating various aspects of the ocular surface; certain therapies have proven to be more beneficial for a particular ocular surface condition or DE subtype.

On the first stage of the treatment algorithm, only environmental modifications, advice, and short-term-palliative aids are offered, which does not treat the root cause of this chronic disease. The first stage provides therapeutical advice for mixed aetiologies, yet no differentiation between disease subtype (EDE and ADDE) is made after this stage. In addition, the created approach does not recommend a specific treatment for a given DE severity stage.

The research questions for the management of this condition aims to answer questions such as: When is it best to use a specific therapy? For which subtype a management strategy is more effective? Should a therapy be changed when the severity of the disease increases? If so, which one is more efficient?

Therefore, the main objectives for this thesis were:

1. To shed light on this non-severity/non-subtype specific treatment classification (Chapter 1:).

2. To understand how clinical DED prescribing and management patterns of eye care practitioners around the globe are in relation to severity and subtype (Chapter 2:).

3. To create more therapeutic supporting evidence for each stage by:

- Compiling a series of experimental studies to provide additional scientific evidence of management strategies and to assess if further modifications of this guideline were needed to improve the treatment of DED.
- Assessing and comparing the effectiveness of AT, liposomal sprays and emulsion drops in the treatment of DED (Chapter 3:).
- Comparing the efficacy of a lipid and a non-lipid based artificial tear supplement for the management of DED (Chapter 4:).
- Determining the temporal-therapeutic profile for clinically significant improvements of signs and symptoms, including the magnitude of change and time to reach maximal clinical benefit (Chapter 4:).
- Exploring whether clinical outcomes are influenced by baseline dry eye disease subtype or severity (Chapter 4:).
- Investigating the efficacy of eyelid warming devices in patients with dry eye disease (Chapter 5:).
- Estimating the effectiveness of a range of common treatments based on the functionality of individual MGs glands classified by their morphological appearance and drop-out extent (Chapter 6:).

Chapter 2: Global survey of clinical practice patterns management of dry eye disease (QUALTRICS)

Published as;

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2.1 Introduction

Dry eye disease (DED) is a multifactorial condition characterised by the loss of tear film homeostasis and perpetuated by a vicious cycle of tear film instability, hyperosmolarity, and ocular surface inflammation (16). The diagnosis of dry eye disease requires the presence of both clinical signs and symptoms. The TFOS DEWS II diagnostic methodology report identified the need for a standardised, minimally invasive approach to DED diagnosis, recommending the use of either the Ocular Surface Disease Index (OSDI) or Dry Eye Questionnaire (DEQ-5) questionnaires for screening dry eye symptomology, in combination with one of the global positive signs of homeostatic breakdown (tear instability, hyper or interocular differences in osmolarity or ocular surface staining) (17).

Following a diagnosis, the subtype of dry eye (16) should be determined as more evaporative (as indicated by lipid layer thickness interferometry, meibomian gland structure imaging and functionality testing) or aqueous deficient (as indicated by tear meniscus height) (17). This subtype, along with the severity of symptoms and identified modifiable risk factors, can then be used to develop an appropriate treatment plan (121).

Previous studies investigating management therapies for dry eye disease subtypes tended to focus on evaporative dry eye disease (112, 114, 268-274). In terms of subtype, Alió and colleagues found little difference in the effectiveness of autologous platelet-rich plasma in the management of aqueous deficient compared to evaporative dry eye (249), whereas, while Essa and colleagues found that artificial tears (AT) performed similarly, osmolarity balanced artificial tears were the preferred treatment in individuals with aqueous deficient dry eye and liposomal sprays were more efficacious for individuals with evaporative dry eye (275). There has been limited research on the efficacy of management therapies across different severity levels of dry eye disease, with one study reporting that cyclosporine is more efficacious in more severe dry eye disease (276).

The TFOS DEWS II reports provided global consensus recommendations for the diagnosis and management of dry eye disease. Nonetheless, there remains limited evidence informing clinicians as to which treatment might work better for differing severities and subtypes of DED. Previous studies have reviewed management practice patterns between specific countries (277), between different professions located within the same country (278-280) and have evaluated differences between clinical patterns and evidence-based guidelines across Australia (281) *(Table 2.1*). To date only a few

studies could be identified, which have compared global trends, such as one on aqueous deficient DED management approaches (233).

The main purpose of the current survey was to examine the clinical DED prescribing and management patterns of eye care practitioners (ECPs) around the globe, in relation to severity and subtype. For this study we hypothesised that the clinical behaviour of ECPs was aligned to TFOS DEWS II guidelines, as it has been 3 years since the new treatment guidelines came out. This approach enables clinicians to benchmark their practice to their peers, identify areas of varying practice across the world, and indicate where further research is required to optimise patient management and inform industry on how best to target product development.

Table 2.1. Previous studies on patterns of clinical diagnosis and management of dry eye disease (all anonymous internet surveys)

Study	Comparison	Topics	Surveyed	Professionals	Results	Comments
Downie et al., 2013 (281)	Australian practice behaviours to internationally recognised guidelines for DED diagnosis and management	- Practitioner demographics - Diagnostic techniques - Management strategies (Mild/Moderate/Severe) - Evidence-base for the practice patterns	144	Optometrists	- DED professionals tend to perform more diagnostic techniques and newer procedures - Mg: - Mild: AT and eyelid hygiene - Moderate: Unpreserved lubricants - Severe: Gel preparations	- Trend of Omega-3 fatty acids recommendation as severity increases. - Corticosteroids and anti-inflammatory eye drop also prescribed for moderate and severe DED.
Downie et al., 2016(277)	UK and Australia practice behaviours for DED diagnosis and management	- Practitioner demographics - Diagnostic techniques - Management strategies (Mild/Moderate/Severe) - Evidence base for the practice patterns	317	Optometrists	- Patient symptoms, MGE and FBUT most important for both UK and Australian practitioners for diagnosis - Mg: - Mild: Eyelid hygiene and lubricant AT - Moderate: Unpreserved gels	- Dx: UK tend to use more TMH, LIPCOF, standardised grading of conjunctival LG staining and OSDI - Dx: Australia tend to use more FBUT - Severity: UK assessment

					- Severe: Topical ointments and PP	through patient symptoms - Mg: UK recommends higher intake of omega-3 and less preserved AT for mild DED. Australia recommends topical corticosteroids for moderate and severe DED
Sy et al., 2015 (233)	Global eye care practitioners	- Practitioner demographics - Management of aqueous deficient patient case study - Access to treatments - Treatment algorithms	115	Cornea specialists (66 %), general ophthalmologists (16 %), non-clinical researchers (6 %), optometrists (6 %) and other (6 %)	The most prescribed topical treatments included cyclosporine A, fluorometholone, loteprednol etabonate and autologous serum eye drops. The most prescribed non-topical medications included essential fatty acid supplements, low-dose oral	Treatment response was monitored with corneal fluorescein staining, foreign body and burning sensation.

					doxycycline and flaxseed supplements as well as PP.	
Van Tilborg et al., 2015 (278)	Dutch optometrists and GPs on symptoms, causes, diagnosis and treatment of DE	- Knowledge - Investigative methods - Therapy preference	231	Optometrists (138) GPs (93)	- Dx: No agreement. GP: rarely use Dx tests. LG and NaFl staining and FBUT Optometrists: mainly FBUT, LG and NaFl staining -Mg: Agreement only in prescription of gel/ointment	- Mg: - Optometrists: Tend to prescribe more unpreserved AT, lid hygiene and heat therapy - GPs: Tend to prescribe more preserved AT
Williamson et al., 2014 (279)	Perception of optometrists and ophthalmologists on DED management in North Carolina	- Knowledge about patient symptoms - Diagnostic - Treatment approaches	100	Optometrists Ophthalmologists	-Dx: BUT, NaFl staining -Mg: Patient history, AT, warm compress, and lid scrubs	- Dx combination BUT, NaFl and LG staining Optometrists: NaFl staining and history Ophthalmologists: Schirmer test
Xue et al., 2017 (280)	Diagnostic and management protocols of New Zealand optometrists and ophthalmologists for DED	- Practitioner demographics - Diagnostic techniques - Management strategies (Mild/Moderate/Severe)	203	Optometrists (174) Ophthalmologists (29)	-Dx: Symptomatology, MGE, FBUT Optometrists: MGE Ophthalmologists: NaFl staining -Mg:	In a severe condition unpreserved gel, topical ointment, cyclosporine A, topical corticosteroids,

	- Evidence base for the practice patterns	Mild: Unpreserved AT, Preserved AT and eyelid hygiene Moderate: Eyelid hygiene, Omega-3, unpreserved AT and gels Severe: Eyelid hygiene, unpreserved AT and gels	systemic tetracyclines, PP and autologous serum prescribed by both professions

AT – Artificial Tears, DED – Dry Eye Disease, GP – General Practitioner, Dx – Diagnose, Mg – Management, MGE – Meibomian Gland Examination, FBUT - Fluorescein Tear Break-Up Time, PP – Punctal Plugs, LG – Lissamine Green, NaFl – Fluorescein, BUT – Break-Up Time (undefined method), TMH – Tear Meniscus Height, LIPCOF – Lid Parallel Conjunctival Folds, OSDI – Ocular Surface Disease Index Questionnaire.

2.2 Methods

2.2.1 Survey design

The survey (*Table 2.2*) was designed by the authors based on the DED management options reported by TFOS DEWS II (121) and sought to examine how management decisions were based according to the severity and subtype of DED (16, 17). The survey was designed in English (), translated into 14 languages (Brazilian Portuguese, Chinese/Mandarin Chinese, Czech, French, German, Italian, Spanish, Polish, Portuguese, Rumanian, Russian and Serbian). In each case the translation was back translated and checked by an independent native-speaking eye care professional to ensure that consistency was maintained in the meaning of the questions. The anonymous, online survey was administered using the Qualtrics platform (Utah/Seattle, Washington, USA).

Table 2.2. Summary of questions presented in the survey

Question Categories	Possible Responses
Practitioner demographics	Mode of practice; Ophthalmologist, optometrist or other Years of clinical experience Country of practice
Type of dry eye patients managed (ranking)	1. No presenting specific symptoms: identified incidentally on questioning 2. Intermittent presenting symptoms: occasional effect on quality of life 3. Intermittent presenting symptoms: occasional effect on quality of life 4. Moderate symptoms: frequent impact on quality of life 5. Severe symptoms: constant debilitating effect on quality of life
Are you LICENSED to use this within your scope of practice in your country? Do you ever PRESCRIBE this option? What SUBTYPE(S) of dry eye disease	1. Advice 2. Essential fatty acid supplements 3. Artificial Tears a. Low viscosity-enhancing lubricant PRESERVED b. High viscosity-enhancing lubricant PRESERVED c. Low viscosity-enhancing lubricant, UNPRESERVED d. High viscosity-enhancing lubricant UNPRESERVED 4. Ointments 5. Lipid containing lubricants (drops/spray) 6. Lid hygiene a. Lid wipes/scrubs

do you consider this treatment appropriate for (select as many as apply)?	b. Demodex cleansing lid wipes c. In-office demodex lid control d. Lid margin debridement e. In-office lid hygiene (e.g. BlephEx) f. Therapeutic meibomian gland expression 7. Moisture chamber spectacles /goggles 8. Punctal occlusion (with plugs) 9. Warm compresses a. Home-made warm lid compress, such as facecloth b. Commercially available warm lid compress/face mask c. In-office thermal pulsation of lids (e.g. LipiFlow) 10. In-office Intense Pulsed Light therapy 11. Topical antibiotics (e.g. azithromycin) 12. Systemic antibiotics a. Systemic azithromycin b. Oral antibiotics (e.g. doxycycline) 13. Topical Anti-inflammatory/ Immunosuppression a. Topical corticosteroids b. Topical cyclosporine c. Topical tacrolimus d. Topical lifitegrast 14. Secretagogues a. Topical secretagogues b. Oral secretagogues 15. Biologics a. Autologous/allogeneic serum b. Amniotic membrane 16. Therapeutic contact lenses 17. Surgical approaches a. Intraductal probing b. Other surgical approaches
What SEVERITY(S) of dry eye disease do you consider this treatment appropriate for (select as many as apply)?	

2.2.2 Ethical approval

This study followed the tenets of the Declaration of Helsinki and was approved by Aston University and University of Auckland ethics committees. The data were collected anonymously and accessible only by the researchers. Prior to completing the questionnaire, a written statement informed the potential participant about the length of the survey, that submission of the questionnaire implied consent to participate, and that, as the data were anonymous, no changes to submitted data could be made. Responses could be submitted only once from a single device.

2.2.3 Participants

A link to the online survey was distributed via e-mail, through TFOS Ambassadors, conference seminars, professional college institutions, and university alumni communities, and by ECPs word-of-mouth advertising. The survey was accessible from February 2018 and the data extracted in August 2019.

2.2.4 Data analysis

For the statistical analysis, countries were grouped into continents; Europe and the United Kingdom (EU), North America (NA), Latin America (LA), Australasia (AA), and Asia/Middle East (AME). Insufficient responses were received from Africa to permit statistical analysis, but these data were included in the global analysis. When comparing countries within a continent, only those countries with a survey response rate ≥30 was included in the statistical analysis.

All analyses were performed using IBM SPSS Statistics version 26 (New York, USA). Only completed surveys were included. Descriptive statistics such as median and range, or mean and standard deviation, were employed to describe the clinical DED severity and subtype when reviewing the therapy approach of practitioners. Due to the ordinal nature of the data, Mann-Whitney test was used to compare data between continents and countries. Fisher's exact test was used to compare categorical data. Based on a Bonferroni adjustment, a p-value of 0.003 or less was considered to denote statistical significance when comparing the continents approach to dry eye management, whilst a p-value of 0.016 or less (EU and AME) or 0.025 or less (NA, LA and AA) was considered significant when comparing the differences between countries.

2.3 Results

2.3.1 Practitioner demographics

Completed questionnaires were collected from a total of 1,136 eye care professionals, (37% ophthalmologists, 58% optometrists and 5% opticians) from 51 countries across 6 continents:

- Europe/UK and Scandinavia (n=459): Austria (n=22), Belgium (n=1), Bosnia and Herzegovina (n=5), Czech Republic (n=9), France (n=16), Germany (n=9), Greece (n=2), Ireland (n=13), Italy (n=18), Montenegro (n=2), Netherlands

(n=25), Norway (n=9), Poland (n=17), Portugal (n=14), Romania (n=30), Serbia (n=26), Slovakia (n=4), Spain (n=84), Switzerland (n=11), United Kingdom (n=142).

- North America (n=126): Canada (n=66), United States of America (n=60).
- Latin America (n=135): Argentina (n=40), Barbados (n=1), Brasil (n=22), Chile (n=9), Colombia (n=18), Dominican Republic (n=1), Mexico (n=41), Peru (n=2), Venezuela (n=1).
- Australasia (n=111): Australia (n=58), New Zealand (n=53).
- Asia and the Middle East (n=288): Georgia (n=2), Hong Kong (n=34), India (n=11), Iran (n=11), Israel (n=1), Kuwait (n=1), Japan (n=1), Malaysia (n=22), Pakistan (n=3), Philippines (n=23), Russia (n=62), Singapore (n=20), Sri Lanka (n=1), South Korea (n=20), Taiwan (n=18), Thailand (n=48), Turkey (n=10).
- Africa (n=17): Cambodia (n=1), Ghana (n=2), Nigeria (n=1), South Africa (n=13).

The average years of experience was 11.8 ± 7.9, being similar between the different professional backgrounds (p=0.101) (Table 2.3).

Table 2.3. Years of clinical experience by profession

Years of clinical experience	1-5	6-10	11-15	16-20	21+
Optometrists	23%	15%	12%	14%	36%
Ophthalmologists	21%	20%	17%	17%	25%
Opticians	31%	19%	2%	17%	31%

2.3.2 Types of Patients Examined

Of the study respondents (n=1139), 1085 eyecare professionals completed the question describing the severities of DED they manage; patients with intermittent to moderate symptoms of DED were most encountered in clinical practice (accounting for 34% of symptomatic patients, n=303/890). While 13% (n=140) reported they most commonly saw patients with severe symptoms, 56% (n=608) reported this was the least common patient they encountered.

2.3.3 Global management and therapies approach

Of those that responded, 85% (n=968/139) reported that they were actively managing DED. Of those who did not prescribe, 96% (n=164/171) reported providing advice to patients on topics such as hydration, healthy eating, and the office environment.

Globally 72% of the overall respondents reported using more than only advice when managing the dry eye condition. Two thirds (67%) of the responding optometrists reported being able to prescribe management and/or treatment in some form to their patients (beyond advice), compared to 73% of ophthalmologists.

As highlighted in Table 2.4, not all eye care professionals reported being licenced to apply any individual management strategy. Respondents were asked to indicate whether they were licensed to use a certain management strategy and if they were prescribing it. Many treatment options for dry eye were observed to be utilised by respondents (*Table 2.4*). On average, 23% of the eye care professionals, who answer positively on managing dry eye disease, responded not to prescribe a treatment they had previously answered as licenced to use (Ophthalmologists 21%, Optometrists 29%, Others 24%). As a result, on average 12% of the professionals reported being licensed to use a certain treatment but are not currently prescribing it on their daily practise.

Many treatment options for dry eye were observed to be utilised by respondents (*Table 2.4*). Independent of DED severity and subtype, the most used management approaches across the globe are advice (87%), low (85%) and high (80%) unpreserved viscosity-enhancing lubricants and lid wipes/scrubs (81%). *Figure 2.1* displays graphically the mean level of severity and subtype reported by practitioners in their adoption of each dry eye management strategy and the average range over which they consider it to be appropriate.

Table 2.4. Proportion of [licenced] practitioners who use the different management options for dry eye based on continent, followed by proportion of practitioners prescribing that management

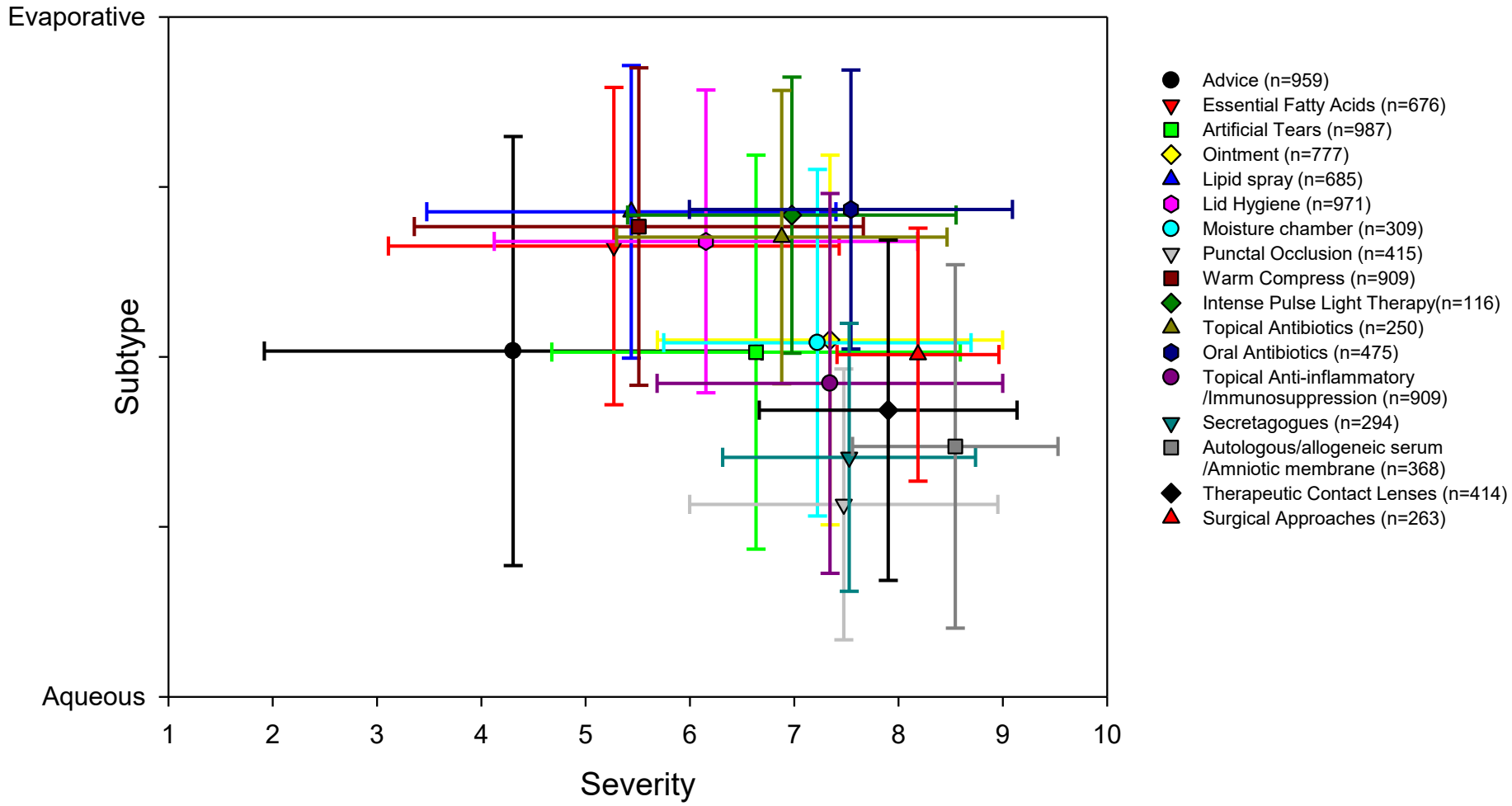
Management approach ⇩	[Licensed practitioners] Prescribing practitioners by continent					
Region studied ⇨	Europe	North America	Latin America	Australasia	Asia and Middle East	**WORLDWIDE**
Advice (e.g., hydration, healthy eating, office environment etc)	[85%] 80%	[98%] 91%	[85%] 85%	[94%] 91%	[90%] 86%	[90%] 87%
Essential fatty acid supplements	[66%] 53%	[94%] 87%	[63%] 60%	[89%] 85%	[66%] 49%	[76%] 67%
Low viscosity-enhancing lubricant PRESERVED	[75%] 48%	[94%] 83%	[80%] 77%	[90%] 75%	[76%] 42%	[83%] 71%
High viscosity-enhancing lubricant PRESERVED	[72%] 45%	[96%] 83%	[76%] 76%	[90%] 72%	[74%] 66%	[82%] 69%
Low viscosity-enhancing lubricant UNPRESERVED	[81%] 80%	[94%] 87%	[79%] 85%	[93%] 94%	[81%] 80%	[85%] 85%
High viscosity-enhancing lubricant UNPRESERVED	[79%] 76%	[94%] 85%	[76%] 73%	[94%] 92%	[76%] 72%	[84%] 80%
Ointment	[64%] 60%	[97%] 92%	[77%] 77%	[92%] 88%	[67%] 61%	[80%] 75%
Lipid containing lubricants (drops/spray)	[74%] 61%	[96%] 75%	[69%] 60%	[90%] 79%	[61%] 46%	[78%] 64%
Lid wipes / scrubs	[78%] 74%	[97%] 93%	[76%] 74%	[95%] 93%	[74%] 72%	[84%] 81%
Demodex treating lid wipes	[48%] 35%	[96%] 74%	[58%] 45%	[88%] 57%	[43%] 27%	[67%] 48%
In-office demodex lid control	[36%] 18%	[90%] 52%	[50%] 35%	[84%] 44%	[32%] 18%	[58%] 33%
Moisture chamber spectacles / goggles	[40%] 21%	[90%] 42%	[41%] 22%	[79%] 38%	[50%] 28%	[60%] 30%
Punctal occlusion (with plugs)	[37%] 26%	[90%] 62%	[61%] 48%	[82%] 46%	[49%] 34%	[64%] 43%
Homemade warm lid compress such as facecloth	[69%] 59%	[92%] 56%	[76%] 75%	[92%] 75%	[72%] 63%	[80%] 66%
Commercially available warm lid compress / face mask	[63%] 56%	[95%] 90%	[56%] 44%	[90%] 76%	[60%] 41%	[73%] 61%
Lid margin debridement	[42%] 30%	[89%] 62%	[45%] 26%	[81%] 48%	[45%] 35%	[60%] 40%
In-office lid hygiene (e.g., microblepharoexfoliation)	[41%] 25%	[87%] 52%	[44%] 26%	[83%] 38%	[37%] 22%	[58%] 33%

Therapeutic meibomian gland expression	[49%] 39%	[96%] 66%	[65%] 62%	[91%] 70%	[42%] 34%	[69%] 54%
In-office thermal pulsation (e.g., LipiFlow)	[20%] 3%	[80%] 44%	[26%] 10%	[63%] 8%	[22%] 8%	[42%] 15%
In-office Intense Pulsed Light (IPL) therapy	[20%] 4%	[40%] 16%	[20%] 7%	[70%] 34%	[16%] 8%	[33%] 14%
Topical antibiotics	[29%] 28%	[94%] 72%	[71%] 64%	[77%] 45%	[46%] 41%	[63%] 50%
Topical azithromycin	[22%] 17%	[77%] 44%	[54%] 38%	[40%] 4%	[31%] 22%	[45%] 25%
Systemic azithromycin	[20%] 13%	[66%] 31%	[50%] 37%	[34%] 30%	[35%] 20%	[41%] 26%
Oral antibiotics (e.g., doxycycline)	[27%] 26%	[75%] 60%	[61%] 63%	[35%] 30%	[44%] 38%	[48%] 43%
Topical corticosteroids	[28%] 28%	[97%] 88%	[71%] 71%	[77%] 55%	[47%] 41%	[64%] 57%
Topical secretagogues	[11%] 3%	[50%] 15%	[34%] 17%	[16%] 2%	[34%] 27%	[29%] 13%
Oral secretagogues	[11%] 3%	[34%] 9%	[29%] 11%	[10%] 1%	[24%] 9%	[22%] 7%
Topical cyclosporine	[23%] 23%	[96%] 87%	[68%] 63%	[22%] 8%	[42%] 36%	[50%] 43%
Topical tacrolimus	[10%] 5%	[41%] 10%	[43%] 26%	[10%] 6%	[20%] 13%	[25%] 12%
Topical lifitegrast	[7%] 2%	[67%] 52%	[22%] 5%	[6%] 1%	[10%] 3%	[23%] 12%
Autologous/allogeneic serum	[16%] 15%	[50%] 25%	[58%] 50%	[10%] 10%	[32%] 27%	[33%] 25%
Therapeutic contact lens approaches	[40%] 30%	[87%] 49%	[72%] 52%	[70%] 37%	[47%] 35%	[63%] 41%
Amniotic membrane	[13%] 9%	[58%] 39%	[44%] 30%	[7%] 4%	[32%] 26%	[31%] 22%
Intraductal probing	[13%] 6%	[40%] 10%	[28%] 14%	[19%] 6%	[17%] 8%	[23%] 9%
Other surgical approaches	[8%] 4%	[13%] 6%	[30%] 17%	[4%] 4%	[23%] 13%	[16%] 9%

Differences in management approaches were identified globally. Artificial tears with low viscosity were more commonly prescribed by practitioners than high viscosity products (by 1.06 times; n=817 vs 768) and unpreserved prescribed by more practitioners than preserved (by 1.27 times; n=697 vs 888). Ointments were prescribed by more practitioners (by 1.13 times; 762 vs 672) than lipid containing lubricants, but high viscosity lubricants were recommended by a similar number of practitioners as ointments (by 1.01 times; n=768 vs 762). The number of practitioners who recommended the use of general lid wipes/scrubs outnumbered those recommending specific demodex cleansing wipes (by 1.89 times; 860 vs 454). Demodex lid cleansing wipes for home use were recommended by more practitioners than those applied in-office (by 1.55 times; 454 vs 293). Home-made warm lid compresses (such as using a facecloth) are still recommended more commonly by practitioners than commercially available warm lid compresses/face masks (by 1.14 times; n=702 vs 603).

When it comes to performing in-office treatments for eyelid management such as Blephex or lid-debridement, the latter is used by more practitioners (by 1.27 times; n=403 vs 323), as is therapeutic meibomian gland expression compared to Blephex (by 1.59 times; n=514 vs 323). For tear preservation, more practitioners used punctal occlusion than moisture chamber spectacles/goggles (by 1.35 times; n=409 vs 302). LipiFlow and intense pulsed light therapy were used by similar numbers of practitioners (n=116 vs 108). More practitioners prescribed topical than oral antibiotics (by 1.14 times; n=471 vs 415). Azithromycin was used by a similar number of practitioners in both oral and topical form, with a slight preference for topical use (by 1.05:1; n=248 vs 235). Secretagogues were prescribed by more practitioners topically rather than orally (by 1.97 times; n=134 vs 68). Topical immunomodulators such as tacrolimus were prescribed slightly more frequently than lifitegrast (by 1.22 times; n=110 vs 90), but corticosteroids were much more frequently prescribed than tacrolimus (by 4.63 times; n=510 vs 110). Practitioners tend to prescribe therapeutic contact lenses more commonly than either autologous/allogenic serum (by 1.62 times; n=406 vs 250) or amniotic membranes (by 1.94 times; n=406 vs 209). Autologous/allogenic serum was reported to be used more abundantly than amniotic membrane (by 1.20 times; n=250 vs 209). Intraductal probing and other surgical approaches were reported to be performed by equal number of practitioners 1:1 (n=90 vs 90).

Figure 2.1: The median severity and subtype for which each DED management is prescribed (symbol) and the average reported range ('error' bars). n = number of practitioners reporting prescribing each management.



Sub-categories of management therapies identified in (*Table 2.2*) were used to reduce the data to make it easier to visualise in (*Figure 2.1),* bringing the number down from 35 to 17 categories.

Of those that prescribed:

1. Artificial tears, 832 (73%) reported prescribing both low and high viscosity, with and without preservatives.
2. Lid hygiene, 360 (32%) reported using the lid hygiene options listed in (*Table 2.2).*
3. Warm compress, 408 (36%) noted working with in-office thermal pulsation of lids as well as prescribing home use warm compresses.
4. Antibiotics, 372 (33%) reported using both topical and oral azithromycin.
5. Topical anti-inflammatory/immunosuppressants, 408 (36%) prescribe two anti-inflammatory options.
6. Secretagogues, 197 (19%) used both topical and oral forms.
7. Biologic products, 243 (21%) used both serum and membranes.
8. Surgical approaches, 145, (13%) performed intraductal probing as well as other surgical approaches.

2.3.4 Severity

The range of treatments offered at different disease severities are shown in (*Table 2.5).* Some treatments were prescribed across all severity levels, such as artificial tears (by ~80% of practitioners) and nutritional supplements (by ~45%). Other products were prescribed mostly for moderate severities such as ointments (from ~35 to ~47%), lipid-based products (from ~54 to ~73%), lid hygiene (from ~12 to ~27%) and warm compresses (from ~55 to ~65%). Some others were prescribed more frequently with increasing disease severity, for instance, in-office lid management, which increased from ~29% for mild DED to ~57%, punctal occlusion (from ~5 to 48%) as were MCG (from ~9 to ~38%). This increase was noted also on pharmacological therapies as the disease progresses, topical antibiotics (increased from ~8 to ~38%), oral antibiotics (from 4% for mild DED to ~43% for severe DED), topical anti-inflammatories/immunosupressants (from ~4 to ~24%), secretagogues (from ~10 to ~56%), biologics (from ~2 to ~47%), therapeutic contact lenses (from ~5 to ~43%) and surgical approaches (from ~3 to ~27%).

Table 2.5. Proportion of practitioners' therapy prescribing for each level of DED severity that they examine

	Mild	Moderate	Severe
Essential fatty acids	43.2%	49.4%	40.2%
Artificial tears	84.1%	88.0%	74.2%
Ointments	34.9%	47.2%	31.1%
Lipid-based products	54.4%	73.1%	50.2%
Lid hygiene	11.8%	26.6%	25.0%
Moisture chamber googles	8.4%	22.8%	38.4%
Punctal occlusion	4.7%	24.5%	47.9%
Warm compresses	55.1%	64.5%	44.3%
In-office treatments	28.6%	56.8%	56.7%
Topical antibiotics	7.5%	31.9%	37.6%
Oral antibiotics	4.0%	26.9%	43.2%
Topical anti-inflammatories/immunomodulators	4.4%	13.3%	24.4%
Secretagogues	10.5%	41.1%	56.2%
Biologics	1.7%	12.6%	46.5%
Therapeutic contact lenses	4.5%	16.0%	42.9%
Surgical approaches	2.9%	7.2%	26.7%

Analysis of the percentage of practitioners choosing a specific management approach according to the different severity levels, identified statistically significant differences between continents and within continents (Table 2.6). Between continents, the main differences were:

- The use of pharmaceuticals at lower levels of severity in North America and Asia/Middle East.
- The use of unpreserved lubricants and homemade warm compresses at higher severities in Latin America compared to other continents (*Table 2.6*).

Within continents, the main differences were:

- Lid hygiene, topical corticosteroids, topical and oral secretagogues and autologous/allogeneic serum were prescribed at lower severities in Romania than in the UK, as was oral secretagogues in Spain compared to the UK.
- Pharmaceuticals were prescribed at lower severities in the USA than Canada.
- Commercially available warm lid compresses/face masks were prescribed at lower severities in Mexico than Argentina.
- Preserved lipid containing lubricants were prescribed for lower disease severity in Thailand than Hong Kong.
- Moisture chamber spectacles/goggles and surgery were prescribed for lower severities in Hong Kong than Thailand.
- In office anti-demodex treatment was prescribed at a lower severity in Russia than in Hong Kong (*Table 2.6*).

Table 2.6. Statistically significant differences in the severity at which therapies were prescribed between continents and between countries within these continents with sufficient data.

	Europe/UK Scandinavia (EU)	North America (NA)	Latin America (LA)	Australasia (AA)	Asia/Middle East (AME)
Europe/UK Scandinavia	LVUL Spain ↑11% UK (p=0.006) OLH UK ↑19% Romania (p=0.009) HWC Spain ↑16% UK (p=0.016); Romania ↑22% UK (p=0.001) Tco UK ↑16% Romania (p=0.005) TS UK ↑52% Romania (p=0.005) OS UK ↑35% Spain (p=0.014); UK ↑63% Romania (p=0.006) AAS UK ↑23% Romania (p=0.05)				
North America	Advice NA ↑8% EU (p=0.001) LVUL NA ↑8% CSA (p=0.001) Tco EU ↑7 NA (p=0.001) TCy EU ↑11% NA (p=0.001) LF EU ↑1% NA (p=0.001) AM NA ↑4% EU (p=0.001)	CAM Canada ↑9% USA (p=0.009) OLH Canada ↑10% USA (p=0.012) OTP Canada ↑13% USA (p=0.001) Tcy Canada ↑12% USA (p=0.001)			

		LF Canada ↑17% USA (p=0.001) AAS Canada ↑9% USA (p=0.010) AM Canada ↑9% USA (p=0.001)		
Latin America	Advice LA ↑8% EU (p=0.002) LVUL LA ↑12% EU (p=0.001) HVUL LA ↑7% EU (p=0.001) HWC LA ↑12% EU (p=0.001) Tco EU ↑7% LA (p=0.002) TCy EU ↑13% LA (p=0.001)	HVUL LA ↑7% NA (p=0.003) HWC LA ↑22% NA (p=0.001)	CAM Argentina ↑23% Mexico (p=0.011)	
Australasia	LcL EU ↑8% AA (p=0.001) HWC EU ↑1% AA (p=0.003)	LVUL NA ↑8% AA (p=0.001) Tco AA ↑8% NA (p=0.001) TCy AA ↑18% NA (p=0.001) LF AA ↑22% NA (p=0.001) AM AA ↑10% NA (p=0.001)	LVUL LA ↑13% AA (p=0.001) HVUL LA ↑8% AA (p=0.001) LcL LA ↑11% AA (p=0.001) HWC LA ↑20% AA (p=0.001) Tco AA ↑9% LA (p=0.001) TCy AA ↑20% LA (p=0.001)	No significant differences

Asia/Middle East	TS EU (p=0.001) ↑7% TCy EU (p=0.001) ↑10%	AME	HWC AME ↑11% AA (p=0.002) TS NA ↑11% AME (p=0.003)	LVUL LA ↑11% AME (p=0.001) HVUL LA ↑6% AME (p=0.002) HWC LA ↑11% AME (p=0.001)	LcL AME ↑15% AA (p=0.001) HWC AME ↑9% AA (p=0.001) TS AA ↑20% AME (p=0.001) TCy AA ↑17% AME (p=0.001) LF AA ↑23% AME (p=0.001)	LVPL HK ↑14% Thailand (p=0.001) HVPL HK ↑17% Thailand (p=0.001) LcL HK ↑17% Thailand (p=0.003) ODC HK ↑26% Russia (p=0.006) MCG Thailand ↑17% HK (p=0.011) OSA Thailand ↑17% HK (p=0.006)

Notes: Amniotic membranes (AM), autologous/allogeneic serum (AAS), commercially available warm lid compress/face mask (CAM),essential fatty acids (EFA), high viscosity enhancing preserved lubricants (HVPL), high viscosity enhancing unpreserved lubricants (HVUL), homemade warm lid compress (HWC), intraductal probing (IP), intense pulsed light (IPL), lipid containing lubricants (LcL), lid margin debridement (LMD), lid wipes/scrubs (LwS), low viscosity enhancing preserved lubricants (LVPL), low viscosity enhancing unpreserved lubricants (LVUL), moisture chamber spectacle/goggles (MCG), therapeutic meibomian gland expression (MGE), oral antibiotics (OAb), in office demodex lid control (ODC), ointment (OI), in office lid hygiene (OLH), oral secretagogues (OS), other surgical approaches (OSA), in office thermal pulsation of lids (OTP), punctal occlusion (PO), oral azythromicin (SAz), topical antibiotic (TAb), topical azythromicin (TAz), therapeutic contact lenses (TCl), topical corticosteroids (TCo), topical cyclosporine (TCy), topical lifitegrast (TLF), topical secretagogues (TS), topical tacrolimus (TT). HK = Hong Kong. **↑ = prescribed at higher severity, ↓ = prescribed at lower severity**

2.3.5 Subtype

Globally, practitioners seem to have a well-defined management behaviour when treating DED patients according to their subtype. While a similar number of practitioners seems to prescribe ointments for each subtype, the principal reported approach for aqueous deficient DED was punctal occlusion by most practitioners, on the other hand, the use of product-containing lipids is the preferred management choice for EDE (*Table 2.7*). On the spectrum from aqueous deficient to evaporative DED, essential fatty acids, lipid based products, lid hygiene, warm compresses, in office treatments such as IPL and antibiotics (topical or oral) were more commonly prescribed for evaporative DED, while punctal occlusion, secretagogues, biologics, therapeutic contact lenses, artificial tears, moister chamber goggles, topical anti-inflammatories/Immunosupressants and surgical approaches were more typically prescribed for aqueous deficient DED (*Figure 2.1).*

Table 2.7. Proportion of practitioners that specifically use each therapy for a particular subtype of DED

	ADDE	EDE
Essential fatty acids	6.7%	26.4%
Artificial tears	26.9%	9.9%
Ointments	18.8%	16.5%
Lipid based products	5.7%	32.8%
Lid hygiene	4.2%	20.5%
Moisture chamber goggles	24.4%	16.3%
Punctal occlusion	66.2%	6.4%
Warm compresses	4.2%	26.4%
In office treatments	5.3%	31.6%
Top antibiotics	3.5%	22.9%
Oral antibiotics	3.5%	31.9%
Topical anti-inflammatories/Immunosupressants	20.3%	5.0%
Secretagogues	52.3%	8.3%
Biologics	41.0%	4.3%
Therapeutic contact lenses	34.6%	10.5%
Other surgical approaches	22.7%	14.7%

Notes: Aqueous deficient dry eye (ADDE); evaporative dry eye (EDE)

Between continents, few differences were identified, but are presented below:

- High viscosity lubricants were more commonly prescribed for predominantly aqueous deficient DED in Australasia than in North America or Europe
- EFAs were more frequently prescribed for DED that is predominantly aqueous deficient DED in Asia/Middle East than in Latin America.

Within continents:

- Autologous/allogeneic serum were more commonly used for managing evaporative DED in Romania than in Spain.
- Antidemodex wipes, topical antibiotics/azythromicin were prescribed for more evaporative DED in Argentina, than in Mexico.
- Lid hygiene with wipes/scrubs was more frequently recommended for aqueous deficient DED in Russia than in Thailand or Hong Kong.
- Antidemodex wipes and topical antibiotics were prescribed for evaporative DED in Thailand more than Russia.
- Topical/systemic azythromicin was more commonly used for the evaporative subtype patients in Thailand than Hong Kong (*Table 2.8*).

Table 2.8. Statistically significant differences in prescribing therapies for aqueous deficient (ADDE) and evaporative (EDE) dry eye subtypes between continents and between countries within these continents with sufficient data.

	Europe/UK Scandinavia (EU)	North America (NA)	Latin America (LA)	Australasia (AA)	Asia/Middle East (AME)
Europe/UK Scandinavia	AAS Romania ↑29% Spain (p=0.008)				
North America	No differences	No differences			
Latin America	No differences	No differences	ADW Argentina ↑26% Mexico (p=0.009) Tab Argentina ↑17% Mexico (p=0.006) Taz Argentina ↑17% Mexico (p=0.007)		
Australasia	HVUL EU ↑5% AA (p=0.003)	HVUL NA ↑9% AA (p=0.001)	No differences	No differences	
Asia/ Middle East	No differences	No differences	EFA LA ↑12% AME (p=0.001)	No differences	LwS Thailand ↑18% Russia (p=0.001) ADW Thailand ↑23% Russia (p=.004) Tab Thailand ↑14% Russia (p=0.006) LwS HK ↑17% Russia (p=0.008) Taz Thailand ↑11% HK (p=0.011) Saz Thailand ↑16% HK (p=0.001)

Notes: Autologous/allogeneic serum (AAS), Antidemodex wipes (ADW), essential Fatty Acids (EFA), unpreserved high viscosity enhancing lubricants (HVUL), lid wipes/scrubs (LwS), oral azythromicin (SAz), topical antibiotic (TAb), topical azythromicin (TAz).
↑ = prescribed more towards the evaporative part of the dry eye spectrum EDE, ↓ = towards the aqueous deficient part of the spectrum ADDE.

2.4 Discussion

The aim of this study was to determine how clinical DED management patterns differed by disease severity and subtype. Data collected from practitioners across the globe has also allowed differences in dry eye management approaches between continents and countries within continents to be identified.

Although previous studies have compared clinical patterns of DED management, this has been limited to comparisons between two countries (277), between professions (282) or exploring the trends within one or two countries only (278-281). The only previous global study had responses from 115 mainly corneal specialists and focused on aqueous deficient DED management (233). Hence, to our knowledge, this is the first study to have compared the current management patterns of eye care professionals across the world in the context of severity and subtype of DED.

The survey respondents are largely reflective of the balance of eye care professions across the globe, in each region (http://atlas.iapb.org/global-action-plan/gap-indicators/), with data from over 50 countries. The online delivery of the survey facilitated the response from eye care professionals with a range of clinical experience from 1 to 21 years.

Globally, eye care practitioners reported predominantly seeing patients with mild symptoms and least commonly those with severe DED. Overall, 85% of the surveyed practitioners were managing DED with a broad range of strategies, and almost all (96%) were providing patient education about dietary modification, local/office environment, and hydration. Environmental and iatrogenic factors can disrupt the homeostasis of the tear film, hence advice is critical with all levels of DED severity, as identified in the TFOS DEWS II Management and Therapy paper (121).

Patient education/advice, dietary advice, artificial tears and warm compress, artificial tears, are classified as “Step 1” interventions in the TFOS DEWS II management algorithm (121) (i.e. they are conventional, low risk, and commonly available management approaches for early stage disease); this study identifies these are the most commonly recommended management approaches, as reported also in other studies (277, 279-281). A slightly higher preference for the use of low viscosity preserved lubricants was noted in this study, which could be related to the overall milder nature of the DED the respondent reported treating and the greater perceived blurring of vision on instillation with ointments (283). No differences were found between the frequency of use

of high viscosity drops and ointments. Among treatments recommended for demodex infestation, less practitioners (by ~1.5x) treat the patient in-office at their consultation, instead preferring to prescribe demodex cleansing lid wipes for at-home use. Also, general lid wipes/scrubs are prescribed by almost twice (~1.9x) as many practitioners as those specifically for treating demodex, despite the high prevalence of these mites, particularly in the elderly (284). Therapeutic contact lenses are more used more frequently than biologic products when treating advanced DED (x1.62 autologous/allogenic serum and x1.92 amniotic membranes), presumably due to availability and difficulty to produce.

2.5 Conclusions

Management patterns according to the severity level of dry eye disease were similar across the different continents. Low severity DED is predominantly managed by advice, while mild dry eye is managed by artificial tear drops and sprays, warm compresses, lipid-containing products, and nutritional supplements. Practitioners use pharmaceutical approaches, ointments, punctal occlusion, in office treatments (in office lid hygiene and demodex control, lid margin debridement, therapeutic meibomian gland expression, thermal pulsation of lids, intense pulsed light) and secretagogues for more moderate DED, while only more severe dry eye is managed with blood/tissue products, therapeutic contact lenses and surgical approaches.

This follows the stepwise approach that is recommended by the TFOS DEWS II management algorithm (121). For the most severe dry eye cases, autologous/allogenic serum is used slightly more commonly (by ~1.2x) than amniotic membranes, perhaps due to their relative availability. Severe dry eye is less commonly managed than mild to moderate dry eye.

There is a consistent drop in the number of practitioners managing DED as the severity of patients increases. The North American and Asia/Middle East regions seemed to differ from other continents due to their tendency for a more pharmacological approach at lower levels of DED severity (especially in the USA). The notable difference within continents was in the use of home-made warm compresses across Europe. The continued use of face-cloths to heat the eyelids is surprising, since they are less effective at heat retention (285) resulting in reduced efficacy (286) relative to commercial products.

On the spectrum from aqueous deficient to evaporative DED, nutritional supplements, lipid drops/sprays, lid hygiene, warm compresses, intense pulsed light therapy, and antibiotics (topical or oral) were reportedly used more commonly for evaporative

subtypes, while punctal occlusion, therapeutic contact lenses, AT, moisture chamber goggles, secretagogues and biologics, were used preferentially for aqueous deficient DED (Figure 1). Even though PP and MCG are both used for ADDE, PP are used x1.35 more than MCG.

As identified in the introduction, there is currently limited research investigating the efficacy of management therapies by DED subtypes, but there is some evidence that liposome containing sprays and drops are more beneficial for patients with evaporative DED (91, 111), yet surprisingly ointments are prescribed x1.14 more than lipid-containing products. More research is needed to explore whether biomarkers and clinical tests can better inform the optimum choice of DED management strategy for an individual patient.

To our knowledge, this is the largest international survey of DED prescribing practices to date and the first to explore how the severity and subtype might influence management choices. Surveys always are subject to selection bias as they are likely to attract practitioners who are more involved in the condition being examined and therefore may be biased to more severe disease management and more actively involved in DED management than the 'average' practitioner. Country and region were based on where respondents were practicing, which may not be where they trained. However, in the absence of comparative and prognostic clinical studies, the results allow clinicians to benchmark their practice against their peers, highlight areas of disparate practice where further research is warranted to ensure optimised patient management, and may be useful in informing industry on how best to target product development.

Having identified current DED management practices across the globe in chapter 2, being similar as those advised by TFOS DEWS II, the next step on this thesis is to try a treatment from each of the recommended therapeutic stages. Starting with the first and most commonly used stage worldwide, Chapter 3:, will study a comparison between different artificial tear formulations on participants diagnosed with DED using the standardised TFOS DEWS II criteria to fill in this gap in the academic literature.

Chapter 3: Effectiveness of Blink Triple Action eye drops compared to a liposomal spray and emulsion drops

3.1 Introduction

Dry eye is a multifactorial disease of the ocular surface, according to the definition & classification subcommittee of the Dry Eye Workshop 2017, which is 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 neuro sensory abnormalities play etiological roles (16).

The evaporative component in DED has been found to be more common than ADDE (20, 21). In EDE thickness of the lipid layer is not continuous or is absent (287). Even though the LLT is a very small portion of the overall tear film thickness, it plays a crucial role in the regulation of the TF evaporation and ocular surface protection. As a consequence of a lipid layer deficiency, either absence or abnormality the TF becomes unstable and the evaporation rate increases four-fold compared to a normal TF (288). Some meta-analyses pointed out that a 2 to 3-fold increase in evaporation could be used as a cut-off value for diagnosing DED (289).

In order to treat DED, restoration of the TF homeostasis is the main aim to protect the ocular surface. Tear film stability has been proven to be subject to the thickness of LLT (287).

To date previous studies have investigated lipid-based drops and liposomal sprays (109, 110, 113, 165, 166, 168, 290). Significant improvement has been noted in several such studies, such as amelioration in lid parallel conjunctival folds (LIPCOF), tear break-up time (TBUT), Schirmer test, LLT, tear evaporation, tear stability and ocular symptoms (21, 109, 168, 171) confirming the efficacy of lipid-based products. Products containing phospholipids, help also enhancing the lipid layer, having been tested and improving subjective comfort as a result of the enhanced LLT as well (168), therefore creating a better TF stability (109, 166, 171).

Topical application of eye drops, sprays and gels are a first stage treatment for DED states (121), with the aim of stabilising or re-building the TF (*Table 3.0.1*), providing minimal or transient relief (21) (*Table 3.0.2*). Different liposomal formulations are now commercially available. Johnson and Johnson Surgical has recently introduced the first Blink Triple Action eye drops combining elements designed to enhance tear film stability (liposomes), longevity (sodium hyaluronate) and ocular surface nutrition (vitamin E). The addition of vitamin E on this product has been reported to have an increase on drug

delivery time onto the surface of the eye by multiple studies (291-293). As a consequence of vitamin E's hydrophobic molecular barrier, the medication to be released needs to travel a further distance which leads to a longer retention of the product on the eye (293).

The aim of the current study was to assess and compare the effectiveness of Blink Triple Action eye drops to a liposomal spray and emulsion drops in the treatment of DED, quantifying the changes resulting from every product, in terms of subjective ocular objective signs and symptoms. For this study we hypothesised that all products would give a clinical benefit but expecting that TAG (liquified-gel eye-drops) might not be as beneficial due to not being lipid-based.

Table 3.0.1. Summary of artificial tear instillation studies on dry eye patients and their respective tear film changes.

Study	Product comparison	Usage	Evaluation	Participants	Results
Korb D et al., 2005	Soothe – STH (one eye) vs Systane - Sy (contralateral eye)	Once	1 minute 5 minutes 15 minutes after instillation	N = 40	LLT at 1 minute: - Sy increased LLT (16%) / Little or no detectable increase of LLT after 15 minutes of Sy. - STH more than doubled LLT (106%).
Scaffidi R et al., 2007	Refresh eye therapy – RET vs. Clinicas Soothe – STH	Once	1 minute 5 minutes 15 minutes after instillation	N = 10	At 1 minute. - RET increased LLT. - STH nearly doubled the LLT. At 15 minutes. - RET identical or worse than baseline. - SO nearly doubled the LLT identical to baseline.
Craig J et al., 2010	Tears again (spray) on one eye - TAS vs Saline solution (spray) on the contralateral eye - SSS	Once	30 minutes 60minutes 90 minutes 135 minutes	N = 22	LLT - TA increase at 30 and 60 minutes. NIBUT increased at 60, 60 and 90 minutes in both groups. TMH no variation over time or groups. Comfort at 30 minutes 50% improvement, at 60 minutes no difference between eyes.
Pult H et al., 2012	Optrex ActiMist (spray) – OAS on one eye vs DryEyesMist - DEM or TearMist - TM on the contralateral eye	Once	10 minutes after instillation	N = 40 OAS + DEM N = 40 OAS + TM	Comfort and NIBUT were significantly better after OAS application improving both by a mean factor of >1.5. DEM and TM seemed to worsen both measurements.
Pult H et al, 2020	Tears again sensitive (spray) - TAS vs	Once	10 minutes 30 minutes	N = 30	NIBUT at 10 and 30 minutes were significant for TAS. NIBUT and comfort scores increased with TA.

	Ocuvers Spray hyaluron - OSH	Comfort scores with OSH were better than TA at 10 and 30 minutes.

DryEyesMist – DEM, LLT – Lipid layer thickness, NIBUT – Non-Invasive breakup time, OAS - Optrex actimist spray, OSH - Ocuvers spray hyaluron, RET – Refresh eye therapy, STH – Clinicas soothe, Sy – Systane, Saline solution spray – SSS, Tears again spray – TAS, TM – Tear mist, TMH – Tear meniscus height.

Table 3.0.2. Summary of artificial tear-supplement studies on dry eye patients

Study	Product comparison	Usage	Evaluation	Participants	Results
Lee S et al., 2004	Tears again (spray) - TAS vs Saline solution (spray) -SSS	3x a day	10 minutes 4 weeks 6 months	N = 191 - TAS N = 191 - SSS	TBUT increase at 6 months - TAS 93.7% longer than 10s. - SSS 36.7% longer than 5s. Lid margin inflammation at 4 weeks - TAS 73.7% decrease. LIPCOF at 6 months - TAS decreased one degree. - SSS remained the same. Schirmer at 4 weeks - TAS increase 5mm.
Wang TJ et al., 2010	Liposic Ophthalmic Liquid gel – LOLG vs Systane Lubricant – Sy	(4 weeks)	2 weeks 4 weeks	N = 15 - LOLG N = 15 - Sy	Symptomatology improvement in both groups. Schirmer improvement at 2 weeks in both groups, and only significant at 4 weeks on LOLG group.
McCann et al., 2012	Sodium hyaluronate – SH vs Hydroxypropyl methylcellulose - HPMC vs Emustil unidose - EUn	4x a day (90 days)	30 days 90 days Protocol compliance at 7 days 60 days	N = 25 - SH N = 25 - HPMC N = 25 - EUn	Tear evaporation reduction in all groups. NIBUT improvement in SH and EUn groups at 60 days. Osmolarity decrease in EUn group. Ocular staining decrease in EUn group at 90 days. Symptomatology decreases with all products.
Tomlinson A. et al., 2013	Refresh eye therapy - RET vs Optive Plus – OP vs Refresh Ultra - RU	3x a day (14 days) 1 week of washout in between	14 days 1 week washout 14 days 1 week washout	N = 19 control N = 18 dry eye sufferers	Tear evaporation reduction greater in OP group followed by RU group. Osmolarity was reduced with all groups, especially in OP group. Symptomatology scores were higher after using OP.

			14 days		
Simmons PA et al., 2015	Refresh Optive advance unidose – ROAU vs Refresh Optive Sensitive unidose– ROSU vs Refresh Optive advance multidose - ROM vs Refresh Optive advance multidose - ROAM	2x a day (30 days)	7 days 30 days	N = 105 - ROAU N = 103 - ROSU N = 51 - ROM N = 56 - ROAM	TBUT no significant differences between groups, but an overall increase at day 7 and 30. Schirmer scores improves at day 30 in all groups Staining decreased at day 7 and 30 for ROAU, ROSU, and conjunctival staining at day 30 with ROAM. Symptomatology decreased after day 7 with all treatments.
Essa L et al., 2018	Clinitas Soothe - STH vs Hyabak -Hy vs Tears Again - TA vs TheraTears - TT	As often as required (4 months, 1 month per treatment)	4 weeks - STH 4 days washout 4 weeks - HY 4 days washout 4 weeks - TA 4 days washout 4 weeks - TT 4 days washout	N = 50	Signs, symptoms, and patient satisfaction were similar after all products. OSDI improved after the 3rd month. LIPCOF after 4th month with all products. Conj. staining after 4th month with all products.

Jerkins G et al., 2020	Sytane Balance - SB vs Optive plus -OP	4x a day (35 days)	15 days 35 days	N = 117 – SB N = 114 - OP	TFBUT increase on day 35 in both groups 1second. Symptomatology improvement at day 35 in both groups equally.
Nosch DS et al., 2021	Tears again (spray) - TAS vs Ectoin Eye Spray (spray) – EES	2x a day (10 days)	10 minutes 10 days	N = 36	NIKBUT increase at 10 minutes with both products. Symptomatology reduced in both groups. LLT no difference in patterns in both groups. Conj. Hyperaemia no difference in between groups. Osmolarity no difference in between groups.
Craig J et al., 2021	Systane Ultra – SU vs Systane Complete - SC	4x a day (6 months)	30 days 60 days 90 days 120 days 150 days 180 days	N = 95	Symptomatology sustained reduction from day 30 in both groups. NIBUT sustained improvement from day 120 in both groups. LWE grade improved from day 60 onwards. Ocular staining grade improved from day 120.

Conj. – Conjunctival, DryEyesMist – DEM, EES – Ectoin eye spray, EUn - Emustil unidose, HPMC - Hydroxypropyl methylcellulose, LIPCOF – Lid-parallel conjunctival folds, LOLG - Liposic Ophthalmic Liquid gel, LLT – Lipid layer thickness, NIBUT – Non-Invasive breakup time, NIKBUT - Non-Invasive keratometric breakup time, OAS - Optrex actimist spray, OP - Optive Plus, OSH - Ocuvers Spray hyaluron, RU - Refresh ultra, RET – Refresh eye therapy, ROAU - Refresh Optive advance unidose, ROSU - Refresh optive sensitive unidose, ROM - Refresh optive advance multidose, ROAM - Refresh optive advance multidose, SH - Sodium hyaluronate, STH – Clinicas soothe, Sy – Systane, SB – Systane Balance, SSS - Saline solution spray, TA – Tears again, TAS - Tears again spray, TFBUT - Tear fluorescein breakup time, TBUT- Tear breakup time, TM – Tear mist, TMH – Tear meniscus height, TT – Thera tears.

3.2 Method

3.2.1 Participants

The study received Ethics a favourable opinion from the Aston University (Birmingham, United Kingdom) ethical committee and was conducted in accordance with the tenets of the Declaration of Helsinki. Participants were recruited from the University's dry eye clinics and participants were enrolled following explanation of the study and after providing written consent. A total of 21 subjects were enrolled and 20 met the inclusion criteria (*Figure 1).*

Twenty adult subjects, ranging in age from 20 to 71 years old (n=20, mean age = 39.6±17.3) (9 males, 11 females) were enrolled in the research study.

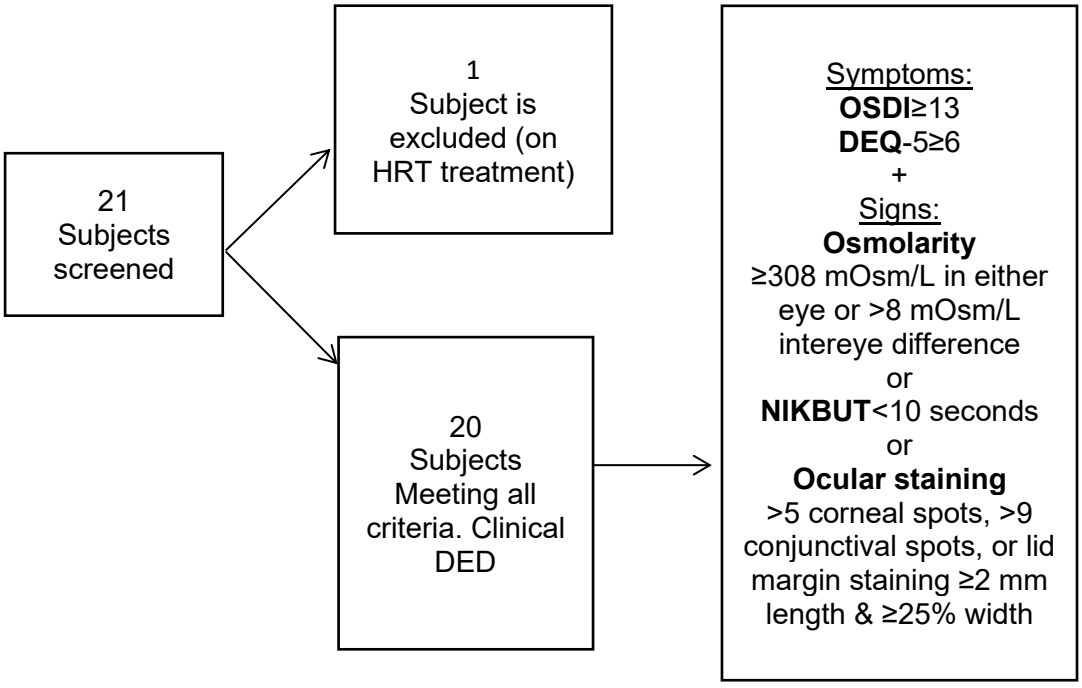


Figure 3.0.1. Consolidated standards of reporting trials 2010 flow diagram. Triple Blink Action.

3.2.2 Inclusion and Exclusion Criteria

Inclusion criteria included a positive response to the following dry eye tests, as recommended by TFOS DEWS II in the screening visit.

Ocular Surface Disease Index ≥ 13 (Ocular Surface Disease Index [OSDI]), osmolarity ≥308mOsm/L or an interocular difference of >8mOsm/L (TearLab, CA USA), average of

3 repeats of non-invasive breakup time <10s (Keratograph 5m), ocular surface corneal staining (> 5 fluorescein spots), conjunctiva staining (> 9 lissamine green spots) or lid wiper epitheliopathy (eyelid margin stain with combined dyes ≥2mm length & ≥25% width) (17). If loss of homeostasis was present in any of the previous tests simultaneously with symptoms, a DED diagnosis was made. In addition, subclassification of dry eye between aqueous deficiency and evaporative DED was informed by Tear Meniscus Height (TMH), meibography, lipid layer thickness (LLT) image pattern (294) with the oculus Keratograph 5m.

Reasons for participant exclusions included presence or undergoing treatment with topical or systemic medications for non-dry eye pathologies, on medication with known dry eye side effects, current contact lens wearers and prior ocular surgery. For patients using any other eye lubricants a washout period of 24hours was required before initiation.

3.2.3 Examination Protocol

The study was a 10-week prospective randomised cross-over investigator-masked, comparative clinical trial. Assessments were conducted in a room where temperature and humidity were controlled, remaining constant between 20-22°C and 30-45% respectively. To ensure that measurements were not affected by ambient humidity, participants spent a minimum of 10 min acclimatising to the room conditions before being tested.
Objective DED measurements were assessed from only the right eye, except for osmolarity.

At the baseline visit with each treatment, anterior eye examinations included the following tests conducted in this sequential order: Symptom Assessment in Dry Eye (SANDE) visual analogue scale (295); osmolarity in both eyes from microliter samples collected from the lower meniscus (296) using a TearLab station (TearLab Ltd, California, USA) with calibration performed every day following the manufacturer's instructions (17); evaporometry (VapoMeter – Delfin technologies average (3 readings)); non-invasive tear breakup time (NIKBUT) average (3 readings after 2 non-forceful blinks) (98, 297), tear meniscus height (TMH) image captured at 25x magnification measured with digital callipers; ocular redness (bulbar and limbal in temporal and nasal areas) lipid layer thickness patterns (LLT) using the Keratograph 5m (Oculus, Wetzlar, Germany) based on the predominant visible lipid pattern during a 15 second assessment-video with unrestricted blinking, graded according to the Guillon classification (294).

Ocular and lid staining was assessed with sodium fluorescein (NaFl) (i-DEW Flo, Mainline, Derby, UK) and lissamine green dye (GreenGlo, HUB Pharmaceuticals, LLC, Rancho Cucamonga, California, USA) were applied using previously recommended techniques (17), in order to evaluate localised areas of corneal and conjunctival epithelial desiccation. Staining was recorded using the modified Oxford grading scheme (105), and lid wiper epitheliopathy (LWE) was evaluated relative to Korb's grading (298).

Baseline tests were repeated at the end of each treatment period, as well as treatment satisfaction (1 being very likely to recommend and 5 being unlikely). An established gap on wash-out periods between treatments was found in the academic literature, therefore in our study a period of 2 weeks of product withdrawal was done to make sure the eye was clear of any product before starting the use of following product.

3.2.4 Intervention

This study involved the use of three commercially available dry eye products. Participants were prescribed with:

- Liposome-based eye drops Blink Triple Action® (Johnson and Johnson®, FL, USA); containing liposomes, sodium hyaluronate, vitamin E, polyethylene glycol succinate (TPGS), polyethylene glycol 400 (PEG 400), disodium edetate, polyhexanide methylbiguanide and purified water.
- Liposomal-based eye spray Optrex Actimist Spray® (AM, Optima-Pharma, Germany); containing soy lecithin, sodium chloride, ethanol, phenoxyethanol, vitamin A palmitate, vitamin E and purified water.
- Preservative-free emulsion liquified-gel eye-drops Tears Again Gel® (Optima Pharmaceuticals GmbH®, Germany); containing sodium hyaluronate, sodium chloride, sodium hydrogen phosphate, chlorides (potassium, calcium, magnesium), sodium hydrogen carbonate and water for injections.

Participants were assigned in a randomised manner to their first product, which was used for two weeks each by either spraying one puff onto each eye with closed eyes four times a day or by instilling one drop per eye four times a day at 10cm (as according to manufacturer). 2 weeks after baseline visit the product container was collected and measured to ensure patient compliance, followed by assessment for treatment 1. 2 weeks after that, baseline visit 1 was booked for after-product-withdrawal and treatment 2 was given, this process was then repeated until the third treatment was completed.

Before giving the products, its use was explained and demonstrated. Afterwards, a new product was given to the participant (labels were removed beforehand by another member of the team) and product was given on a sealed envelope, hence, investigator was blinded to the product type.

3.2.5 Data analysis

A total of 21 eligible participants were eligible at the Aston University site, but only 20 were finally enrolled, (exclusion due to HRT treatment). During the course of the study no participant discontinued participation. The final 20-subject group exceeded the minimum sample size requirement for the desired study power.

Sample size was determined from non-parametric adjusted power calculations conducted using G*Power Version 3.1.9.2 2014 (Universitat Kiel, Germany) (*Figure 3.0.2*). All analyses were performed using IBM SPSS Statistics version 26 (New York, USA) and Sigma Plot version 11.0 (Germany). The distributions of all parameters were assessed using one-sample Kolmogorov-Smirnov test and non-parametric statistics applied to those not normally distributed. Using a repeated measures ANOVA with between (artificial tears) and within time (time) variables, a sample of 20 participants allowed 80% power to detect significance at a level of $p<0.05$.

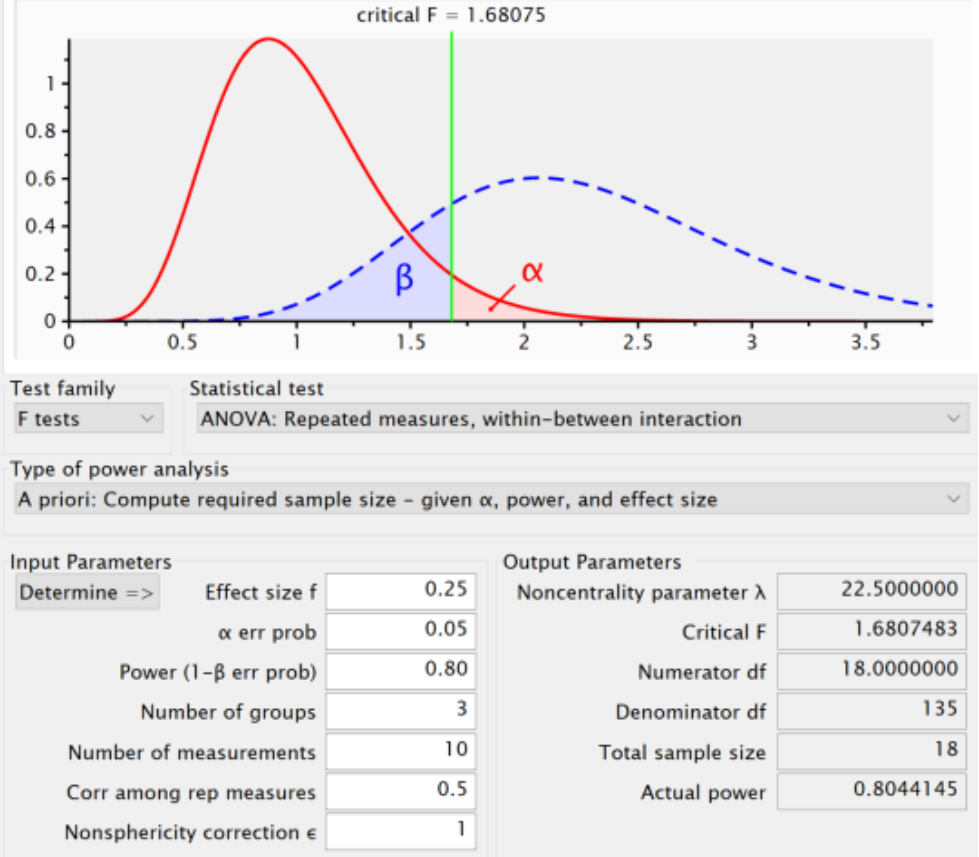


Figure 3.0.2. Adjusted power calculations. BTA-DEC.

3.3 Results

(*Table 3.0.3)* displays comparative analysis of the clinical data obtained before and after the application of each treatment.

Table 3.0.3. Average ± SD of dry eye signs and symptoms at each individual baselines and after 2 weeks of treatment.

	Artificial Tears (after 2 weeks of use)					
	Baseline	BTA	OAS	TAG	F value	p-value
Age (years)	39.6±17.3					
SANDE Frequency	5.74±2.06	4.30±2.04	3.85±1.85	3.48±2.37	0.104	0.901
SANDE Severity	5.75±2.03	4.19±2.00	3.62±1.95	3.51±2.47	0.239	0.789
Osmolarity	305±11.95	299±7.16	300±8.88	297±10.89	0.762	0.474
Evaporation	80.59±48.58	71.51±38.02	78.76±39.37	59.83±31.04	1.437	0.250
Bulbar redness	0.90±0.26	0.79±0.26	0.81±0.34	0.83±0.31	2.909	0.067
Limbal redness	0.58±0.19	0.53±0.21	0.58±0.28	0.56±0.25	1.559	0.224
TMH	0.32±0.16	0.31±0.16	0.31±0.15	0.32±0.15	1.162	0.324
LLT	1.45±0.69	2.15±1.14	1.70±0.86	1.7±1.30	1.852	0.171
NIKBUT	8.44±4.48	8.70±3.85	8.66±3.99	6.78±3.78	0.918	0.408
Corneal staining	1.10±0.85	0.95±0.88	0.95±0.76	0.85±0.81	0.495	0.613
Conjunctival staining	1.0±0.79	0.8±0.77	0.95±0.82	0.90±0.72	0.880	0.423
Lid margin staining	1.0±0.32	0.85±0.67	1.05±0.51	1.0±0.56	2.422	0.102
Future use of the product		2.15±1.09	2.55±1.05	1.85±0.93	1.080	0.139
Product recommendation		2.10±1.07	2.40±1.05	1.85±0.88	1.617	0.212

Blink Triple Action (BTA), Oprex Actimist Spray (OAS), Tears Again Gel (TAG). N=20. SANDE = Symptom Assessment in Dry Eye, TMH = Tear Meniscus Height, LLT = Lipid Layer Thickness, NIKBUT = Non-Invasive Keratometric Break-Up Time.

3.3.1 Efficacy

3.3.1.1 Subjective evaluation of Symptoms

At baseline, the mean Symptom Frequency (SF), scores were 5.74±2.06, and 5.75±2.03 for Symptom's Severity (SS) respectively, in between treatments patient subjective SF and SS were similar and marginally better than pre-treatment *(Figure 3.0.3*). In between group comparisons did not show statistically significant differences after the 2-week period of treatment use.

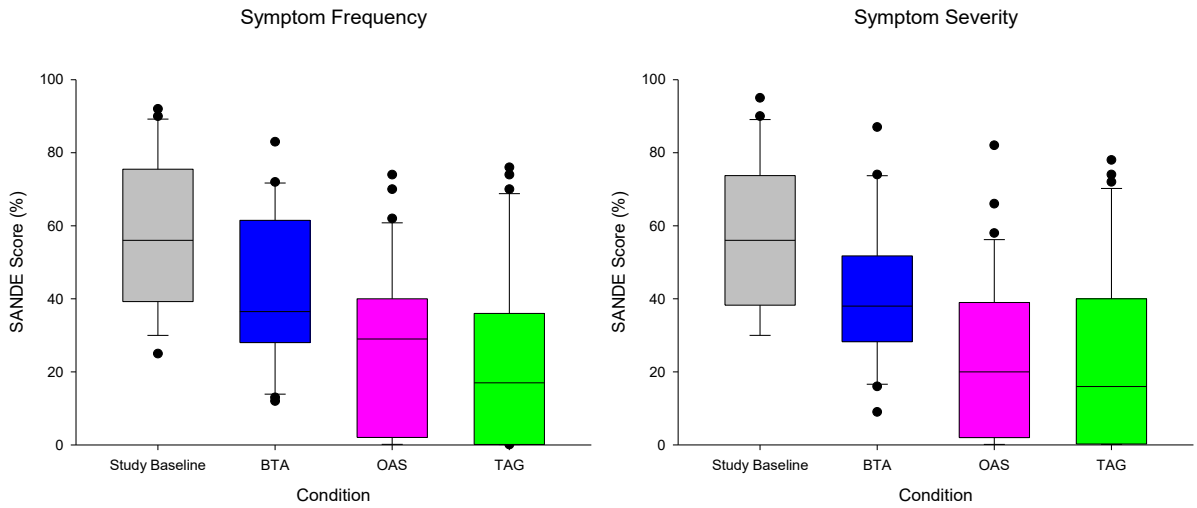


Figure 3.0.3.Ocular Symptomatology measured by SANDE Visual Analogue Scale. Frequency & Severity.
(Box boundaries display the 25th and 75th percentile, the solid line within the box represents the mean, error bars mark the 90th and 10th percentiles and the outer dots the outliers).

3.3.2 Homeosis markers

Homeostatic regulation markers did not show a statistically difference after the use of any of the 3 products. At baseline, osmolarity values were 305±11. 95mOsm/L, after the treatments the highest was 300.6±8.88 (OAS), but a slightly decrease was present after all three treatments up to 299.5±7.16 (BTA) and to 297.8±10.89 (TAG) *((Figure 3.0.4).*

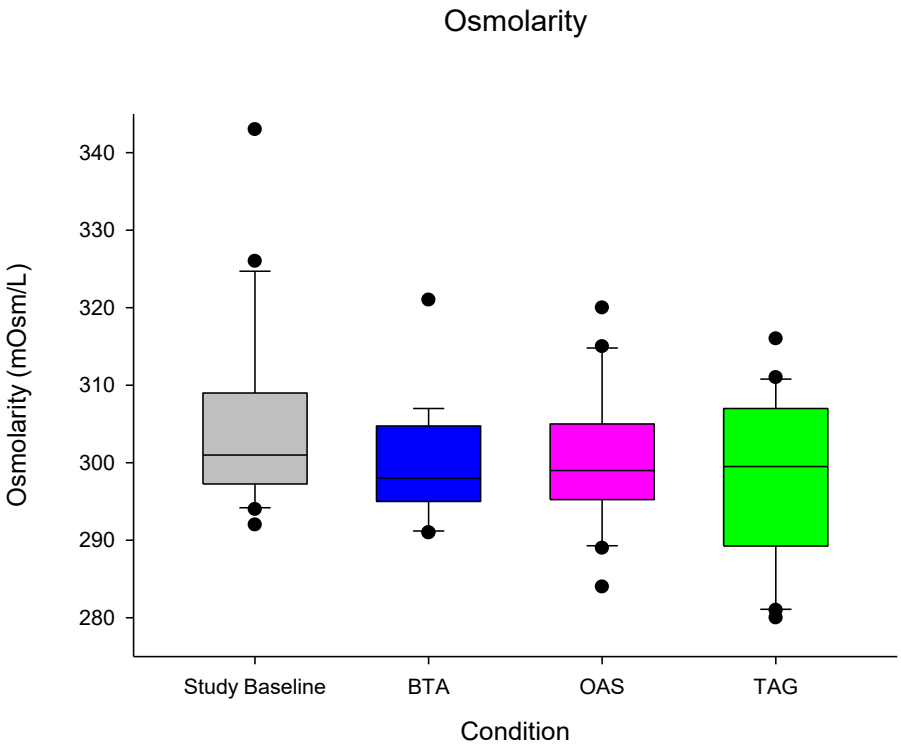


Figure 3.0.4. Homeostasis marker - Osmolarity scores during the BTA clinical trial. (Box boundaries display the 25th and 75th percentile, the solid line within the box represents the mean, error bars mark the 90th and 10th percentiles and the outer dots the outliers).

BTA and OAS maintained NIKBUT stability levels comparable except for TAG which had a slight reduction of TF stability to 6.78±3.78 s (*Figure 3.0.5*), yet the differences found were not significant.

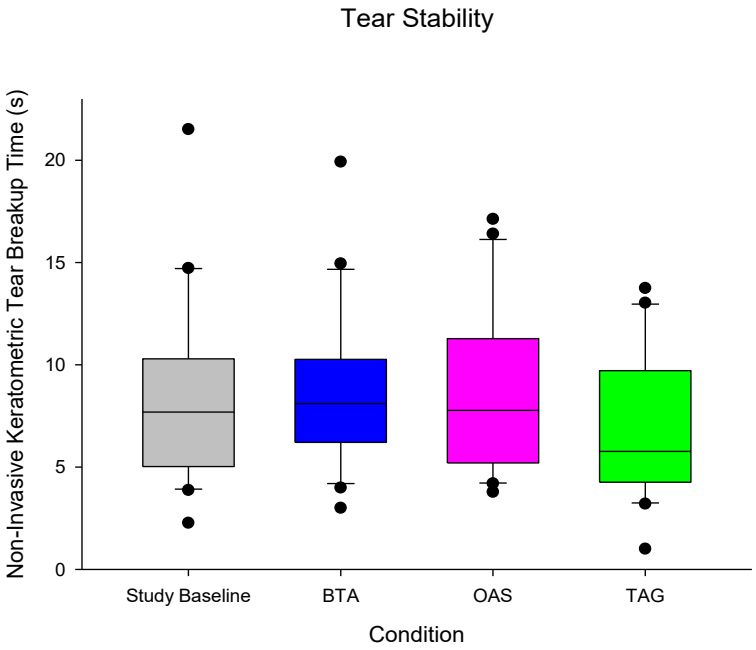


Figure 3.0.5. Homeostasis marker - Non-Invasive Keratometric Break-Up Time during the BTA clinical trial. (Box boundaries display the 25th and 75th percentile, the solid line within the box represents the mean, error bars mark the 90th and 10th percentiles and the outer dots the outliers).

Corneal, conjunctival and lid margin staining did not show any statistically relevant changes after any treatment, but they all had significantly better results than the pre-treatment (Figure 3.*0.6*, Figure 3.*0.7*, Figure 3.*0.8*).

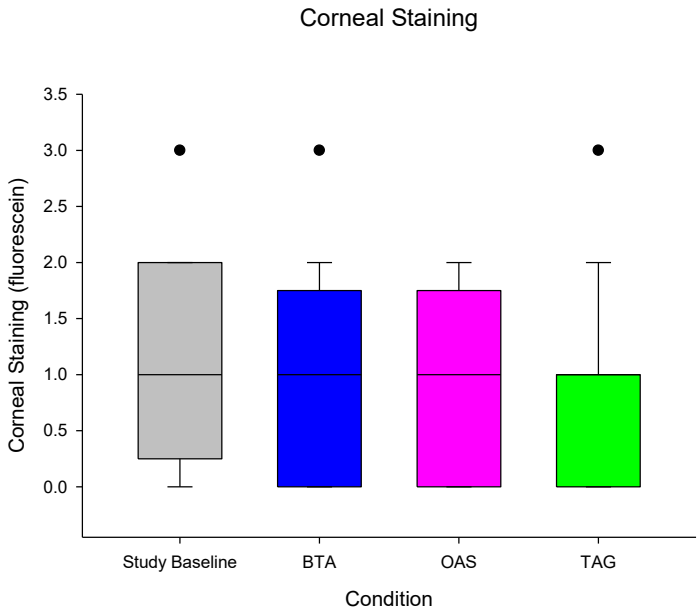


Figure 3.0.6. Homeostasis marker – Corneal staining scores during the BTA clinical trial. (Box boundaries display the 25th and 75th percentile, the solid line within the box represents the mean, error bars mark the 90th and 10th percentiles and the outer dots the outliers).

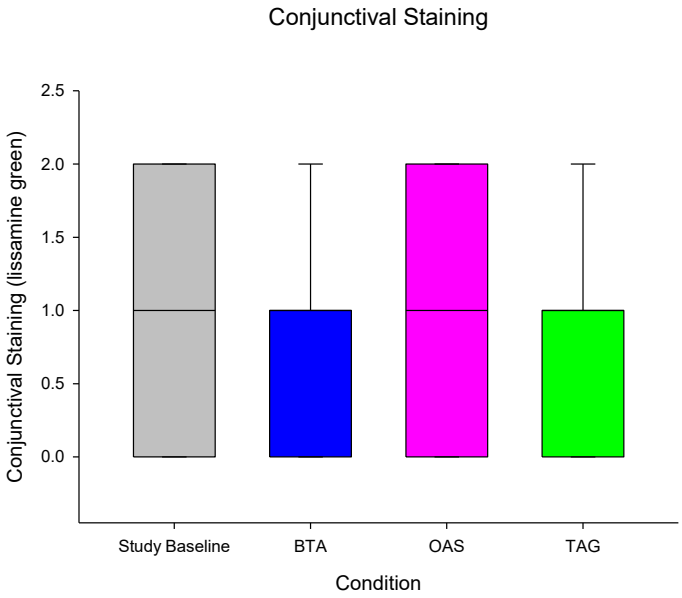


Figure 3.0.7. Homeostasis marker – Conjunctival staining scores during the BTA clinical trial. (Box boundaries display the 25th and 75th percentile, the solid line within the box represents the mean, error bars mark the 90th and 10th percentiles and the outer dots the outliers).

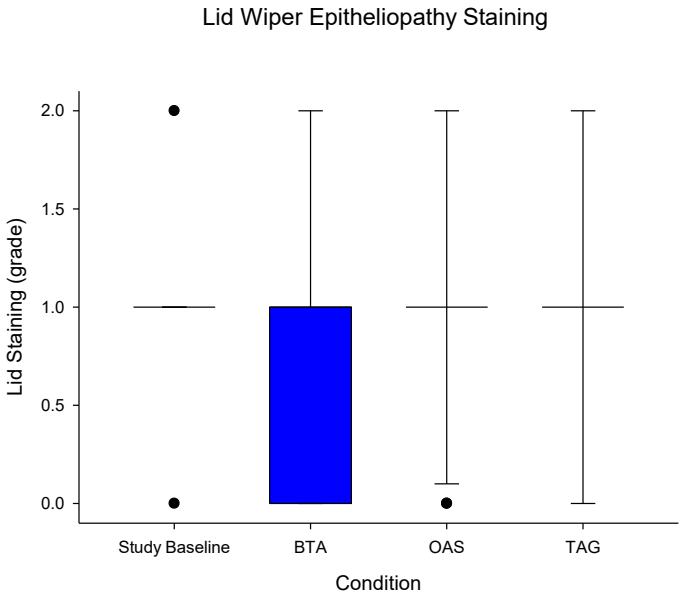


Figure 3.0.8. Homeostasis marker – Lid Wiper Epitheliopathy staining scores during the BTA clinical trial. (Box boundaries display the 25th and 75th percentile, the solid line within the box represents the mean, error bars mark the 90th and 10th percentiles and the outer dots the outliers).

3.3.3 Treatment preference

After trialling the three products, on a scale from, 1 - being very likely to 5 - being undecided, 45% preferred TAG over the other 2 products for both using it again and recommending it to a friend (*Figure 3.0.9*).

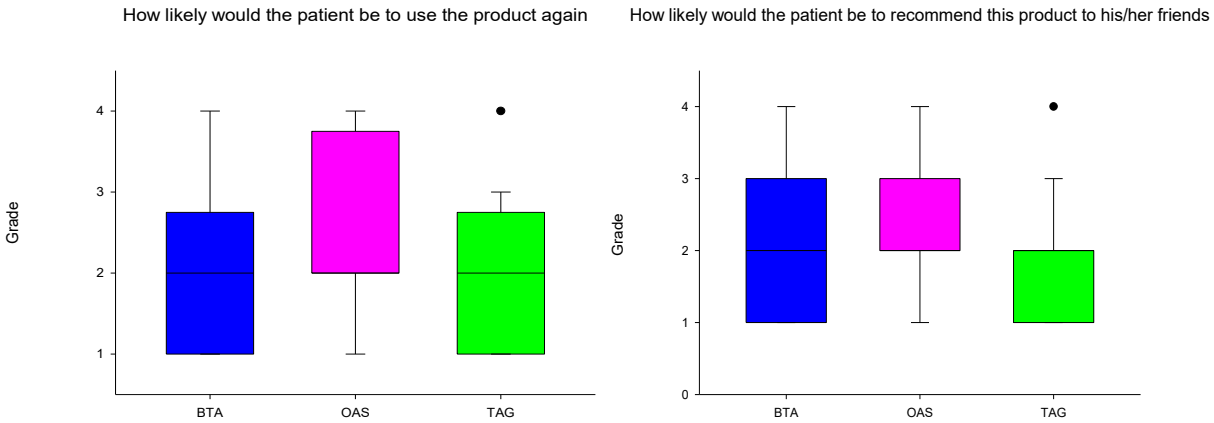


Figure 3.0.9. Product preference during the BTA clinical trial.

(Box boundaries display the 25th and 75th percentile, the solid line within the box represents the mean, error bars mark the 90th and 10th percentiles and the outer dots the outliers).

Safety profile of the 3 product was assessed by instillation tolerance, reactions, and events upon questioning at week 2 of each product. Parameters of safety included, increased hyperaemia, unusual eye-discharge, blurred vision and burning sensation upon product instillation.

No treatment-related events or reactions were reported during this study.

3.4 Discussion

Based on the literature, the current clinical study, is the first to compare different product formulations (spray, drop and liquified-gel) with similar compositions.

Baseline TF stability was below the cut-off value as suggested by (TFOS DEWS II Diagnostic methodology report) (17) giving us a DED diagnosis. In most cases the average of the tear volume (TMH) was higher than 0.2mm (17), indicating that the general DED condition of most participants had a tendency towards the non-ADDE end of the spectrum (2 ADDE:18 EDE). TMH remained consistent after treatment use, same as bulbar and limbal hyperaemia which remained the same or were slightly reduced with all product types.

In this study, an improvement in subjective comfort for both frequency and severity were observed after the use of all 3 product types. OAS and TAG did reduce subjective reported symptomatology more than BTA, yet none were statistically significant. The symptomatology outcomes align with most studies comparing tear supplementation (91, 110-112, 114, 115, 290), yet in our trial the results were not significant which could be due to only using the treatment for 2 weeks, which (as seen in Chapter 4) might not be enough time to see a therapeutic treatment effect. The use of artificial supplementation has been reported to resolve symptomatology in approximately one in five participants after a consistent use during 6 month (17).

Even though osmolarity values modestly decreased after 2 weeks of use with all products, there were no differences between them and there were not statistically significant, this was also reported in previous studies studies (115). Even though clinically all test results improved after any of the treatments, it was not statistically significant for any. These findings could be due to the short period of time that the product was prescribed for. In Chapter 4: and studies with longer therapeutic profiles AT use, denotes significant benefits after 2-4months of use.

Evaporation rates decreased with all products, these results being consistent with those of McCann et al., in 2015 (112). As expected, and due to its formulation and increased viscosity upon contacting with the ocular surface, the liquified-gel TAG showed the highest reduction on evaporation, yet none of the changes post-treatment were significant.

LLT has been seen to thicken after using any of the three products (not statistically significant). The direct transfer of liposomes onto the TF, BTA, seemed to thicken the lipid layer the most. Which in turn, protected more the ocular surface. Some more recent papers have shown differences in LLT even within classes of AT such as commercially available liposomal sprays (109, 169). Craig et al, 2020 suggested that lipid-based AT can be useful for both subtypes (91, 110). Lipid layer grade (LLG) suggests that on a wave pattern (number 3) or lower, on and Keeler-Guillon scale (91, 92) (evaporative tendency), only a lipid-based product will be efficient to treat this subtype. With slow fortification of the lipid layer through repeated lipid supplementation seems to impact ocular surface physiology, restoring tear film homeostasis (91).

Surface staining was clinically reduced with all products, in our study ocular surface staining was not clinically significant, similarly to (114). In other studies comparing ATs containing sodium hyaluronate, such as BTA, clinical evidence on surface staining decrease was evident (112, 299), which could possibly be attributed to the fact that the instillation of AT in these studies was 6 times a day compared to the 4 times a day used in our study.

Even though there was no statistically significant difference between products, therefore our hypothesis cannot be proven, TAG clinically decreased NIKBUT and all three products improved patient reported symptomatology. On the contrary of what would have been expected, due to its ease of installation, the spray formulation was the last product the participants would choose to use. Tears Again Gel (TAG) was preferred specifically in participants with thinner LLT at baseline due to its longer exposures time and increased viscosity.

Some of the limitations for this study could be that there was no control group, the comparison was done with each individual itself after 2 weeks of treatment withdrawal, also our treatment only lasted 2 weeks, for which the full potential of the therapeutic effect might have not been reached (21, 91, 111, 112).

3.5 Conclusion

Subjective symptom frequency and severity decreased with the use of any of the 3 products used.

Homeostasis markers such as osmolarity and ocular surface staining means decreased after any of the three products use, as well as ocular redness. The products used help by creating a protective layer onto the tear film by increasing the thickness of the LLT. The observed improvement of symptomatology and clinical changes, even if not significant, could feasibly be associated to a more protected ocular surface which lead to an increase in comfort.

This study showed that when used in a general DED population, artificial tear formulations are similarly effective, but the cohort was too small to examine the difference in effectiveness with the subtype classification of DED. The two weeks use followed by two weeks cross-over design failed to find statistically significant effects so the study in chapter 4 was designed to examine the longer-term use of drops as part of a multicentre study (for which the author was part of the design, writing up and implementation team).

Chapter 4: Developing evidence-based guidance for the treatment of dry eye disease with artificial tear supplements: A six-month multicentre, double-masked randomised controlled trial

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4.1 Introduction

Topical eye drops that supplement the natural tear film are the mainstay therapy for dry eye disease (DED) (121). The recent focus on meibomian gland dysfunction and lipid deficiency has driven a substantial evolution of artificial tear supplement formulations. While still presenting a largely palliative solution to managing dry eye, lipid components have been incorporated to address tear lipid deficiency, and aqueous-based viscous supplementation continues to be used to target lacrimal gland insufficiency, as seen in (*Chapter 2:*). However, practitioners seeking guidance in their choice of artificial tear supplements for the treatment of dry eye disease are faced with a dearth of sound scientific evidence, as comparative efficacy studies on lipid and non-lipid formulations across the breadth of dry eye subtypes are limited and the quality of evidence is generally low (110, 112, 113, 290, 300-302). Only one study to date has attempted to predict which of a range of artificial tears (used in a 4 week cross-over sequence) a patient would prefer, showing some logic to lipid containing drops assisting those with evaporative DED and osmoprotectants preferred by those with high baseline osmolarity (111). The need for more robust, level 1 comparative efficacy randomised controlled trials for lipid and non-lipid-based formulations, to guide targeted treatment according to individual presenting patient characteristics, dry eye subclassification and severity, is widely acknowledged (16, 121, 301).

Another area important to clinicians and their patients, but which is similarly devoid of sufficient attention in the literature, is the temporal profile or clinical course of artificial tear supplement efficacy and equally the wash-out period after a treatment. In a Cochrane review of 43 randomised controlled trials on artificial tear use for DED treatment (301), the average study follow-up duration was six weeks; three trials featured a three-month follow-up and only a single study attempted to investigate drop use over 12 months (303). Many studies focus on the immediate or short-term effects of a single instillation. Longer-term efficacy studies that more closely resemble intended clinical use are necessary to inform clinicians and patients about the recommended length of treatment regimes. An evidence-based approach may assist practitioners in encouraging patient compliance by setting realistic expectations on the time course of clinically significant improvements in signs and symptoms, and around the anticipated maximal treatment effect.

The objectives of this six-month, international multicentre, double-blind, randomised controlled trial on dry eye disease, diagnosed using contemporary global consensus criteria (17), were to:

1) Compare the efficacy of a lipid and a non-lipid based artificial tear supplement for the management of DED. For this study we hypothesised that lipid-containing products would be more beneficial on EDE cases.
2) Determine the temporal-therapeutic profile for clinically significant improvements of signs and symptoms, including the magnitude of change and time to reach maximal clinical benefit.
3) Assess whether clinical outcomes are influenced by baseline dry eye disease subtype or severity (16, 17).

4.2 Materials and methods

4.2.1 Subjects

This prospective, multicentre, randomised, double-masked, parallel group, six-month efficacy trial adhered to the tenets of the Declaration of Helsinki and was approved by the Human Participant Ethics Committees of Aston University, the University of Auckland, University of New South Wales (UNSW) Sydney, and the University of Waterloo.

The study was registered as a clinical trial (ACTRN12619000390189) and abided by the Consolidated Standards of Reporting Trials (CONSORT) statement (Figure 4.1) (304). The study was conducted between March 2019 and March 2020 at clinical academic sites in Australia, Canada, New Zealand, and the UK. On this chapter the data obtained from the thesis author at the Aston University site was compared to the overall study results.

Participants were required to be 18 years or older, with manifest symptoms and signs of dry eye disease according to the Tear Film and Ocular Surface Society Dry Eye Workshop II (TFOS DEWS II) diagnostic criteria; these were an Ocular Surface Disease Index (OSDI) score ≥13 or 5-Item Dry Eye Questionnaire (DEQ-5) ≥6, with at least one positive indicator of homeostatic imbalance based on non-invasive tear film break up time (NIBUT), tear osmolarity and/or ocular surface staining) (17). In addition, participants were required to be non-contact lens wearers; not be pregnant or planning to become pregnant in the next 12 months; to self-report having experienced dry eye

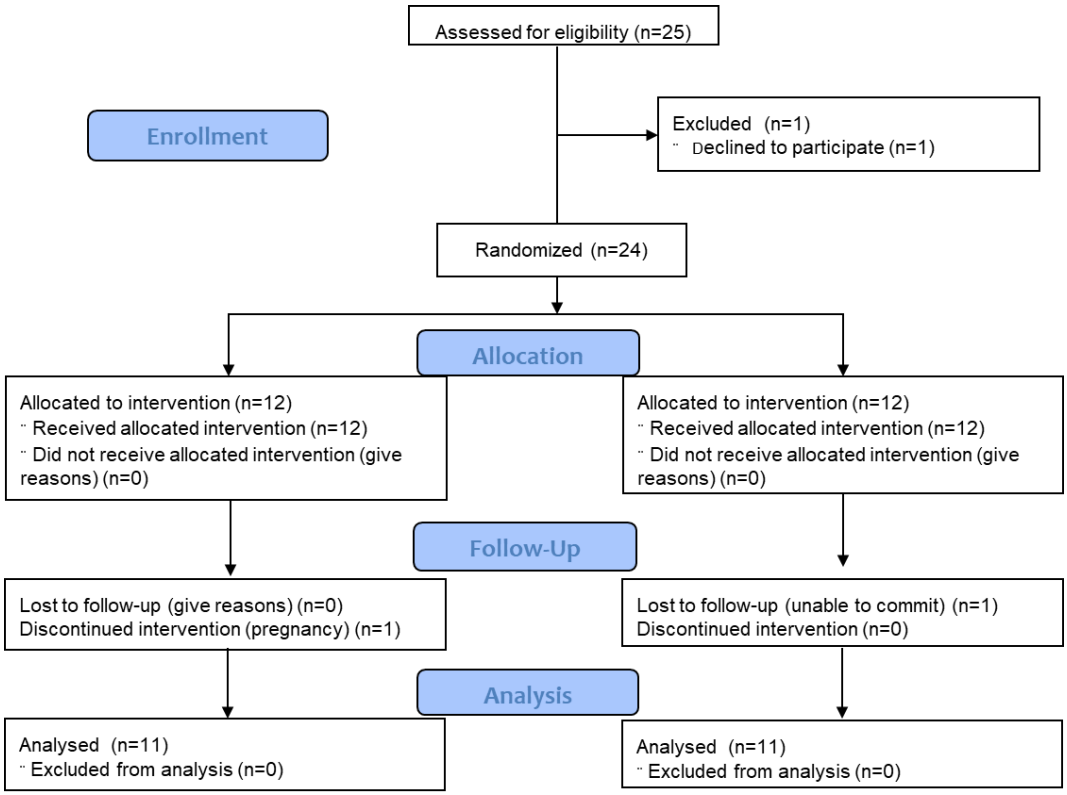

Figure 4.1. Consolidated standards of reporting trials 2010 flow diagram for the multisite JASMINE-DEC study.

symptoms for a minimum of six months; report no history of major systemic or ocular conditions; report no ophthalmic surgery in the previous three months or during the treatment period; and report no use of systemic or topical medications known to affect the eye two weeks prior to baseline assessment or during the treatment period. Ongoing therapeutic measures, such as warm compresses, were permitted to continue during the study period, as long as no changes to the treatment routine occurred. Eligible participants were enrolled for baseline screening after providing written informed consent to participate.

A total of 25 eligible participants were eligible at the Aston University site, but only 24 were finally enrolled, (exclusion due to fear of AT causing further ocular damage). During the course of the study one participant discontinued participation due to pregnancy and another subject was not able to commit the commute to the study location. The final 22-subject group exceeded the minimum sample size requirement for the desired study power.

4.2.2 Interventions

The study compared an aqueous-based drop and a combination lipid-aqueous nano emulsion. The aqueous-based drop (Systane Ultra, Alcon, Fort Worth, TX, USA) contains; aminomethylpropanol, boric acid, hydroxypropyl guar, POLYQUAD (polyquaternium-1) 0.001% preservative, and sorbitol. The lipid-aqueous drop (Systane Complete, Alcon, Fort Worth, TX, USA) contains; boric acid, dimyristoyl phosphatidylglycerol, edatate disodium, hydroxypropyl guar, mineral oil, polyoxl 40 stearate, POLYQUAD 0.001% preservative, sorbitan tristearate, and sorbitol.

Participants were randomised to four times (minimum) daily topical application of either the non-lipid drop (n = 11 in total) or the lipid drop (n = 11 in total) in both eyes for a six-month period, independently of their DED subtype. Randomisation was conducted by computer-generated random number allocation and applied to sequentially enrolled participants. The randomisation schedule was determined prior to participant recruitment, such that the investigator involved in baseline participant assessment was not involved in treatment allocation. Before giving the products, its use was explained and demonstrated. Afterwards, a new product was given to the participant on a sealed envelope, product labels were removed, and customised labels applied to obscure contents. Hence, the study was double-masked. Outcome measures were evaluated at 30, 60, 90, 120, 150 and 180 days after the baseline visit. Returned eyedrop bottles were weighed at each visit to determine patient compliance. Treatment success at six months was judged as an improvement of > 4s in NIBUT and/ or a ≥4.5-point reduction in OSDI symptom score (305, 306). Participants were instructed to avoid eye drop instillation for at least 90 min prior to measurements being collected at review appointments. Any other treatments (such as warm compresses or lid hygiene) were not permitted on the day of testing.

4.2.3 Measurements

Participants were assessed on a room with consistent temperatures of 21.8 ± 1.5 ◦C and relative humidity of 45.0 ± 8.1% (mean ± SD). Ocular measurements were conducted on the right eye only of each participant (except for osmolarity where the manufacturer's recommendations require both eyes to be assessed). Clinical tests were administered in accordance with the recommendations of the TFOS DEWS II Diagnostic Methodology subcommittee (17). To reduce the impact on tear film physiology, the tests were ordered from least to most invasive at each study visit (Table *4.1*).

Table 4.1. Order of clinical assessments conducted at Days 0, 30, 60, 90, 120, 150 and 180.

Assessments	Day 0	Day 30	Day 60	Day 90	Day 120	Day 150	Day 180
OSDI	x	x	x	x	x	x	x
SANDE	x	x	x	X	x	x	x
Best corrected visual acuity	x	x	x	x	x	x	x
Conjunctival hyperaemia	x	x	x	x	x	x	x
TMH	x	x	x	x	x	x	x
NIKBUT	x	x	x	x	x	x	x
LLT	x	x	x	x	x	x	x
Tear osmolarity	x	x	x	x	x	x	x
SL examination	x						x
Ocular surface staining	x	x	x	x	x	x	x
Meibomian gland expressibility	x						x
Infrared meibography	x						x

LLT: Lipid Layer Thickness; NIKBUT: non-invasive keratometric breakup time seconds; OSDI: Ocular Surface Disease Index questionnaire; Osmolarity: Inter eye difference; SANDE: Symptom Assessment in Dry Eye; TMH: Tear Meniscus Height.

Ocular comfort was assessed using the OSDI, DEQ-5, and the Symptom Assessment in Dry Eye (SANDE) questionnaires during the treatment period (307). The overall SANDE score was calculated as the geometric mean of the frequency and severity scores (308). Participants were advised to contact the study investigators during the study period to report adverse events at any time.

Bulbar conjunctival hyperaemia, tear meniscus height, NIBUT, and lipid layer grade (LLG) were assessed using the Keratograph 5 M (Oculus Optikgeräte, Wetzlar, Germany). Bulbar conjunctival hyperaemia was evaluated by automated objective evaluation of high magnification digital imaging, benchmarked against the JENVIS grading scale from 0 to 4 (309). The lower tear meniscus height was assessed using high magnification, pre-calibrated digital imaging, and three measurements within 1 mm of the pupil centre at the lower meniscus were averaged. NIBUT was measured using automated detection of first breakup, while the subject-maintained fixation and was requested to refrain from blinking. Three breakup time readings were averaged in each case (17). Tear film lipid layer interferometry was graded in a masked fashion by a single researcher across all participants according to the modified Guillon-Keeler system: grade 1, open meshwork; grade 2, closed meshwork; grade 3, wave or flow; grade 4,

amorphous; grade 5, coloured fringes; grade 0, non-continuous layer (non-visible or abnormal coloured fringes) (92, 294, 310).

Tear film osmolarity measurements were performed with a clinical osmometer (TearLab Ltd, California, USA), from 50 nL tear samples collected from the lower lateral canthus tear meniscus. A measurement was taken for each eye, and the higher reading and the inter-ocular difference recorded (17).

Lid margin and eyelash abnormalities, including lid margin thickening, rounding, notching, foaming, telangiectasia, meibomian gland capping, staphylococcal and seborrheic lash crusting, and Demodex blepharitis based on eyelash cylindrical dandruff were assessed by slit lamp biomicroscope examination (311). Grading of the clinical features was based on a four-point scale: grade 0, absent; grade 1, mild; grade 2, moderate; grade 3, severe (309).

Sodium fluorescein (i-DEW Flo, Mainline, Derby, UK) and, where pharmaceutical regulations permitted, lissamine green dyes (GreenGlo, HUB Pharmaceuticals, LLC, Rancho Cucamonga, California, USA) were applied using previously recommended techniques (17), in order to evaluate localised areas of corneal and conjunctival epithelial desiccation. Staining was recorded using the modified Oxford grading scheme (105), and lid wiper epitheliopathy (LWE) was evaluated relative to Korb's grading (298).

Meibum expressibility of the inferior meibomian glands was assessed using the Meibomian Gland Evaluator (TearScience/Johnson & Johnson, Morrisville, NC, USA), with a standardised pressure of 1.2 g/mm2 applied just inferior to the nasal, central, and temporal aspects of the eyelid margin. The number of meibomian orifices yielding lipid secretion was graded on a five-point scale: 0, more than 75%; 1, 50%–75%; 2, 25%–50%; 3, less than 25%; 4, none. The quality of expressed meibum was graded according to appearance as: grade 0, clear; grade 1, cloudy; grade 2, cloudy with debris (granular); grade 3, thick, toothpaste-like; grade 4, waxy, inexpressible (312). Infrared meibography was performed with the Oculus Keratograph 5 M, whereby the superior and inferior eyelids were everted and imaged in turn. From the captured images, the proportion of meibomian glands visible within the tarsal area was graded by a single researcher across all participants according to the five-point Meiboscale (313).

Best spectacle corrected visual acuity was recorded as a safety measure at each visit on a 6 m logMAR chart.

4.2.4 Statistics

Sample size requirements were determined from non-parametric adjusted power calculations conducted using NCSS PASS 2002 (Utah, USA), with non-invasive tear film breakup time as the designated outcome, and the standard deviation estimated to be around 6 seconds. On the multisite study the power calculation showed that a minimum of 42 participants per treatment group (Systane Complete or Systane Ultra), or 84 participants in total, were required to detect a clinically significant difference of 4 seconds, with 80% power ($\beta = 0.2$), at a two-sided statistical significance level of 5% ($\alpha = 0.05$). Allowing for a 10% participant dropout rate or loss to follow-up by 3-month review, this would mean that a total of 92 participants would be needed to be recruited (23 per site).

Recruitment of 23 participants per site was deemed optimal, allowing for possible drop out at each site with a minimal of 21 patients per site. The randomisation was stratified into 4 blocks to plan for an approximately equal mix of participants at each of the sites.

A minimum of 84 completed participants (at the 3-month time point) will form the study group (intention-to treat analysis). It was anticipated that 92 participants would be recruited to obtain the necessary sample size by 3 months, allowing for a 10% drop out. It was acknowledged that, due to the nature of the study, further drop out by the 6-month time point could occur. The statistics were then adjusted per site accordingly.

Mixed-effects model two-way analysis of variance (ANOVA) testing was conducted to examine the significance of treatment, time and interaction (treatment-by-time) effects on measurements over the six-month period, where continuous variables with a normal distribution had been confirmed (Shapiro-Wilk test $p > 0.05$). Post-hoc analysis for the significance of treatment effects at each time point, and intra-group comparisons relative to baseline, was conducted using the Kruskal-Wallis and the multiplicity-adjusted non-parametric Dunn's test.

In our site a sample of 22 participants allowed 90% power to detect significance at a level of $p<0.05$, yet Bonferroni adjustment was applied due to the multiple comparisons between time visits (considering $p \leq 0.003$ to be significant) and due to multiple comparisons between treatments (considering $p \leq 0.025$ to be significant).

Data are presented as mean ± SD, or median (IQR) unless otherwise stated.

4.3 Results

A total of 22 participants (18 females and 4 males) with a mean ± SD age of 36 ± 14 years (range, 21–75 years), completed the study. Analysis was conducted for n = 22, according to the intention-t-treat analysis (Table 4.2).

Table 4.2. Demographic characteristics of participants randomised to lipid and non-lipid containing eye drops. Data are presented as mean ± SD, or number of subjects (% of subjects).

Characteristic	Lipid drop (n = 11)	Non-lipid drop (n = 11)	p-value
Demographic			
Age (years)	39.09 ± 14.39	36.36 ± 14.84	0.681
Female sex	9 (82%)	9 (82%)	1.000
Ethnicity			
Caucasian	7 (64%)	5 (45%)	-
Asian	4 (36%)	4 (36%)	1.000
Others	-	2 (18%)	-

Demographic characteristics of the participants are summarised in (*Table 4.2*). Baseline characteristics did not differ between treatment groups (all p > 0.05). Returned eye drop bottles at each visit averaged a weight reduction of 6.6 ± 3.4 g, with no significant difference between study groups (p = 0.71), equating to the application of approximately four drops daily, indicating good product administration adherence. Out of the 22 participants enrolled, 68% had a lipid deficiency and 23% a tendency to a more ADDE.

All participants fulfilled the TFOS DEWS II criteria for dry eye disease, based on signs and symptoms, at baseline; by the end of the study, 48% of all participants no longer fulfilled these criteria (*Table 4.4*). Participants showing an improvement from baseline of > 4s in NIBUT and/or a ≥ 4.5-point reduction in OSDI were classified as ‘responders’ to treatment [13,14]. By days 30, all participants responded to treatment, with no difference between treatments (*Figure 4.2*).

Throughout the study period, responders showed an average improvement of 19.44 ± 4.05 in OSDI symptomology score and of +1.59 ± 1.75 s in NIBUT. Non-responders, however, registered an overall worsening of 5.23 ± 9.74 in OSDI and of 0.82 ± 2.46 s in NIBUT. In our study patients under Systane Ultra, an OSDI symptomatology reduction from baseline to 6-months of 20.51±14.06 vs a decrease of 18.42 ± 5.74 with Systane Complete, NIKBUT increase with Systane Ultra of 2.75 ± 2.43 s vs an increase with Systane Complete of 0.58 ± 0.57 s.

3.2. Dry eye symptomology

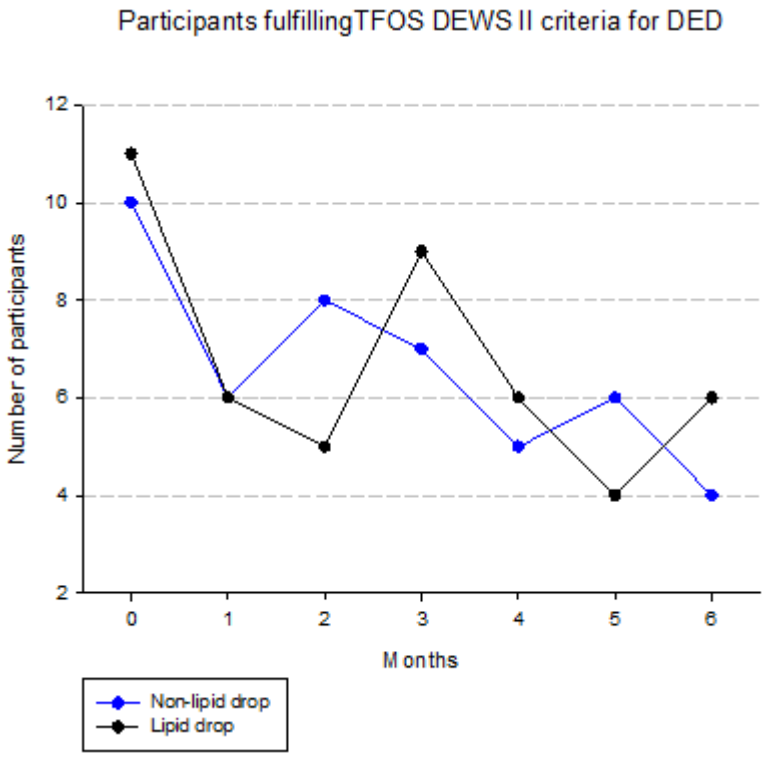

Figure 4.2. Recovery of DED status according to TFOS DEWS II criteria in the lipid and non-lipid groups at each study visit.

Mixed-effects model ANOVA demonstrated significant time effects for OSDI, DEQ-5, and SANDE dry eye symptomology scores (all $p < 0.001$, Table 4.3), although treatment and interaction effects were non-significant (all $p > 0.40$, Table 4.3). Multiplicity-adjusted post-hoc testing demonstrated sustained reductions in OSDI, DEQ-5, and SANDE scores from the 4th month onwards (all $p \leq 0.002$, Table 4.4) (Figure 4.3).

4.3.1 Tear film quality and quantity

A significant time effect was detected for TMH ($p = 0.003$), although treatment and interaction effects were non-significant (both $p > 0.60$). Time was also significant for tear film lipid layer grade (LLT) ($p > 0.04$).

Treatment, time, and interaction effects for NIKBUT and tear osmolarity were not statistically significant (all $p > 0.20$).

4.3.2 Ocular surface characteristics

Time effects were significant for both inferior and superior lid wiper epitheliopathy grade, as well as sodium fluorescein staining (all $p < 0.001$). Multiplicity-adjusted post-hoc analysis demonstrated significant decreases in superior lid wiper epitheliopathy grade

from the 2nd month onwards ($p < 0.001$), for inferior LWE at 6 months' time only (all $p < 0.002$), and for corneal staining from the 5th month onwards ($p < 0.001$). No significant treatment, time, or interaction effects were detected for conjunctival hyperaemia and staining, eyelid margin and eyelash characteristics, meibomian gland dropout, and meibum expressibility and quality (all $p > 0.05$) (*Table 4.3)*.

4.3.3 Safety

Safety profile of the 2 product was assessed by instillation tolerance, reactions, and events upon questioning at week 2 of each product. Parameters of safety included, increased hyperaemia, unusual eye-discharge, blurred vision and burning sensation upon product instillation.

Stinging sensation upon drop instillation was reported by one participant, the ocular reaction was recorded, assessed, and transferred to the PI. No damage was seen upon NaFL and SL anterior health inspection.

No other treatment-related events or reactions were reported during this study.

Table 4.3. Mixed-effects model analysis of variance of measurements for treatment, time, and interaction (treatment-by-time) effects. Ordinal data were analysed using multiple ordinal regression. Data are presented as p-values.

Measurement	p-value		
	Treatment	Time	Interaction
Dry Eye symptomatology			
OSDI score	0.434	<0.001*	0.583
DEQ-5 score	0.519	<0.001*	0.836
SANDE score	0.434	<0.001*	
Tear film quality			
Tear meniscus height	0.946	0.003*	0.696
Tear film lipid layer grade	0.351	0.044*	0.056
Non-invasive keratometric tear film breakup time	0.240	0.579	0.395
Tear osmolarity	0.762	0.212	0.341
Inter-ocular difference in osmolarity	0.947	0.084	0.907
Ocular surface characteristics			
Bulbar conjunctival hyperaemia	0.451	0.375	0.557
Limbal conjunctival hyperaemia	0.658	0.874	0.908
Sodium fluorescein staining score	0.523	<0.001*	0.496
Lissamine green staining score	0.593	0.002*	0.546
Lid wiper epitheliopathy grade SUP	0.159	<0.001*	0.137
Lid wiper epitheliopathy grade INF	0.415	<0.001*	0.298
Baseline to Final visit			
Lid anomaly	1.000	0.173	1.000
Lid margin thickening grade	0.528	0.582	0.260
Lid margin rounding grade	0.340	0.748	0.833
Lid margin notching grade	-	0.112	0.718
Lid margin foaming grade	0.498	0.498	0.666
Lid margin telangiectasia grade	0.748	0.340	0.837
Meibomian gland capping grade	0.503	-	0.676
Staphylococcal lash crusting grade	0.042*	0.291	0.530
Seborrheic lash crusting grade	0.528	0.528	0.067
Demodex lash cylindrical dandruff grade	-	-	-
MG secreting	0.582	0.276	0.828
Superior lid meibography grade	0.849	0.570	0.253
Inferior lid meibography grade	0.631	0.268	0.703
Meibum expressibility grade	0.349	0.530	0.679
Expressed meibum quality grade	0.433	0.059	0.727

Mixed-effects model analysis of variance of measurements for treatment, time and interaction (treatment-by-time) effects. Data are presented as p-values.
**Asterisks denote statistically significant effects ($p < 0.05$)*

Table 4.4. Dunn's test for significant time visits. Ordinal data were analysed using Kruskal-Wallis test. Data are presented as p-values. Data presented is each time-point compared to baseline. Asterisks denote statistically significance after Bonferroni adjustment (significance at p≤0.003)

Measurements	Time visits					
	1 month	2 months	3 months	4 months	5 months	6 months
DEQ-5	0.195	0.167	0.015	0.002*	0.001*	0.001*
SANDE	1.000	0.407	0.013	0.008	0.004	<0.001*
OSDI	0.155	0.043	0.013	0.003*	0.001*	<0.001*
TMH						
TLL						
NaFl	0.004	0.007	0.038	0.051	0.001*	0.001*
LWE SUP	0.067	0.001*	0.003*	<0.001*	<0.001*	<0.001*
LWE INF	1.000	1.000	1.000	1.000	1.000	0.002*

DEQ-5 – Dry Eye Questionnaire – 5, LWE – Lid wiper epitheliopathy, NaFl – Flourescein sodium, OSDI – Ocular surface disease index, SANDE – Symptom assessment questionnaire in dry eye, TLL – Tear lipid layer, TMH – Tear meniscus height.

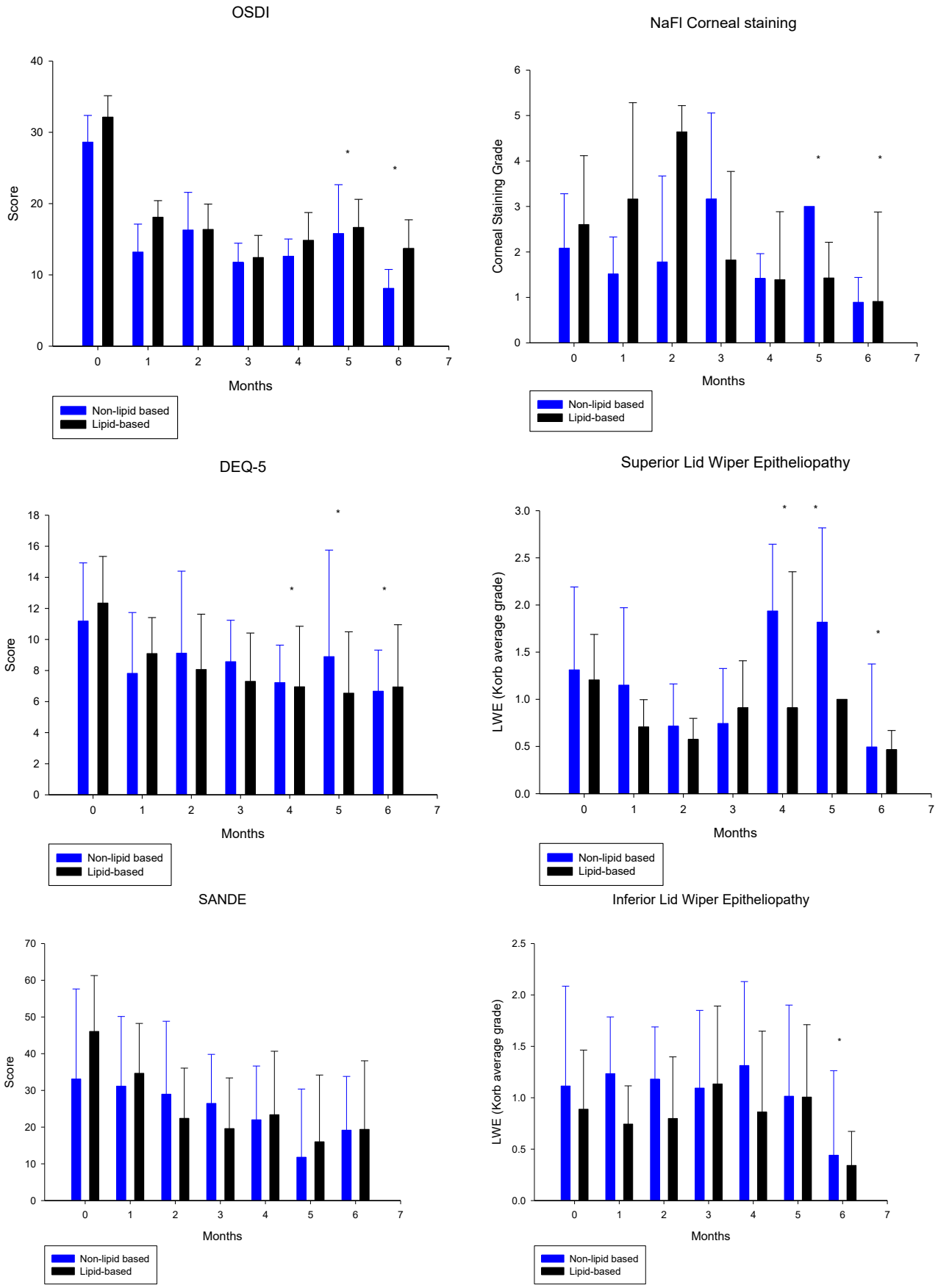

Figure 4.3. Clinical measures of participants randomised to lipid and non-lipid containing eye drops showing statistically significant improvements at the indicated timepoint relative to baseline. Asterisks denote significant changes observed for both drops. Data presented as mean ± SD.

4.4 Discussion

This study aimed to compare two drop formulations to find when the most efficient its use is for a therapeutic effect. This study was a sub-part of a multisite project, the data presented in this thesis is from Aston University only. Even though the number of patients were just a subset of the complete study, the analysis shows very similar outcomes to the multinational study (91).

The multi-centre study provided level 1 clinical evidence for the long-term efficacy of lipid and non-lipid ocular lubricants for the management of dry eye disease. The level-1 study proved that both artificial tear formulations performed similarly, which this study also found in every measurement taken. Patients on both subgroups reported significant symptomatology improvements, which was also seen on ocular surface signs. The decrease of lubrication creates direct ocular surface damage by friction, this then creates inflammation and further destabilisation of the tear film. According to our study this can be treated and ameliorated by both drop formulations; after 2 months of conscientious use at least 4 times a day, on lid wiper, and after 5 months on the corneal surface. An apparent decrease on the effects of friction was observed after the second month as from that time-point onwards the lid wiper decreased significantly, and so did the corneal staining, which aligns with the symptomatic improvement at the same time-point, and with the findings of the multisite study.

Even though a change on tear film lipid layer could be expected as other studies have reported, after the use of lipid-based drops, specifically for evaporative dry eye (91, 111, 112, 301, 314), our study only found a statistically insignificant improvement, yet the multisite study improvements on LLT were exclusively associated with the nano emulsion product. Its mechanism is poorly understood as some researchers found that the application of emulsion-based formulations does not guarantee a change on the thinning rate of the lipid layer, specifically on medium thickness lipid layers (89, 288). It should also be noted that not only thinner lipid layers are at risk of evaporation, but abnormal lipid patterns could also experience increase evaporation (288). Another study also claimed that lipid-based products could only change temporarily the lipid layer thickness, but no effects were seen after 1 month of consistent drops instillation, despite an improvement in symptoms (315). Not only was our hypothesis proven, but it was also discovered on the multisite study that lipid-containing drops are also beneficial to ADDE sufferers.

The inconclusive LLT result from the Aston cohort of patients alone could be due to our small sample or because of the nature of the condition; Out of the 22 participants enrolled, 68% had a lipid deficiency and 23% a tendency to a more ADDE, by the end of the study 64% had a lipid deficiency, 14% of those had a tendency to a more ADDE. Previous studies reported improvements in TLL, from 3 months onwards after consistent daily use of lipid-based products (283, 301).

Linked to an increase on lipid supplement one would expect a more stable tear film, but in our study cohort, those results were insignificant. In the multicentre project which this study took part, it was suggested that the slow fortification of the lipid layer through repeated lipid supplementation seemed to impact ocular surface physiology, restoring tear film homeostasis. As a result of this multicentre study having lasted a six-month period instead of the more traditional 4–6-week duration, the current study has further allowed exploration of longer-term effects and provided evidence to support recommending lipid-based products for patients identified as tear lipid deficient.

From the Multisite study, on *(*Figure 4.4), we can see an efficacy diagram of the lipid-based formulation across all the spectrum of dry eye subtypes from mild to moderate cases, suggesting that its use can be useful for both subtypes (91, 110). Lipid layer grade (LLG), in this figure suggests that on a wave pattern (number 3) or lower, on and Keeler-Guillon scale (91, 92) (evaporative tendency), only a lipid-based product will be efficient to treat this subtype. On the other hand, artificial tears without the lipid component improve ocular signs and symptoms but are unable to provide the lipidic component needed on evaporative dry eye cases. Yet, a standardised criterion indicating which treatment is best suited to alleviate a precise dry eye subtype is still unavailable. In our site this difference was not found to be significant. This could be limitation due to low numbers on our site.

Not found on our site, but on the multisite project, the drop efficacy exhibited a distinct time course with respect to the onset of each of the clinical benefits. The conflict between signs and symptoms in an individual still remains in most of the cases (4, 78, 108), which puts the clinicians in the difficult position of having some aspects measured from the patient which indicated high severity, while others suggested low severity (81). It was also identified that while there is lots of strong evidence that many treatments work compared to a placebo helping reduce symptomatology, there are few evidence-based comparative studies that would inform when to change from one treatment type to another either with disease severity or sub-classification of the disease, only a couple of

studies had compared the clinical effect between classes of artificial tears (91, 109-111, 169). Some more recent papers have shown differences in effectiveness even within classes of AT such as commercially available liposomal sprays (109, 169).

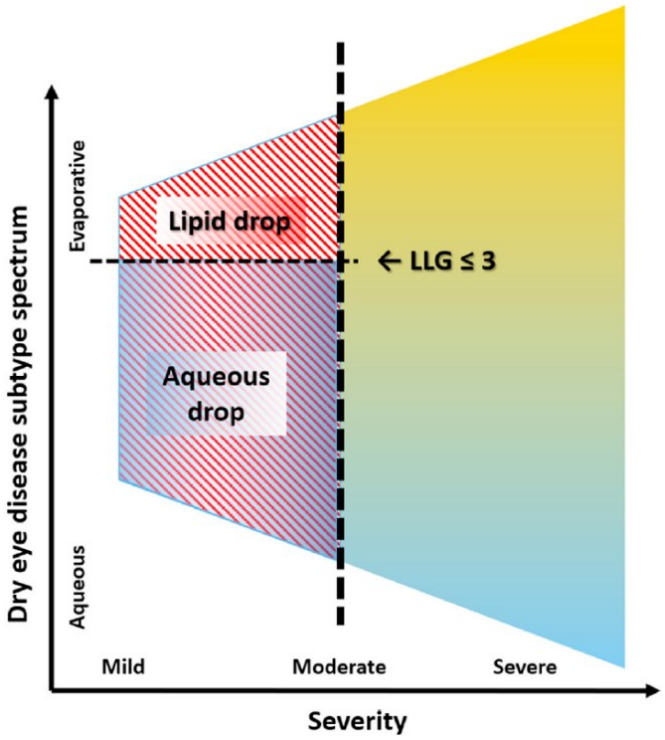

Figure 4.4. Schematic representation of the differential suitability of lipid and non-lipid drop formulations by DED subtype and severity. This article was published in "Developing evidence-based guidance for the treatment of dry eye disease with artificial tear supplements: A six-month multicentre, double-masked randomised controlled trial "The Ocular Surface, Vol. 20, Craig, J.P., et al. Page No. 68, Copyright Elsevier (2021). Reprinted with permission.

In our study the symptomatic relief started after 4 months and it was sustained until the end of the study, but the multicentre study outcome show an improvement of symptoms by the one-month visit with both drops, which plateaued between 1 and 6 months; this rapid improvement in symptoms is consistent with studies with shorter follow-up times (74, 290, 302, 315), in our case it could be due to the decreased number of participants in comparison to the multisite study. The use of artificial supplementation has been reported to resolve symptomatology in approximately one in five participants after a consistent use during 6 month (17).

Possible limitations on this study would be the sample size, which upon gathering the data from multiple sites, more clear changes are observable such as slightly more gradual improvements in signs than symptoms, and the severity of participants, which were mostly mild to moderate cases.
Future work is needed on accommodating both dry eye severity and subtype within clinical trials, to provide better understanding of treatment efficacy at each stage of DED.

4.5 Conclusions

This study found that both formulations of artificial tears provide a rapid symptomatology benefit, for both dry eye subtypes. According to multicentre study results, a lipid-based supplement might provide a more sustained relief for specifically evaporative DED cases.

Prolonged daily compliance led to not only palliative tear film supplementation and comfort, but a therapeutic restoration of structural changes by at least 4 months, which was reported to come all together with symptomatology benefits. In the multicentre study, the higher the number of participants demonstrated only two-thirds of the mild to moderate DED participants received symptomatology relief from the artificial tears used in the study and this was evident by the 1-month visit; hence clinicians can use this information to promote patient compliance for a month and to confidently alter the treatment approach after this time if no benefit is perceived.

Acknowledgements

This study was conducted by a multinational consortium and funded by Alcon, but the author was part of the study team and active in the study design as well as coordinating the patients at the Aston University site.

Having addressed some of the gaps in the academic literature with the most commonly prescribed management for DED, being artificial tears (as identified in chapter 2), Chapter 5 examines the effectiveness of new warm compress technology, another less invasive (conservative) management strategy for DED.

Chapter 5: Efficacy of a novel automatic heating eye mask massager on tear film and ocular adnexa (AURAI)

Published as;

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Efficacy of a novel water propelled heating eye mask massager on tear film and ocular adnexa.

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5.1 Introduction

Meibomian Gland Dysfunction (MGD) has been defined as "*chronic*, *diffuse abnormality of the meibomian glands*, *commonly characterized terminal duct obstruction and/or qualitative quantitative changes in the glandular secretion*. *It may result in alteration of the tear film*, *symptoms of eye irritation, clinically apparent inflammation*, *and ocular surface disease*" (23, 316). It is a subset of dry eye disease (16, 317).

As a chronic condition that requires ongoing management, patient compliance has a significant impact on treatment efficacy (186, 318). Many studies have assessed different efficacies of lid heating treatments and there is an established relationship between the use of eyelid warming devices and improvement of tear film stability and MGD (319). In MGD, the meibum is altered to consist of lower levels of unsaturated fatty acids and non-polar lipids) (320, 321), raising the melting point, causing the lipid secretions to solidify and become inspissated. The mechanism of action appears to be a clearing of the ducts through raising the temperature of the glands beyond the melting point of the altered meibum, followed by physical massaging of any remaining gland plugs (322). Many management and therapy options had been developed to warm the lids. Such treatments include infrared devices (322, 323), warm compresses (269, 324), disposable eyelid warming devices (325) and warm moist air devices (326) (Table 5.1).

The efficacy of warm compresses' studies is presented in (*Table 5.1*). Studies regarding what the most optimal temperature to melt the meibum of the glands might be suggest reasonable variability (327), perhaps greater in patients with MGD (328), affecting the efficacy of lid warming treatments that are in place (329, 330). Massaging in general has many health benefits and few known risks, so could massage of the eyelids and adnexa benefit sleep or blood circulation? (187). Hence, new approaches to lid warming and massage are of great interest to manage this chronic, debilitating condition.

The aim of this study was to investigate the efficacy of a novel water propelled eyelid warming device after 2 weeks use in patients with dry eye disease. For this study we hypothesised that the use of warm masks would give the TF and ocular surface a clinical benefit apart from a symptomatology benefit.

Table 5.1.Comparison of dispensing warm compress efficacy studies. Symptom improvement reported for dry eye group from warm compress therapy.

Study	Participants	Warm Compress	Comparison	Duration	Sign Improvements	Symptom Improvement with warm compress	Measures Unchanged	Additional Comments
Murphy et al., 2020 (331)	42 MGD	MGDRx EyeBag & OPTASE Moist Heat Mask	Warm face cloth	8 weeks, 10 minutes 2x/day for 2 weeks	MG quality & expressability, demodex (OPTASE only)	-23.1 from 39.8 OSDI EyeBag -21.5 from 39.0 OPTASE mask	Osmolarity, NIBUT, Schirmer I test	Face cloth cohort OSDI baseline was 24 vs 40 in commercial compress group
Wang et al., 2019 (331)	20 DED	MGDRx Eyebag	Contralateral eyelid massager vs manual massage	2 weeks, 10 min a day	LLT, NIBUT	Not measured	Visual acuity, TMH, redness, corneal staining, MG dropout	Measures 15 minutes post treatment
Tichenor et al., 2019 (332)	51 contact lens dry eye	Bruder moist heat compress	Single vs two applications vs washcloth	4 weeks, 10 min applications	Comfortable wear time and MG expressability with Bruder mask regardless of frequency of use	-8.9 from 22.9 OSDI 1x/day, -16.3 from 33.8 2x/day	TBUT	
Gao et al., 2019 (333)	82 MGD	Tobramycin/dexamethasone + warm compress	IPL	4 weeks, 10 min compress nightly	TBUT, MG expressability (1 month) and cytokines (1 week) with IPL	-16.7 from 38.1 OSDI	OSDI, corneal staining, MG dropout	Warm compress not as good as IPL Both groups applied sodium hyaluronate eye drops 4x/day

Badawi, 2018 & 2019 (334, 335)	24 DED, 12 in extension	Heat mask	Tearcare system	4 weeks, 5 min daily followed up after 6 months	TBUT, MG expression, corneal & conjunctival staining with Tearcare, worsening TBUT with heat mask	-8.4 from 33.0 OSDI. SPEED & SANDE also improved	None reported	No changes in IOP at 6 months. Tearcare retreatment at 6 months boosted benefits. Sole author employee of device company
Arita et al., 2017 (336)	35 DED, 20 controls	Heat mask	Contralateral eyelid with or without menthol	2 weeks, 10 min 2x/day	MG quality improved in all patients, but TMH and TBUT only increased with menthol mask	-25.5 from 49.7 with heat, -17.8 from 41.0 with heat & menthol DEQS	Corneal staining	Effects were also seen after a single 10 min application
Zhao et al., 2016 (337)	50 DED	Washcloth & lid scrubs n=25	Lipiflow n=25	12 weeks, 2x/day vs single session Lipiflow	TBUT (at 4 but not 12 weeks) in both groups, Schirmers in the washcloth group, MG expression (only examined in Lipiflow group)	-15.9 from 52.4 modified SANDE	Corneal staining, LLT	Not randomised & baseline staining higher in washcloth group. Lid hygiene encourage in both groups. Duration of warm compress and frequency of use not reported
Blackie et al., 2016 (203)	200 MGD + DED	Warm compress & lid hygiene n=99	Lipiflow n=99	12 weeks, 10 min 2x/day vs single session Lipiflow	MG expression, greater with Lipiflow	-17.8 from 51.8 OSDI	None reported	Lipiflow followed for up to 12 months and positive benefit still apparent. Baseline OSDI 6.2 points greater in warm compress group

Villani et al., 2015 (338)	50 MGD	Warm compress	Blephasteam	3 weeks warm compress 10 min 2x/day followed by 3 weeks Blephasteam	18 had no improvement in BUT or acinar diameter with warm compress but did with Blephasteam. No further improvement in warm compress responders	-13.6 from 36.3 OSDI in n=32 responders: ~-8.7 across all patients	None reported	Corneal staining, Schirmer, MG expression in methodology but not further reported
Bilkhu et al., 2014 (269)	25 MGD	MDGRx Eyebag	Contralateral unheated MDGRx Eyebag	2 weeks, 5 min 2x/day followed for 6 months	NIBUT, LLT, osmolarity, MG expression, hyperaemia and staining	-33.0 from 52.5 scaled to 100	Visual acuity & corneal topography	Improvement in OSDI from day 1 but continued to improve daily
Lane et al., 2012 (339)	139 MGD	iHeat warm compress	Cross-over Lipiflow	2 weeks, 5min/day	MG expression and TBUT only with Lipiflow	-7.8 from 34.7 OSDI. Syptom improvement greater with Lipiflow	Staining, IOP, visual acuity	
Matsumoto et al., 2006 (326)	10 MGD, 10 controls	Warm moist air compress	Hot towel	2 weeks, 10 min 2x/day	LLT (more with warm moist compress) TBUT (warm moist compress only)	-53.8 from 77.3 in ocular fatigue out of 100	Staining	
Mori et al., 2003 (325)	17 MGD, 8 controls	Eye Warmer prototype	-	2 weeks, 5 min 2x/day	BUT, symptoms, LLT, MG expressability	-24 from 52 scaled to 100	None reported	

MGD meibomian gland dysfunction; IPL intense pulse light therapy; LLT lipid layer thickness; NIBUT non-invasive breakup time; TBUT fluorescein tear breakup time; MG meibomian gland; OSDI ocular surface disease index; SANDE symptom assessment in dry; SPEED standard patient evaluation of eye dryness; DEQS dry eye–related quality-of-life score; TMH tear meniscus height; IOP intraocular pressure.

5.2 Methods

This prospective, randomised cross-over trial adhered to the tenets of the Declaration of Helsinki and was given a favourable opinion by Aston University Ethics Committee and governance approval. It was registered as a clinical trial on www.researchregistry.com UIN #5167.

Participants were required to be 18 years or older, having been diagnosed with DED as per TFOS DEWS II guideline (17, 340). Exclusion criteria included use of contact lenses, current eyelid warming therapies, artificial drop use and ocular pathologies. Prior to commencement participants had signed the informed consent and were enrolled if eligible. Fifteen participants (67% female, age 25.8 ± 5.45 years, range 20-37 years) were recruited, based on the minimum sample size recommendation for repeated measure analysis of variance (341). Data were obtained from the right eye only.

The Aurai Eye Massager (AEM) has an integral silicon mask which contains water which can be warmed/cooled and vibrated *(Figure 5.1*) The water-propelled massager is marketed as relaxing muscular tension around the eyes, to improve blood circulation and relaxation. All measurements were performed by the same clinician in the same order. The assessment was conducted in a room where temperature and humidity remained constant between 20-22°C and 30-45% respectively, to ensure that the measurements were not affected by ambient humidity. Participants spent a minimum of 10 min acclimatising to the room conditions before being tested.

The baseline measurements, conducted in the following order, were: Ocular Surface Disease Index (OSDI); Symptom Assessment iN Dry Eye (SANDE)(342); Osmolarity, highest value from 2 eyes collected from the lower meniscus (296) using a TearLab station (TearLab Ltd, California, USA) with calibration performed every day following the manufacturer's instructions and chips were placed beside the station for humidity and temperature regulation; Ocular Hyperaemia in the nasal and temporal bulbar and limbal regions (OH); Tear meniscus height (TMH) illuminated with infrared light, calculated from a calibrated digital image; Non-Invasive Breakup Time (NIKBUT) - average of 3 readings after two non-forceful blinks; and Lipid layer thickness (LLT) evaluated by tear film interferometry and graded as: 0 (absent), 1 (open meshwork), 2 (closed meshwork), 3 (wave), 4 (amorphous), or 5 (coloured fringes), all evaluated using the Keratograph 5M (Oculus, Wetzlar, Germany) (343).

Ocular staining with fluorescein, corneal staining (CornS) was assessed by wetting a fluorescein strip with saline, shaking off the excess, and instilling it at the outer canthus. Lissamine green, conjunctival staining (ConjS), was assessed by wetting a Lissamine strip (GreenGlo, HUB Pharmaceuticals, LLC, Rancho Cucamonga, California, USA) with a single drop of saline solution, keeping the drop on the strip for 5 seconds to elude the dye, and instilling it at the outer canthus. Assessment was performed with a slit lamp and the number of punctate spots counted (17, 340).

Participants were required to wear a watch (IP68 Fitness Activity Tracker, Smart Watch, Teepao.com), 24h a day, which captured Heart Rate and Blood Oxygen (O2 – using red and infra-red light pulse oximetry) and sleeping time (ST – through inactivity of movement). For 2 weeks no intervention was undertaken and for the other 2 weeks (in random sequence), participants were instructed to use the AEM 6 minutes twice daily for 2 weeks, using the warm and vibration cycle as recommended in the manufacturer's instructions. After each two weeks, participants returned, and the baseline measures were repeated. After returning the AEM, participants were asked how often they had used the AEM.

Heart rate, Blood Oxygen (O2) and Sleeping Time (ST) were gathered from the watch. Intraocular pressure was measured at each visit with Ocular Response Analyser (ORA) (Reichert Technologies, Germany). In addition, IOP was measures before and at minute intervals after eye massager use for 3 minutes at the end of the study to examine for any immediate pressure spikes.

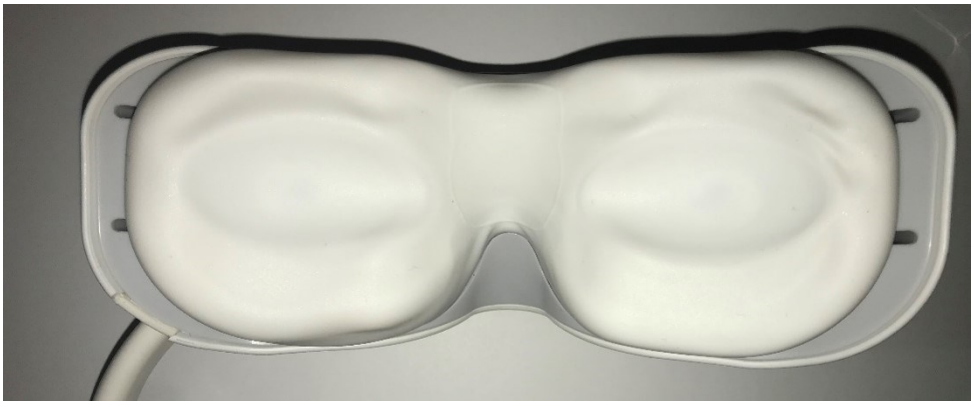

Figure 5.1. AURAI Mask Massager

5.2.1 Data analysis

Sample size was determined from non-parametric adjusted power calculations conducted using G*Power Version 3.1.9.2 2014 (Universitat Kiel, Germany), with NIBUT and OSDI as the designated primary outcome measures. This number of participants (n=15) has been shown to be adequate to detect a clinically significant difference (one lipid layer grade) even for the inter-group comparisons at 80% power with an alpha of 0.05 (344).

All analyses were performed using IBM SPSS Statistics version 26 (New York, USA). The distributions of the data were assessed using one-sample Kolmogorov-Smirnov test. Symptomology, intraocular pressure, heart rate, blood oxygen and sleeping time were found to be normally distributed and therefore analysed with a repeated measured Analysis of Variance, while the other metrics were assessed with a related sample Friedman's Analysis of Variance by Ranks. All tests were two-tailed and $p<0.05$ was considered significant.

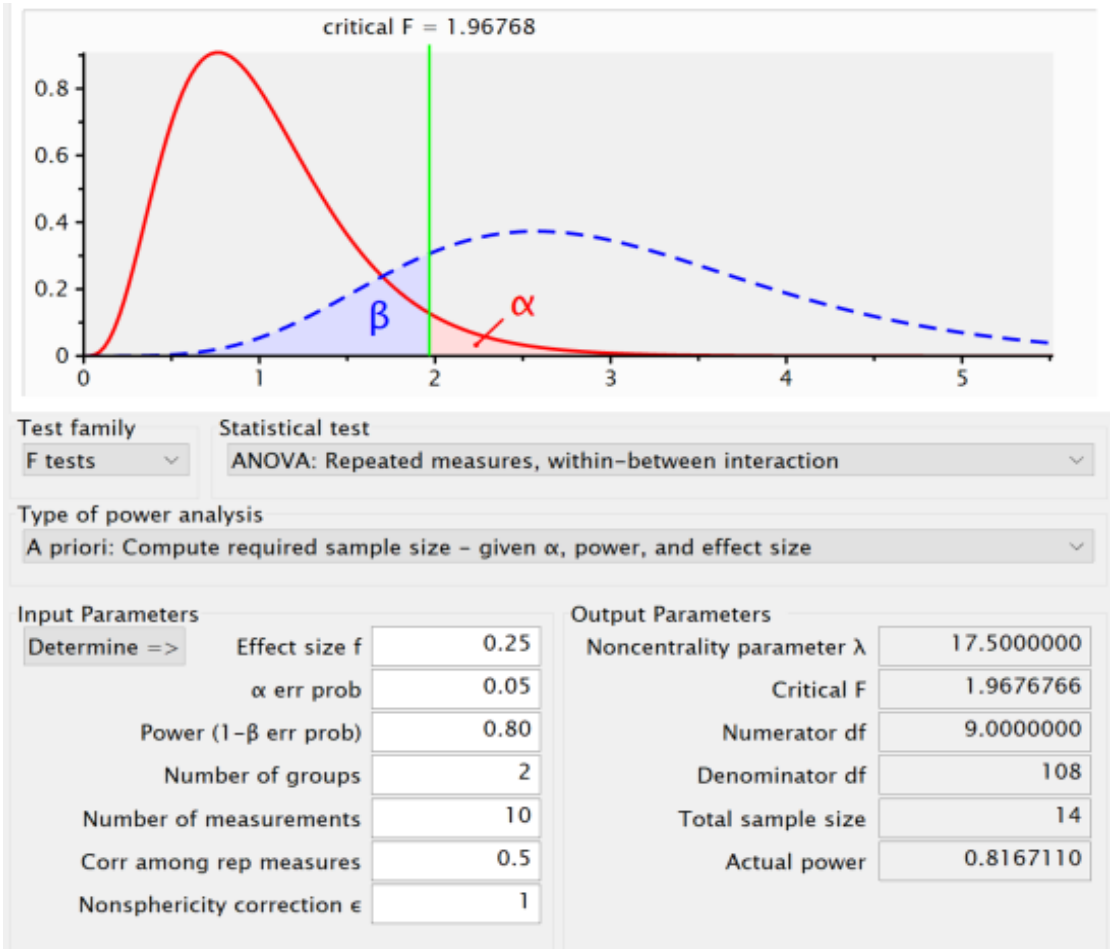

Figure 5.2. Adjusted power calculations. AURAI-DEC

5.3 Results

Summary statistics of clinical measurements at baseline, after 2 weeks of no treatment, and 2 weeks post-treatment are presented in (*Table 5.2*). Dry eye symptoms decreased with treatment (OSDI and SANDE severity metric; p<0.001). Interestingly, there was an apparent placebo effect of just wearing the fitness monitoring watch (OSDI: p=0.042; SANDE severity; p=0.006).

Table 5.2. Measurement Pre and post treatment. Average ± SD or median (range) of dry eye symptoms and signs at baseline, after 2 weeks of watch control, and after 2 weeks of treatment. Asterisks denote statistically significant effects (p < 0.05).

	Baseline	Post control period	Post AEM	Significance (p-value)
OSDI	34.3±19.5	26.5±19.8	18.8±17.5	0.001*
SANDE Frequency	4.05±2.20	4.03±2.46	2.55±1.61	0.262
SANDE Severity	5.70±2.43	4.35±2.40	3.65±2.12	0.001*
Osmolarity (mOsm/l)	293 (284-348)	291 (285-312)	291 (280-307)	0.888
Bulbar redness temporal (grade)	0.6 (0.0-1.1)	0.5 (0.3-1.5)	0.6 (0.4-0.8)	0.534
Bulbar redness nasal (grade)	0.6 (0.4-1.7)	0.6 (0.3-2.1)	0.5 (0.0-1.7)	0.775
Limbal redness temporal (grade)	0.3 (0.0-1.1)	0.3 (0.0-1.4)	0.3 (0.2-1.7)	0.971
Limbal redness nasal (grade)	0.4 (0.2-0.7)	0.4 (0.2-2.0)	0.3 (0.2-0.6)	0.472
TMH (mm)	0.22 (0.12-0.63)	0.24 (0.11-0.45)	0.23 (0.13-0.42)	0.247
NIKBUT (s)	6.3 (4.2-10.8)	5.4 (3.8-13.7)	5.3 (1.0-12.7)	0.430
LLT (grade)	2.0 (1.0-3.0)	2.0 (1.0-3.0)	2.0 (1.0-3.0)	0.975
Corneal staining (grade)	0.0 (0.0-3.0)	0.0 (0.0-3.0)	0.0 (0.0-1.0)	0.949
Conjunctival staining (grade)	1.0 (0.0-3.0)	1.0 (0.0-3.0)	1.0 (0.0-3.0)	0.575
Lid margin staining (grade)	1.0 (0.0-3.0)	1.0 (0.0-3.0)	1.0 (0.0-3.0)	0.298
IOP (mmHg)	13.91±1.89	13.29±2.02	13.83±2.59	0.349
Heart rate (beats per minute)	N/A	81.33±9.16	81.10±9.84	0.828
O2 (%)	N/A	98.87±36.11	97.37±3.69	0.268
ST (hours)	N/A	6.94±1.89	7.38±1.30	0.529

N=15. AEM = Aurai Eye Mask, SANDE = Symptom Assessment in Dry Eye, TMH = Tear Meniscus Height, NIKBUT = Non-Invasive Keratometric Break-Up Time, LLT = Lipid Layer Thickness, IOP = Intra Ocular Pressure, O2 = Blood Oxygen, ST = Sleeping Time

Intraocular pressure did increase immediately after use of the AEM (F=4.113, p=0.017) by 1.5 ± 2.0 mmHg, but this reduced rapidly (difference from baseline: 2 minutes, +1.3 ± 2.3mmHg; 3 minutes, -0.49 ± 2.3mmHg).

5.4 Discussion

This study investigated the efficacy of a novel water propelled eyelid automated warming device after 2 weeks use in patients with dry eye disease. The OSDI scores at baseline ranged from 13 to 66 indicating mild to moderate DED severity.

Two weeks use, twice a day, has previously been shown to be an effective duration of treatment of the meibomian glands to observe improvements in symptoms and signs such as tear stability, lipid layer thickness and meibomian gland expressability, but generally not ocular surface staining and meibomian gland drop-out (*Table 5.1*). Several studies have shown immediate patient benefit from even a single session of eyelid warming (269, 336, 344) including in non-dry eye individuals (186, 324), making this an attractive treatment option.

There was a clear reduction on symptoms having used the device for 2 weeks. Comfort was the only subjective measure and in the repeated measures design it also appeared to improve in the period when there was no treatment (the control condition), hence a possible 'placebo' effect of being monitored. However, when just the participants who were randomised to have the no treatment period monitoring for 2 weeks before using the AEM device are considered, no improvement in symptoms was seen (the difference in symptomatology was only -0.4±6.5 for the OSDI and 0.1±1.6 for the SANDE Severity score [minus indicates worsening symptoms]); this suggests that the improvement in symptoms following use of the AEM was a real and sustained effect, as the participant randomised to use the AEM device first followed by 2 weeks of no treatment had little reason to believe that the benefit in symptomatology would occur even when assessed two week after last using the device. Compliance was reported as being good by all participants which may have been aided by the reduction in symptoms resulting from the AEM use.

Unlike previous studies (*Table 5.2*), no improvement was seen in ocular surface signs. It is well known that the signs & symptoms of dry eye disease do not correlate well (345), however the findings do not allow the mechanism of the improvement in symptoms to be explained. Perhaps the massaging of the skin around the eyes might be contributing to the benefit on symptomatology. Surprisingly our hypothesis was only valid and significant

on symptomatology benefit. Clinically signs improved after the treatment, yet not enough to be statistically significant. These findings could possibly because of the short-term therapy or lack of compliance.

In this study only the morphological shape and size of the glands and the TF parameters were studied, not the excreta of MGs. This could have been a limitation if we were to study the excreta of the glands and will be included in future trials.
Previous studies had not looked at other possible general health benefits such as improved lower levels of stress as indicated by heart rate, blood oxygen and sleep duration. Most participants, unprompted, reported a feeling of being relaxed after the use of the mask, but the number of hours to sleep prior or after the use of AEM did not change statistically. In this study, no difference was seen in these possible general health benefits, but that could be because of the short-term use of the eye mask. Future longer duration studies could examine these potential general health benefits more in depth.

5.5 Safety

It is known that rubbing the eyes can increase the IOP (346); the lack of any change seen on this study suggests that there are no immediate or long-term safety concerns from the massaging action.

5.6 Conclusion

In conclusion a considerable improvement in subjective symptom severity has been shown after the use of the AEM device even though no improvement in clinical signs were detectable in this study. Further randomised studies with longer term use of the AEM are required to fully explore the potential benefit of novel eye warming and massaging devices.

As identified in section 5.1, although there is good evidence that warm compresses can improve the signs and symptoms of DED, there is little information on how structure and function of the meibomian glands are linked and whether this relationship can be enhanced by treatments such as a warm compress and therapeutic expression. That is why (*Chapter 6:*) is looking onto the next step of treatment for DED and Meibomian gland dysfunction.

Chapter 6: Effect of Meibomian gland morphology on functionality with treatment

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6.1 Introduction

Management of MGD typically involves eyelid warming to melt the pathologically altered meibum and eyelid massage to remove obstructed material from within to restore normal functionality (347). Eyelid warming methods range from simple hot wet towel to specially made devices such as eye masks delivering infrared irradiation and moist-air goggles (269, 348). Likewise, eyelid massage includes manual manipulation and electric toothbrush-like devices, to in-office forcible expression procedures (318, 349). Other procedures include debridement of abnormal tissue from MG orifices to remove any obstruction (210). While forcible expression and debridement is performed in-office, this can be supplemented by patient self-care using warm compresses and eyelid massage (318, 347). However, advice regarding these techniques is not standardised, compliance is variable at best, and it remains unclear how they should be tailored for individuals (347).

Diagnosis of MGD is mainly clinical (347) - focussing on detecting signs of altered MG secretions, abnormal eyelid margin morphology, and MG drop-out (atrophy of acinar tissue) (93). Meibography is a technique used to visualise the MG acini, traditionally utilising a white light source applied to the eyelid skin to detect drop-out in silhouette (350). More recently, infrared technology has permitted a non-contact method to image the MGs from the mucosal side (351), allowing assessment of MG morphology with respect to shortening, dilatation or tortuosity (352).

Grading or scoring MG drop-out is traditionally based on the proportion of the lower eyelid that exhibit total and/or partial acinar loss (353, 354). Overall drop-out is considered a major factor in MGD, where significant correlations with altered meibum, tear film stability, tear film lipid layer pattern (a surrogate for thickness) and dry eye symptoms have been reported (352, 355-357). Finis et al. (2015) has shown that the meiboscore (system used to quantify drop-out (351)) significantly inversely correlates with the proportion of expressible MGs (322). These studies suggest that where drop-out is observed, MG and tear film function are impaired (322). MG tortuosity is less well studied, but greater MG bending has been observed in the upper eyelid which also correlated to tear film stability, while MG bending in the lower eyelid was correlated to dry eye symptoms (352).

Given the important role of MG morphology in MGD diagnosis, it is currently not well understood how affected glands will respond to treatment. Turnbull et al. (2018) show

increased tear film lipid layer thickness and stability with a variety of treatments regardless of MGD severity (based on drop-out extent), but direct MG functionality was not assessed (344). A case report on the impact of 3 week's treatment in an MGD patient (eyelid warming, massage, and scrubs) showed improved symptoms and ocular signs, but no change in drop-out as measured with meibography (356). This raises an important consideration of which treatment to advise MGD patients, as the presence of drop-out may render certain options ineffective. Furthermore, it is unknown which particular treatment, from patient-applied to in-office based, is more or less effective based upon initial MG morphology.

The aim of this study was therefore, to investigate the effectiveness of a range of common treatments based on the function of individual MGs glands classified by their morphological appearance and drop-out extent. For this study we hypothesised that all MGs irrespective of its shape or length would benefit from all treatments.

6.2 Materials and methods

The study was designed as an interventional case series. The study was conducted in accordance with the Declaration of Helsinki and the protocol received positive opinion and governance approval from the Southeast Scotland NHS Research Ethics Committee (REC reference: 15/SS/0113) prior to study commencement.
Inclusion criteria required participants to be aged ≥18 and have diagnosis of dry eye (based on TFOS DEWS II criteria) (17). Exclusion criteria were active ocular disease or systemic disorder known to affect the eye except for a diagnosis of MGD and self-reported dry eye symptoms (confirmed via prior dry eye clinic assessment); medications known to affect the eye; and contact lens wear (if worn, they were removed 7 days prior to the study visit). Participants were enrolled with written informed consent following adequate time to read and understand the participant information leaflet.

Eligible participants attended for one visit where the following procedures were conducted on the lower eyelid of the right eye:

1. Video slit lamp examination to determine the location and number of the MG orifices (CSO Phoenix, Firenze, Italy). Each MG was identified at the slit lamp (diffuse white light, x16 mag) by placing a mark on the eyelid skin adjacent to the associated orifice with a surgical pen to ensure the same gland was identified each time; the glands were then numbered manually from the captured image.

2. Meibography images were captured (313, 358) (Oculus Keratograph 5M, Wetzlar, Germany) to determine the morphological appearance of MGs detected in step 1. Glands were divided into nasal, central, and temporal locations respectively by dividing the total number of glands into thirds. Visible MG length (black line on *Figure 6.1*), calculated as proportion of the vertical length of the palpebral surface on full lid eversion (red line on *Figure 6.1*), (359) was measured with ImageJ (https://imagej.nih.gov/ij/). They were also classified as either complete (C, 100-75%), partial (P, 75-25%), or minimal/absent (M, <25%). Again, using ImageJ software, the tortuosity of each MG was assessed by measuring the difference between the maximum horizontal width of the MG (green line on *Figure 6.1*) and the maximum horizontal width of the region bound by the MG (yellow line on *Figure 6.1*), expressed as a percentage.

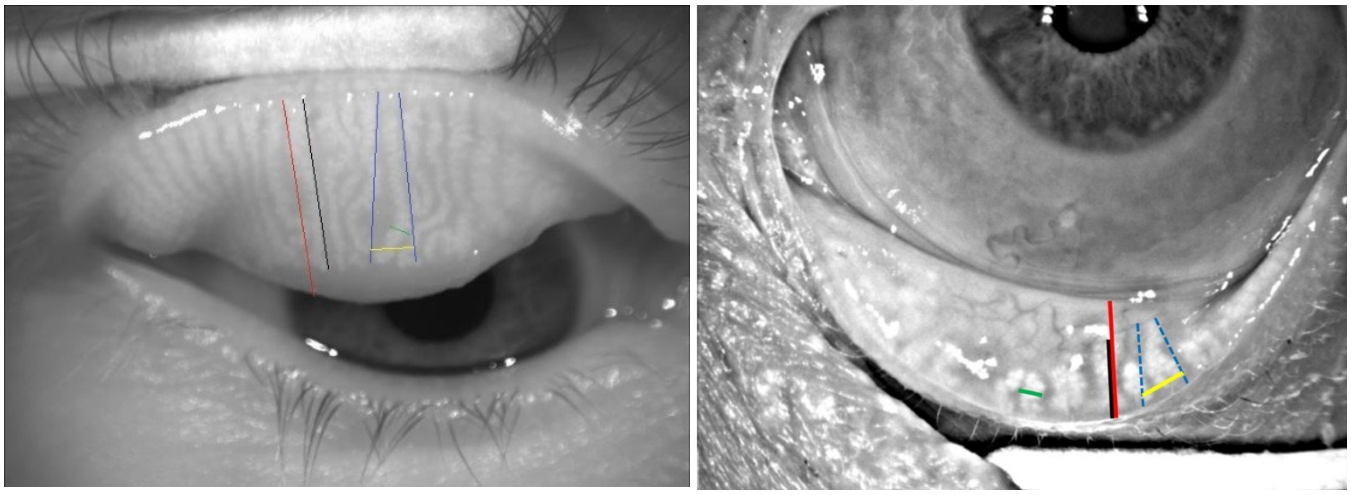

Figure 6.1. Meibography image to demonstrate ImageJ analysis procedure.

Red line = palpebral surface length adjacent to an MG; black line = the MG length; green line = maximum observed width of an MG; blue lines – boundaries of region covered the MG; yellow line = maximum observed width of region by the MG.

3. Assessment of meibum quality using the MGD Workshop Meibomian Gland Function scale (quality: 0=clear fluid, 1=cloudy fluid, 2=cloudy particulate fluid, 3=inspissated like toothpaste) following a standardized and repeatable pressure to the lower eyelid margin with the Korb Meibomian gland evaluator (MGE) from TearScience (360). If no expression was possible, this was recorded (grade = 4).

Where any MG was identified as having MG function score ≥2 (i.e. abnormal); participants received in sequence of increasing invasiveness with the step 3 evaluation conducted 5 minutes after each intervention:

A. Eyelid warming therapy using the MGDRx Eyebag (5 minutes duration after heating in 800W microwave for 40 seconds on full power followed by manual massage) (269).

B. Debridement of keratinised tissue (stained with lissamine green; GreenGlo, HUB Pharmaceuticals, Iowa, USA) over the MG orifices using a corneal epithelial spatula (Melosa; BVI Medical Limited, Yorkshire, UK) following application of topical anaesthetic (proxymetacaine hydrochloride 0.5%; Bausch & Lomb, UK) to the ocular surface and eyelid margin (brushed with a soaked cotton bud mainly to soften the tissue).

C. Forcible expression of each gland (with movement from proximal to distal end) using MG forceps (Melosa medical) following application of topical anaesthetic to the ocular surface and eyelid margin.

6.2.1 Statistical Analysis

All analyses were performed using IBM SPSS Statistics version 26 (New York, USA). Data was checked for normality using Kolmogorov-Smirnov test. Due to the ordinal nature of the grading system applied, data were found to be significantly different from a normal distribution (D=2.50, p<0.001). Thus, differences between MG function grade for each MG classification (C, P, or M) with each treatment over time was assessed using the Friedman test.

G*Power Version 3.1.9.2 2014 (Universitat Kiel, Germany) identified a sample size of 24 glands in each group (72 glands overall) and with this statistic power it could identify a 0.5 change in grade (with a grade standard deviation of 0.5) with $p \leq 0.05$ with 95% power (*Figure 6.2*).

Where changes with treatment were determined to be statistically significant (p <0.05), post-hoc analysis was performed using Wilcoxon signed-rank tests with Bonferroni correction applied (6 test pairs per analysis: thus, new threshold for statistical significance between treatments = $p \leq 0.008$). Relationships between MG function and morphology metrics was assessed using Spearman's rank correlation analysis due to ordinal nature of MG function grade; with statistical significance at $p \leq 0.05$.

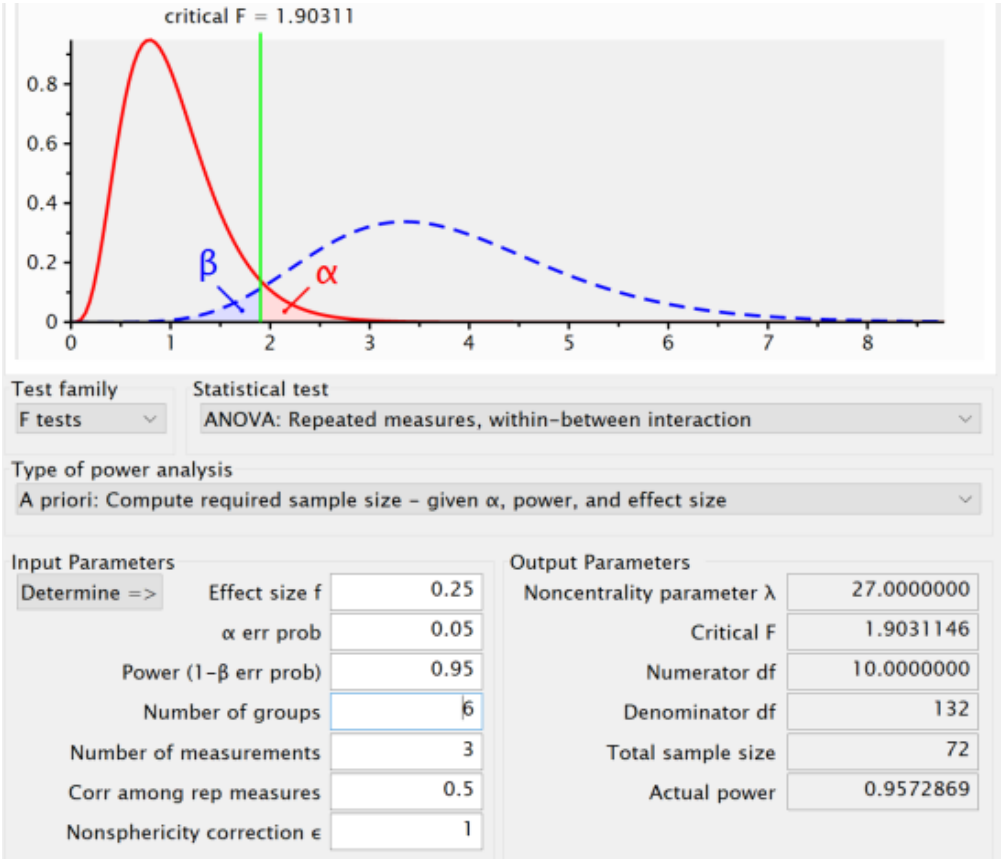

Figure 6.2. Adjusted power calculations. MGDtx-DEC

6.3 Results

The MGs of 15 participants were assessed (n=9 female; age 31.6±13.1 years; range 18-66), with a combined total of 365 individual MGs identified and examined. Of these, 55.1% (n=201) were classified as complete, 35.9% (n=131) partial, and 9.0% (n=33) minimal/absent. The median number of MGs per participant was 24 (interquartile range: 23 – 26; range: 21 – 28).

6.3.1 Meibomian Gland Function & Morphology

Only just over 10% of complete length MGs gave clear expression, while about 5% did not express at all, with a peak of particulate expression (*Figure 6.3)*. In contrast, the majority of partial length glands gave inspissated expression (38%), with one third (32%) not expressing at all, and nearly 70% of glands of <25% length expressed (Figure 2,3). None of those glands with <10% length expressed (Figure 3). MG function was correlated with MG length (r=-0.507, p<0.001; Figure 4, Table 1) and MG tortuosity (r=-0.129 p<0.001; Table 1), but not MG width (r=-0.090, p=0.167; Table 1).

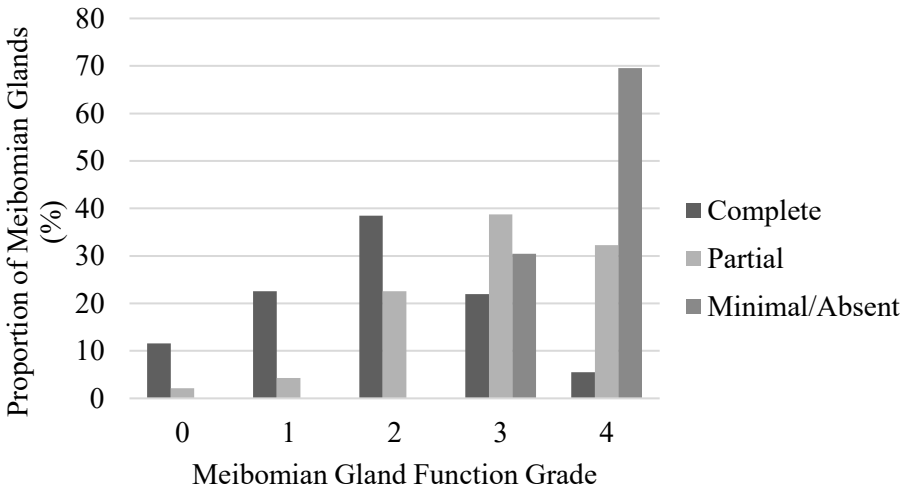

Figure 6.3. Proportion of meibomian gland classification (complete, partial, and minimal/absent) based on meibomian gland function grade (0 = clear, 1 = cloudy, 2 = particulate, 3 = inspissated, 4 = no expression) at baseline.

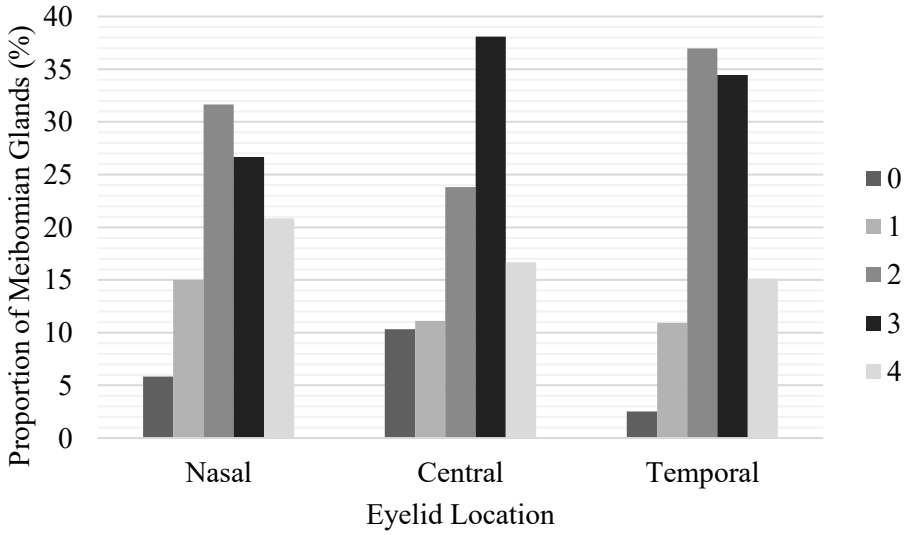

Figure 6.4. Proportion of meibomian glands at each eyelid location by meibomian gland function grade (see legend). Nasal n = 120 glands, central n = 126, temporal n = 119.

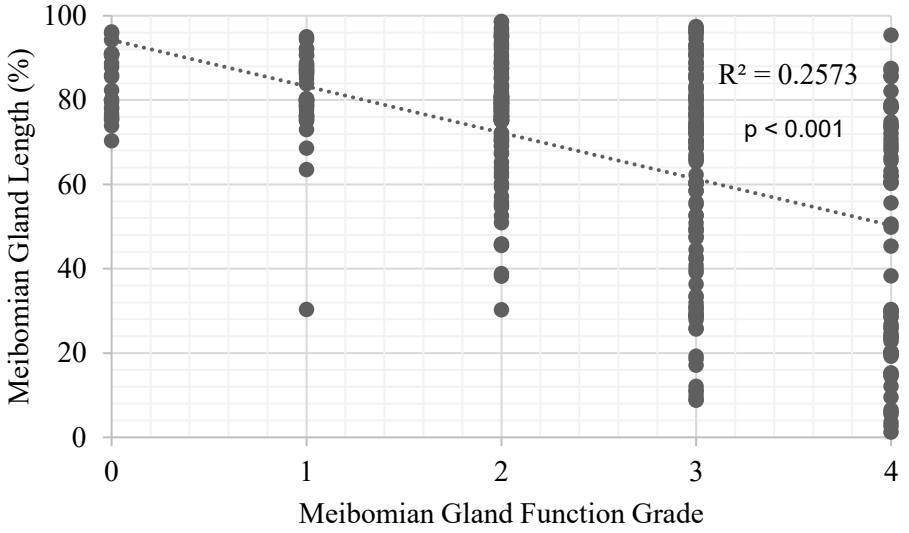

Figure 6.5. Meibomian gland function grade versus digitally measured meibomian gland length (% = proportion of vertical palpebral length) at baseline. N=365 glands.

Table 6.1. Spearman's rank correlation (r) and associated p-values between meibomian gland (MG) function grade and MG length, MG tortuosity, and MG width.

Correlation between MG Function and MG Morphology			
	MG Length	MG Tortuosity	MG Width
MG Function	r = -0.507	r = -0.129	r = -0.090
	p < 0.001	p < 0.001	p = 0.167

6.3.2 Treatment Effectiveness

Table 6.2 Median (interquartile range; IQR) meibomian gland (MG) function values for complete, partial, and minimal/absent MGs at baseline, and after treatment with warm compress, debridement, and forcible expression.

MG Function - Median, (IQR)				
	Baseline	Warm Compress	Debridement	Forcible Expression
Complete MGs	2 (1 – 3)	2 (1 – 2)	1 (1 – 2)	1 (1 – 2)
Partial MGs	3 (2 – 4)	3 (2 – 3)	2 (1 – 3)	2 (1.5 – 3)
Minimal/Absent MGs	4 (3 – 4)	3 (3 – 4)	3 (3 – 4)	4 (3 – 4)

6.3.2.1 Complete Meibomian Glands

There was a statistically significant difference in MG function between the different treatments for full length (complete) MGs (χ^2=144.1, p <0.001). There were significant differences between baseline and treatment with warm compress (mean grade

reduction: 0.31±0.78 (95% Confidence Interval: 0.19, 0.42); *Z*=-4.79, *p* <0.001) and between debridement and forcible expression (0.25±0.62 (95% CI: 0.16, 0.34); *Z*=-5.05, *p* <0.001); but not between warm compress and debridement (0.08±0.56 (95% CI: -0.02, 0.16); *Z* =-1.97, p =0.049).

6.3.2.2 Partial Meibomian Glands

There was a statistically significant difference in MG function between the different treatment types for partial MGs (χ^2=90.5, *p* <0.001). There was significant difference between baseline and treatment with warm compress (mean grade reduction: 0.46±0.58 (95% CI: 0.34, 0.39); *Z* =-6.01, *p* <0.001) and between warm compress and debridement (0.22±0.68 (95% CI: 0.08, 0.36); *Z* =-2.92, p= 0.003); but not between debridement and forcible expression (0.04±0.61 (95% CI: -0.08, 0.16); *Z* =-0.63, *p* =0.529).

6.3.2.3 Minimal/Absent Meibomian Glands

There was a statistically significant difference in MG function between the different treatment types for minimal/absent MGs (χ^2=14.42, *p* =0.002). There was a statistically significant difference between baseline and warm compress (mean grade reduction: 0.42±0.51 (95% CI: 0.21, 0.63); *Z*=-3.16, *p* =0.002) – but thereafter there was no statistically significant difference between the treatments (warm compress and debridement: *Z*=-1.73, *p* =0.083; debridement and forcible expression: *Z*=-1.07, *p* =0.285).

6.4 Discussion

This study examined how meibography assessed MG morphology affects MG functionality (meibum expression). It also assessed whether this could be improved in the short term by eyelid warming, debridement and forcible expression approaches. As might be expected, at baseline when a patient is first examined, expression is dependent on residual gland length, with no gland <70% of the lower lid length giving clear meibum expression. It should also be noted that for even full-length glands expression can be inspissated or absent (*Figure 6.3).* Glands of less than 10% of the lid length did not express and those less than 25% did not express or the meibum was inspissated. It has previously been shown that even ‘healthy’ MGs do not always express and appear to need time to recharge (88, 361, 362) which will affect the correlation between MG morphology and function.

Regarding calculation of MG length as a proportion of vertical palpebral length, this was chosen rather than absolute length due the obvious variation that a MG can extend along

the roughly semi-circular inferior tarsal plate. This anatomical feature may also explain the weak correlation between MG tortuosity and MG function (*Table 6.1)*, where an MG may exhibit bending to simply fit within the physically available space, rather than a pathological change in MGD (361, 363). However, this relationship was statistically significant, with more tortuous glands exhibiting reduced functionality with respect to expression. This has also been reported elsewhere – Adil et al. (2019) also found weak but statistically significant correlation between tortuosity and meibum expression grade (r=-0.107, p <0.05) (364). However, a recent prevalence study detected 37% of young, asymptomatic participants have measurable MG tortuosity (365); suggesting this parameter does not influence tear film and symptom measures. In contrast, MG width displayed no significant correlation with MG function (*Table 6.1*). Although obstructed material in MGD can lead to dilatation of the central duct, this widening may be offset by acinar atrophy that results from the same process (361, 363, 366). More recently, Pucker et al. (2019) observed that narrower MGs were associated with worsening expressibility in successful and unsuccessful contact lens wearers; however, there was no significant difference in MG tortuosity and width between the two groups (367). Together with the present results, this suggests that width does not influence MG function, nor correspond to tear film parameters or symptoms and therefore can be ignored as an informative metric by clinicians. Tortuosity, however, requires further study to determine its role in MGD pathogenesis due to the variable presentation between normal and symptomatic patients.

The results demonstrate that despite the extent of MG dropout, warm compresses produce a statistically significant improvement in MG function. This may be explained by the presence of pathologically altered meibomian secretions which result in higher melting temperatures (361, 366) such that even those with significant dropout may still respond to eyelid warming treatment and manual expression. Reduced MG expressibility consistent with MGD diagnosis, has been observed even in the absence of dropout (322). This abnormal, more viscous meibum is proposed to result from an initial MG obstruction causing changes in lipid composition and subsequently raises the melting temperature (361, 366). While these improvements were modest and not clinically significant, (mean reduction in grade for all gland classes =0.39±0.64), this effect was observed after only one standardised application, whereas traditional MGD management requires regular long-term use. Thus, a cumulative effect is likely to be more pronounced and this study therefore supports their widespread recommendation by clinicians for all severities of MGD (347).

Glands with complete/intact acini exhibited the smallest improvement in function, but these glands were initially near normal at baseline (*Table 6.2*) so this was not unexpected. Indeed, those glands which were classified as partial length or minimal/absent had a higher proportion of impaired function/reduced meibum quality (Table 6.2); this supports findings observed by Finis et al. (2015) described above (322). However, glands that are ≥10% (of vertical palpebral length) are still able to be expressed without treatment (*Figure 6.5).* Thus, unless there is complete loss of the MG acini, improvement in function may still be possible particularly where the distal portion is intact. The commonly held belief of MG morphology is that after MG atrophy regeneration or reactivation does not take place, yet a study has recently suggested that the absence of visible gland structure might not be due to absolute atrophy or loss of function, instead the loss of activity (224). This same study suggest that MGs can be improved upon treatment (both IPL and LipiFlow), indicating gland reactivation and a possible regeneration (224) which requires further investigation.

Debridement alone, did not appear to further improve MG function for glands with complete and minimal/absent acini, but did so for glands with partial length acini, although this was not clinically significant. This suggests that occlusion of the orifice may be precursor to MG acinar tissue atrophy - complete glands have the ability to express normally, while minimal/absent glands do not, such that debridement has no effect on either state. However partial glands, which may be undergoing continued atrophy, responded to debridement - likely as the blockage which can result in dropout has been removed. Indeed, the pathophysiology of obstructive MGD is based on hyperkeratinisation of the orifices and excretory ducts, which in turn may lead to acinar tissue degeneration and atrophy due to increased intra-glandular pressure caused by continually produced meibum (361).

Surprisingly, forcible expression did not further improve MG function in partial length MGs, but this may be due to prior debridement removing the obstruction and releasing meibum and a return to near normal MG function grade (*Table 6.2*). Forcible expression was also ineffective for glands exhibiting minimal/absent acinar tissue, which was not unexpected due to the very limited or complete absence of meibum production capability; this corroborates with an earlier case report where MG dropout was not affected even by longer term treatment (356).

Unexpectedly not all MGs had the same response to treatments, with shorter glands reacting better to more invasive treatments. Also disproving our hypothesis is the fact

that WCs help the release the meibum excreta better than debridement or forcible expression. A potential limiting feature of this study is the reported observation that a proportion of MGs, whether or not they exhibit dropout, may not be actively secreting at the time of MG function measurement; in a group of young normal, Korb and Blackie (2008) observed that across the entire lower eyelid length, approximately only 10 MGs yielded liquid secretion at the time of measurement (88). Further, this temporal variation also varies with location along the eyelid (88, 362). Further, nasal glands appear more active, and this reduces toward the temporal margin (88, 362). Thus, individual glands may not have responded to treatment simply because they were not active at the time of measurement, or they were located in a region along the eyelid that is known to be less active. However (*Figure 6.4)* reveals that both the proportion of MG function grade and the proportion of expressible glands (nasal = 79.2%, central = 83.3%, temporal = 84.9%) was very similar between eyelid locations.

Further, this study examined MG function on an individual basis and in relation to the extent of individual MG dropout detected by meibography; hence it is more likely that a gland did not secrete or respond to treatment due to the reduction or absence of MG acini.

In contrast, a study of the ability of meibography to predict MG function by means of therapeutic expressibility and secretion volume in MGD patients found that while nasal and central MGs had significantly more functional MGs, these regions also exhibited the highest level of dropout; and no correlation was observed between overall dropout and either MG function or secretion volume (368). However, this group of patients was significantly older (mean age 48.0±12.1 years) and may have developed compensatory mechanisms to maintain meibum secretion in the remaining glands, given that aging leads to loss of MGs even in healthy asymptomatic patients (369, 370). In addition, there may have been non-obvious causes of MGD where orifice/ductal obstruction is observed rather than dropout. This study by Murakami et al. (2014) suggests that meibography should not be used alone to determine MG function due to the temporal nature of MG activity and potential non-obvious causes of MGD (368, 371). Indeed, it is well recognised that morphological examination, such as through meibography, should be used to detect dropout in conjunction with measures of MG function (meibum expression with the force of a blink and lipid thickness over the ocular surface), to help guide therapy (322, 371, 372). The present study supports this but adds the importance of individual contribution of MGs to meibum production as well as supporting the use of warm compress therapy and in office debridement but questions the role of forcible expression;

therapeutic expression seems to excrete similar meibum to diagnostic expression (373), but causes considerable discomfort (215). A recent randomised controlled trial demonstrated beneficial effects of therapeutic expression alone over a sham/placebo treatment (374), but it did not include the less invasive stages of warm compress therapy and in office debridement which were just as effective without forcible expression in this study.

6.5 Conclusion

Using meibography to examine dropout of individual glands is an important tool to help target appropriate therapy (based on MG length rather than width or tortuosity) as well as to communicate the damage sustained contributing to their dry eye.

Eyelid warming therapy, after a single application, significantly improves meibum expression quality, regardless of the extent of MG dropout. This effect is likely to be further enhanced with long-term patient usage, and so clinicians should encourage this treatment in all patients with MGD and to check compliance at all subsequent patient visits. In addition, debridement is useful to perform in office in patients with MG drop-out to help remove orifice and/or excretory duct obstruction as a preventative measure of MGD progression, with little additional benefit of forcible expression.

Chapter 7: Summary, discussion & future work – addressing the title

The management of dry eye has been studied for a long time, but due to the multifactorial aetiologies, nature of the condition, disease severity and its complexity, a single treatment is not useful to manage all the subtypes, instead a tailored-managed-therapy plan is needed to lead to an effective treatment. The clinical tests needed to diagnose and subclassify dry eye between evaporative, aqueous, and mixed dry eye has been thoroughly researched and with all the possible treatment studies obtained before and during 2017, TFOS DEWS II (121), created guidelines to treat this disease.

The proposed management approach by TFOS DEWS II (121) is more of an organisation tool for eye care professionals for when it might be best to initiate a treatment. This recommended treatment algorithm progresses from more generalised to more advanced-specific treatments, also bearing in mind the aetiology of dry eye disease. As a result of the different presentations of dry eye disease, this condition can be managed with multiple therapies treating various aspects of the ocular surface; despite this, certain therapies are more indicated for a particular ocular surface condition or dry eye subtype. Some of the treatments offer a more short-term-palliative aid, but in persistent chronic cases, continuous management of sequelae might be needed to decrease or eliminate signs and/or symptoms.

Even if there has been a lot of research on managing this disease, there is still a lack of level 1 studies looking at the effectivity of a specific treatment across different severities and subtypes. Due to this lack of studies, only the very first stage provides a therapy for mixed aetiologies, yet no differentiation between disease subtype (EDE and ADDE) is made after this stage. In addition, the created approach does not recommend a specific treatment for a specific dry eye stage. Therefore, in this thesis, the creation for more supporting evidence to understand when a therapeutic change might be needed to provide a better treatment and outcome was the main aim.

The research questions for the management of this condition aims to answer; When is it best to use a specific therapy? For which subtype a management strategy is more effective? Should a therapy be changed when the severity of the disease increases? If so, which one is more efficient? Yet, a subtype/severity-specific algorithm for when a specific treatment is more efficacious has yet to be agreed upon.

Consequently, one of the aims of this thesis was to compile a series of experimental studies to provide additional scientific evidence of management strategies and to assess if further modifications of this guideline were needed to improve the treatment of dry eye

disease. The research projects that were carried out also aimed to identify the effect of different treatment strategies from all different stages of the TFOS management and therapy report guidelines (121) and to disseminate the current prescribing patterns of therapies across the globe, identifying areas of uncertainty where further research is warranted.

In this thesis a comprehensive literature review of current dry eye disease therapies was conducted in Chapter 1:. The information was sourced from all levels of evidence from the last 50 years according to relevance to the topic, citations, journal impact factor, research centres and authors that have influenced the field of ocular surface diseases with an emphasis on management strategies.

The review of the literature in Chapter 1:, identifies the challenges and gaps in the field of dry eye treatment strategies, together with the current knowledge around the clinical application of each treatment. Dry eye treatments during 2020/2021 had a cost to the NHS of over £9.2 million (375), in England alone. Over the counter interventions if not used appropriately and consistently, might only have a palliative effect, and in some cases, there is a need of a pharmaceutical intervention, which for ECPs entails either a referral within the NHS or a treatment by a qualified independent eye care practitioner.

The TFOS DEWS II dry eye disease management and therapies report (121), published in mid-2017, identified that while there was strong evidence for the effectiveness of some treatments for dry eye disease against a placebo, there were few comparative randomised control trials within (such as different artificial tears) or between (such as artificial tears compared to a warm compress or pharmaceutical) treatment approaches. In addition, few studies had examined treatments stratified for severity or subtype to aid clinician in their selection of a management therapy likely to be most efficacious for an individual patient in their practice. Therefore, the survey reported in chapter 2 aimed to uncover current dry eye disease management practices by eye care practitioners and the differences in between continents and countries. Most regions were found to be practicing similarly and following the TFOS DEWS II guidelines, as close as possible depending on their regulatory rights and/or product availability.

Explanation, prognosis, and advice was given for patients with most severities of dry eye disease, as a good tool to help patients understand the condition and to get them started with environmental changes. Strategies to manage mild to moderate severities was by prescribing the use of artificial tear drops and sprays, warm compresses, lipid-containing

products, and essential fatty acids, as suggested by TFOS DEWS II (121). For more moderate cases, the least encountered by the survey cohort, practitioners used pharmaceutical approaches, ointments, in-office treatments and secretagogues. For the most severe dry eye cases, blood/tissue products, therapeutic contact lenses and surgical approaches were reported to be used. The availability of products could be a factor by which practitioners reported prescribing a certain treatment over another one, for example, the use of home-made warm facecloth instead of commercially available warm lid compresses/face masks.

A differentiation between products used for EDE and ADDE was noted on this study, by which, lipid drops/sprays, lid hygiene, warm compresses, treatments such as intense pulsed light and antibiotics (topical or oral) were reportedly used more commonly for evaporative subtypes. Ointments were still prescribed x1.14 more than lipid-containing products for EDE, perhaps due to the latter being less available and on the market for a shorter period of time. Meanwhile, punctal occlusion, secretagogues, biologics, therapeutic contact lenses, artificial tears and moisture chamber goggles were used preferentially for aqueous deficient DED. Even though punctal plugs and moisture chamber googles are both used for ADDE, punctal plugs are used x1.35 more than moisture chamber googles, which could be due to the lack of standardisation, availability, and evidence of studies on both management strategies.

North American and Asia/Middle Eastern regions differed from the rest of regions surveyed by their tendency to use a more pharmacological approach at lower levels of dry eye disease severity (especially in the USA). The notable difference within continents was in the use of home-made warm compresses across Europe. A surprising finding was that the prescription of face-cloths, to heat the eyelids, was still quite high, even after the heat retention was proven to quickly decrease (285, 376), resulting in reduced efficacy of this therapy (332).

Having identified the limited number of randomised controlled trials of dry eye treatments (Chapter 1:) and the clinician and patient over-the-counter quandary of which artificial tear to prescribe/chose, Chapter 3: compared the effectiveness of different commercial artificial tears (stage 1 products). Different ‘categories’ of artificial tears were selected, comparing a liposomal-based drop, a liposomal-based spray and an emulsion-drop, assessing changes on both signs and symptoms from baseline. Even though a decrease in symptoms was present was observed with all the 3 products, improvements in LLT and osmolarity were not statistically significant. Participants with thinner LLT at baseline

preferred the use of Tears Again Gel, a liquified gel, contrary to what was expected, followed by the emulsion drop and the spray formulation which placed last. The study was a cross-over design with 2 weeks of treatment followed by 2 weeks of treatment withdrawal and a treatment change. This is a powerful clinical study design, but due to the length of the trial and results found it was decided that the next study (Chapter 4:), was going to be designed to be a parallel group randomised controlled trial experiment for a period of 6 months.

Chapter 4 sought to obtain data which would help to address these research gap of the temporal-therapeutic profile for a clinically significant improvement of both signs and symptoms by comparing a lipid and a non-lipid based artificial tear. Aston University was one of the sites in a multi-national clinical trial which showed that 4 times a day use of both formulations provided a rapid (within 1 month) symptomatology benefit for both dry eye subtypes. Signs assessed took longer to improve, taking 3-4 months. In agreement with a previous study (111), the lipid-based supplement provided enhanced relief for patients specifically with the more evaporative subtype of the disease. Also, in the overall multi-site study, one-third of the patients did not perceive a therapeutical benefit from the artificial tears. Because of this multisite study, if a subjective change cannot be perceived by 1 month of artificial tear use, that will give the clue to the ECPs to try the next step of the treatment stage.

Having examined the difference between dry eye artificial tear ‘categories’, the time course of their benefits and how they perform with dry eye disease subtypes, the aim of chapter 5 was to investigate the efficacy of a novel water propelled eyelid warming device. While no improvement in clinical signs was detectable, a significant improvement in symptomatology, specifically on severity was observed after 2 weeks of use, and surprisingly even 2 weeks after stopping the mask-treatment.

As evaporative dry eye disease is the most common subtype, but the association between the structural appearance of the glands and function is not yet clear in the academic literature, Chapter 6: wanted to address this gap in the literature. This was achieved by examining the effectiveness of different common treatments based on the function of individual Meibomian glands, in relation to its structural integrity.

The more tortuous glands exhibited a reduced functionality with respect to meibum expression, yet its width displayed no significant correlation with its function. Therefore, our study, as found recently (367) suggested that width does not influence MG function,

nor correspond to tear film parameters or symptoms and can therefore be ignored as an informative metric by clinicians.

The results demonstrated that eyelid warming therapy, after a single application, produced a statistically significant improvement in MG expression quality, regardless of the extent of MG dropout. This effect is likely to be further enhanced with long-term patient use, for which clinicians should encourage this treatment in all patients with MGD. In addition, debridement performed in office, in patients with MG dropout to help remove orifice and/or excretory duct obstruction was beneficial as a preventative measure of MGD progression, yet forcible expression provided little to nonadditional benefit on partial MGs.

7.1 Redesigning the management and treatment algorithm for dry eye clinicians

Taking all the studies from this thesis into account, a newly subtype management algorithm is proposed. This algorithm aims to separate the treatments given by ECPs after a careful examination of subtype. (Chapter 4:) provided evidence that artificial tears are only beneficial in about two-thirds of mild to moderate dry eye cases and this can be determined in compliant patients by one month of treatment use. If AT use does not seem to have that effect by that time, the ECPs should move to the following step on the TFOS DEWS II guideline list to a more advanced treatment (121). This management algorithm also separates ADDE from EDE treatments, which ECPs worldwide seem to know and use accordingly to the latest evidence (Chapter 2:).

Severity classification is also present in the suggested treatment approach. Whenever one treatment is not functional enough after 1 month, a more invasive treatment applies in an iterative way until either signs or symptoms of improvement are seen. Normally a more severe condition requires a more invasive procedure and further training is required from ECPs. In this case the more severe the disease is, the more invasive / therapeutical the treatment will turn to as in the previous guidelines (121).

Even though there is not enough evidence to back all the treatments up at each stage, further temporal therapeutic product profiles and treatment effect evidence is warranted. This is the first known redesign of the 2017 global management and treatment algorithm taking into account the latest evidence on ECP clinical behaviours.

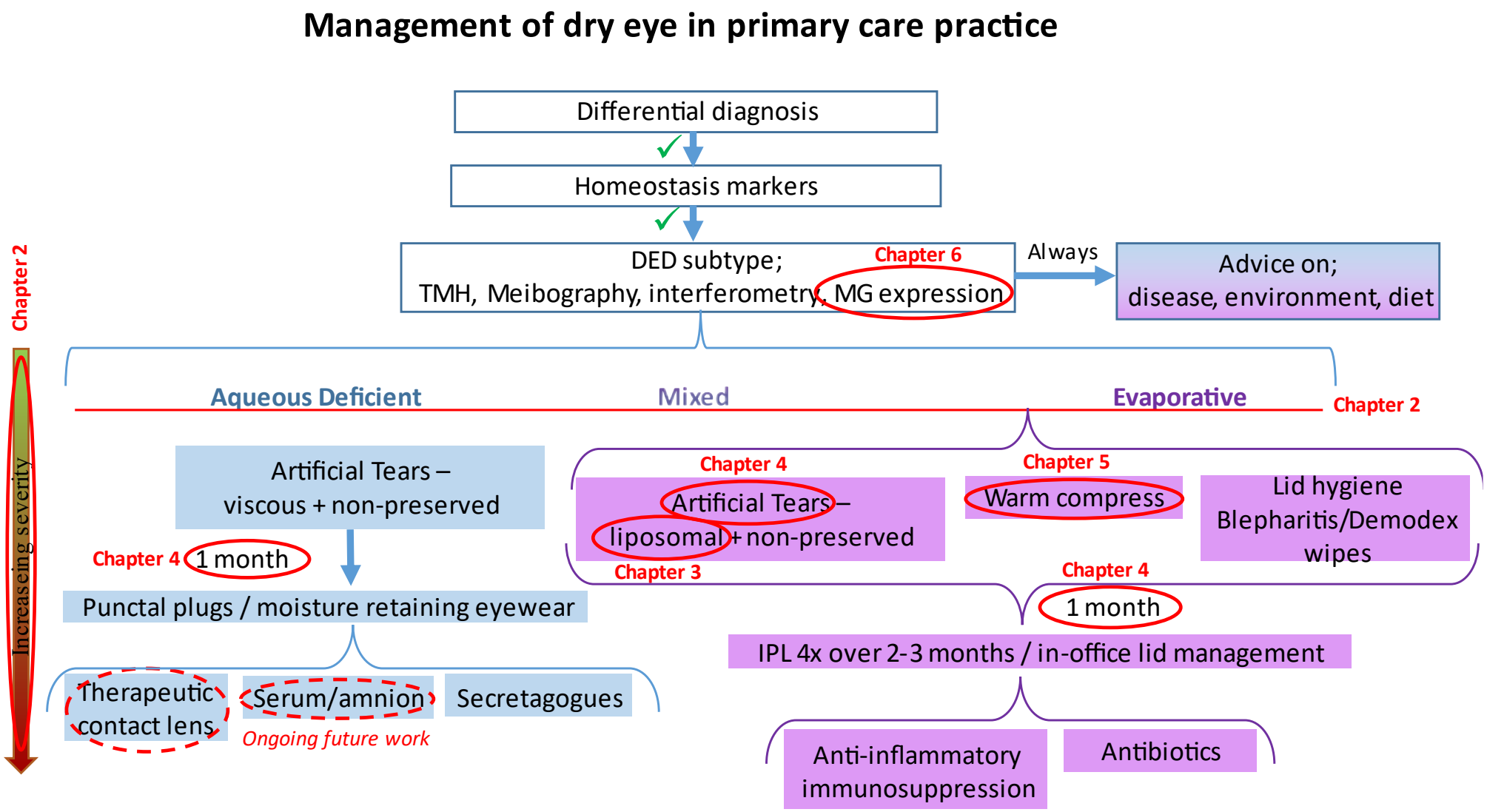

Figure 7.1. Proposed new algorithm for DED management and treatment according to PhD findings (relevant chapters highlighted in solid red ring and on-going future studies in dashed red ring)

7.2 Future work

An increasing number of publications on treatment strategies for dry eye disease have been observed, in recent years, yet much more research is necessary to inform clinical treatment plans based on the severity and subclassification of the disease. In particular, (*Chapter 4:*) proved that the effect of an artificial tear by one month demonstrated its long-term effectiveness and that a significant proportion of mild to moderate dry eye patients did not benefit from artificial tear use (at least the two formulations used). Further research is needed to see whether this effect can be determined in less time than 1 month (to speed up clinical optimisation) and what features predict the ineffectiveness of artificial tears so these patients can be prescribed more efficacious treatments at diagnosis.

During this PhD, one study was conducted where the efficacy of 6-month of AT, followed by heating eye masks, debridement & expression and 4 sessions of IPL was tested, but due to COVID most of the 9 month of follow-up data was lost, so only the 6 month of AT use was used on (Chapter 4:). Another study was to be conducted, but due to product development delays, have started at a later date, and we were not able to include them in this thesis.

One ongoing study involves the use of scleral lens wear for the treatment of dry eye disease as the evidence for this approach is currently limited; this product is on step 3 on the TFOS DEWS II treatment algorithm (121).

Another product was tested and developed by the thesis author to be part of a clinical trial, but it was not included in this thesis as the project is still underway due to product development (September 2021). This product is within step 4 on the TFOS DEWS II treatment algorithm (121), the use of a human amniotic membrane, which has been used for many ophthalmic indications including many related to inflammation of the ocular surface. The human amniotic membrane has anti-bacterial, anti-angiogenic, anti-inflammatory, and anti-fibroblastic properties. These characteristics play a role in the use of amniotic membrane in the treatment of DED. This dehydrated amniotic membrane derivative has been reported to be efficient on reconstructing a corneal surface severely damaged by chemical agents, promoting the healing of persistent corneal epithelial defects, enhancing the success of corneal surface reconstruction surgery, and substituting for conjunctival autografts after excision of pterygium or removal of conjunctival lesions (377, 378).

A recent study published in 2018 retrospectively reviewed the outcome of 84 patients (97 eyes) receiving cryopreserved amniotic membrane treatment (Prokera); an improved ocular surface along with a notable reduction of the severity of the overall symptomatology score up to three months post treatment was demonstrated (261). The current one is prospective randomised, patient-masked clinical trial, and the membrane is carried by a bespoke bandage contact lens (BCL) designed to enable the application of the amniotic membrane without the need for either sutures or glue. Our aims are to expand the work done by McDonald et al. (261) and assesses several clinical assessments of the ocular surface including confocal microscopy and tear sampling to understand the mechanism of action of any benefits observed. Currently, this product has been used on a clinical trial (NCT04553432) for 10 months with preliminary data showing very promising results.

7.3 Concluding statement

In conclusion, the research studies in this thesis have examined the current management strategies for dry eye disease and its efficiency, adding to the academic literature by conducting randomised controlled trials and examining the effectiveness of new treatment from different stages from the TFOS DEWS II guidelines (121).

From the management strategies studied, they all offered a benefit on either symptoms, signs or both, but some of them seemed to work better for a specific subtype of DED. Automated warm compresses, lid debridement and AT were proven to be more beneficial for evaporative cases due to the direct impact on Meibomian Glands, its openings or the overall surface protection. In patients with evaporative dry eye (a deficiency in the lipid layer thickness, expression of the meibomian glands or having structural damage of the Meibomian glands), reported more benefit after using lipid-based drops, warm masks and after treatments such as debridement, Meibomian gland expression and IPL. Some of these effects lasted 4 months or more post-treatment.

Therefore, a first redesign of the known 2017 treatment algorithm for dry eye disease management, was created in this thesis as an outcome of each chapter results. Further research is needed to provide background evidence on the effects of every treatment on each subtype and severity, since there is not a specific treatment that will work for everyone, as it all depends on the nature and aetiology of the disease. Therefore, a thorough examination and subclassification is needed to be able to tailor a personalised treatment with regular checks and follow-ups to encourage patient compliance and to understand if the management strategy used is providing a benefit.

7.4 COVID-19 Statement

This statement refers on how COVID-19 pandemic affected the fieldwork, analysis and writing up stage of this thesis.

The main backlash that COVID-19 had on this thesis was on (Chapter 4:). The idea behind this chapter was to provide treatment to the patients on the trial continuously with a treatment from every single stage of the TFOS DEWS II guidelines.

Starting as it explains in Chapter 4:, assessing the therapeutical-temporal effects of AT use 6 months, followed by warm compress given for 2 weeks used twice a day, continued with lid debridement and MG expression, finishing with 4 sessions of IPL. A baseline visit was done at every appointment to assess the changes in between treatments.

On 16 March 2020, UK's Prime Minister Boris Johnson announced the Government would be implementing measures intended to halt the spread of the SARS COVID-19 virus, and the regulations were applied right after, with the first lockdown beginning in England on 23rd March 2020. Consequently, all the future treatment visits had to be cancelled. As the subjects taking part on the study and the data taken by then was not powered enough to formulate a full thesis chapter. This can be seen on (Table *7.1*).

Therefore, it was decided to only include the data for the 6-month use of AT trial and compare it to the multisite study.

The idea was to finish data collection by July 2020 and submit in September 2020, but due to the lockdown restrictions, Aston University gave a year extension to Postgraduates in need, which gave more time for further analysis, writing up time and a full-time position as a Postdoctoral researcher.

Table 7.1. Calendar date cancellation for JASMINE/DEC follow-up study due to COVID-19 lockdown

AT Warm eye-mask	Lid debridement MG expression	IPL 1st	IPL 2nd	IPL 3rd	IPL 3rd	1m FUP	3m FUP	6m FUP
01/10/2019	01/11/2019	01/12/2020	16/12/2020	06/01/2020	06/01/2020	30/02/2020	X	X
21/02/2020	21/03/2020	X	X	X	X	X	X	X
14/01/2020	04/02/2020	27/02/2020	19/03/2020	X	x	x	x	X
12/08/2019	16/12/2019	06/01/2020	30/01/2020	14/02/2020	14/02/2020	27/03/2020	x	X
21/01/2020	10/02/2020	10/03/2020	24/03/2020	x	x	x	x	x
08/01/2020	28/01/2020	18/02/2020	10/03/2020	31/03/2020	x	x	x	x
07/01/2020	28/01/2020	28/02/2020	13/03/2020	27/03/2020	x	x	x	x
11/02/2020	17/02/2020	28/02/2020	27/03/2020	x	x	x	x	x
15/01/2020	11/02/2020	17/3/2020	27/03/2020	x	x	x	x	x
15/01/2020	11/02/2020	17/03/2020	27/03/2020	x	x	x	x	x
24/02/2020	21/03/2020	x	x	x	x	x	x	x
25/02/202	17/03/2020	27/03/2020	x	x	x	x	x	x
21/01/2020	25/02/2020	17/03/2020	07/04/2020	x	x	x	x	x
04/02/2020	03/03/2020	24/03/2020	x	x	x	x	x	x
03/03/2020	24/03/2020	x	x	x	x	x	x	x
21/01/2020	10/03/2020	24/03/2020	x	x	x	x	x	x

Treatment dates cancellations for JASMINE/DEC follow-up study.

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Appendices

1. Blink Test Enhances Ability to Screen for Dry Eye Disease

Wolffsohn J, Vidal-Rohr M, **Travé Huarte S**, Craig J, Ah-Kit I, Wang M. (2018) Blink test for DED. New Zealand Optics. Sept, 26.

Blink Test Enhances Ability to Screen for Dry Eye Disease

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Abstract

Aim: To evaluate the patient-applied Optrex™ Dry Eye Blink Test against established clinical criteria for the diagnosis of dry eye disease and to evaluate its benefit in enhancing screening for DED.

Methods: Eighty-seven participants aged 38 ± 17 years, (44 female) were screened for dry eye disease using the Tear Film and Ocular Surface Society (TFOS) Dry Eye Workshop II diagnostic criteria. In addition to symptoms screening with the Ocular Surface Disease Index questionnaire (≥13 cut-off for DED), a sign of loss of homeostasis of the tear film in the form of a non-invasive tear breakup time (NIBUT) <10s (Oculus Keratograph; K5m), an osmolarity reading ≥308mOsm/L or an interocular difference in osmolarity of >8 (Tearlab), or ocular surface staining (>5 fluorescein corneal spots, >9 lissamine green spots or lid wiper staining [≥2mm length & ≥25% width]) was required to confirm a diagnosis of DED. The self-administered Blink Test, which requires the participant to observe an image on a computer screen and report the length of time (in seconds) that they can refrain from blinking without discomfort, was repeated three times.

Results: Using a cut-off of 10s, the Blink Test demonstrated sensitivity of 66%, specificity of 88%, and an area under the curve of 0.77 ($p<0.001$), in predicting a diagnosis of DED according to the TFOS DEWS II criteria. The correlation between the Blink Test and NIBUT was $r=0.47$, $p<0.001$). When combined with the screening questionnaire, the sensitivity and specificity of the Blink Test increased to 71% and 90% respectively.

Conclusion: The Blink Test offers health professionals without advanced instrumentation, as well as patients, themselves, a rapid method of identifying possible DED.

Introduction

“Dry eye disease (DED) 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] The Tear Film and Ocular Surface Society (TFOS) Dry Eye Workshop (DEWS) II provided this updated definition, focusing on the loss of homeostasis in addition to symptoms, as the central pathophysiological concept of dry eye disease (DED). Tear film instability is considered to be a principal factor in the vicious cycle of disease, perpetuating tear film hyperosmolarity and ocular surface inflammation leading to ocular surface damage.[2]

The prevalence of DED has been estimated to be between 5% and 50%,[3] although the presence of signs and symptoms has been a prerequisite of few studies to date and none have yet been published conforming to the new global consensus on DED diagnosis. Globally, the incidence has been found to be higher in Asian, than in Caucasian, populations. DED prevalence increases approximately linearly with age, with a steeper rise by decade for clinical signs than for the prevalence of symptoms. Sex-related differences become significant only with increasing age.[3]

DED causes both an economic and social burden on those who suffer with this chronic condition.[4] It has been shown that DED can reduce the everyday quality of life of those with the disease.[5] Mild to severe DED has been associated with anxiety and depression.[6-8] In particular, patients suffering from DED secondary to Sjögren’s syndrome reportedly have a high prevalence and severity of depression.[9]

The prevalence of ocular symptoms related to DED is difficult to quantify, as symptoms are not spontaneously reported by many patients unless specifically asked. Hence, non-selective use of validated questionnaires such as the Dry Eye Questionnaire 5-item [10] or Ocular Surface Disease Index (OSDI)[11] can aid in screening for DED. It’s also important, through differential diagnosis, to exclude non-dry eye conditions that can mimic some of the DED signs and symptoms such as ocular allergy, infection and even binocular vision anomalies.[12, 13] Exclusion of such non-dry eye cases, together with a positive symptom score, prompts testing to identify whether there is an accompanying loss of homeostasis, and this is most often undertaken in a specialist clinic due to the time and equipment requirements. Tests recommended by TFOS DEWS II for identifying the loss of homeostasis of the tear film are those that evaluate non-invasive breakup time (with a cut-off time of <10s deemed to indicate DED),

osmolarity (with a value of ≥308mOsm/L or a difference of >8mOsm/L between the two eyes), and ocular surface corneal staining (>5 fluorescein spots), conjunctival staining (>9 lissamine green spots) or lid wiper epitheliopathy at the inner eyelid margin (stained zone width with the combined dyes of ≥25% and length ≥2 mm).[12] If any one of these tests identifies a loss of homeostasis then, in conjunction with the previously confirmed symptomology, a diagnosis of DED is made.

Since pharmacists and general practitioners most often lack access to the technology/dyes for diagnosing dry eye or monitoring treatment effects, availability of a rapid self-assessment test for dry eye could allow non-eyecare practitioners to give better advice to patients and make more appropriate referrals for a full diagnosis or differential diagnosis. Raising awareness of possible dry eye in patients themselves, might also encourage affected individuals to seek appropriate advice and management. The present investigation thus sought to validate a patient-self-assessed test (Optrex^{TM} Dry Eye Blink Test) and determine the level of certainty with which a diagnosis of DED might be made in the absence of specialist equipment against an established diagnosis, according to TFOS DEWS II.

Methods

This study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the institutional ethics committees of Aston University, Birmingham, United Kingdom and the University of Auckland, Auckland, New Zealand. Study participants were recruited from both centres and were enrolled following explanation of the procedures and after providing written informed consent. Eighty-seven adults, ranging in age from 19 to 87 years old (mean age 38±17) (43 males, 44 females) were enrolled in the study. All subjects were in good general health and were able to participate in the test sessions without difficulty. Subjects with any active ocular disease or currently using ocular medications were excluded. None of the subjects were contact lens wearers. In particular subjects with a range of DED were recruited to test the discriminatory ability of the Blink Test.

Room conditions were maintained at a temperature of 20.8 ± 2.1ºC and relative humidity of 46.1 ± 9.2%, and subjects spent a minimum of 10 minutes acclimatising to the study room conditions before measurements were captured. The participants were asked to answer the 5 item DEQ questionnaire which scores eye discomfort and eye dryness frequency from 0 (never) to 4 (constantly) and intensity from 0 (never have it) to 5 (very intense) along with watery eye frequency. In addition they scored the 12 item OSDI questionnaire, which scores experience of 3 ocular symptom questions, 6 visual function-related questions and 3 environmental trigger questions during their previous week from 0 (none of the time) to 4 (all of the time). The final score is calculated with the following formula;

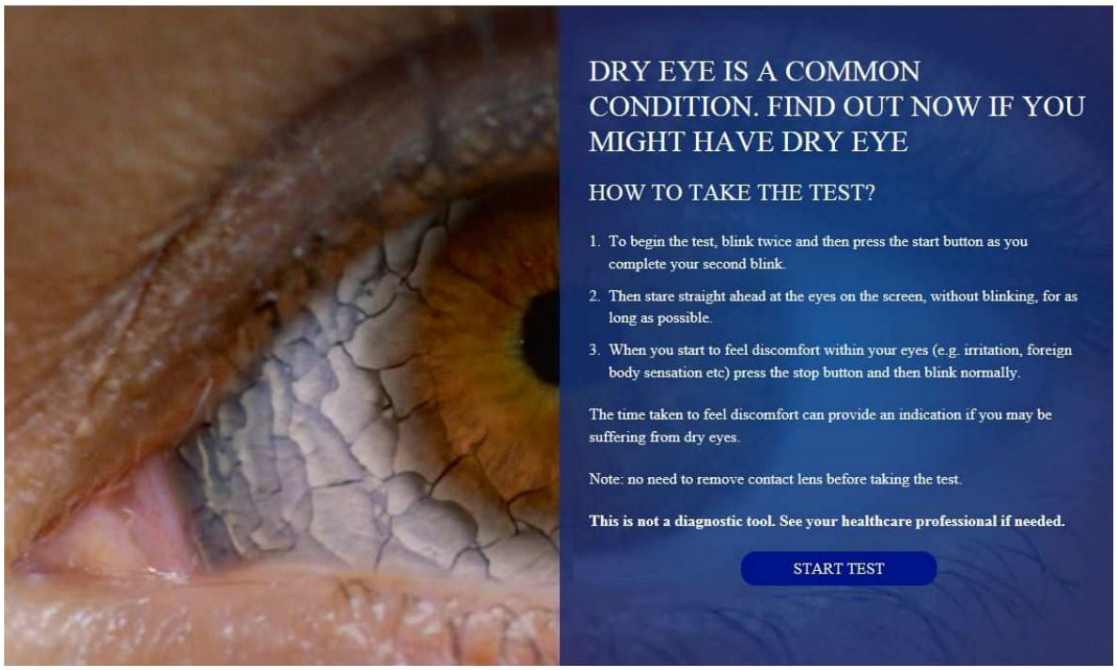
$$\text{OSDI} = \frac{(sum\ of\ scores) x\ 25}{(number\ of\ questions\ answered)}$$

DED signs were assessed from the right eyes of subjects only in the following order. Non-invasive tear film stability (NIBUT) was assessed objectively with the Keratograph 5M (Oculus, Wetzlar, Germany) using the infrared light setting, after two non-forceful blinks, from automated detection of disruption of a projected mire pattern reflected from the tear film surface, during a period of non-blinking.[14, 15] An average of three ‘average breakup time’ measurements was recorded each time.

The mean values of three consecutive Blink Test measurements were obtained (the manufacturer’s instructions does not specify repetition). Test instructions were displayed on a 15-inch thin-film-transistor liquid-crystal display digital screen positioned at ~40cm from the participant. The patient was requested to make 2 non-forceful

blinks, before ceasing blinking, and to press a timer button on the screen when discomfort was noted (Figure 1).

Figure 1: The Optrex™ Dry Eye Blink Test (with permission from Reckitt Benckiser)



Tear film osmolarity was measured at the inferior tear meniscus with an impedance-based, low volume osmometer (TearLab, San Diego, USA).[16] Daily instrument calibration was performed and stability in temperature ensured in accordance with the manufacturer's instructions.[12] Measures were collected non-invasively from each eye, and the maximum osmolarity and interocular difference recorded.

Conjunctival damage was evaluated with the aid of lissamine green dye (Green Glo, HUB Pharmaceuticals LLC, Rancho Carcamonga, CA, USA). The strip was wet with saline and the lissamine green solution instilled at the temporal canthus, after a period of 5s to ensure an adequate concentration of lissamine green. Care was taken to avoid conjunctival or lid wiper tissue damage. Grading was conducted between 1 to 4 minutes after instillation (ocular staining score 0: 0-9 dots; 1: 10-32 dots; 2: 33-100 dots; 3: >100 dots). Corneal damage was evaluated using sodium fluorescein strips. The strip was wet with saline solution and the excess shaken off to minimise instillation volume. Grading (ocular staining score 0: 0 dots; 1: 1-5 dots; 2: 6-30 dots; 3: >30 dots)[17] was conducted between 1 and 3 minutes after instillation.[18] Lid wiper epitheliopathy was observed after corneal and conjunctival grading, and was graded (0:

length <2mm, <25% of the lid wiper; 1: length 2-4mm, 25% - <50% of the lid wiper; 2: length 5-9mm, 50% - <75% of the lid wiper; 3: length >10mm, ≥75% of the lid wiper)[19] 3 – 6 minutes after instillation of a further drop of lissamine green from a new strip by one of three trained researchers.[12]

Data analysis

Statistical analysis was performed using Graph Pad Prism version 6.02 (California, USA) and IBM SPSS Statistics version 23 (New York, USA). Due to the Blink Test having a maximum duration of 15s, all NIBUT durations >15s were recorded as 15s. The distributions of all continuous variables were assessed using the Kolmogorov-Smirnov normality test, and non-normally distributed measurements (blink test value and non-invasive tear film breakup time) underwent logarithmic transformation before further analysis. The mean blink test and non-invasive tear film breakup time were compared using the paired t-test, variances assessed using the F-test, and Bland-Altman analysis then performed to determine the level of agreement between the two measurements. Correlation analysis of ocular measurements with the blink test value was conducted using Spearman's rank correlation coefficient. A receiver operative characteristic (ROC) curve was constructed to assess the diagnostic ability of the Blink Test measurements in discriminating whether or not subjects fulfilled the TFOS DEWS II dry eye diagnostic test battery. The area under the curve (C-statistic) and diagnostic accuracy values at the Youden's optimal diagnostic cut-off were then calculated. All tests were two-tailed and $p<0.05$, considered significant.

A sample size of 85 was calculated to significantly power an association of $r \geq 0.30$ between the Blink Test result, NIBUT and other DED variables.

Results

Of the 87 participants enrolled, 62 (71%) subjects fulfilled the TFOS DEWS II diagnostic criteria for DED (Table 1).

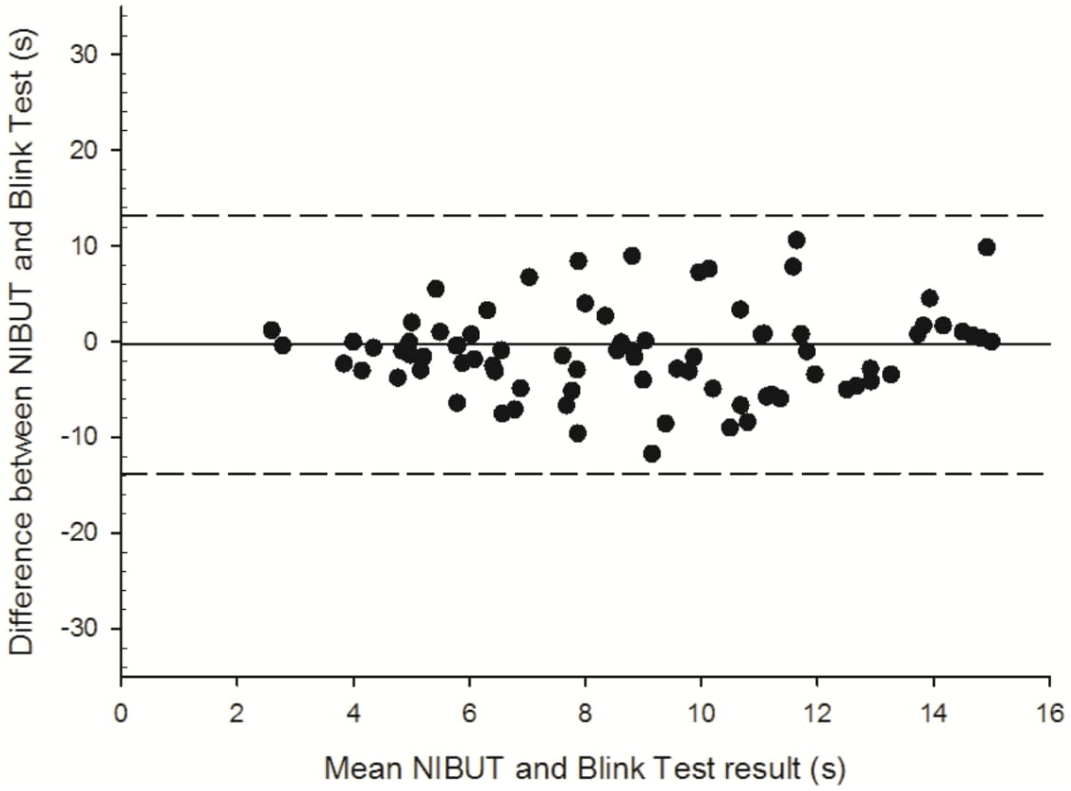
Table 1: Demographic, ocular surface, and tear film characteristics of the study participants. Data are presented as mean ± SD, median (IQR), or the number of participants (% of participants).

Characteristic	Values
Age (years)	38±17
Female sex	44 (51%)
Ethnicity	
European	38 (44%)
East Asian	40 (46%)
South Asian	4 (5%)
Other	5 (6%)
TFOS DEWS II dry eye diagnostic criteria met	62 (71%)
OSDI score	19±16
DEQ-5 score	9±4
Tear film osmolarity (mOsm/L)	305±14
Inter-ocular difference in osmolarity (mOsm/L)	9±7
Non-invasive tear film breakup time (s)	9.5±7.3
Optrex Blink Test value (s)	9.8±4.0
Corneal staining score	0 (0-1)
Conjunctival staining score	0 (0-1)
Superior lid wiper epitheliopathy grade	0 (0-0)
Inferior lid wiper epitheliopathy grade	0 (0-2)

Comparative analysis between the Blink Test and non-invasive tear film stability

The strength of the association between the Blink Test, as a patient-reported 'equivalent' to NIBUT, and NIBUT itself, was evaluated. Following logarithmic transformation, the mean Blink Test and non-invasive tear film stability measurements did not differ significantly ($p=0.15$). The F-test of variances showed that the Blink Test had a narrower distribution than non-invasive tear film breakup time ($p<0.001$). A significant positive correlation was observed between the two measurements ($r_s=0.47$, $p<0.001$). Bland-Altman analysis (Figure 2) showed a mean bias (95% limits of agreement) of -0.3 (± 13.5)s. Adjusting the Bland-Altman mean biases to the pre-transformed equivalents, the blink test values were on average 1.13 (0.36 to 3.55) times those of non-invasive tear film stability.

Figure 2: Bland-Altman analysis of difference between non-invasive tear breakup time and Blink test. Solid line represent the bias and dotted lines the 95% limits of agreement. N=87.



Correlation analysis of DED signs and symptoms with the Blink Test

Correlation analyses of dry eye signs and symptoms with the Blink Test value are presented in Table 2. Significant negative correlations with OSDI, DEQ-5, conjunctival staining, and inferior lid wiper epitheliopathy were observed (all $p<0.05$).

Table 2: Correlation analysis of dry eye signs and symptoms with the Blink Test. Data are presented as Spearman's rank correlation coefficients. Asterisks denote statistically significant values ($p<0.05$).

	Correlation with Blink Test value	
Ocular measurement	**Coefficient**	**p**
OSDI Score	–0.290	0.006*
DEQ-5 Score	–0.364	0.004*
Tear film osmolarity (higher value) (mOsm/L)	–0.066	0.55
Interocular difference in osmolarity (mOsm/L)	–0.010	0.93
Corneal staining score	–0.163	0.13
Conjunctival staining score	–0.237	0.03*
Superior lid wiper epitheliopathy grade	0.018	0.87
Inferior lid wiper epitheliopathy grade	–0.251	0.02*

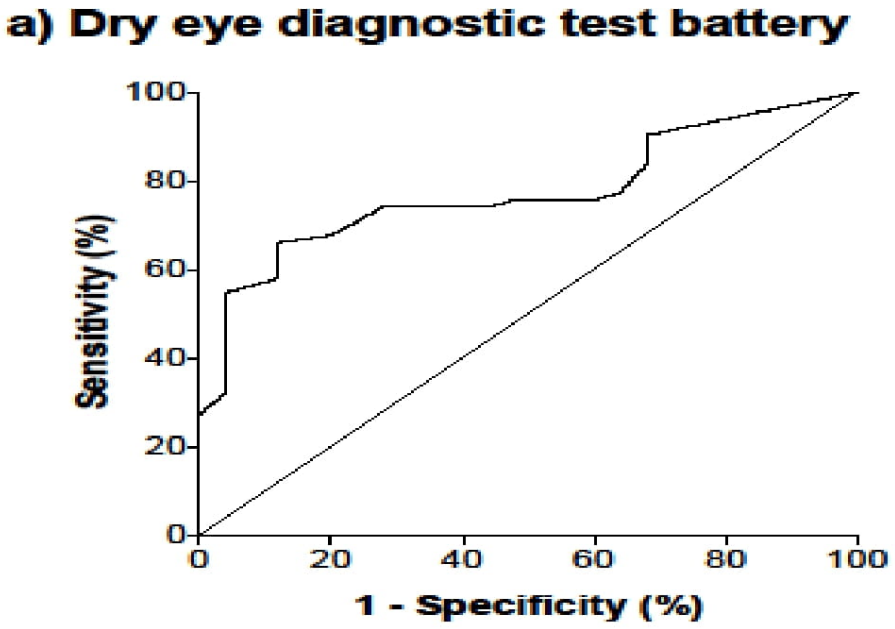
Diagnostic ability of the Blink Test

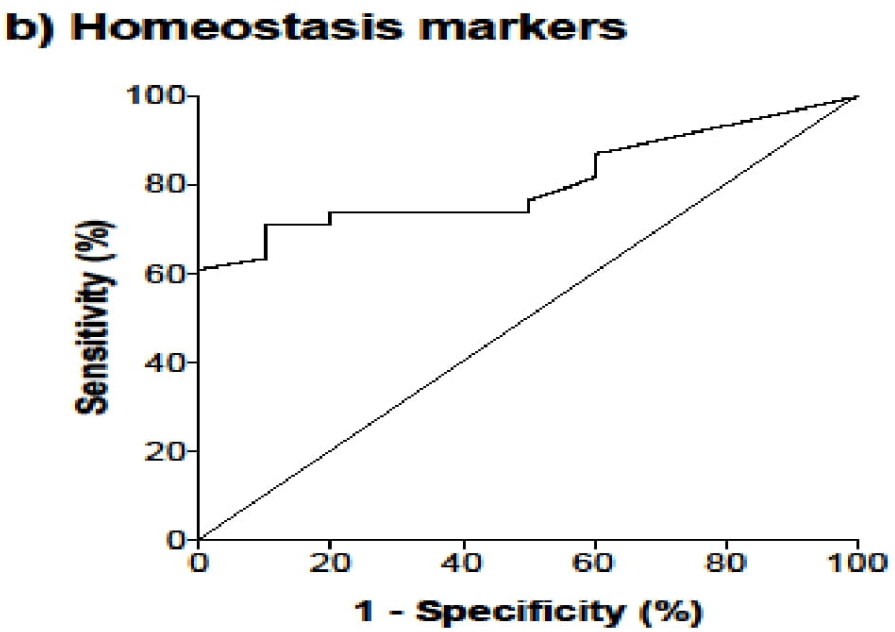
The Blink Test was a moderately strong indicator of a DED diagnosis according to TFOS DEWS II (Table 3; Figure 3). The average of three repeats of the Blink Test was more sensitive than a single measure and resulted in a greater area under the receiver operating curve.

Table 3: Diagnostic accuracy indicators of the Blink Test (single measure or average of 3 values) versus the TFOS DEWS II diagnostic criteria or signs of homeostasis.

	TFOS DEWS II diagnostic criteria		TFOS DEWS II signs of homeostasis	
Diagnostic accuracy value	**Blink Test (averaged value)**	**Blink Test (single value)**	**Blink Test (averaged value)**	**Blink Test (single value)**
C-statistic, 95% CI	0.77 (0.68 to 0.87)	0.70 (0.59 to 0.82)	0.81 (0.71 to 0.91)	0.70 (0.55 to 0.85)
C-statistic p-value	<0.001*	0.003*	0.001*	0.04*
Youden's optimal diagnostic cut-off	≤10	<8	≤11	≤8
Sensitivity, 95% CI	66% (53% to 78%)	53% (40% to 66%)	71% (60% to 81%)	52% (40% to 63%)
Specificity, 95% CI	88% (69% to 97%)	88% (69% to 98%)	90% (56% to 100%)	90% (56% to 100%)
Positive predictive ability, 95% CI	93% (82% to 98%)	92% (79% to 97%)	98% (90% to 100%)	98% (86% to 100%)
Negative predictive ability, 95% CI	51% (42% to 60%)	43% (36% to 51%)	29% (21% to 38%)	20% (15% to 25%)
Positive likelihood ratio, 95% CI	5.51 (1.88 to 16.17)	4.44 (1.50 to 13.15)	7.14 (1.11 to 46.10)	5.19 (0.80 to 33.77)
Negative likelihood ratio, 95% CI	0.38 (0.26 to 0.56)	0.53 (0.39 to 0.72)	0.32 (0.21 to 0.48)	0.53 (0.39 to 0.73)

Figure 3: Receiver operative characteristic curves assessing the diagnostic ability of the Blink Test in discriminating whether subjects fulfilled the (a) TFOS DEWS II dry eye diagnostic test battery or (b) homeostasis marker signs.





Discussion

The Optrex™ Dry Eye Blink Test is advertised as a rapid online test to indicate whether an individual might have dry eye. Like the clinical tear breakup test, it reflects the stability of the tears, however, in this test the time taken for the eyes to sense ocular discomfort is the measure for dry eye rather than the clinical assessment of when the tear film first 'breaks'. However, the diagnostic potential of the self-reported Blink Test approach has not been investigated previously. In order to test the validity of this approach, the current study examined how well the Blink Test performed against the TFOS DEWS II diagnostic criteria for dry eye disease.

The results showed that the mean blink test and NIBUT results were not significantly different. The Blink Test has a narrower distribution than NIBUT, even when NIBUT is capped at 15s, hence in its current form, the disagreement beyond this point will increase. Bland-Altman analysis showed fair levels of clinical agreement between the two tests, however the limits of agreement were moderately wide. As expected, there was also a positive correlation between the tests, with the blink time increasing as NIBUT increased. Tear film breakup is associated with increasing ocular discomfort and this is well documented in the literature.[20, 21] It is thought that tear breakup during the blink-interval results in a transient, localised, increase in tear osmolarity, which in turn stimulates the corneal nociceptors, driving the blink reflex to replenish the tear film.[21] If the breakup of tears on the ocular surface led directly to a rapid loss of comfort, it would be expected that the correlation between NIBUT and the Blink Test would have been greater than moderate. Hence the results suggest that other individual factors, such as the patient's tolerance to pain, the area of breakup and osmolarity levels, affect when patients report discomfort. A more frequent blink rate has been associated with a shorter NIBUT and this may be a compensatory mechanism to mitigate the effects of reduced tear stability.[22] It is known that blink rate diminishes during tasks involving concentration, which may explain why ocular discomfort is commonly experienced by computer users.[23] The blink test, being an online tool, is a relevant tool for such individuals, amongst whom evaporative dry eye is a rising issue. Although it does not simulate a truly natural state, it may reflect the typical stresses on the tear film that occur during the day. The Blink Test also demonstrated a significant negative correlation with conjunctival staining levels such that longer Blink Test time were associated with higher symptomology scores and a lower level of ocular surface

staining. While other ocular measures of dry eye showed similarly negative trends with higher Blink Test scores, these were not statistically significant.

Poor correlation is not infrequently reported between objective clinical test outcomes and symptom severity.[24] This may be due partly to a degree of compensation by blinking more frequently which would serve to reduce the time the tear film needs to maintain its interblink homeostasis. The instantaneous and quantitative Blink Test has the potential to be a more reliable indicator of dry eye risk, by overcoming issues associated with inaccurate patient recall of discomfort symptoms over the previous month, as posed by typical dry eye questionnaires. According to the Blink Test instructions, contact lenses do not need to be removed before taking the test, widening its applicability to a broader patient group. The validity of the test in this demographic was not addressed in the present study, but requires independent evaluation. Contact lenses may act as a partial barrier to corneal sensation,[20] and therefore their wear during the test might be expected to overestimate the Blink Test time (giving a false negative result).

Test sensitivity is a measure of how well a test correctly identifies those with disease, whereas specificity evaluates how well a test can correctly identify those without disease. A trade-off typically exists between the two with the precise values for each influenced by the selected cut-off value for a positive diagnosis. Maximal sensitivity and specificity for the Blink Test was achieved with a cut-off of ≤10s. This offered a sensitivity and specificity of 66% and 88%, respectively. The ROC curve (Figure 3) shows this graphically, with the area under the curve demonstrating high diagnostic ability (AUC=0.77, $P<0.001$). Interestingly the optimal cut-off for the Blink Test aligned closely with that reported by TFOS DEWS II, for NIBUT.[12]

As questionnaires are rapid and simple to administer outside a clinical setting, the sensitivity and specificity of the Blink Test as a surrogate for detecting a loss of homeostasis as classified by TFOS DEWS II,[12] was also assessed. Screening out non-symptomatic individuals first, demonstrated improved discrimination (71% sensitivity, 90% specificity and an AUC of 0.81), compared with the Blink Test alone. It was further examined whether a single assessment of the Blink test would be adequate to save testing time, however, it was clearly demonstrated that, like NIBUT measurement in clinical practice, an average of three repeats of the Blink Test improves diagnostic sensitivity and therefore should be recommended.

All procedures on a participant were carried out by a single examiner, therefore investigator-masking was not possible. However, all were objective or participant completed so operator bias in the results is unlikely. Ocular surface grading was completed subsequent to the Blink Test and without reference to the other clinical findings, and so was effectively masked. As the Blink Test is designed for completion in non-clinical settings, the working distance used was only approximately 40cm and the gaze angle will have differed depending on patient height and sitting stature. Screen illumination and screen size could also affect the blink rate and induce reflex tearing so are other factors that could affect the result. However, despite this, the Blink Test was demonstrated to be a useful indicator of the presence of DED that can be easily applied in non-specialist eye-care settings to aid in the identification and management monitoring of this chronic, debilitating disease, with a view to improving patient outcomes.

Declaration of Interests

No funding was received to support this study. None of the authors have any proprietary interests relating to this research.

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2. How to implement the DEWS II outcomes into your eye care services

Travé Huarte S, Wolffsohn JS. (Nov. 2018). How to implement the DEWS II outcomes into your eye care services. Optometry Today. In press. p. 78.

How to implement the DEWS II outcomes into your eye care services

Sònia Travé Huarte BSc, MSc and **Prof James Wolffsohn** BSc, MBA, PhD, FCOptom

This article outlines the keys points from the TFOS DEWS II report, which drew consensus from over 150 experts in the field of dry eye disease, providing guidance on the definition, causes, diagnosis and management of the condition.

Introduction

The Tear Film and Ocular Surface Society (TFOS) Dry Eye Workshop (DEWS) reports are a summary of the latest evidence-based clinical research in dry eye.[1,2] The reports are intended for use as a tool for disseminating knowledge among the eye care practitioner community in a transparent and clear way. The aim of TFOS DEWS is to achieve a consensus concerning multiple aspects of dry eye disease (DED). This article will discuss the outcome of the TFOS DEWS II from 2017 compared to the initial DEWS report from 2007, and how to apply its outcomes to a practical routine.

1 CET POINT

Definition and classification

In the 2007 DEWS report, the definition of dry eye started to become more specific regarding the pathophysiologic basis of the disease, including tear hyperosmolarity and ocular inflammation, alongside the influence on visual function. Symptoms were considered as the central feature of the disease. It was the first time that dry eye was formally described as a disease with a clear definition:

'Dry eye is a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface. It is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface.'

By comparison, the 2017 TFOS DEWS II report, which besides mentioning neurosensory abnormalities, introduces the new term 'loss of homeostasis' clarifying that hyperosmolarity of the tear film and inflammation of the ocular surface play aetiological roles, considering all aspects of tear imbalance:

'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.'

Classification 2007

Aqueous-deficient dry eye (ADDE) and evaporative dry eye (EDE) are the two main sub-classifications for dry eye (***see Figure 1***). EDE may have the following causes:

- Intrinsic, where the tear film is directly affected by the regulation of evaporative loss, that is to say, meibomian gland dysfunction (MGD), lid disorders, low blink rate, drug action, for example, Accutane
- Extrinsic, where the tear film is affected by factors that impact upon evaporation, such as vitamin A deficiency, preservatives, contact lens wear, ocular diseases or allergies.

ADDE may have causes that include:

- Sjögren symptom: lacrimal hyposecretion and inflammatory changes in the tears and conjunctiva
- Non-Sjögren symptom: lacrimal dysfunction, most common age-related dry eye.

ADDE and EDE are not mutually exclusive, that is to say, both conditions may coexist or even lead to one another amplifying the severity.

Classification 2017

In the TFOS DEWS II 2017 report, the continuum of ADDE and EDE diagnoses is emphasised and the classification drawn to demonstrate that they are not separate entities. Therefore, elements of each should be considered when managing and treating DED. Another differentiation from the past report is that the new classification accommodates patients with a conflict between signs and symptoms, including even a diagnosis

Figure 1

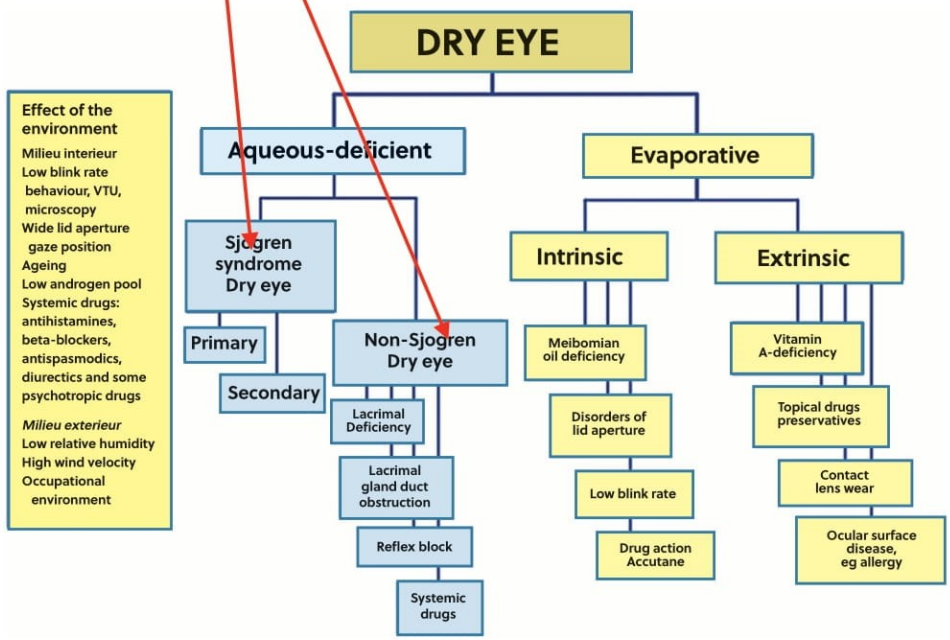


Figure 1 2007 Dry eye disease classification

of 'normal' (***see Figure 2, page 82***). It highlights the fact that EDE is significantly more common than ADDE.

DED can be differentiated from other types of ocular surface disease (OSD) with the use of triaging questions, which are part of a normal history and symptoms (along with assessing dry eye risk factors which help in patient management if they are diagnosed with dry eye). However, eye care practitioners may not be the first port of call for patients with ocular symptoms, so the triaging questions introduced for the first time in TFOS DEWS II will aid pharmacists and general practitioners to more appropriately manage such patients. As shown in ***Figure 2***, the upper part of the oriented flow chart helps to differentiate between symptomatic and asymptomatic patients:

- Symptomatic patient with signs → proceed to identification of other comorbidities, and further testing
- Symptomatic patient without demonstrable clinical signs → do not fall into the DED group, but are separated into:
  - Early stage of DED - monitor condition as it could develop dry eye signs with time
  - Neuropathic pain, which is not OSD related (~5% of the patients).[3] Lesion within the somatosensory nervous system may cause pain associated with but not triggered by the ocular surface. Treatment would be ineffective, and general pain management referral is needed
- Asymptomatic patient with signs →
  - Predisposition to DED (prodromal signs) - risk of developing manifest DED with time and provocation
  - Reduced corneal sensitivity - DED management is required
- Asymptomatic without signs → normal.

Epidemiology

In both TFOS reports epidemiology remains a challenge due to a lack of standardisation of a diagnosis. In the 2007 report the prevalence of dry eye was reported to be from 5% to 35%, on those aged 50 years or more but in the latter report the range has been extended to be between 5–50%. In terms of OSD in general, with the presence of signs, the prevalence increases even more, reaching up to 75%.

Risk factors

As in the 2007 TFOS DEWS report, TFOS DEWS II reports the risk factors divided into 'conclusive,' 'probable' and 'inconclusive.' This article outlines the

Figure 2

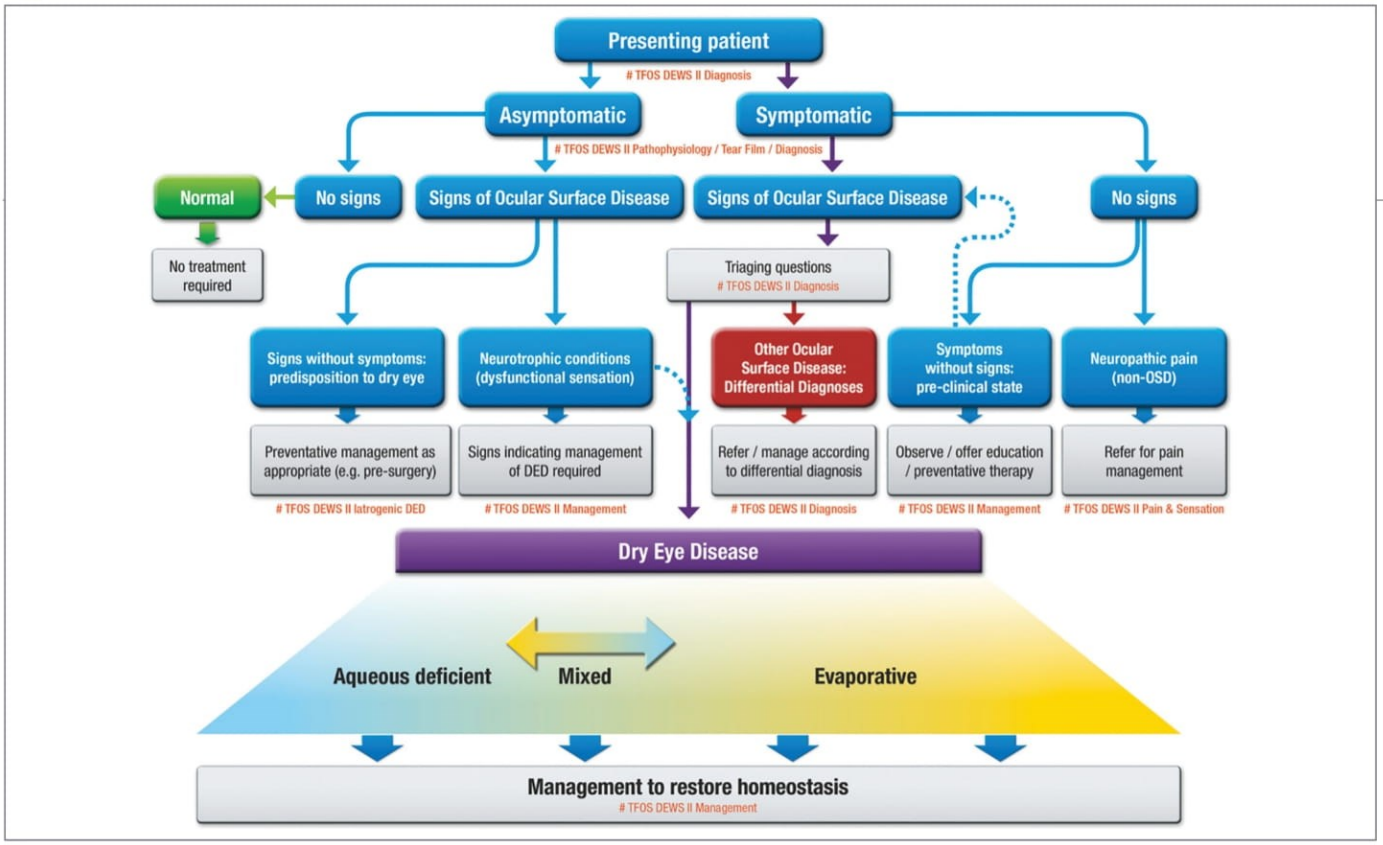


Figure 2 2017 dry eye disease classification

conclusive ones, which can be divided into:

- Non-modifiable:
- Advancing age increases the risk of DED, but also that signs exhibit a greater gain per decade than symptoms, peaking at 40-50 years of age
- A significantly higher percentage of women than men have DED, especially in older age groups
- Asian ethnicity carries an increased risk of 1.5–2-2x, although the reason remains unclear
- MGD
- Sjögren syndrome
- Modifiable:
- Androgen deficiency
- Hormonal imbalance replacement
- Computer use
- Contact lens (CL) wear
- Environment (pollutions or low humidity)
- Medication (antihistamines, isotretinoin, anxiolytics and antidepressants)
- Haematopoietic stem cell transplantation (graft attacks the host following transplantation).

Quality of life (QoL) is affected in some cases where there is impact on visual function, transient changes resulting in reduction to contrast sensitivity and visual acuity prior to the next blink, culminating in decreased workplace productivity.

DED is associated with CL intolerance and discontinuation of lens wear and can adversely affect refractive surgery outcomes. In some patients with decreased corneal sensation, wound healing can be impaired. A possible risk of infection and complications with ocular surgery can be associated with ocular morbidity, especially in patients with connective tissue disorders, as corneal biomechanical properties may be altered.[4]

Sex, gender and hormones

The second TFOS DEWS report emphasised more about why DED has a higher impact on women than men, suggesting that there are sex-related differences underlying the DED aetiology.

It has been demonstrated in the epidemiology section of the report that female sex is a significant risk factor, alongside gender (a person's self-representation as a man or woman). The difference in prevalence has been attributed mostly to the effects of sex steroids (androgens and oestrogens), hypothalamic-pituitary hormones, glucocorticoids, insulin, insulin-like growth factor 1 and thyroid hormones, as well as the sex chromosome complement, sex-specific autosomal factors and epigenetics (microRNAs). However, it is identified that the presentation of the disease, immune responses, pain, care-seeking behaviours, service utilisation, and a myriad of other facets of eye health are also different between both sexes.

It is known that hormones, sex and gender play a significant role in the pathogenesis of aqueous-deficient and evaporative DED, regulating the ocular surface and

Figure 3

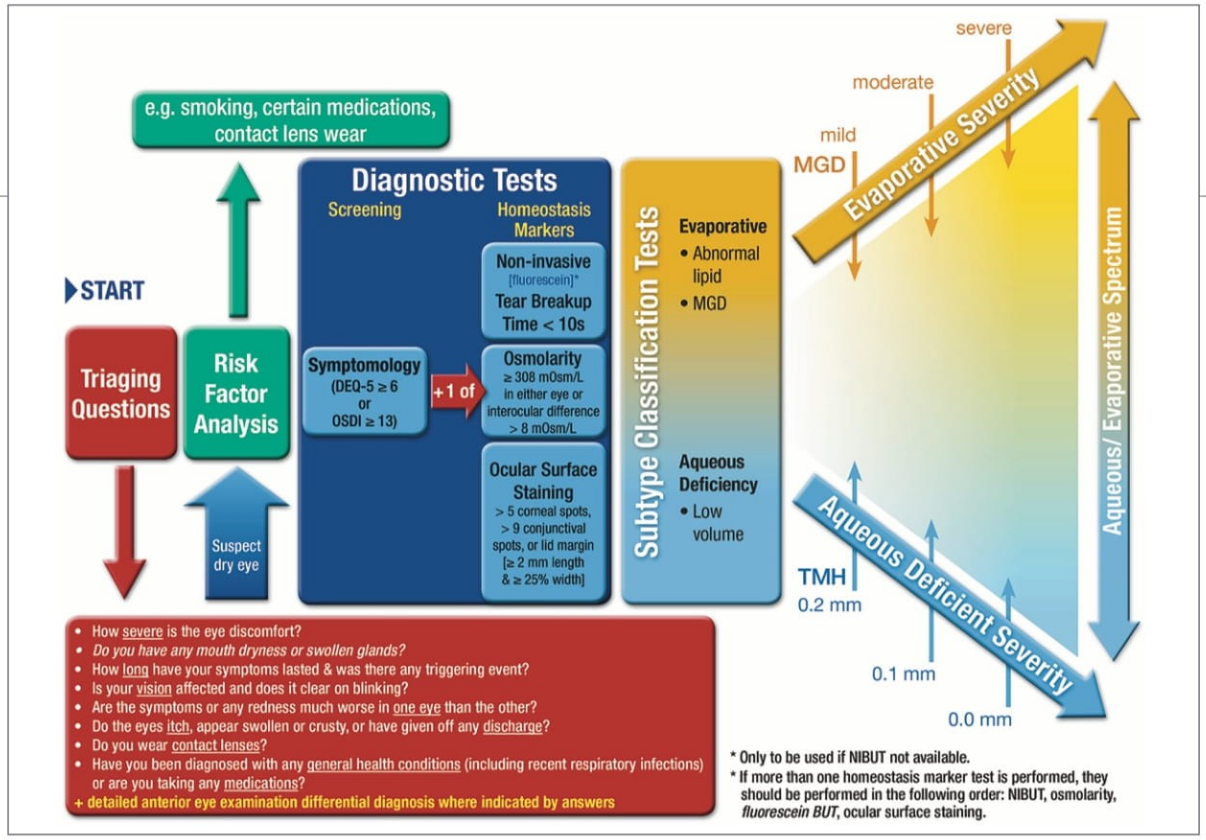


Figure 3 Test battery for diagnosing dry eye disease

adnexal tissues; however, further research needs to be conducted to clarify the precise nature and extent of this cause.

Pathophysiology

The first TFOS DEWS report has demonstrated that dry eye has a chain of inflammatory events and tear hyperosmolarity, which leads to a vicious circle of disease. Events such as surface inflammation (allergic disease), topical preservative toxicity (loss of goblet cells) or altered mucin expression (xerophthalmia) all have different entry points.

The 2017 report substantiated that the main cause of EDE is still believed to be caused by MGD, while Sjögren and non-Sjögren lacrimal gland dysfunction principally cause ADDE, although many hybrid forms of DED exist. In both conditions, the main indicator of DED is tear film hyperosmolarity, which arises from tear evaporation. This hyperosmolarity directly damages the ocular surface by starting a cascade of events in the surface epithelial cells and initiating inflammation, creating symptomatology. When inflammatory mediators and hyperosmolarity of the tear film coexist, goblet cell loss and epithelial cell apoptosis occur by damaging the expression of glycocalyx mucins. The inflammatory mediators gathered in the ocular surface reinforce the epithelial damage causing tear film instability. Once the tear film stability is compromised, the friction between the lids and the globe increases after each blink, which causes the characteristic punctate epitheliopathy and further symptomatology. The reduction in tear volume and decrease in surface wettability, leads to an early tear breakup, which exacerbates tear hyperosmolarity, completing the cycle of vicious events causing ocular surface damage. If left untreated, the condition stimulates corneal nerve endings, generating symptoms of discomfort, increased blinking rate and possibly a compensatory increased reflex drive in lacrimal tear film stimulation and secretion.

Tear film

Traditionally the tear film has been viewed as a three-layered model with distinct lipid layer (LL), aqueous and mucin layers. Evidence is giving way to the more preferred model of a two-phase tear film structure, lipid layer (~42nm),[5] overlying a muco-aqueous gel layer (~2-6μm):[6]

- Lipid layer: containing polar and non-polar lipids
- Muco-aqueous layer: containing at least four major mucins and over 1500 different proteins and peptides, overlying the carbohydrate-rich glycocalyx of the apical epithelium.

Substances such as lipids, proteins, mucins and electrolytes compose the tear film, all contributing to its integrity.

In DED, major changes to the tear film occur both in structure and function as tear film osmolarity increases. Proteins and mucins are reported to change in DED

Figure 4

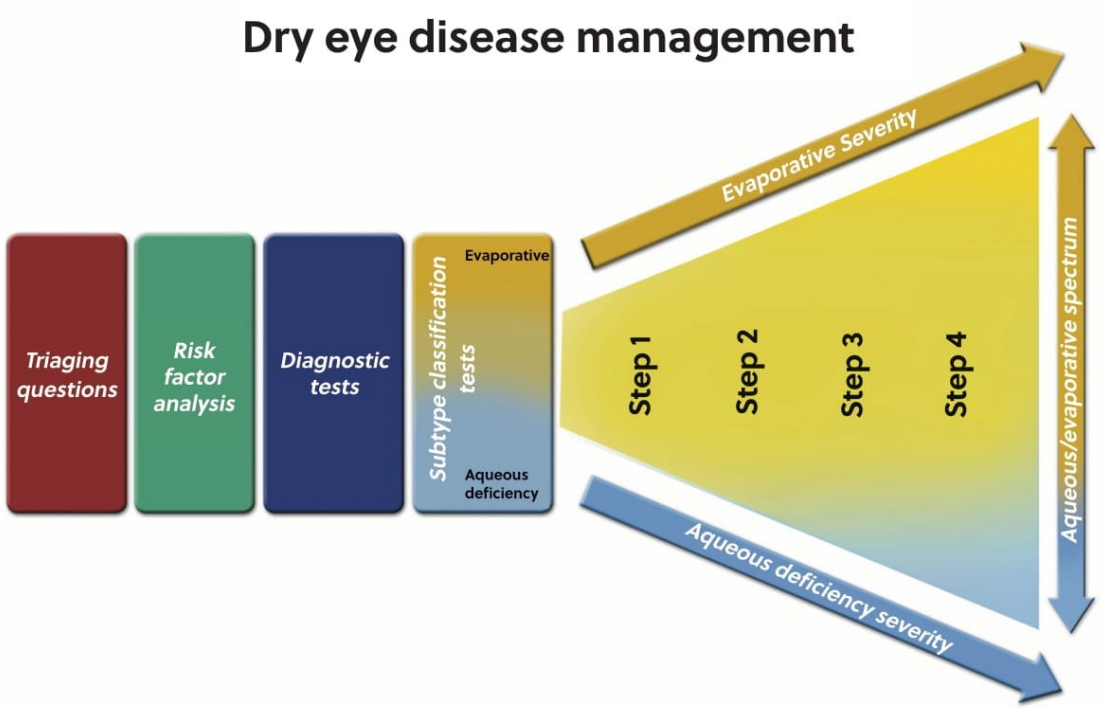


Figure 4 Diagrammatic representation of the process associated with the management of DED

as well, but no definitive set of proteins or changes in protein levels have been validated for aiding in diagnosis. Changes in biomarkers need to be further characterised and investigated.

Diagnostic methodology

Despite the TFOS DEWS 2007 definition of DED requiring symptoms to always be present in DED, it has often been overlooked in its reporting. DED is a subset of OSD and TFOS DEWS II has further emphasised that DED *always* has symptoms. As noted in the classification, practitioners may still choose to treat patients with just ocular signs as they may develop DED if they are having refractive surgery for example. In these cases there is no expectation of a subjective improvement as the patient is asymptomatic.

With the appropriate designed tests/questionnaires and using cut-off values for each test, diagnosing, monitoring and assessing treatment impact is much easier. For this reason, DEWS 2007 recommended a preferred screening and diagnostic approach for dry eye:

1. Symptom questionnaires – administration of structured questionnaires provides an opportunity for screening patients with potential DED
2. Grading ocular surface staining - fluorescein used to assess corneal damage, and lissamine green for assessing conjunctival staining. It is possible to detect and score staining on both cornea and conjunctiva using fluorescein alone if fluorescence is viewed through a yellow barrier filter (Wratten 12)
3. Tear film break-up time (TFBUT): applying a standard volume of fluorescein while using a yellow barrier filter. The cut-off value has been stablished for <10 seconds since the 1973 report of Lemp and Hamill[7]
4. Reflex tear flow: Schirmer Test without anaesthesia keeping the patient's eyes closed. Cut-off value of <5mm
5. Tear osmolarity: test inaccessible to most practitioners. Cut-off value of 316mOsm/l
6. Best performance combining different tests, as provided by the American-European consensus group.[8] For example, diagnosing dry eye as a component of Sjögren syndrome the criteria requires a minimum of a single ocular symptom and a single ocular sign.

The original TFOS DEWS report identified certain tests with high sensitivity and specificity for a presumed DED gold standard but some of the recommendations were too complicated to be easily conducted in clinical practice. With the revised 2017 TFOS DEWS II report, a much clearer path on decision making helps practitioners consistently diagnose whether a patient has dry eye or not.

The test battery shown in ***Figure 3*** starts with:

1. Screening: the latest research evidence suggests that the best clinical approach involves the use of triaging questions and risk factor analysis as part of a traditional patient history and symptoms. Based on the answers, a detailed anterior eye assessment and a differential diagnosis can be made, triggering some more diagnostic tests. Either the DEQ-5, cut-off value ≥6; or OSDI, cut-off value ≥13 is recommended for the screening stage with a positive result leading to the testing for a loss of homeostasis
2. Homeostasis markers:

a. Non-invasive breakup time (NIBUT): cut-off value <10 seconds
b. Osmolarity (measured prior to breakup time if fluorescein BUT used): cut-off value ≥308mOsm/L in either eye or interocular difference >8mOsm/L
c. Ocular surface staining with fluorescein and lissamine green (observing the cornea, conjunctiva and eyelid margin). Cut-off values:

i > five corneal spots
ii > nine conjunctival spots
iii ≥2mm length and ≥25% width

If diagnosed with dry eye, further non-invasive tests should be conducted to clarify the subclassification of DED (EDE – such as meibography, diagnostic meibomian gland expression, lipid thickness interferometry; ADDE – tear meniscus height. The tests aid selection of the appropriate therapeutic choices with a main goal of restoring the homeostasis.

Management and therapy

As expected, more DED targeted therapies have become available over the last decade. The evidence for their efficacy is reported in TFOS DEWS II. The severity matrix has been dropped as signs and different symptoms do not correlate well together. It was also identified that while there is lots of good evidence that many treatments work compared to a placebo, there are few comparative studies that would inform when to change from one treatment type to another either with disease severity or sub-classification of the disease. Therefore, the best approach that could be taken was to place the treatments for DED into four stages to indicate what might be considered as first line and later treatments (***see Figure 4***).

A global survey of current practice is being conducted to benchmark practice approaches currently adopted and to inform research needs to provide better evidence on how to optimally treat DED.[9]

It is important for the practitioners to take time to permit therapies to work; typically around three months of compliant use is needed to determine its efficacy. Some therapies might be more successful when combined. Consequently, a staged algorithm for managing dry eye has been proposed, starting with simple techniques and progressing to more complex therapies.

Pain and sensation

In DED, reduced tear secretion results in epithelial exposure to the environmental conditions, which may result in inflammation and peripheral nerve damage. Once the inflammation is detected, it causes some nerve endings to be more sensitised. Thermoreceptors respond to changes in temperature and osmolarity increases, contributing to the reflex control of basal tear production and blinking.

Long-term inflammation and nerve injury alters receptors at terminals and cell bodies of trigeminal ganglion and brainstem neurons, changing their excitability, connectivity and impulse firing, eventually leading to dysesthesias and neuropathic pain associated with the eye surface. Dryness-induced nerve damage dominates over inflammation.

Different questionnaires can be used to assess pain and sensation. Functional status of the corneal nerves can be assessed with aesthesiometry and *in vivo* confocal microscopy allows nerve visualisation of inflammatory cells in the corneal surface.

Iatrogenic dry eye

Iatrogenic dry eye is an adverse clinical condition resulting from medical treatment by a health professional, which can be caused by numerous factors:

- Topical medication, causing allergic, toxic and immuno-inflammatory effects on the ocular surface
- Systemic medications, culminating in decreased tear production, altered sensory input, and reflex tear secretion
- Contact lens-induced dry eye
- Surgical and non-surgical procedures, due to corneal nerve transection, or postoperative topical drugs

- Preservatives in some formulations which can enhance DED resulting in toxic and pro-inflammatory effects
- Cosmetic and functional eyelid surgeries, botulinum toxin injections and refractive surgeries.

Future plans

Ongoing research is needed to fully understand the nature of each condition including:

- Further knowledge of tear biochemistry to help diagnose, predict and treat DED
- Other ways to measure inflammation and osmolarity over the whole ocular surface
- More work on the early stages of DED and its subclinical inflammation
- Epidemiological studies in different locations, ages, socioeconomical and environmental factors and impact or electronic devices
- Further awareness of DED natural history could help in treating each condition.

Conclusion

The TFOS DEWS II report has updated the knowledge on DED. As practitioners, introducing the changes related to testing and managing dry eye on a daily basis helps to improve professional development and deliver patient satisfaction. With the help of triaging questions, questionnaires, dry eye markers and a stepwise approached for dry eye treatments, TFOS DEWS II aims to introduce an evidence-based standard of practice to diagnose, manage and treat DED.

Exam questions

Under the enhanced CET rules of the GOC, MCQs for this exam appear online at www.optometry.co.uk. Please complete online by midnight on 7 December 2018. You will be unable to submit exams after this date. Please note that when taking an exam, the MCQs may require practitioners to apply additional knowledge that has not been coveredin the related CET article.

CET points will be uploaded to the GOC within 10 working days. You will then need to log into your CET portfolio by clicking on 'MyGOC' on the GOC website (www.optical.org) to confirm your points.

References

Visit www.optometry.co.uk, and click on the 'Related CET article' title to view the article and accompanying 'references' in full.

About the authors

Sònia Travé Huarte graduated in Optics and Optometry at the Faculty of Optics and Optometry in Terrassa FOOT (Barcelona). After completing her degree in 2016 she proceeded to work in private clinics, hospitals and private stores and completed a Master's in visual sciences with expertise in contact lenses in 2017. She is currently undertaking postgraduate research at Aston University on dry eye management and treatment.

Professor James Wolffsohn is deputy executive dean for life and health sciences at Aston University, teaching and researching in the field of anterior eye. He has been awarded fellowships from the International Association of Contact Lens Educators, the American Academy of Optometrists, the British Contact Lens Association, and the College of Optometrists.

Course code: C-60651 Deadline: 7 December 2018

Learning objectives

General Optical Council – Approved CET For Optometrists

- Be able to explain treatment options for dry eye to patients (Group 1.2.4)
- Be able to assess patients presenting with dry eye disease (Group 3.1.8)
- Be able to manage patients presenting with dry eye disease (Group 6.1.3)

General Optical Council – Approved CET For Therapeutic Optometrists

- Understand the pharmacological and non-pharmacological treatments for dry eye disease (Group 1.1.2)
- Be able to assess patients presenting with dry eye disease (Group 2.1.2)

General Optical Council – Approved CET For Dispensing Opticians

- Be able to explain treatment options for dry eye to patients (Group 1.2.4)
- Understand the management of patients presenting with dry eye disease (Group 8.1.3)

General Optical Council – Approved CET For Contact Lens Opticians

- Be able to explain treatment options for dry eye to contact lens patients (Group 1.2.4)
- Be able to assess patients presenting with dry eye disease (Group 3.2.2)
- Be able to manage contact lens patients presenting with dry eye disease (Group 5.4.2)

3. Clinical signs of ocular surface disease

Travé Huarte S, Wolffsohn JS. (2020). Clinical signs of ocular surface disease. VRICS, Visual Recognition test 1 CET point. Optometry Today. April 2020. Pg. 76.

VRICS

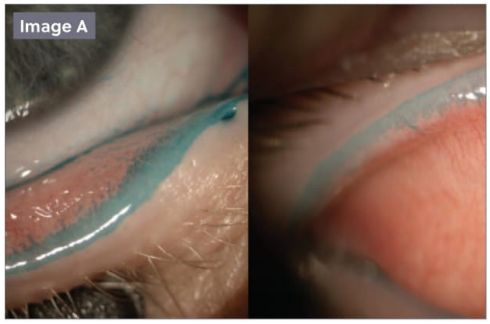
Clinical signs of ocular surface disease

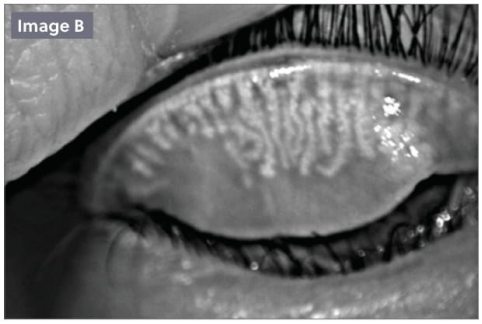
1 CET POINT

Sònia Travé Huarte BSc, MSc and **Professor James Wolffsohn** BSc, MBA, PhD, FCOptom

Readers are invited to review the images in this feature on ocular surface disease and use their clinical knowledge to answer the questions, accessing additional resources where required.

Optometrists | Therapeutic optometrists | Contact lens opticians





01 The staining in this image shows:
a) Marx line
b) Abnormal roughness of tarsal conjunctiva
c) Lid wiper epitheliopathy
d) Meibomian glands openings

02 Which of the following statements in relation to the sign displayed in the image is *true*?
a) It can be only found in patients with punctal plugs
b) It can be seen in patients where there is friction between the lid and the ocular surface
c) It is caused by an increase in tear meniscus height
d) It is caused by a thick lipid layer

03 In the first instance, patients with this type of presentation should:
a) Be referred to secondary care for further investigation
b) Be monitored with photography for future changes
c) Avoid screen time
d) Have artificial tears prescribed to lubricate the eye

04 What is the diagnosis?
a) Keratinisation of the lid margin
b) Obstruction of the meibomian gland orifices
c) Meibomian gland shortening or dropout
d) Increased lid roughness

05 When viewing this sign:
a) It will appear exactly the same under white or infrared light
b) It can only be seen under infrared light
c) It can only be seen under white light
d) The contrast of the glands will be seen better with white light than infrared light

06 Why does this image appear to have differently sized white grapevine-shaped structures?
a) Due to contrast of the image
b) Due to overexposure of the picture
c) Due to a shortening and loss of glands
d) Due to madarosis

About the authors

Sònia Travé Huarte graduated in optics and optometry at the Faculty of Optics and Optometry in Terrassa FOOT (Barcelona). After completing her degree in 2016 she completed a Master's in visual sciences with expertise in contact lenses in 2017. She is currently undertaking postgraduate research at Aston University on dry eye management and treatment.

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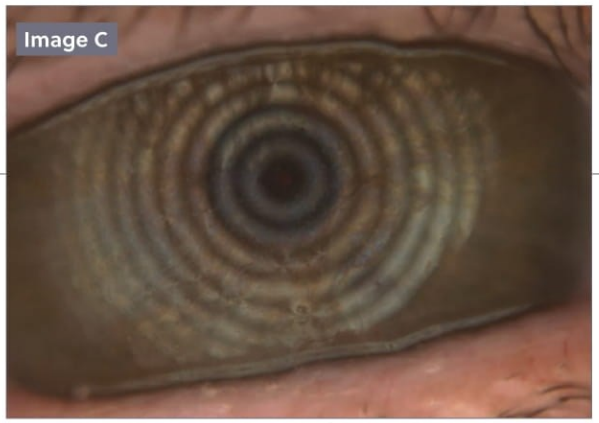

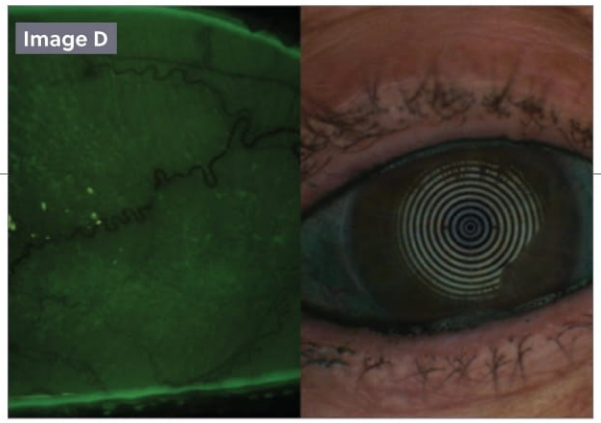

07 Which of the following is *not* observable in the image?
a) Distorted Placido rings
b) Tear film debris
c) Colour fringe lipid layer
d) Corneal irregularity

08 If this patient was fitted with contact lenses what might we expect?
a) Lipid deposits on the lens
b) Complications because of the irregular lid margin
c) Lens discomfort because of blocked meibomian glands and telangiectasia
d) Excellent lens comfort due to the tear meniscus height

09 How thick might the lipid layer of this patient be?
a) Less than 13–15nm
b) Between 13–15nm
c) Between 90–140nm
d) More than 90–140nm

10 What do the images show?
a) Lid parallel conjunctival folds (LIPCOF)
b) Conjunctival abrasion
c) Punctate conjunctival staining
d) Lipid fluorescence

11 Which subtype of dry eye disease does this indicate?
a) Aqueous deficient dry eye (ADDE)
b) Evaporative dry eye (EDE)
c) Mixed dry eye
d) More tests would be needed to make this decision

12 Which statement is *incorrect*? Corneal and conjunctival staining:
a) In severe dry eye is an informative marker related to the severity of the disease
b) Is a marker to diagnose dry eye
c) Correlates to the symptomatology of the patients
d) Occurs when there is a disruption of epithelial cell-tight junctions

Exam questions

Under the enhanced CET rules of the GOC, MCQs for this exam appear online at www.optometry.co.uk. Please complete online by midnight on 22 May 2020. You will be unable to submit exams after this date.

Course code: C-74029 Deadline: 22 May 2020

Learning objectives

- Be able to identify signs of ocular surface disease (Group 6.1.4)
- Be able to assess the ocular surface to identify abnormalities using appropriate techniques (Group 2.1.2)
- Be able to identify ocular surface abnormalities in contact lens patients (Group 5.4.1)

4. Dry eye disease is associated with retinal microvascular dysfunction and possible risk for cardiovascular disease

Shokr H, Wolffsohn S. J, **Trave Huarte S**, Scarpello E, Gherghel D. (2021). Dry eye disease is associated with retinal microvascular dysfunction and possible risk for cardiovascular disease. Acta Ophthalmologica. In press. IF3.153

Acta Ophthalmologica

Acta Ophthalmologica 2021

Dry eye disease is associated with retinal microvascular dysfunction and possible risk for cardiovascular disease

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ABSTRACT.

Purpose: **To explore the presence of microvascular endothelial dysfunction as a measure for early cardiovascular disease in individuals diagnosed with dry eye disease (DED) as compared to age-matched normal controls.**

Methods: **Systemic blood pressure, Body Mass Index, intraocular pressure, blood levels of glucose (GLUC), triglycerides, cholesterol (CHOL), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C)] as well as retinal and peripheral microvascular function were assessed in twenty-five 35–50 year olds with diagnosed with DEDa (using the TFOS DEWS II criteria) and 25 age and sex-matched controls.**

Results: **After controlling all the influential covariates, individuals diagnosed with DED exhibited significant lower retinal artery baseline ($p = 0.027$), artery maximum diameter ($p = 0.027$), minimum constriction ($p = 0.039$) and dilation amplitude ($p = 0.029$) than controls. In addition, the time to reach the vein maximum diameter was significantly longer in the DED patients than in normal controls ($p = 0.0052$). Only in individuals diagnosed with DED, artery maximum constriction correlated statistically significantly and positively with HDL-C blood levels ($p = 0.006$). Similarly, artery $slope_{AD}$ correlated positively with T-CHOL and LDL-C ($p = 0.006$ & 0.011 respectively). Additionally, artery baseline diameter and maximum constriction were significantly and negatively correlated to T-CHOL/HDL-C ratio ($p = 0.032$ and $p = 0.013$ respectively) in DED individuals only.**

Conclusions: **Individuals with positive diagnosis of DED exhibit abnormal retinal microvascular function and possible higher risk for CVD.**

Key words: cardiovascular disease – dry eye disease – microvascular function – retinal vessels

Acta Ophthalmol.

doi: 10.1111/aos.14782

Introduction

Dry eye disease (DED) represents a, multifactorial, chronic and debilitating pathology of the ocular surface characterized by loss of homeostasis of the tear film and accompanied by ocular symptoms. Tear film instability and hyperosmolarity, ocular surface inflammation and damage as well as neurosensory abnormalities play etiological roles in this disease (Craig et al. 2017).

In addition to other risk factors, DED has previously been associated with dyslipidaemia, a group of metabolic abnormalities characterized by any or a combination of the following: raised low-density lipoprotein cholesterol (LDL-C), raised total cholesterol (TC), raised triglycerides (TG) and low high-density lipoprotein cholesterol (HDL-C) (Musunuru 2010). Indeed, as lipid homeostasis is important for the stability of the tear film, the association between dyslipidaemia and DED is entirely justified. Moreover, disruptions of cholesterol biosynthesis are also associated with sebaceous/Meibomian gland (MG) dysfunctions (Bu et al. 2019), another cause of tear film instability and dry eye. Dyslipidaemia also represents a significant risk factor for cardiovascular disease (CVD) especially due to its contribution in the pathogenesis of atherosclerosis in medium-sized and large arteries but also at the microvascular level (Pereira 2012; Padró, Vilahur & Badimon 2018). This affects not only the anatomy of these vessels but, most importantly, their function. Indeed, at the functional level, it impairs endothelium-dependent vasodilation because of defects on nitric oxide (NO) bioavailability (Padró, Vilahur & Badimon 2018). This has catastrophic effects on the balance between the physiological vascular dilatory and constrictory states, which, in turn, will also affect other important circulatory

functions and, most importantly, vascular protection against oxidation, inflammation and thrombosis (Mudau et al. 2012). This imbalance characterises the so-called endothelial dysfunction (ED), an initial reversible step in the development of atherogenesis; nevertheless, it is also one of the most important stages in the development of CVD. Therefore, its identification, as early as possible, represents a key factor in CVD prevention (Mudau et al. 2012).

Besides having common risk factors, other relationships between DED and CVD are not clearly understood. However, as both CVD and DED disease are common and important health problems encountered frequently in the general population, a closer look at their other possible links is warranted. The present study explores the presence of microvascular ED (as a measure for early CVD) in individuals diagnosed with DED as compared to age-matched normal controls.

Methods

Study participants

Healthy individuals aged between 35 and 50-year-old were recruited for this case-control study through advertisements at the Vascular Research Laboratory, and the Dry Eye Clinic, Aston University (Birmingham, UK). Ethical approval was sought from the relevant local ethics committees, and written informed consent was received from all participants prior to study enrolment. The study was designed and conducted in accordance with the tenets of the Declaration of Helsinki, and all study-related procedures adhered to institutional guidelines.

Study exclusion criteria were defined as the positive diagnosis of hypertension, CVD, cerebrovascular disease, peripheral vascular disease, dyslipidaemia, diabetes, as well as other metabolic disorders or chronic diseases that required treatment. Individuals using any vasoactive medications were also excluded from the study. Potential participants were also screened for ocular diseases and were excluded from the study if they had a refractive error of more than ± 3DS and more than ± 1DC equivalent, intra-ocular pressure (IOP) >21 mmHg, cataract or any other media opacities, as well as history of intra-ocular surgery or any form of retinal or neuro-ophthalmic disease affecting the ocular vascular system.

Quality of the retinal vascular images was assessed after the analysis and participants with poor image quality was excluded from the study.

General investigations

Standard anthropometric measures of height and weight were recorded to determine body mass index (BMI = weight/height). Systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) were measured using an automatic Blood Pressure monitor (UA-767; A&D Instruments Ltd, UK) to determine mean arterial pressure (MAP = 2/3 DBP + 1/3 SBP). Intra-ocular pressure (IOP) readings were obtained using non-contact tonometry (Pulsair; Keeler Ltd, UK).

In addition, blood and plasma samples drawn from the antecubital fossa vein were assessed immediately for fasting GLUC, TG, total cholesterol (T-CHOL), and HDL-C using the Reflotron Desktop Analyzer (Roche Diagnostics, UK). Low-density lipoprotein cholesterol (LDL-C) values were calculated as per the Friedewald equation (Friedewald, Levy & Fredrickson 1972).

DED diagnosis

All subjects underwent dry eye assessment using a digital slit lamp and the Keratograph K5m (Oculus, Wetzlar, Germany) including objective non-invasive breakup time, taking the average of three readings and ocular surface staining with fluorescein (i-DEW Flo, Mainline, Derby, UK) and lissamine green (GreenGlo, HUB Pharmaceuticals, Plymouth, MI, USA) using the TFOS DEWS II recommended methodology (Wolffsohn et al. 2017). Osmolarity was assessed in each eye using the Tearlab (Dallas-Fort Worth, TX, USA).

Diagnosis of dry eye was based on the latest TFOS DEWS II criteria (Wolffsohn et al. 2017) which involves a positive symptoms screening with the Ocular Surface Disease Index (cut-off >+13) and one or more of non-invasive tear breakup time (<10 s), hyperosmolarity (>+308 mOsm/l in the higher eye or an intereye difference .8mIsm/l) and ocular surface staining (>5 corneal, >9 conjunctival punctate spots or lid margin (≥2 mm length and >+25% width).

Dynamic retinal microvascular function vessel analysis

Retinal microvascular function was assessed using the dynamic retinal vessel analyser (DVA, IMEDOS GmbH, Jena, Germany) in accordance with an established protocol (Nagel, Vilser & Lanzl 2004) Using a validated in-house algorithm, the following vessel reactivity and time-course parameters were determined: the average baseline diameter and range of maximum and minimum baseline vessel diameters (baseline diameter fluctuation, BDF); the maximum vessel dilation diameter during flicker stimulation expressed as a percentage change relative to baseline diameter (MD%) and the time taken in seconds to reach the maximum diameter (tMD); the maximum vessel constriction diameter during the postflicker recovery period expressed as a percentage change relative to baseline diameter (MC%) and the time taken in seconds to reach the maximum vessel constriction diameter (tMC); the overall dilation amplitude (DA) calculated as the difference between MD and MC; and the baseline-corrected flicker response (BCFR) used to describe the overall DA after normalizing for fluctuations in baseline diameters (DA-BDF). In addition, the arterial (A) and venous (V) dilation slopes ($Slope_{AD/VD}$ = (MD − baseline diameter)/tMD) and constriction slopes ($Slope_{AC/VC}$ = (MC − MD)/tMC) were also calculated (Fig. 1) (Shokr, Dias & Gherghel 2020).

Digital thermal monitoring

The peripheral microvascular function was assessed using VENDYS 5000 BCE digital thermal monitoring (DTM) system (Endothelix, Inc, Houston, TX, USA) according to an established protocol (Schier et al. 2013).The following parameters were measured and calculated (Fig. 2): temperature rebound (TR): maximum temperature (T_{MAX}); minimum temperature (T_{MIN}); adjusted TR (aTR); and area under the curve TR. The post-occlusive aTR determined by the software algorithm

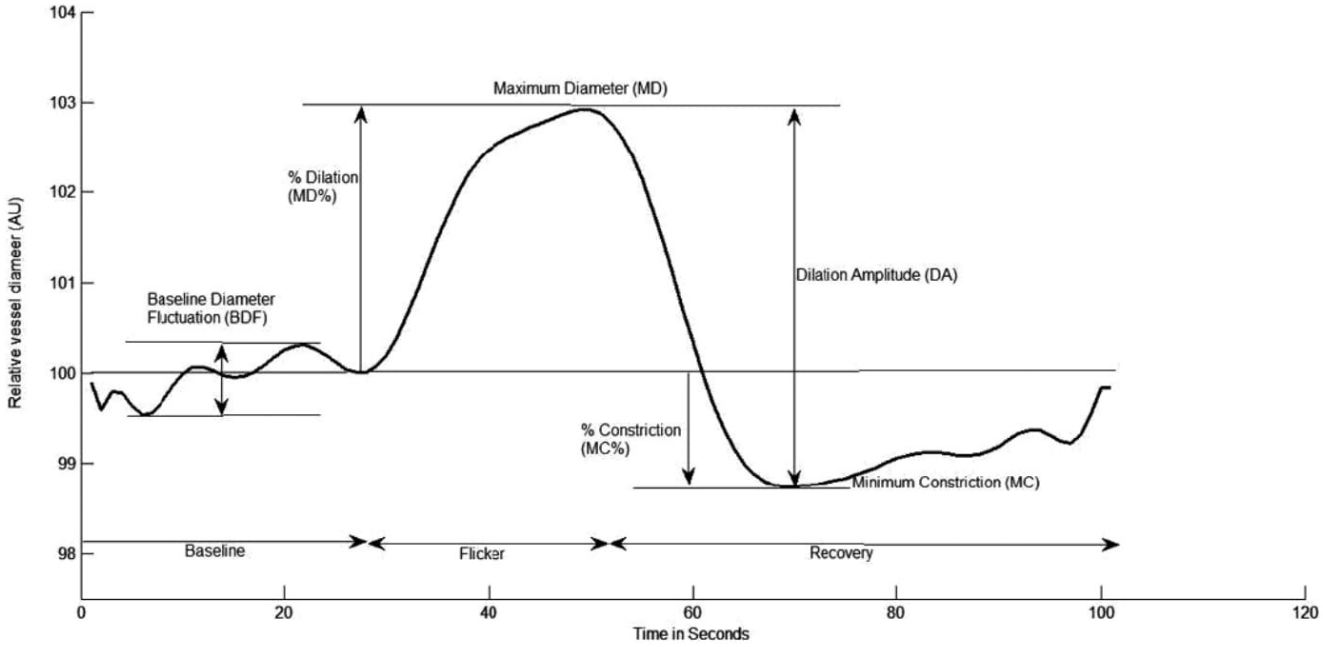

Fig. 1. Graphical presentation of the dynamic vessel response profile displaying the parameters calculated and used in analysis. (DA) calculated as (MD − MC). (MD%) calculated as the percent increase from baseline to MD. (MC%) calculated as the percent constriction below baseline following MD.

is directly associated with the extent of the subjects vascular reactivity. An aTR below 1 was considered poor cardiovascular reactivity (high risk), whereas an aTR between 1 and 2 was considered intermediate vascular reactivity (medium risk). An aTR of >2 was considered a sign for normal peripheral vascular reactivity (Karimzad, Shokr & Gherghel 2019).

Statistical analysis

Based on previous studies, a change of 30% with a SD of 2.5% in retinal vessel reactivity was shown to be significant. As the study design was multi-factorial in nature it was calculated that $n = 25$ in each group was sufficient to provide 90% power with an alpha of 0.05.

All data are reported as mean (SD) unless otherwise indicated. The Shapiro–Wilk test was used to determine the distribution of the data. Univariate associations were determined using Pearson's (normally distributed data) or Spearman's method (non-normally distributed data), and forward stepwise regression analyses were performed to test the influences of

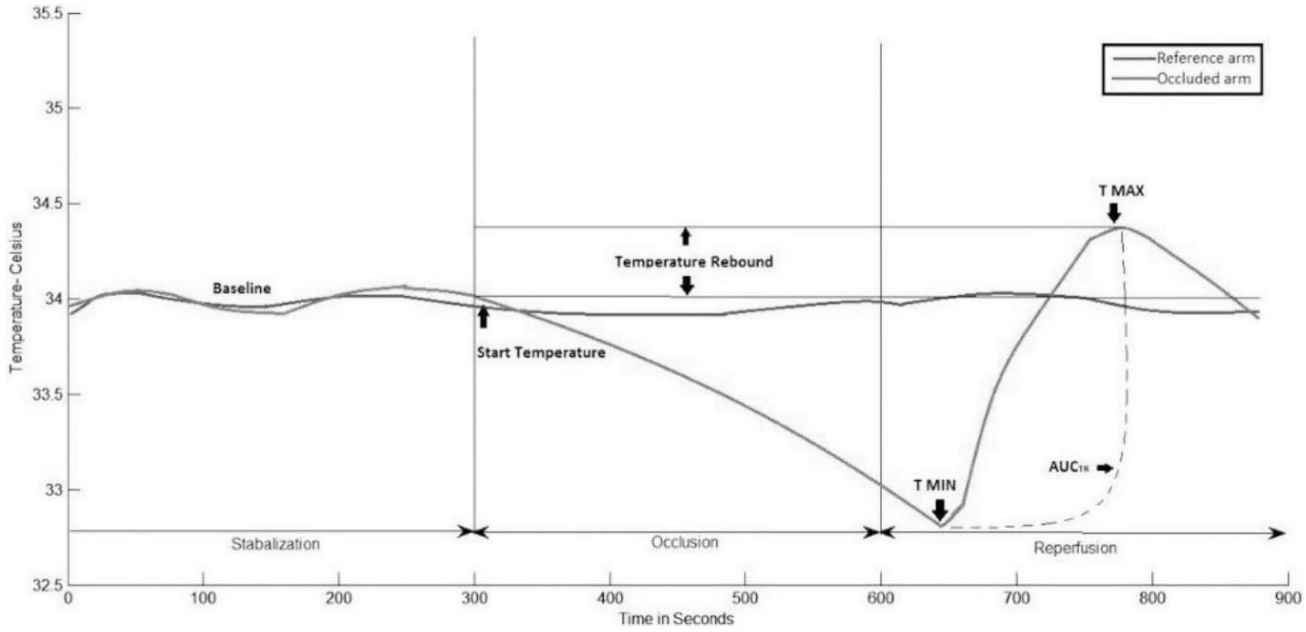

Fig. 2. Graphical representation of the Digital Thermal Monitor software analysis; AUCTR = area under the curve temperature rebound; T_{MAX} = maximum temperature, T_{MIN} = minimum temperature.

systemic and circulating markers on the measured variables. In multivariate regression models the β coefficient value was considered to answer the question of which of the independent variables has a greater effect on the dependent variable as β refers to the SD change in the dependent variable per SD increase in the predictor variable, and is particularly useful when variables are measured in different units. Differences between groups were subsequently assessed using one-way analysis of variance (ANOVA) or analysis of covariance (ANCOVA).p-values of <0.05 were considered significant. All analyses were performed using Statistica® (version 13.3; StatSoft Inc., Tusla, OK, USA) software.

Results

A total number of 66 participants were initially screened for study inclusion, of which 16 individuals were excluded based on the quality of retinal vascular image analysis. The remaining 50 participants were included in the final analysis and classified into two study groups based on a positive diagnosis for DED: 25 individuals (12 men and 13 women) and negative diagnosis: 25 individuals (15 men and 10 women).

General characteristics of the study population are presented in Table 1. There were no significant differences in age, sex, BMI, HR, IOP, GLUC, TG, and LDL-C, between the study groups (all $p > 0.05$). Although still within the normal range, the individuals with DED had a statistically significant higher T-CHOL (5.0 versus 4.44 mmol/l) and lower HDL-C (1.21 versus 1.58 mmol/l) values than those without DED ($p = 0.0356$ and 0.0137 respectively), albeit close to the upper limit for these parameters (Table 1).

There were no significant differences between the study groups with regard to the peripheral microvascular function parameters as measured using the DTM method (all $p > 0.05$, Table 1). In addition, after controlling all the influential covariates identified using multivariate analysis, there were no significant differences between the study groups with regards to the retinal microvascular parameters BDF, BCFR, MD%, MC%, tMD and tMC, $Slope_{AD}$ and $Slope_{AC}$ (all $p > 0.05$, Table 2). However, individuals diagnosed with DED exhibited significant lower artery baseline diameter, artery MD, MC and DA than individuals in the non-dry eye group ($p = 0.0269$, $p = 0.0273$, $p = 0.0386$ and $p = 0.0291$, respectively, Fig. 3). In addition, vein tMD was significantly longer in the DED patients than in normal controls ($p = 0.0052$; Table 3).

Only in individuals diagnosed with DED, artery MC correlated statistically significantly and positively with HDL-C blood levels ($p = 0.006$). Similarly, artery $slope_{AD}$ correlated

Table 1. General characteristics of the study population.

	Dry eye diagnosis	None dry eye	p-value
Number	25	25	–
Sex	12 Male : 13 Female	15 Male : 10 Female	–
Age (years)	44.1 (2.5)	37.6 (2.5)	0.073
BMI (kg/m^2)†	26.03 (1.03)	26.71 (1.03)	0.645
SBP (mmHg)	116.90 (4.96)	120.5 (4.96)	0.611
DBP (mmHg)	68.94 (3.43)	71.3 (3.43)	0.629
HR (bpm)	62.14 (2.10)	66.00 (2.98)	0.366
IOP	13.48 (0.42)	13.31 (0.44)	0.776
GLUC (mmol/l)	4.81 (0.15)	4.54 (0.16)	0.228
TG (mmol/l)	1.01 (0.07)	0.94 (0.07)	0.502
T-CHOL (mmol/l)	5.0 (0.172)	4.44 (0.19)	0.036*
HDL-C (mmol/l)	1.21 (0.109)	1.58 (0.09)	0.014*
LDL-C (mmol/l)	3.15 (0.23)	2.81 (0.23)	0.301
T-CHOL/HDL-C	4.36 (0.377)	3.086 (2.34)	0.020*
aTR	1.52 (0.14)	1.76 (0.14)	0.237

Data are presented as mean (SD) unless otherwise indicated.
aTR = adjusted temperature rebound, BMI = body mass index, DBP = diastolic blood pressure, GLUC = glucose, HDL-C = high-density lipoprotein cholesterol, HR = heart rate (in beats per minute), IOP = intraocular pressure, LDL-C = low-density lipoprotein cholesterol, SBP = systolic blood pressure, T-CHOL = total cholesterol, TG = triglycerides.
* Significant p-values are indicated where $p < 0.05$ was considered significant.
† Calculated as weight in kilograms divided by height in metres squared.

Table 2. Summary of retinal arterial vascular functional parameters.

	Mean (SD)		
Parameter	Dry eye	None-dry eye	p-value
Artery baseline	110.59 (2.52)	112.72 (2.51)	0.027*
Artery-BDF	5.47 (0.47)	5.434 (0.47)	0.954
Artery-DA†	8.29 (0.77)	10.73 (0.77)	0.029*
Artery-BCFR‡	3.84 (0.51)	4.33 (0.51)	0.502
Artery-MD	118.75 (2.64)	123.28 (2.85)	0.027*
Artery-tMD	16.73 (0.92)	15.67 (0.92)	0.414
Artery-MD%	4.95 (0.54)	5.56 (0.53)	0.434
Artery-MC	109.50 (2.38)	111.67 (2.38)	0.039*
Artery-tMC	23.95 (1.59)	25.71 (1.58)	0.436
Artery-MC%	−3.05 (0.38)	−3.63 (0.38)	0.287
Artery-$Slope_{AD}$§	0.41 (0.04)	0.39 (0.043)	0.764
Artery-$Slope_{AC}$¶	−0.52 (0.08)	−0.41 (0.085)	0.361

Unless otherwise indicated, all values are expressed in arbitrary units, which approximately correspond to micrometres (μm) in a normal Gullstrand eye.
Baseline = baseline diameter, BCFR = baseline-corrected flicker response, BDF = baseline diameter fluctuation, DA = dilation amplitude, MC = Maximum constriction, MC% = percentage constriction below baseline, MD = artery maximum dilation, MD% = percentage change in diameter from baseline to maximum dilation, $Slope_{AC}$ = slope of arterial constriction, $Slope_{AD}$ = slope of arterial dilation, tMC = reaction time to maximum constriction diameter from maximum dilation diameter, tMD = reaction time to maximum dilation diameter.
* Significant p-values are indicated where $p < 0.05$ was considered significant.
† Calculated as MD − MC.
‡ Calculated as DA − BDF (Nagel, Vilser & Lanzl 2004).
§ Calculated as (MD − baseline)/tMD (Mroczkowska et al. 2012).
¶ Calculated as (MC − MD)/tMC (Mroczkowska et al. 2012).

positively with T-CHOL and LDL-C ($p = 0.006$ & 0.011 respectively). Additionally, artery baseline diameter and MC were significantly and negatively correlated to T-CHOL/HDL-C ratio ($p = 0.032$ and $p = 0.013$ respectively) in DED individuals only (Fig. 4).

Discussion

This study examined the link between DED and CVD risk, as assessed using known circulatory markers as well as measurements of microvascular function at the retinal and peripheral level. Through this approach, it identified that individuals diagnosed with DED exhibit abnormal retinal, but not peripheral microvascular function and these abnormalities correlate with plasma levels of circulating cholesterols. To our knowledge, this is the first study to reveal that middle-aged individuals with DED have increased retinal microvascular dysfunction compared to normal, sex and age-matched controls.

Similar to other research (Dao et al. 2010; Wang et al. 2012; Chun et al. 2013), this study also demonstrated that individuals with DED exhibit higher, albeit at the upper normal levels of circulating T-CHOL when compared to those without DED. It has been previously proposed that the relationship between hypercholesterolemia and DED can be explained as increased levels of cholesterol in the meibomian lipid would increase its melting point, thus leading to increased viscosity and plugging of the meibomian orifice (Butovich, Millar & Ham 2008). It is also important to note that individuals with DED included in our study also exhibited statistically significant lower, albeit still normal levels of HDL-C and higher T-CHOL/HDL-C ratio than the normal controls group. It is well known that HDL-C transports cholesterol from the tissues to the liver to be disposed, making it beneficial in the prevention of CVD. Moreover, decreases in HDL-C have also been linked with ED and a

Table 3. Summary of retinal venous vascular function parameters.

Parameter	Mean (SD)		p-value
	Dry eye	None-dry eye	
Vein-baseline	133.62 (3.92)	139.94 (3.92)	0.260
Vein-BDF	5.37 (0.43)	5.23 (0.43)	0.822
Vein-DA[†]	9.11 (0.61)	8.96 (0.62)	0.867
Vein-BCFR[‡]	3.73 (0.43)	3.72 (0.43)	0.988
Vein-MD	140.04 (4.58)	149.92 (4.58)	0.134
Vein-tMD	19.46 (0.70)	16.40 (0.70)	0.005*
Vein-MD%	4.94 (0.38)	4.50 (0.38)	0.415
Vein MC	130.94 (4.35)	140.96 (4.35)	0.110
Vein-tMC	33.49 (1.61)	33.29 (1.61)	0.930
Vein-MC%	−2.0 (0.19)	−1.76 (0.19)	0.371
Vein-slope$_{AD}$[§]	0.49 (0.07)	0.48 (0.07)	0.908
Vein-slope$_{VC}$[¶]	−0.52 (0.08)	−0.41 (0.086)	0.361

Unless otherwise indicated, all values are expressed in arbitrary units, which approximately correspond to micrometres (μm) in a normal Gullstrand eye.
Baseline = baseline diameter, BCFR = baseline-corrected flicker response, BDF = baseline diameter fluctuation, DA = dilation amplitude, MC = Maximum constriction, MC% = percentage constriction below baseline, MD = vein maximum dilation, MD% = percentage change in diameter from baseline to maximum dilation, Slope$_{VC}$ = slope of venous constrictionSlope$_{VD}$ = slope of venous dilation, tMC = reaction time to maximum constriction diameter from maximum dilation diameter, tMD = reaction time to maximum dilation diameter.
* Significant p-values are indicated where $p < 0.05$ was considered significant.
[†] Calculated as MD − MC.
[‡] Calculated as DA − BDF (Nagel, Vilser & Lanzl 2004).
[§] Calculated as (MD − baseline)/tMD (Mroczkowska et al. 2012).
Calculated as (MC − MD)/tMC (Mroczkowska et al. 2012).

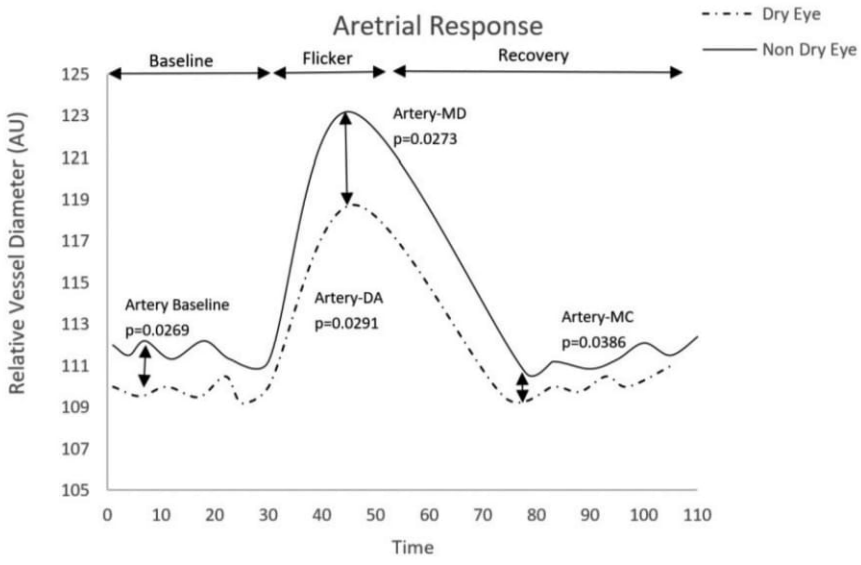

Fig. 3. Comparison of retinal arterial response profile across groups. AU = arbitrary units, BDF = baseline diameter fluctuation calculated as the maximum range in vessel diameter during first 30 s of baseline readings, MD% = calculated as the percentage change in vessel diameter from baseline to maximum following onset of flicker, slope$_{AC}$ = calculated as (MC – MD)/(tMC), tMC = time to reach maximum constriction post flicker, tMD = time to reach maximum diameter during flicker.

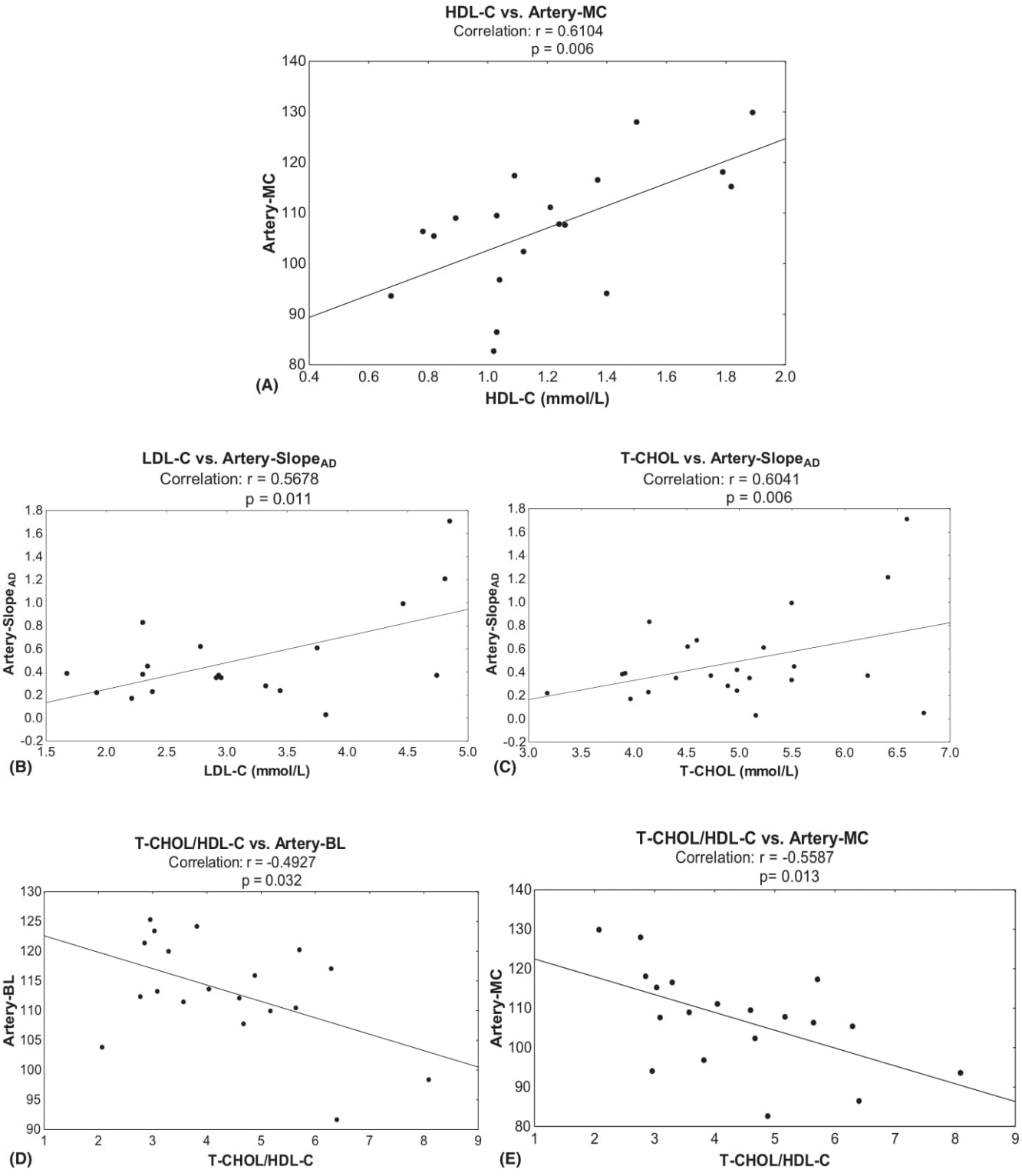

Fig. 4. Correlation between retinal arterial responses and systemic blood lipids. Arterial maximum constriction, Artery- MC = Arterial maximum constriction, Artery- MC, Artery- SlopeAD = slope of arterial dilation, Artery-BL = artery baseline diameter, HDL-C = high density lipoprotein cholesterol, LDL-C = low density lipoprotein cholesterol, T-CHOL = total cholesterol, T-CHOL/HDL = total cholesterol/high density lipoprotein ratio. Normalized unit values used on *y*-axes.

reduction in the bioavailability of NO (Campbell & Genest 2013). In addition, T-CHOL/HDL-C ratio has previously been specified as a better indicator of premature CVD risk in than T-CHOL levels (Tewari et al. 2005), making these observations very important.

The present study revealed a novel finding that, independent of other characteristic, individuals with DED exhibit abnormal retinal arterial and venous microvascular dysfunction. To date, little research looked at microvessels in individuals with DED and only at the level of conjunctival vessels. It is

interesting to mention that patients with DED have been previously shown to exhibit abnormal microvascular response and reduced blood flow at the conjunctival vessels level after trigeminal stimulation, suggesting that these patients suffer of an imbalance in the autonomic nervous system (ANS) (Chen et al. 2017). Indeed, conjunctival vessels have a dual autonomic innervation (Ruskell 1985). Moreover, DED has been proposed to be associated with various ANS dysfunctions as autonomic nerves are abundant in MG tissue and play an important role in regulating the secretory activities of MG in animals (LeDoux et al. 2001; Li et al. 2012). Endothelial dysfunction (ED) and ANS imbalance often co-exist in the development of various CVD processes (Amiya, Watanabe & Komuro 2014). At the retinal microvascular level, in the absence of autonomic innervation, metabolic and myogenic stimuli are more involved in retinal autoregulation of the microvascular calibre (Kur et al., 2012). Although the function of retinal microvessels are not under the influence of ANS, this study suggests that both the ANS and endothelial dysregulation co-exist in individuals with DED and the results of this imbalance are evident and can be measurable at different vascular levels. In addition, abnormalities in the retinal venous functionality have also been found in the DED group. Since retinal veins typically incite a more passive regulatory contribution to increases in blood flow whether this observation may reflect some kind of reconciliation of alterations in arterial outflow to the venous side via downstream autoregulatory mechanisms (Kotliar et al. 2004) is unclear at present.

The positive correlation between the retinal microvascular function parameters and the levels of circulating HDL-C in DED individuals reinforces the fact that the HDL-C has an important role in prevention of CVD. Indeed, retinal vascular calibres have been shown to be independently associated with risk factor variables such as age, blood pressure, HDL-C, and LDL-C. Our DED group, however, also exhibited negative relationship between T- CHOL/HDL-C ratio and retinal artery MC. As this circulatory parameter is a strong indicator of risk for CVD (Davidson et al. 2008; Agirbasli et al. 2015), this observation is very important and, in addition to the above mentioned microcirculatory abnormalities, points to the fact that DED individuals could exhibit higher risk for CVD than age- and sex-matched normal individuals. Further follow-up studies, to confirm the actual development of CVD in these individuals, are warranted.

Conclusion

Significant attenuations in retinal vascular function exist and can be detected in persons diagnosed with DED. Moreover, these abnormalities correlate with known circulatory markers for CVD. Functional retinal assessments could therefore be useful for early vascular screening, possibly contributing to a reduced risk for CVD morbidity in these individuals.

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5. Phospholipid Concentration on the Tear Film and Ocular Comfort

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Effect of Eye Spray Phospholipid Concentration on the Tear Film and Ocular Comfort

Heiko Pult, Ph.D., Farzana S. Khatum, Sonia Trave-Huarte, B.Sc., and James S. Wolffsohn, Ph.D.

Objectives: To evaluate the effect of eye spray phospholipid concentration on symptoms and tear film stability.

Methods: High-concentration (Tears Again, Optima Pharma GmbH, Hallbergmoos, Germany) and low-concentration (Ocuvers, Innomedis AG, Germany) phospholipid eye sprays were sprayed onto the closed eyelids of 30 subjects (33.2±1.8 years; 20 women) in a multicentered, prospective, crossover study. Ocular comfort (visual analog scale) and non-invasive tear film stability (NIBUT) of each eye were evaluated before application (along with the Ocular Surface Disease Index), 10 min after application, and 30 min after application.

Results: Comfort (high concentration: 68.5±16.4 vs. low concentration: 70.7±14.5 phospholipid) and NIBUT (high concentration: 11.5±4.6 sec vs. low concentration: 11.2±6.0 sec phospholipid) were not different ($P>0.3$) between sprays before application, but comfort (by 12 points, $P=0.001$) and NIBUT (by 5 sec, $P=0.016$) were significantly better with a high-concentration phospholipid spray at both 10 min and 30 min time points than those with the low-concentration phospholipid spray.

Conclusions: The liposomal eye spray with higher concentration of phospholipids significantly improved ocular comfort and tear film stability in contrast to the eye spray with lower concentration of phospholipids, hence practitioners need to choose an appropriate eye spray to maximize the patient benefit.

Key Words: Phospholipids—Tear film stability—Comfort—Tear film.

(*Eye & Contact Lens* 2021;00: 1–4)

Dry eye is a common disease, described as aqueous-deficient and/or evaporative dry eye,[1] the latter being the most common type (78%).[2,3] The lipid layer of the tears plays an important role in inhibiting tear film evaporation and in spreading the tears across the ocular surface.[4] The lipid layer is a complex structure with an inner polar layer, interfacing with the aqueous phase and reducing surface tension, and a thicker, outer, nonpolar layer.[5] The lipid layer stabilizes the tear film providing a surface tension decrease and a 90% to 95% aqueous evaporation reduction.[6,7]

Phospholipid liposomal spray is suggested to improve the polar properties of the lipid layer (such as its surface tension and solubility), enhancing lipid spread over the tear film.[8–14] The spray is applied to the closed eyelids and supplements phospholipid liposomes to the lid margins, where the phospholipids mix with the present lipid reservoir from the meibomian glands. Improvements in symptomatology, visual acuity, lipid layer thickness, tear film stability, eyelid margin inflammation, tear production, and lid parallel conjunctival folds have been documented with the use of the original liposomal spray in patients with dry eye.[8–15] However, as other sprays have become available with different concentrations of phospholipids, contradictory studies have been published on their relative effectiveness.[14,16,17] These studies were based on a contralateral eye design, limiting the accurate reporting of symptomology.[18] It is well established that normal eyes do not function as independent units, but rather there is cross-communication between right and left eyes.[18] Hence this study aimed to determine the relative effectiveness of two liposomal eye sprays with different concentration of phospholipids on dry eye symptoms and tear film stability in a multicentered, prospective, crossover trial.

METHODS

Based on laboratory analyses of phospholipid concentrations of different sprays, one high-concentration and one low-concentration phospholipid eye spray was selected (Table 1).

Participants gave written informed consent before participating in the study. Ethical approval was given by the Research Ethics Committee of Aston University. All procedures were conducted in accordance with the Declaration of Helsinki (1983). Potential participants were excluded if they were contact lens wearers or had worn lenses within the last year, were younger than 18 years, had any ocular surface defect or pathology, were pregnant, or had used any ocular medications or drops on the study visit days.

The high-concentration (Tears Again Sensitive, Optima Pharmazeutische GmbH, Hallbergmoos, Germany) and low-concentration phospholipid (Ocuvers, Innomedis AG, Germany) formulations were sprayed from a distance of 10 cm onto each closed eyelids of 30 participants in a multicentered, prospective, double-blind, crossover study. Participants were randomized as to which spray they received bilaterally first, and there was a gap of 24 to 120 hr between trials.

Before application, each participant's symptoms were evaluated using the Ocular Surface Disease Index (OSDI) questionnaire.[19] Total OSDI scores were calculated as recommended by Schiffman et al.[20] Ocular comfort ("How is the current comfort of your eye?") of each

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Table 2: *Comfort and Noninvasive Breakup Time (NIBUT) Mean and SD*

	Ocular Comfort			NIBUT		
Phospholipid Concentration	Low	High	Significance	Low	High	Significance
Time point	Mean±SD	Mean±SD		Mean±SD	Mean±SD	
Baseline	70.7±14.5	68.5±16.4	0.794	11.3±6.0	11.5±4.6	0.934
10 min	67.4±14.8	84.0±19.1	0.006	11.8±5.3	16.7±8.8	0.038
30 min	68.7±14.5	87.3±17.3	0.001	11.9±5.6	16.7±7.8	0.027

lipid layer suppresses the vicious cycle of dry eye disease, thus improving the epithelium and consequently the mucin layer and wettability of the cornea.[7] This might be an important factor to be considered in the long-term treatment of patients with the evaporative form of dry eye.

This study showed that a high-concentration phospholipid spray improved tear film stability and also enhanced ocular comfort. Phospholipids are recognized to be important components within the tear film. They are vital in surface monolayer formation and for surfactant properties. Ninety-two percent of the meibum consists of neutral lipids, and the remaining 8% of polar lipids.[27] The polar lipids consist of 70% phospholipids; the most predominant of which is phosphatidylcholine. Deficiency of these components prevents formation of a stable, continuous lipid layer, which, in turn, causes an increased tear evaporation rate.[27,28] The liposomal spray Tears Again is a tear film supplement containing phosphatidylcholine derived from highly purified soy lecithin. The major phospholipid, phosphatidylcholine, is delivered in a stable form of liposomes to the closed eyelid. From there, they migrate, with blinking, across the eyelid margins to combine with the tear film.[14] Improvement of tear film stability after application of a spray with a higher concentration of phosphatidylcholine confirms the positive effect this phospholipid has on the tear film.

CONCLUSION

The liposomal eye spray with a high concentration of phospholipids significantly improved ocular comfort and tear film stability in contrast to the eye spray with a lower concentration of phospholipids, which had no effect; hence, practitioners need to choose an appropriate phospholipid eye spray to maximize the benefit to patients with dry eyes due to a deficient lipid layer.

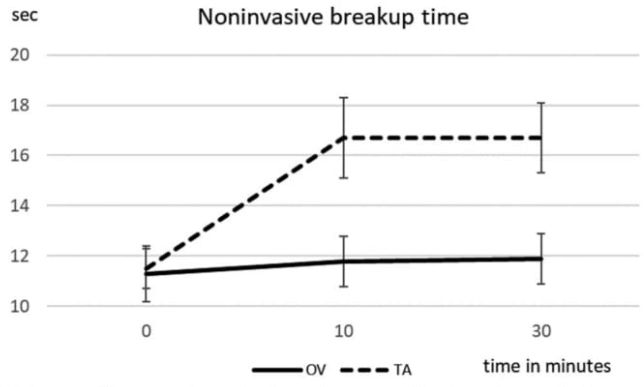

FIG. 2. Mean noninvasive breakup time [sec] and standard error over the observation time of 0 to 30 min (high-concentration [HC] and low-concentration [LC] phospholipid spray).

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6. Original QUALTRICS Survey in English

Dry_Eye

Start of Block: Default Question Block

Q10 This Tear Film and Ocular Surface Society (TFOS) survey focuses on your current practice in diagnosing and managing dry eye. We are very grateful for your time taken to complete this. By completing this survey you are giving your consent for us to use your anonymous data for research purposes. Once submitted, this data is untraceable to you and therefore cannot be withdrawn.

Q4 What type of dry eye patients do you predominantly manage (please rank from 1 to 5 where 1 is the most common and 5 is the least common type of patient you see, leaving blank if not seen)? Patients who present with:
_____ No presenting specific symptoms: identified incidentally on questioning (1)
_____ Intermittent presenting symptoms: occasional effect on quality of life (2)
_____ Mild presenting symptoms; low impact on quality of life (3)
_____ Moderate symptoms: frequent impact on quality of life (4)
_____ Severe symptoms: constant debilitating effect on quality of life (5)

Q5 Your Current Management of Dry Eye Disease [NOTE on some devices you may need to scroll to the right to see the whole question]

	Do you ever PRESCRIBE this option?	What SUB-CLASSIFICATION(S) of dry eye disease do you consider this treatment appropriate for (select as many as apply)?					What SEVERITY(S) of dry eye disease do you consider this treatment appropriate for (select as many as apply)?										Are you LICENSED to use this within your scope of practice in your country
	Prescribe (1)	Aqueous Deficient (1)	... (2)	Mixed (3)	... (4)	Evaporative (5)	Low (1)	2 (2)	3 (3)	4 (4)	5 (5)	6 (6)	7 (7)	8 (8)	9 (9)	Extreme (10)	Licensed (1)
Advice (e.g. hydration, healthy eating, office environment etc) (1)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Essential fatty acid supplements (2)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Low viscosity-enhancing lubricant PRESERVED (3)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
High viscosity-enhancing lubricant PRESERVED (4)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Low viscosity-enhancing lubricant UNPRESERVED (5)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
High viscosity-enhancing lubricant UNPRESERVED (6)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Ointment (7)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Lipid containing lubricants (drops/spray) (8)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Lid wipes / scrubs (9)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Anti-demodex lid wipes (10)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
In office demodex lid control (11)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Moisture chamber spectacles / goggles (12)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Punctal occlusion (with plugs) (13)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Home made warm lid compress such as face-cloth (14)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Commercially available warm lid compress / face mask (15)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Lid margin debridement (16)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
In office lid hygiene (e.g. BlephEx) (17)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Therapeutic meibomian gland expression (18)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

In office thermal pulsation of lids (e.g. LipiFlow) (19)																	
In office Intense Pulsed Light therapy (IPL) (20)																	
Topical antibiotics (21)																	
Topical azithromycin (22)																	
Systemic azithromycin (23)																	
Oral antibiotics (e.g. doxycycline) (24)																	
Topical corticosteriods (25)																	
Topical secretagogues (26)																	
Oral secretagogues (27)																	
Topical cyclosporine (28)																	

Topical tacrolimus (29)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Topical lifitegrast (30)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Autologous/allogeneic serum (31)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Therapeutic contact lens approaches (32)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Amniotic membrane (33)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Intraductal probing (34)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other surgical approaches (35)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Q6 Please add any further comments on your dry eye disease management philosophy.

____

Q7 What tests do you currently use to diagnose dry eye? [NOTE on some devices you may need to scroll to the right to see the whole question]

	Critical to diagnosis of dry eye	Aids diagnosis of dry eye	Aids classification of dry eye sub-type	Give details such as brand/model	Give value (e.g. 3mm) OR grade (e.g 3 out of 5)
	Critical to diagnosis (1)	Aids diagnosis (1)	Aids Sub-classification (1)	Instrument Used (1)	Cut-off for positive result (1)

Symptoms (verbal) (1)	☐	☐	☐		
Symptoms (questionnaire) (2)	☐	☐	☐		
Visual disturbance questions (3)	☐	☐	☐		
Aberrometry (4)	☐	☐	☐		
Light scatter (glare) (5)	☐	☐	☐		
Thermography (25)	☐	☐	☐		
Non-invasive breakup time (8)	☐	☐	☐		
Fluorescein tear breakup time (9)	☐	☐	☐		
Osmolarity value (10)	☐	☐	☐		
Osmolarity difference between the eyes (11)	☐	☐	☐		
Tear evaporation rate (12)	☐	☐	☐		

Tear volume (including Schirmer and Phenol red test) (13)	☐	☐	☐		
Tear ferning (14)	☐	☐	☐		
Corneal staining - fluorescein (15)	☐	☐	☐		
Conjunctival staining - lissamine green (16)	☐	☐	☐		
Lid margin staining (17)	☐	☐	☐		
Impression cytology (18)	☐	☐	☐		
Lid parallel conjunctival folds (19)	☐	☐	☐		
Confocal imaging (20)	☐	☐	☐		
Ocular surface sensitivity (21)	☐	☐	☐		
Ocular/conjunctival redness (22)	☐	☐	☐		
Matrix metalloproteinase level (23)	☐	☐	☐		

Cytokines and chemokine level (24)	☐	☐	☐		
Lipid layer appearance (Interferometry) (26)	☐	☐	☐		
Meibography (27)	☐	☐	☐		
Meibomian gland expressability (28)	☐	☐	☐		
Meibomian gland orifice assessment (29)	☐	☐	☐		
Blink/lid closure analysis (30)	☐	☐	☐		

Q11 Do you use any tests, other than those listed above, for diagnosing dry eye disease (please give details)?

_____

Q8 In your opinion, which tests need to show a positive result, in order to constitute a diagnosis of dry eye?

_____

Q9 Demographics

	Profession	Years of Clinical Experience	Country
			Name your Country of Practice (1)
Please complete (1)	▼ Ophthalmologist (1) ... Other (3)	▼ 0-5 (1) ... 21+ (5)	

Q13 Thank you for completing this survey. Click >> to submit

End of Block: Default Question Block

7. Publications

Wolffsohn JS, **Travé Huarte S**, Jones L, Craig JP, Wang MTM (2021). Clinical practice patterns in the management of dry eye disease: A TFOS international survey, The Ocular Surface, Volume 21, Pages 78-86, ISSN 1542-0124, https://doi.org/10.1016/j.jtos.2021.04.011. IF 5.033

Pult H, Khatum FS, **Trave-Huarte S,** Wolffsohn JS (2021). Effect of eye spray phospholipid concentration on the tear film and ocular comfort. Eye & Contact Lens 2021;00: 1–4). In Press. IF 1.521.

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Travé Huarte S, Evidence-based dry-eye therapies for clinical use – General Optical Council CET approved (*Chapter 1:).*

Awaiting submission

Dry Eye association to cardiovascular diseases and systemic lipid levels.